Neural Mechanisms of Selective Auditory Attention in Rats

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Abstract of the Dissertation

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How does attention modulate sensory representations? In order to probe the underlying neural mechanisms, we established a simple rodent model of modality-specific attention. Rats were trained to perform distinct auditory two-tone discrimination and olfactory odor discrimination in a two alternative choice (2AC) paradigm.

To determine auditory cortex's role in this frequency discrimination task, we used GABA-A receptor agonist muscimol to transiently and reversibly inactivate auditory cortexes bilaterally in rats performing simple interleaved auditory and olfactory discrimination. With olfactory discrimination performance serving as internal control for motivation and decision making capability, we found only auditory two-tone discrimination was selectively impaired in these rats. This shows the auditory cortex is involved in this two-tone discrimination task.

To investigate the neural correlate of modality-specific attention in the auditory cortex, we trained rats to perform interleaved auditory and olfactory blocks (of 50~70 trials each) in a single session. In auditory blocks, pure tones were either presented with or without a neutral odor (caproic acid, n=2 and 3 respectively), and subjects were rewarded for discriminating auditory stimuli. In olfactory blocks, both task odors and pure tones were presented simultaneously, and subjects were rewarded for discriminating olfactory stimuli. We recorded neural responses in primary auditory cortex (area A1) in freely moving rats while subjects performed this behavior. Single unit responses to tones were heterogeneous, and included transient, sustained, and suppressed. We found 205 of 802 units recorded responsive to the stimuli we used. Of these 205 units, 18.5% showed modality-specific attentional modulation of the anticipatory activity before tone onset. In addition, we also observed in smaller proportion of units (11.2%) modality-specific attentional modulation of the tone-evoked responses; in most cases, the responses to a particular auditory stimulus was enhanced in the auditory block (or, equivalently, suppressed in the olfactory block). Attention increased choice probability of the population in the auditory block. We have also observed significant behavior choice probability in small proportions of units.

Our results suggest that shifting attention between audition to olfaction tasks can modulate the activity of single neurons in primary auditory cortex.

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List of Symbols and Abbreviations

2AC	two-alternative choice
A	auditory
AI	primary auditory cortex
AII	secondary auditory cortex
ACSF	artificial cerebral spinal fluid
AM	amplitude modulation
BOLD	blood-oxygenation-level-dependent
CBF	cerebral blood flow
CPU	caudate putamen
dB	decibel
ERF	event related magnetic field
ERP	event related potential
FEF	frontal eye fields
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
Hz	hertz
IC	inferior colliculus
LFP	local field potentials
LIP	lateral intraparietal area
MGN	medial geniculate nucleus
µl	micro-liter

MI	modulation index
ms	milli-second
O	olfactory
PET	positron emission tomography
PID	photo-ionization detector
RF	receptive field
ROC	receiver operating characteristic
RT	reticular thalamic nucleus
SC	superior colliculus
SG	supragenicuate nucleus
SNR	signal-to-noise ratio
SPL	sound pressure level
SEM	standard error of the mean
STD	standard deviation
STRF	spectral-temporal receptive field
TE2	temporal cortical area 2
TE3	temporal cortical area 3

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Lastly, I would like to thank my parents for raising me and supporting my choice of being a scientist along the way.

Chapter 1

Introduction

The ability to selectively process relevant sensory information depending on the animal's internal state and to respond with appropriate action is essential to an animal's survival. Selective attention refers to the preferential processing of sensory information at many different scales, from modalities, objects, features to spatial locations (Egeth, 1967; Treisman, 1969; Posner and Boies, 1971). What are the general neural mechanisms underlying selective attention? This question has intrigued many neuroscientists. Selective attention in the auditory and visual system has been extensively studied with a variety of paradigms in both humans and animals. With the advance of single-unit recording and brain imaging technologies, we have begun to understand the neural correlates of selective attention (Reynolds and Chelazzi, 2004; Raz and Buhle, 2006; Fritz et al., 2007b; Hromadka and Zador, 2007; Knudsen, 2007).

Studies of visual cortical responses with single-unit recordings from monkeys have provided us great opportunities to peak into the general principle of how attention operates. For example, when attention is directed to locations within a neuron's receptive field (RF), it increases the neuron's sensitivity to visual stimuli presented in the neuron's RF (Spitzer et al., 1988; Motter, 1993; Treue and Maunsell, 1996; Ito and Gilbert, 1999; McAdams and Maunsell, 1999). When attention is directed to one of two competing stimuli inside the RF, the neuron's response is either increased or decreased depending on the neuron's selectivity for the two stimuli (Moran and Desimone, 1985; Treue and Maunsell, 1996). It is proposed that visual attention increases the effective saliency of the attended stimuli (Gottlieb et al., 1998; McAdams and Maunsell, 1999; Reynolds et al., 2000; Reynolds and Desimone, 2003). However, little is known about whether this principle also applies to activity of single neurons in the other cortical areas. In this thesis, I tried to address this question by studying attentional modulation of single neuron activity in the auditory cortex. Since attention can operate on many dimensions, I decided to focus on intermodal selective attention, i.e. preferential processing of sensory information from the attended modality.

Single-unit recordings from monkeys have provided evidence showing that attention modulates responses of auditory cortical neurons while the monkey is performing an intermodal selective attention task that uses combined auditory and visual stimuli. This modulation can affect even the earliest short-latency responses (Hocherman et al., 1976; Miller et al., 1980). Selective attention can either enhance or suppress the neuronal responses based on the relevant stimulus modality or whether stimuli in both modalities contradict each other (Hocherman et al., 1976). With this information in mind, I would like to extend our previous knowledge of intermodal selective attention to a well established rodent model, rats.

Rats offer several advantages as experimental preparations in auditory research. First, rats are relatively inexpensive to maintain. Second, the auditory system of rats has been extensively studied (Kelly and Masterton, 1977; Kelly and Sally, 1988; Sally and Kelly, 1988; Heffner et al., 1994; Kilgard and Merzenich, 1999; Doron et al., 2002; Polley et al., 2007). Third, the rapid development of new electrophysiological, molecular and imaging techniques in rodents provides us exciting opportunities to examine the auditory circuit at great detail, such as sub-threshold synaptic events (Wehr and Zador, 2003; Zhang et al., 2003; Wehr and Zador, 2005), viral-mediated molecular dissection of gene function (Rumpel et al., 2005), or even manipulating neuronal activity in-vivo (Boyden et al., 2005; Han and Boyden, 2007; Zhang et al., 2007) at the level of single neurons.

Before the main body of the thesis, I'll briefly review what we know from previous work about attentional modulation of neuronal activity in auditory cortex.

1.1 Attentional modulation in human auditory cortex

Our early knowledge on the neural bases of auditory selective attention in humans comes from event related potential (ERP) studies. The major components of human auditory ERP are N1, a negative wave peak at around 100 ms (thus also called N100) and P2, subsequent positive wave peak at 160-200 ms. In an intermodal attention task, the click-evoked N1-P2 response is significantly larger when the subject is attending to the click comparing to reading (Picton et al., 1971). In a dichotic listening task, the stimuli delivered to the attended ear elicited considerably larger N1 than the responses elicited by the same stimuli to the same ear when unattended (Hillyard et al., 1973). Later using event related magnetic fields (ERF), the equivalent current dipole for N100 in the attended condition, though significantly larger, was found to co-localize with the dipole moment for N100 in the non-attended condition within the auditory cortex (Woldorff et al., 1993; Fujiwara et al., 1998). This indicates attentional modulation of N100 amplitude come from enhanced neural activities within the auditory cortex. Recently, it has been shown that the N100 dipole distance between high and low frequency tones can undergo dynamic change during a pitch discrimination task, indicating short-term plastic changes in the primary auditory cortex by attention (Ozaki et al., 2004).

Studies using positron emission tomography (PET) in similar dichotic listening tasks also found increased cerebral blood flow (CBF) to auditory cortex in the hemisphere contralateral to the attended ear (Tzourio et al., 1997; Alho et al., 1999). Several functional magnetic resonance imaging (fMRI) studies found that increased auditory attention load caused proportional activation in the primary (AI) and second (AII) auditory cortex (Pugh et al., 1996; Jancke et al., 1999). In some studies, increased auditory attention load also recruited some areas in inferior frontal and inferior parietal cortices (Pugh et al., 1996; Jancke et al., 2003).

In several fMRI studies using intermodal selective attention task with competing auditory and visual stimuli (Woodruff et al., 1996; Laurienti et al., 2002; Petkov et al., 2004; Shomstein and Yantis, 2004), the general finding is that attending to an auditory task enhances the responses in auditory cortex to auditory stimuli. Conversely, attending to a visual task tends to suppress the activity in auditory cortex in response to the same auditory stimuli.

1.2 Auditory attention studies in animals

Ever since the use of tungsten microelectrode for recording from single units (Hubel, 1957), it is known that attention strongly modulates neuronal responses in the auditory cortex (Hubel et al., 1959). Hocherman et al. (Hocherman et al., 1976) trained Rhesus monkeys in an intermodal selective attention task in which monkeys have to detect visual (light from left or right) or auditory (tone or noise) stimuli and respond with lever pressing to corresponding left or right side to get rewards. They reported that two-thirds of units showed differences in response strength depending on whether sound or light was the relevant cue. About equal proportions of these units showed either enhanced or suppressed responses when sound was relevant. However, they also observed generally stronger unit responses when both modalities agree on the same motor response required for rewards. In both type of changes, they found that even the short latency onset response (15~20 ms) can be strongly modulated, which implies that the modulation effect may have already begun at the preceding stages of information transfer. Miller et al. (Miller et al., 1980) trained monkeys in an intermodal selective attention task using a key-release reaction time paradigm. They found that a shift from a visual to an auditory task caused a small but consistent increase in firing rate and a shortening of initial latency of responses in auditory cortical neurons.

In other studies, Hocherman et al. (Hocherman et al., 1981; Hocherman and Yirmiya, 1990) showed that responses of auditory cortical neurons as well as thalamic neurons in medial geniculate nucleus (MGN) can be modulated by anticipation of auditory signals (*intramodal* selective attention). Two different modulation effects were found in both auditory cortex and MGN. One involved facilitation of the responses to anticipated sounds and suppression of the responses to unanticipated sounds. The other involved facilitation of the responses to unanticipated sounds and no change of base line responses to anticipated sounds. The similarity of the modulation effects between cortex and the thalamus indicates that intramodal selective attention may affect subcortical auditory signal processing.

In line with the notion that attention may be able to influence early stages of auditory sensory pathway, several early study in cats (Hernandez-Peon et al., 1956; Oatman, 1971, 1976; Oatman, 1988) and a recent study in chinchillas (Delano et al., 2007) showed that visual attention during a visual discrimination task can significantly decrease cochlear sensitivity, measured by sound-evoked auditory-nerve compound action potentials and cochlear microphonics, and cochlea nucleus responses. In contrast, when the animal is performing an auditory discrimination task, little effect on the early auditory processing pathway was observed. However, these results do not rule out the possibility that auditory attention may still modulate neural activity at later stages in the auditory pathway.

Receptive-field properties can also undergo rapid changes under the influence of attention. Fritz et al. (Fritz et al., 2003; Fritz et al., 2005, 2007a) have shown that auditory stimulus under different behavior contexts can induce receptive-field plasticity based on the meaning of the stimulus at that context. They found that spectral-temporal receptive field (STRF) can be enhanced at target frequency that the animal is attending to while suppressed at reference frequency that the animal is supposed to ignore. This change is fast (in minutes) and sometimes long lasting.

1.3 Top-down versus bottom-up attention control

The deployment of attention can be driven by salient sensory input (bottom-up, involuntary) or guided by the subject’s intention (top-down, voluntary). In real life, the dynamic interaction between bottom-up and top-down attention determines what sensory information enters into our consciousness from moment to moment. The bottom-up process captures attention when a salient and/or singleton stimulus appears in the environment, while the top-down process directs attention to goal/task-relevant aspects of the sensory stream. How do these two attention processes interact? Psychophysical and neurophysiologic studies have shown that these two processes have different time course following the appearance of a salient and/or singleton stimulus (Kim and Cave, 1999; Bisley and Goldberg, 2003; Lamy et al., 2003; Ogawa and Komatsu, 2004). Bottom-up attention enhances neuronal sensitivity to the salient stimulus immediately after the onset response and fades away in a short time. Top-down attention then modulates neuronal sensitivity to the task-relevant feature in a slower time course. Therefore, examining the time course of neural activity modulated by attention may tease apart different components of attention mechanism.

1.4 The possible sources and mechanisms of attentional modulation

Studies of visual spatial and object-based attention in humans using fMRI have identified regions in the frontal and parietal cortex as potential sources for controlling the initiation and/or the maintenance of a desired attentive state. In a visual spatial attention task, sustained blood-oxygenation-level-dependent (BOLD) responses in regions of parietal and frontal cortex were found during a delay period following a spatial attention cue, suggesting that parietal and frontal areas may be involved in tonically maintaining the current locus of attention (Corbetta et al., 2000; Corbetta and Shulman, 2002). Similar parietal and frontal regions also showed sustained increase of activity in a study that requires subjects to covertly track a small dot appearing at different locations in the visual periphery (Beauchamp et al., 2001). In an object-based visual attention task, transient activity during shifts of attention between objects were found in frontal and superior parietal regions (Serences et al., 2004); functionally similar parietal areas were also found in previous studies of spatial attention shifts (Vandenberghe et al., 2001; Yantis et al., 2002). Since the demand for attention shifting and maintenance of attention differs from task to task, it's possible that parietal and frontal regions engage in both the initiation and the maintenance of attention depending on the task demand.

In monkey neurophysiology studies, feedback from parietal and frontal regions of cortex has been implicated in modulating the responses in the visual cortex. Elevated responses in the lateral intraparietal area (LIP) are associated with increased saliency or behavioral relevancy of stimuli (Colby et al., 1996; Gottlieb et al., 1998; Toth and Assad, 2002) and with increased contrast sensitivity in the receptive field at the behavioral level (Bisley and Goldberg, 2003). In the frontal eye fields (FEF), subthreshold microstimulation causes spatially localized increases in sensitivity both at the behavioral level and in visual cortical neurons (Moore and Fallah, 2001; Moore and Armstrong, 2003; Moore and Fallah, 2004).

How does the feedback from these potential sources of attentional modulation change neuronal sensitivity to attended stimuli in a way similar to increasing the effective contrast of the stimulus? Increased baseline spontaneous activity has been observed in some cases (Luck et al., 1997; Chawla et al., 1999; Reynolds et al., 2000). Increases in synchronization among afferent activities may also account for such attentional modulation of neuronal responsiveness. In monkey somatosensory cortex, Steinmetz et al. (Steinmetz et al., 2000) found a higher degree of synchronous firing between pairs of neurons in the secondary somatosensory cortex during a tactile discrimination task than a visual task. In macaque V4, Fries et al. (Fries et al., 2001) found increases in high frequency synchronization among small clusters of neurons when attention was directed to their overlapping receptive fields. It is still not clear how attention causes neuronal firing to become more oscillatory and better synchronized.

1.5 Thesis outline

This thesis is divided into five chapters. In chapter 2, I'll describe in detail the behavior setup and protocols I used to train animals. Chapter 2 also contains detailed descriptions of surgery, cortical inactivation, and electrophysiological recording as well as analytical methods used to analyze data.

Since this is the first time our lab adapted two-alternative choice (2AC) paradigms to train rats to discriminate sounds, in chapter 3, I'll describe characterization of rat behavior such as learning speed, reaction time and psychophysical measurement of discriminability regarding basic stimulus parameters such as stimulus frequency, duration and signal to noise ratio (SNR).

In chapter 4, I set out to investigate the role of auditory cortex in auditory two-tone discrimination task using 2AC paradigm. One advantage of using this paradigm is that there is internal control in each animal for motivational state and movement related issues using discrimination task in a second modality (olfaction) interleaved with auditory trials. I found that transient inactivation of auditory cortex selectively impaired the animals' ability to discriminate tones of different frequencies while leaving their ability to discriminate different odorants intact. This finding indicates that auditory cortical neural activity is involved in rats' frequency discrimination behavior in this task.

In chapter 5, I'll describe the results from tetrode recordings in search for neural correlates of selective attention in rat primary auditory cortex. I found that attention can modulate the activity of small portions of neurons in the auditory cortex. In most cases, the responses to a particular auditory stimulus was enhanced in the auditory block (or, equivalently, suppressed in the olfactory block). I also found that attention specifically increased population choice probability in the auditory block.

Finally, in chapter 6, I'll briefly summarize the results from chapter 3-5 and provides some perspective.

Chapter 2

Experimental Procedures

2.1 Experimental animal

I used male Long-Evans rats as experimental animals. The body weight at the beginning of training is 150~200gm. The body weight is 250~350gm at the time of surgery.

2.2 Behavior training setup

Animal training is performed in a 30 cm cubic box containing 3 conical nose cones (38 mm inner diameter, 38 mm depth). The floor composed of 17 stainless rod grids is raised at 65mm from the bottom of the box. The stimulus port is located at the middle of one wall at a height of 32 mm from the floor to center. Two choice ports are located 57 mm left and right of the center port (center-to-center). A pair of infrared photodiode and phototransistor was placed on either side of the nose poke at 15 mm depth from the surface to measure the occurrence and timing of nose pokes. The acoustic speaker (Stax SR-303, Stax Ltd., Japan) is positioned outside of the box at 65 mm from the wall with nose cones. Sound intensity was calibrated with Brüel & Kjær type 4939 free-field microphone, Type 2670 1/4-inch Microphone Preamplifier and Type 2690A0S2 2-Channel Microphone Conditioning Amplifier (Brüel & Kjær Sound & Vibration Measurement A/S, Denmark). The microphone was positioned in front of the stimulus port at rat ear’s position. The training box with water delivery valves are maintained in a single walled sound booth (Industrial Acoustics Company, NY). Odorant delivery is controlled by a custom built olfactometer consist of 3-way and 2-way air valves (Neptune Research, FL) located outside of the sound booth to minimized acoustic signal from the valves during odorant delivery.

A custom Matlab program (The MathWorks, Inc., MA) developed by Mainen and Tai controls two RP2 or RM1 real time processors units (Tucker-Davis Technologies, FL). One RP2 or RM1 is responsible for monitoring the nose poke signals and control the odor delivery and water delivery system. A second RP2 or RM1 is used for acoustic stimulus generation with sampling rate of 100Hz. All auditory stimuli were multiplied by an envelope with 5 ms cosine square rise and fall time. Training protocols are written in way of modules that allow ample flexibility to change parameters on the fly and display graphic feedback of the animal performance and related analysis.

2.3 Behavior training procedures

Animals were first habituated to the behavioral apparatus and experimenter (1 week). Animals were then mildly water deprived under a protocol approved by the Cold Spring Harbor Laboratory Animal Committee. Training and testing were conducted in a custom three-port computer-controlled behavioral apparatus as shown in figure 2.1A. Animals were initially trained to perform an auditory task following similar steps as described in Uchida and Mainen (Uchida and Mainen, 2003) shown in figure 2.1B. The task consisted of the discrimination between two different pure tones, at 60-65 dB SPL, free-field: a low frequency one and a high frequency one. Subjects initiated trials with a center-poke, which triggered presentation of a tone (either low or high frequency) after a random delay of 100~150 ms. Subjects responded by moving to the left or right port. Correct responses were rewarded with ~25 µl of water.

For experiments in chapter 3.1, seventeen animals were trained to discriminate 2 kHz and 15 kHz 200 ms pure tones at 60dB SPL. For experiments in chapter 3.2, six animals were first trained to discriminate 1 kHz and 15 kHz pure tones at 60dB SPL embedded in broadband white noise (60dB SPL, 20 Hz ~ 48 kHz). For experiments in chapter 3.3, four animals were first trained to discriminate 6.3 kHz and 10 kHz 200 ms pure tones at 60dB SPL. After the animals reached a performance of >85%, the stimulus parameters were systematically varied to allow us to measure the effect of SNR, stimulus duration or frequency on psychometric functions of this discrimination behavior.

For experiments in chapter 4, four animals were first trained to discriminate 1 kHz and 15 kHz 200 ms pure tones at 60dB SPL. After animals reached a performance of >85%, they were trained to perform an olfactory discrimination task that consisted of discrimination between caproic acid and hexanol. After stable olfactory performance is reached, auditory and olfactory trials were interleaved so that a center-poke would trigger presentation of either a tone or an odor. These animals were then tested in the same trail structure with cortical inactivation as described in chapter 2.5.

For experiments in chapter 5, four animals were first trained to discriminate 6.3 kHz and 14.1 kHz pure tones at 60dB SPL. After animals reached a performance of >85%, five animals were trained to perform an olfactory discrimination task that consisted of either the discrimination between caproic acid / hexanol (2 animals), R(-)-2-octanol /

S(+)-2-octanol (1 animal), or R(-)-2-octanol / S(+)-2-octanol mixture (ratio of 70/30 vs 30/70, 2 animals). I hypothesize that discrimination between odorant mixtures of stereo-isomers will increase the olfactory task difficulty. I then trained these animals to perform both auditory (A) and olfactory (O) tasks in alternating blocks (AAA.../OOO...). After a few sessions, I then trained the animals to perform in alternating auditory-only and olfactory with sound distracters (Oa) blocks (AA.../OaOa.../AA...), which is called the asymmetrical task. The first three animals performed the asymmetrical task. For the other two animals, I introduced a non-meaningful null odor (caproic acid) in the auditory block and the animals performed a pseudo-symmetric task (AoAo.../OaOa.../AoAo...). Results from these five rats were similar and were pooled together. The blocks lasted for ~50-70 trials. The animal performance was 80.75% during the auditory blocks and 90.73% during the olfactory blocks, indicating that the animals understood the block structures of the task

2.4 Surgery

All surgical procedures were approved by the Cold Spring Harbor Laboratory Animal Committee. Animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (60mg/kg) and medetomidine (0.51 mg/kg). Wounds were infiltrated with lidocaine. For bilateral inactivation of auditory cortices, the temporal muscles were recessed and craniotomies, 2mm in diameter, were performed to expose both primary auditory cortices stereotaxically. The dura mater was left intact. A custom made plastic well was implanted around each craniotomy and the craniotomy was covered with

KWIK-CAST Silicone Casting Compound (World Precision Instruments, Inc., FL). For tetrode implants in the left primary auditory cortex, the temporal muscle was recessed and a craniotomy and a duratomy were performed. Tetrodes were implanted under anesthesia into left auditory cortex with stereotaxic coordinate: 4.5~5.5 mm posterior to Bregma and 6.3mm left from midline (Paxinos and Watson, 1998). After surgery, animals were left to recover for several days before resuming water deprivation.

2.5 Cortical inactivation

After the animals' performance reached criteria (85%), bilateral access wells were implanted over the auditory cortexes. GABA-A receptor agonist muscimol was used to reversibly inactivate neuronal activity bilaterally in the rat auditory cortex while animals performed auditory frequency discrimination and odor discrimination tasks in randomly interleaved trials. Artificial cerebral spinal fluid (ACSF) or muscimol (12.5µl, 3.44µg/µ1) was topically applied through the dura with gelfoam on alternating days. After two days of recovery, behavior tests were conducted an hour after the application of ACSF or muscimol for up to 10 days. I found that after 10 days the connective tissue on top of the dura mater became thickened so that I could not get reliable inactivation effects on the discrimination behavior. Olfactory performance served as an internal control for whether general ability of the rats to perform a similar discrimination task is compromised.

2.6 Tetrode recording

I implanted polyimide-coated nichrome wires (H.P. Reid, Inc., FL; wire diameter 12.5 μm) twisted in groups of four as tetrodes. Each wire was gold plated with impedance ranging between 300 and 400 kΩ at 1 kHz. I implanted six independently movable tetrodes using a custom-built drive designed by Gonzalo Otazu (see appendix 2). I used a nearby stationary nichrome wire implanted at the same time as a reference. One skull screw on the right hemisphere was used as ground. I recorded spiking activity and local field potentials (LFP) with a Cheetah32 32 Channel System (Neuralynx, Inc., AZ). To detect the spiking activity, I filtered the signal between 900 Hz and 6 kHz. When a threshold crossing event in any of the four leads was detected, a 1 ms waveform was acquired at 32 kHz.

The sampled waveforms were manually clustered using MClust (A. D. Redish et al.; http://mclust.sourceforge.net). Clusters were included in the analysis only if the following criteria were met: (1) <1% refractory period violations; (2) an isolation distance (ID; (Schmitzer-Torbert, Jackson et al. 2005)) of more than 15, calculated based on Peak, Valley, Energy, FFT and PC1 (Principal Component 1); (3) clusters were stable for at least 150 trials to include at least one auditory and olfactory block. The ID is defined as the Mahalanobis distance from the center of an identified cluster within which as many spikes belong to the specified cluster as others.

Each day, each tetrode was independently advanced until I could observe stable spiking activity. I did not specifically sample for sites that were responsive to the stimulus ensemble. I advanced the electrodes at least 40 μm every day to avoid having multiple recording sessions with the same subset of cells. Tetrode tracks were labelled by making small lesions right before the end of the experiments and recovered from brain histology slices. I confirmed the recording sites were in the primary auditory cortex (appendix 3).

2.7 Data analysis

For experiments in this thesis, all plots are mean±standard error of the mean (SEM). All statistical tests was measured using student test with $p<0.05$ as criteria for significance unless otherwise specified.

For figure 3.1, I analyzed 178~578 trials (mean 339 trials) per session. For figure 3.2A, I analyzed 1409~1687 trials per rat. For figure 3.3, I pooled data from all rats and analyzed 5267~9207 trials per SNR. For figure 3.4A-B, I pooled data from all rats and analyzed 1453~2974 trials per SNR for mixed SNR condition. Same SNR condition is the same data from figure 3.3. For figure 3.5A I analyzed 787~3997 trials per stimulus duration. For figure 3.5C, I analyzed 1901~4199 trials per SNR for 25 ms stimuli and 3062~5626 trials per SNR for 200 ms stimuli. For figure 3.5D, I analyzed 2099~2900 trials per SNR for 25 ms stimuli and 2268~3152 trials per SNR for 200 ms stimuli.

For figure 3.6A, each high-low frequency combination is probed with 1~11 sessions with a total of 170 sessions and an average of 4.5 sessions for each combination. Each session has an average of 807 trials. For figure 3.6B, the performance-frequency difference curve was fitted with a logistic function. To calculate Weber ratio, the frequency difference that gives rise to 25% and 75% of trials reported high were derived from the logistic function. Weber ratio if the average of the distance of the two frequency difference to zero as shown by the following equation:.

$$Weber\_ratio = \frac{|FD@25\%| + |FD@75\%|}{2}$$ FD: frequency difference

For experiments in chapter 5, the responsiveness of single units to each of the two stimuli was determined by comparing the spike count in a 40 ms window before the onset of the stimulus with the response of another 40 ms window between 10 and 50 ms from the onset of the sound. I used a Wilcoxon signed rank test and set the significance level at $p<0.05$. For attentional modulation of either the anticipatory spontaneous activities or the onset responses, I compared firing rate in the corresponding time windows with a Wilcoxon rank sum test and set the significance level at $p<0.05$. For table 5.2, I corrected the significant level of Wilcoxon rank sum test for multiple comparisons assuming all events are independent. For two events I set $p<0.0253$ and for three events $p<0.017$ in order to get the combined $p<0.05$ significance level. For figure 5.10, modulation index (MI) was calculated by taking the difference in firing rate between auditory block and olfactory block and divide it by the sum of the firing rate in both blocks. Modulation Index ranges from -1 to 1.

$$Modulation\_Index = \frac{|response\_in\_auditory\_block| - |response\_in\_olfactory\_block|}{|response\_in\_auditory\_block| + |response\_in\_olfactory\_block|}$$

Choice probability is obtained by calculating the area under the ROC curve when comparing firing rate for preferred choice to non-preferred choice. Preferred choice is the corresponding reward port of the stimulus that evoked higher onset response in correct trials. For figure 5.12, the significant level for ROC analysis was accomplished using permutation of 1000 repeats and set the significant level to $p<0.05$.

Figure 2.1

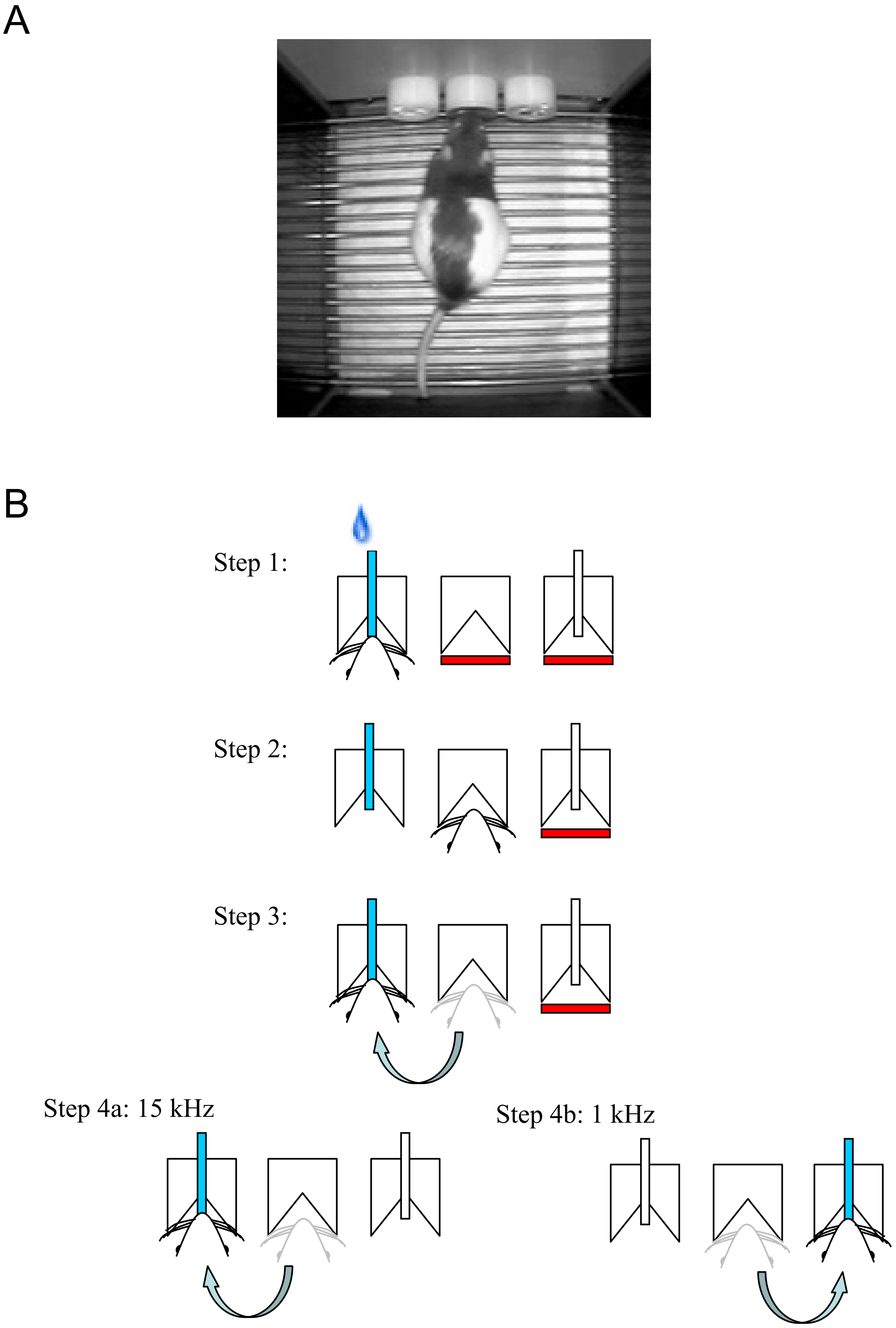

Figure 2.1. **A**. Snapshot of a rat performing a two-alternative choice two-tone discrimination task. The rat is shown making a nose poke at the central port to trigger the delivery of a tone. After making a center poke, the rat is rewarded for making a nose poke at the correct choice port depending on the frequency of the tone. **B**. Example training steps: *Step 1*: Water is delivered when the rat makes a poke into water port. The other ports are blocked. *Step 2*: The tone paired with the open water port is played when the rat makes a center poke. Water is then delivered to the water port. *Step 3*: Same as step 2, only water is delivered after the rat makes a second poke into the open water port. *Step 4*: After making a center poke, the rat is rewarded with water when making a nose poke into the correct port depending on the tone frequency.

Chapter 3

Two-tone discrimination behavior in rats

The ability of rats to discriminate fine frequencies has been demonstrated in earlier studies (Syka et al., 1996; Talwar and Gerstein, 1998). In these studies, they asked the rats to detect a frequency change in two sequential tones or a series of tones. By measuring the amount of change in frequency for a tone to be perceived as a different stimulus, one can derive the psychophysical Weber ratio. Weber ratio in these studies refers to the ratio of the change in frequency that has 50% chance of being detected to the original frequency. This measure is quite similar across the rats' hearing range at modest sound intensity. The average Weber ratio for detecting frequency change is 5.7% in two sequential tones, and 3.1% in a series a tones.

In an ideal selective attention study, one would like to record neural activity in response to the same stimulus when the subject is either attending or not attending to the stimulus. In the above mentioned studies, frequency discrimination involves presentation of multiple tones separated by short durations (300~350 ms) of silence. The forward masking effect between sequential tones ((Harris and Dallos, 1979; McFadden and Wright, 1987; Wright and McFadden, 1992; Calford and Semple, 1995; Brosch and Schreiner, 1997; Tai and Zador, 2002; Wehr and Zador, 2005)) also has to be considered in addition to any attentional manipulation one would like to study using these paradigm.

I adapted a two alternative choice paradigm developed by Uchida and Mainen (Uchida and Mainen, 2003) to train rats to discriminate different acoustic stimuli. This task requires the presentation of only one stimulus per trial. When a tone, or any other auditory stimulus, is simultaneously presented with a stimulus from another modality, one can cue the rats to attend and respond to the auditory or the other modality and study the effect of modality specific attention on the response to the same auditory stimulus.

In this chapter, I first demonstrate that rats can learn to discriminate two tones of different frequency in very short time (~2 week). They can achieve high accuracy performance in this 2AC two-tone discrimination paradigm. The fast reaction time allows large number of trials (>300 trials) to be recorded in a single session. Then I characterize how stimulus parameters such as signal-to-noise ratio and duration affect two-tone discrimination behavior in rats. I also compare the rats' ability to discriminate two tones of different frequencies under this 2AC paradigm to previous studies by measuring the Weber ratio.

3.1: Fast learning of two-tone discrimination task

I trained seventeen rats to discriminate two pure tones (2 kHz and 15 kHz 200 ms at 60dB SPL) following the steps in figure 2.1B. After initial shaping for about a week, when the animals reached step 4, I started to record their performance as shown in figure 3.1. Most rats can reach a performance of 85% with in 7 ~ 9 days. Therefore the total training time is about two weeks.

3.2: Discrimination of two tones in noise

I trained six rats to discriminate two pure tones embedded in 60dB broad band white noise (20Hz~48kHz). This allows me to change the task difficulty through manipulating the signal-to-noise ratio (SNR) by changing the intensity of the tones embedded in the white noise. The frequencies of the two tones are 1 kHz and 15 kHz respectively. The initial intensity of the two tones is 65dB SPL. The rats were trained to near asymptotic performance before testing began. Rats were initially allowed to control the stimulus duration (100, 200 or 300 ms) by varying their center nose poke duration. The idea is to see whether the rats will increase their "sampling" time to stimuli with low SNR. Later the stimulus duration was specifically controlled by the experimenters. Stimuli of the same SNR were first presented in a session and the SNR was gradually lowered. Later tones of different SNR are randomly interleaved in a session. Each of six rats was tested in one session (>300 trials) per day and 5–8 sessions per condition, yielding a total of 100,068 trials. Among these, I discarded ambiguous trials where the rat did multiple center pokes and the resulting 88478 trials were used for final analysis.

I begin by describing the discrimination performance of experiments with rat-controlled stimulus duration. I then describe the discrimination performance in experiments with systematically varied stimulus duration.

3.2.1 Fast and accurate performance at high SNR

In order to acquire basic parameters for this discrimination behavior, all six subjects were first tested with the highest SNR, 65dB SPL pure tones in white noise. Performance was very good for all subjects (mean ± SEM across animals: 95.12% ± 0.23%, figure 3.2A). Tone poke duration is defined as the interval between an in and an out events at center port (figure 3.2B). Typical tone poke duration for correct trials is around 124~244 ms (mean ± STD: 184.71 ± 60 ms, figure 3.2C). Reaction time is defined as the interval between the stimulus onset and the first in event at any water port. The reaction time for correct trials typically ranges from 323~546 ms (mean ± STD: 434.7 ± 111.4 ms).

3.2.2 Performance decreases with decreasing SNR

In order to probe the subjects' performance under different SNR and estimate the difficulty of each SNR, the subjects were subsequently tested with lowered SNR: 60dB, 55dB and 50dB SPL pure tones in white noise. Animals were tested at a single SNR on any given session. The performance declines with decreasing SNR (figure 3.3A). Tone poke durations for false trials are slightly shorter than those of correct trials (figure 3.3B). There is also a slight increase of tone poke duration as SNR decreases. Since tone poke duration also controls the stimulus duration, I also observed that the performance was slightly better for trials with longer (200 and 300 ms) tone duration (figure 3.3C).

In order to further probe the animals' performance at even lower SNR level without compromising the ratio of rewarded trial in a session, one key factor for maintaining the animals' motivation to perform this task, I mixed stimuli with different SNR within a session (figure 3.4). Performance showed similar declining trend with decreasing SNR. At high SNR (60~65dB SPL), performance is even higher than that for the same SNR levels presented alone in a session. This enhancement of performance indicates that even the performance has reached asymptote for the same-SNR sessions, mixing different SNR levels in a session can make high SNR stimuli even easier to discriminate. At 0dB

SNR, while only white noise is presented, the performance is close to chance level.

3.2.3 Reaction time for error trial is long

In the experiment where animals were tested at a single SNR on any given session, the reaction time for correct trials does not change significantly with decreasing SNR (figure 3.3D). Since increased tone poke duration is correlated with improved performance, one would predict that slower movement and longer reaction time for correct trials. However, just the opposite is observed, reaction time for error trials is generally longer than correct trials. This is more obvious at high SNR. This indicates that after withdrawal from the center port, the movement to reward port is much faster and perhaps more "ballistic" in correct trials than in error trials.

In the experiment where animals were tested with mixed SNR in a session, consistent with previous result, I also found the reaction time of error trials in this experiment is longer than that of correct trials (figure 3.4B).

3.2.4 Performance decreases with shorter stimulus duration

Most of the stimulus duration in rat-controlled sessions fell between 100 ms and 300 ms. Sorted performance according to stimulus duration in these sessions shows that the performance for 200 ms and 300 ms trials are in general higher than that of 100 ms trials (figure 3.3C). However, we can not rule out the possibility that the tone poke duration can by itself affect the performance. In order to understand the effect of stimulus duration on rats' performance in this task, I specifically controlled the duration of the tones delivered in the following task.

Animals were first tested with single stimulus duration at the highest SNR (65dB SPL tones) in any given session. The performance did not change with shortening stimulus durations I tested (figure 3.5A). The reaction time of error trials is consistently longer than that of correct trials (figure 3.5B).

Without significant effect on the performance with the shortest tone duration at the highest SNR level, the subjects were subsequently tested with 200 ms or 25 ms stimuli respectively at lowered SNR: 60dB, 55dB and 50dB SPL tones embedded in white noise. Each stimulus duration and SNR is presented in a given session. Compare to the performance for 200 ms stimuli sessions, the performance of 25 ms stimuli sessions showed steeper decline with decreasing SNR (figure 3.5C).

In order to compare the performance under even lower SNR, I tested the animals' performance with mixed SNR in a given session for stimulus duration 200 ms and 25 ms respectively (figure 3.5D). The performance for 200 ms stimuli is significantly higher than the performance for 25 ms stimuli at mid-SNR range (45~55dB SPL tones). This indicates that integration through longer stimulus duration can indeed help the animals do better in this task.

All subjects were then tested with mixture of all the possible SNR and stimulus durations in a given session (data not shown). Surprisingly, I did not find significant difference in the performance for the stimulus durations used in these experiments. One possible explanation is that the rats adapted their performance to difficult stimulus parameters in a session, in this case, shorter stimulus duration, and did not exploit the information from longer duration stimulus. Indeed, the performance in these sessions at mid SNR (45~55dB SPL tones) is lower than the performance at the same SNR in the experiment where only 200 ms stimuli were presented.

3.3: Weber ratio of frequency discrimination in rats

In order to compare the frequency discriminability in rats using my paradigm with previous studies, I trained another four rats to discriminate pure tones of close-by frequencies within 1/2 octave. The subjects at first learned to discriminate 6.3 kHz and 10 kHz 200 ms pure tones at 60dB SPL. After the animals have reached asymptote performance, the frequencies were adjusted gradually toward the center frequency at 7.9 kHz. In any given session, the frequencies of the two pure tones were kept constant. I probed the animals' ability to discriminate nearby frequencies with the low and high frequency combinations shown in figure 3.6A. On average each combination is tested in 4.5 sessions. I than sorted the performance according to the frequency difference between the two tones in each frequency combination (figure 3.6B). Weber ratio was calculated from a logistic function fitted to the performance-frequency difference curve. The Weber ratio in this task is 0.1 octave or 7.2% in frequency near 7.9 kHz at 60dB SPL. This Weber ratio is higher than previous studies using a frequency change detection task (Syka et al., 1996; Talwar and Gerstein, 1998). This is probably due to the fact that the animals do not have perfect memory of the perceptual boundary. And in this task they are required to make their judgment based on the comparison between the perceived tone and the memorized perceptual boundary instead of immediately preceding reference tones as in previous studies.

The strain of rats I used (Long-Evans) is the same as in the study by Syka et al. (Syka et al., 1996) while Talwar et al. (Talwar and Gerstein, 1998) used Wistar rats in their study. It is unlikely that the difference in frequency discriminability between my result and previous studies is due to rat strain differences.

Figure 3.1

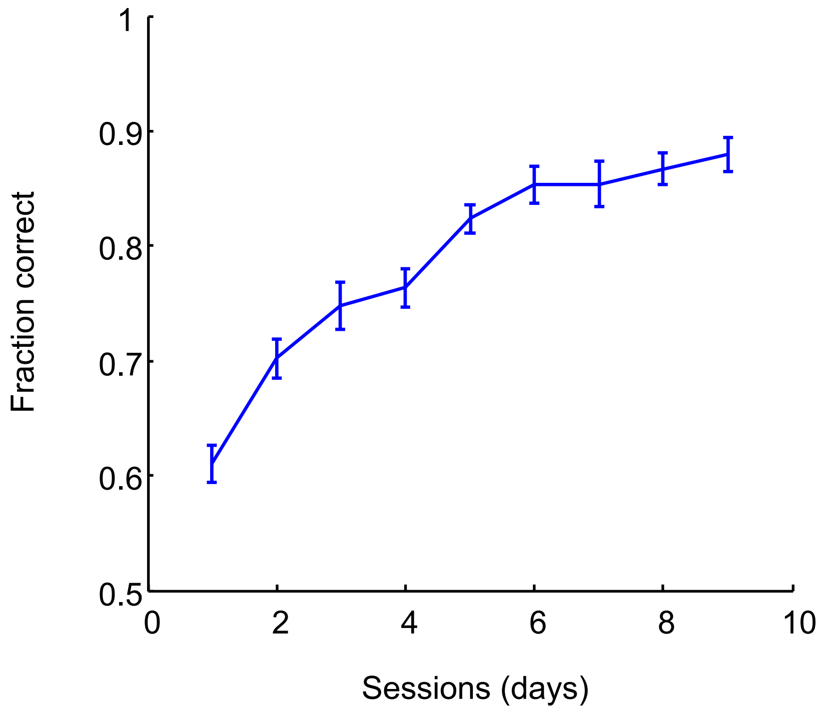

Figure 3.1. The two-tone discrimination performance as a function of training time (step 4 in figure 2.1) after initial shaping. Data pooled from 17 rats.

Figure 3.2

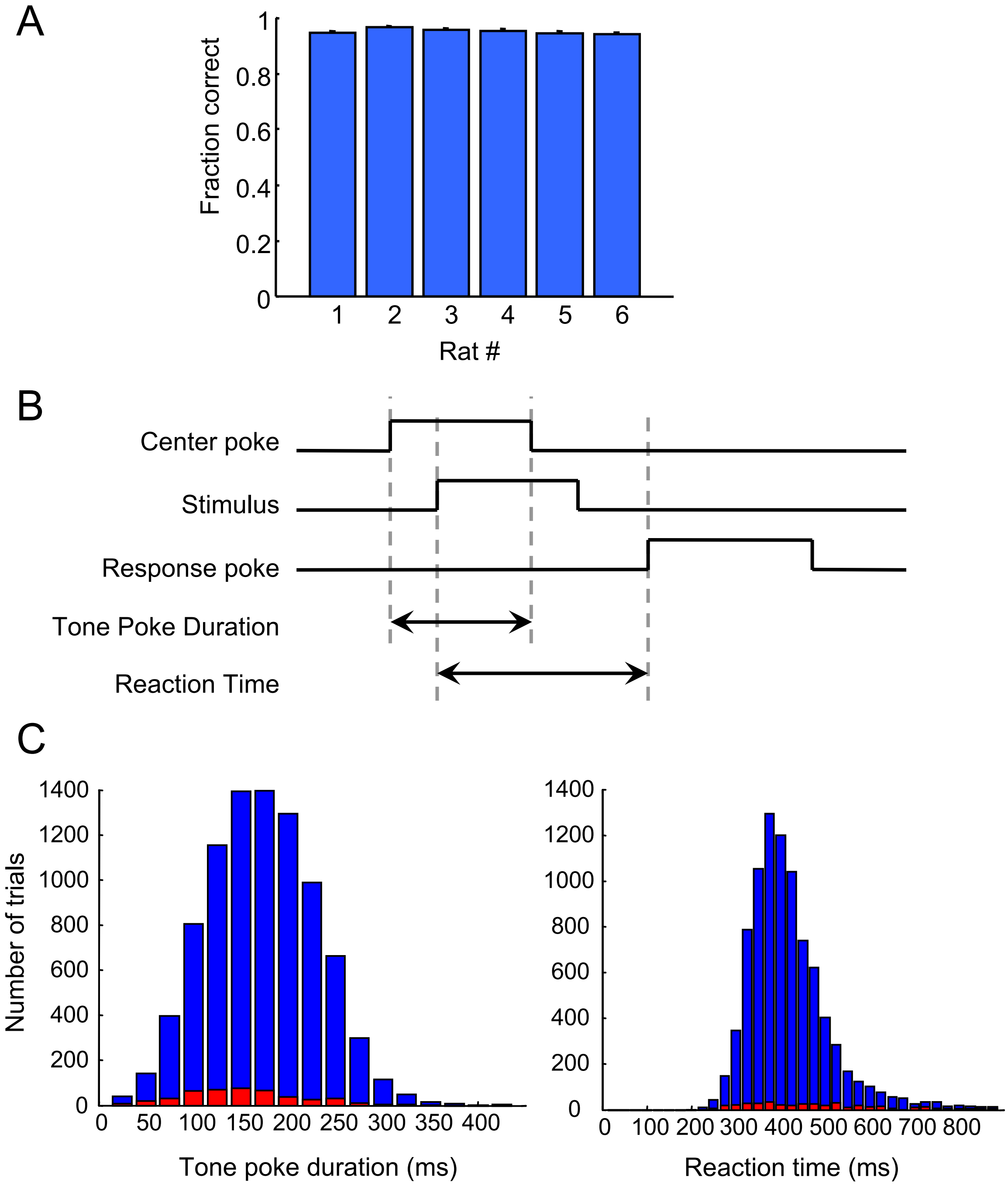

Figure 3.2. **A**. All six rats performed extremely well in high SNR condition. **B**. Event timing diagram showing the definition of tone poke duration and reaction time. **C**. Distribution of tone poke duration and reaction time. Blue: correct trials. Red: Error trials.

Figure 3.3

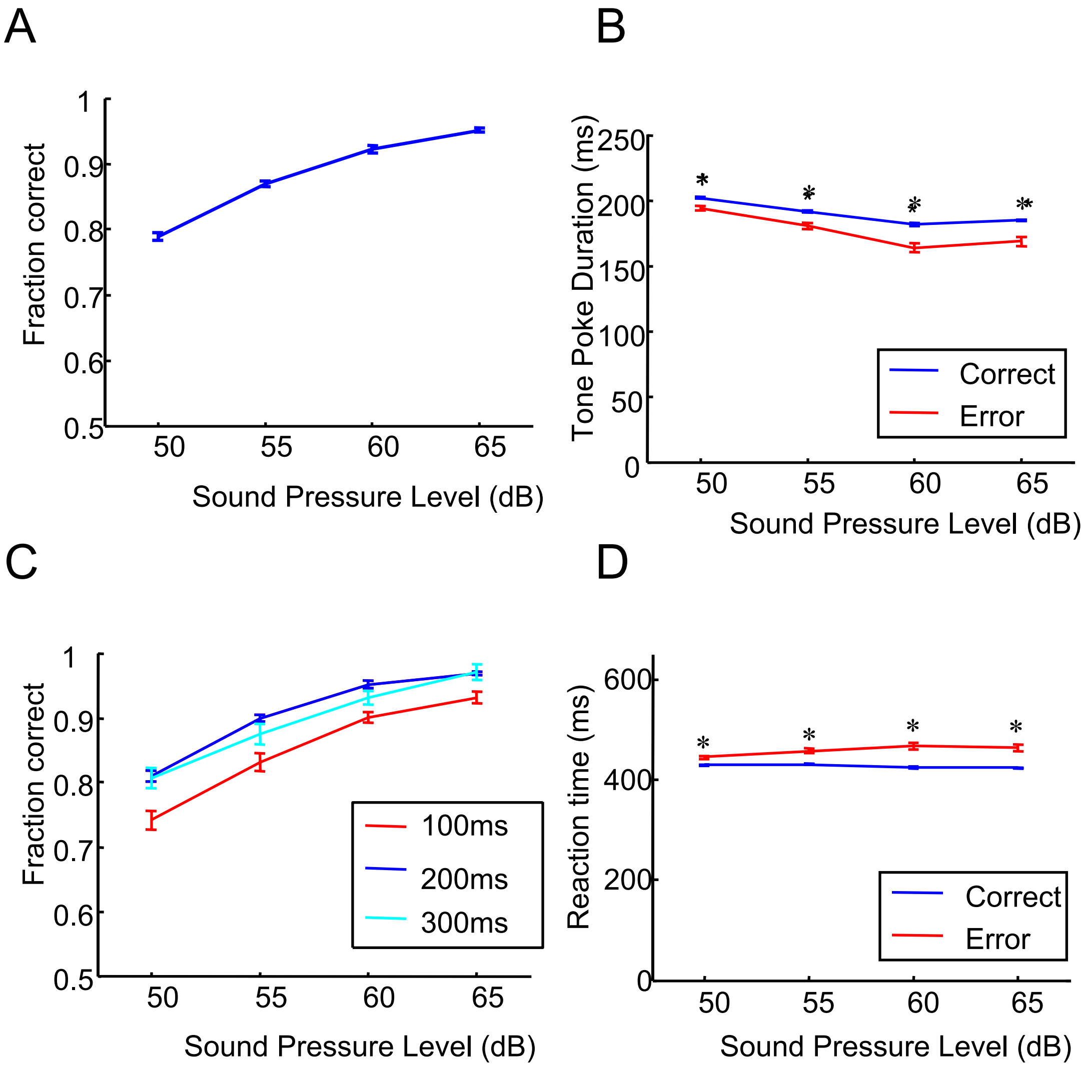

Figure 3.3 Results from experiments with same SNR per session and rat-controlled stimulus duration **A**. Performance drops as the SNR decreases. **B**. Tone poke durations for false trials are slightly shorter than those of correct trials at all SNR tested. **C**. Performance sorted according to stimulus duration. The performance of 200ms- and 300ms-trials is higher than that of 100ms-trials. **D**. Reaction time is quite constant for correct trials across SNR. Reaction time for error trials is generally longer than correct trials.

Figure 3.4

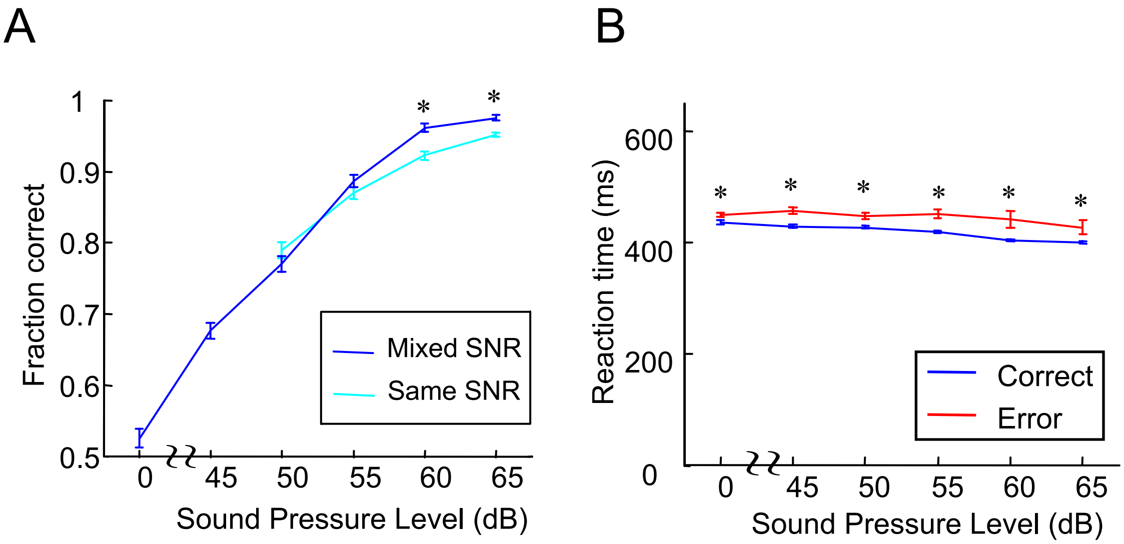

Figure 3.4 Results from experiments with mixed SNR per session and rat-controlled stimulus duration **A**. Performance drops as the SNR decreases. At high SNR, the performance from mixed-SNR sessions (blue) is even better than from same-SNR sessions (cyan). **B**. Reaction time is slight longer for low SNR trials. Reaction time for error trials is generally longer than correct trials.

Figure 3.5

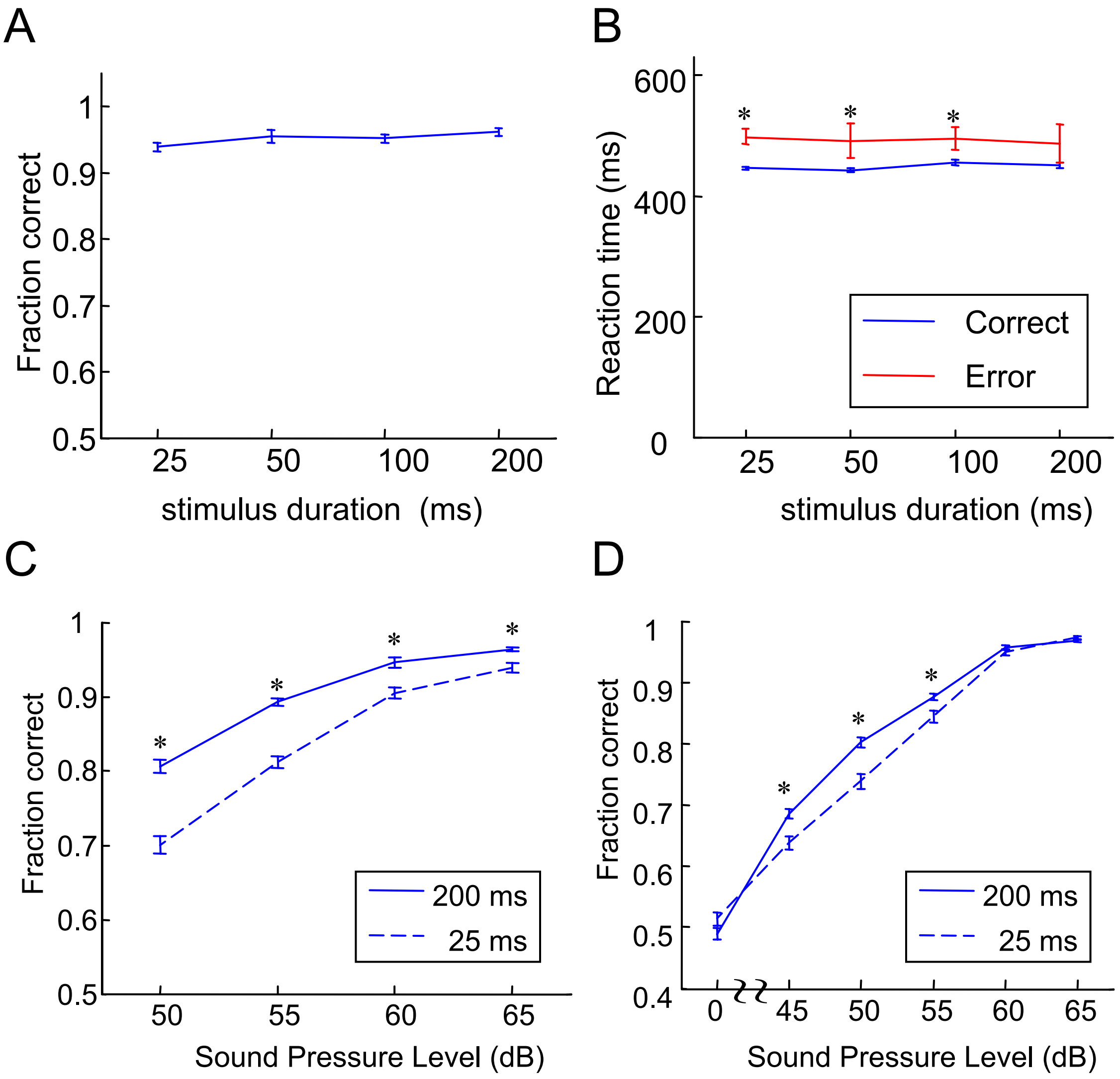

Figure 3.5 **A**. Performance as a function of stimulus duration at high SNR (65dB SPL tone). **B**. Reaction time for error trials is consistently longer than correct trials in sessions with various stimulus duration. **C**. Performance from fixed-SNR and fixed-stimulus duration sessions. **D**. Performance from mixed-SNR and fixed-stimulus duration sessions.

Figure 3.6

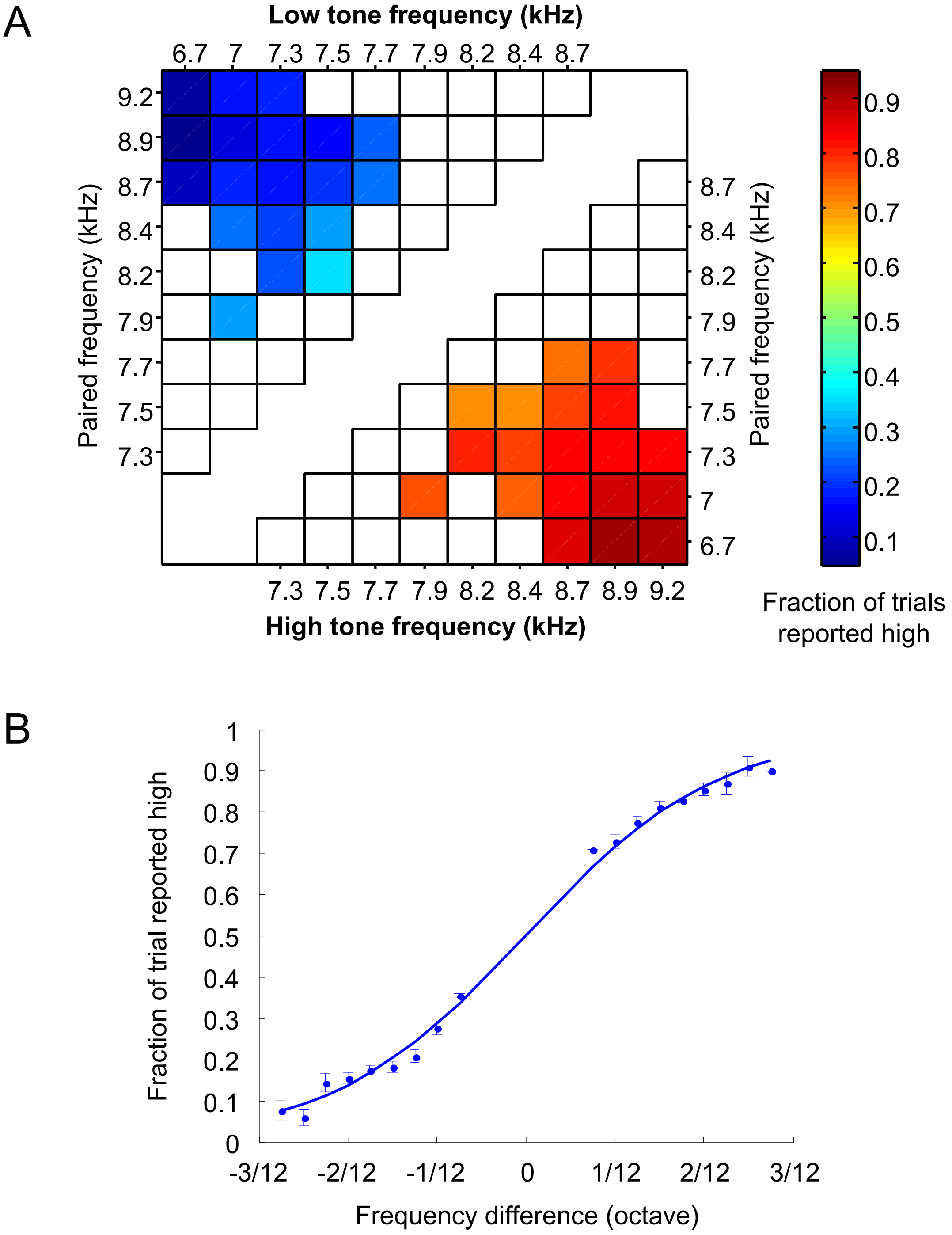

Figure 3.6 **A**. Frequency discrimination performance for tested (colored) frequency combinations. Each combination results in two colored panels. **B**. Psychometric curve for frequency discrimination.

Chapter 4

The role of auditory cortex in two-tone discrimination behavior in rats

The role of auditory cortex in rats' ability to discriminate basic sound properties has been studied with lesions (Kelly, 1970; Kelly, 1980; Cooke et al., 2007). In the sound properties studied, frequency discrimination thresholds, sound sensitivity and sound localization ability seems to be unaffected by extensive ablation of auditory cortical areas. Only the threshold for detecting amplitude modulation (AM) of a broadband noise carrier at fast rate (100~1000Hz) was affected. This indicates that the rat auditory cortex is more concerned with temporal modulation of an auditory stimulus than other basic sound properties.

However, one can not rule out the possibility that cortical reorganization after surgery restores in animals the ability to perceive these basic sound properties. Talwar et al. (Talwar et al., 2001) have shown that after acute inactivation of the auditory cortex by GABA-A receptor agonist muscimol, the rats exhibited profound inability to detect tones. The hearing slowly recovered after a few hours and the rats regained the ability to detect tones followed by the ability to discriminate frequencies. This result argues that the auditory cortex is both involved and necessary for any sound perception.

The frequency discrimination paradigm used in the study by Talwar et al. requires the animal to self-report detected change in tone frequency. Therefore, any change in the animals' internal state that affects self-reporting behavior may also be mistaken as a change in the ability to detect frequency change. Since we have no way of knowing the effect of muscimol on these animals' internal motivational state or decision making capability without proper controls, one should be more conservative in interpreting the result.

4.1 Inactivation of auditory cortex impaired two-tone discrimination

To determine auditory cortex's role in two-tone discrimination while at the same time controlling for animals' internal state, I trained four rats to perform a simple auditory and olfactory discrimination task in randomly interleaved trials. I then used muscimol to transiently and reversibly inactivate auditory cortexes bilaterally in rats performing this discrimination task in both modalities. With olfactory discrimination performance serving as an internal control, I found muscimol application specifically impaired the performance of auditory two-tone discrimination in these rats but not olfactory discrimination (figure 4.1). This shows that muscimol application did not interfere with the normal decision making capability or motivational state of the animal. Under my experimental conditions, however, some residual ability to discriminate the two tones still persisted after inactivation of the auditory cortex.

My result shows that the auditory cortex is involved in this two-tone discrimination task. This result supports the conclusion of Talwar et al. (Talwar et al., 2001) that the auditory cortex is involved in sound frequency perception. However, I did not find a total loss of two-tone discrimination performance as would be predicted by the observation of Talwar et al. Their rats exhibited profound inability to detect tones soon after muscimol application and lasted for up to 5 hours. It is unlikely that I did not inactivate the auditory cortex completely. In my experiment, I used even higher dose of muscimol (43 μg versus 20 μg) and waited an hour after muscimol application for it to diffuse into the cortex before testing the animals. Therefore it is possible that tone detection is not explicitly required for frequency discrimination in my task.

Another difference between the two studies is the stimuli I used, 1 kHz and 15 kHz tones versus 8 kHz and 9.6 kHz tones. The fact that the animals can still perform two-tone discrimination at above chance level argues that some ability to discriminate broader frequency difference is still preserved after muscimol inactivation. This could be achieved by functional compensation from other brain areas or the animals may also use other sound properties such as perceived loudness difference to help them perform the two-tone discrimination. In any case, my results do not support the broader claim by Talwar et al. that the cortex appears to be both involved in and necessary for any sound perception.

Figure 4.1

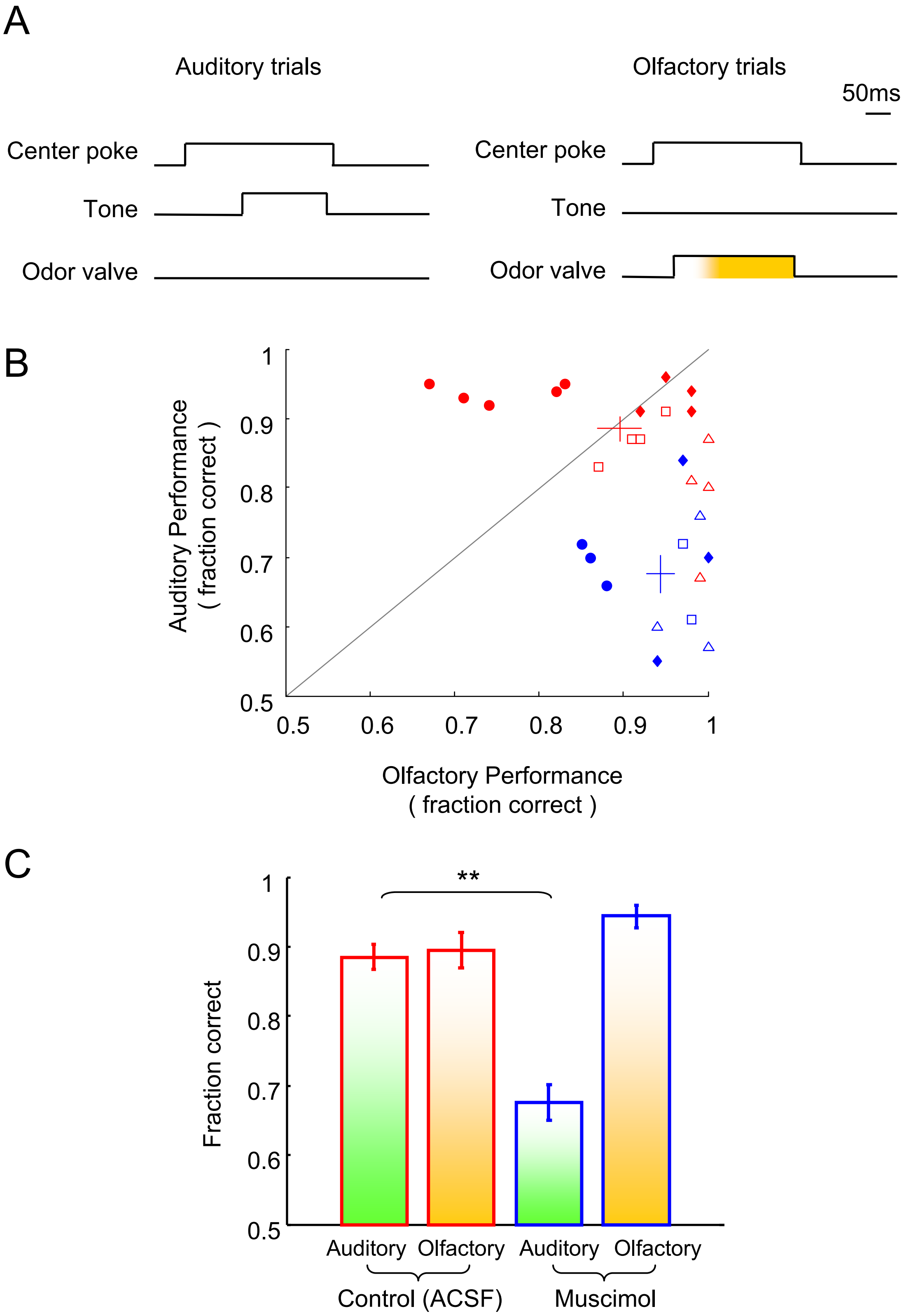
A
Auditory trials
Olfactory trials
50ms
Center poke
Tone
Odor valve
Center poke
Tone
Odor valve
B
Auditory Performance
(fraction correct)
1
0.9
0.8
0.7
0.6
0.5
0.5
0.6
0.7
0.8
0.9
1
Olfactory Performance
(fraction correct)
C
Fraction correct
1
0.9
0.8
0.7
0.6
0.5
**
Auditory
Olfactory
Auditory
Olfactory
Control (ACSF)
Muscimol

Figure 4.1 **A**. Timing diagram of behavioral events in randomly interleaved auditory and olfactory trials. The color gradient under odor valve activation indicates the odor delivery latency. **B**. Scatter plot of auditory and olfactory performance of control (red symbols) or muscimol (blue symbols) treated animals. Each symbol represent one animal. The crosses indicate the mean ± SEM. **C**. Bilateral inactivation of auditory cortex by muscimol selectively impaired auditory discrimination performance but left olfactory performance intact ($p<0.001$).

Chapter 5

Neural correlates of intermodal selective attention in the rat auditory cortex

Single-unit recordings from monkeys have provided examples showing how attention modulates the responses of neurons in the auditory cortex (Hocherman et al., 1976; Hocherman et al., 1981; Hocherman and Yirmiya, 1990). However, there have been very few reports in rodents on how higher cognitive functions affect the neuronal activities in the sensory cortices. Recently Shuler and Bear (Shuler and Bear, 2006) reported that through repeated conditioning, neural activities in the rat primary visual cortex can express activity patterns that resemble the reward timing even in the absence of reward. This shows that in rats, just as in primates, even the activities in the “primary” sensory cortices can be modulated by behaviorally meaningful events.

Rats offer several advantages as experimental models in attention research. First, rats are relatively inexpensive to maintain. Second, due to their small size, high throughput behavioral training can be done by computer programs to automatically train multiple rats in parallel. Third, the rapid development of new electrophysiological, molecular and imaging techniques in rodents provides us exciting opportunities to examine the underlying mechanisms of attention at the circuit and cellular level (Wehr and Zador, 2003; Zhang et al., 2003; Wehr and Zador, 2005; Kitamura et al., 2008). With the advances of technology in cell-type specific and pathway specific labeling (Borrell et al., 2005; Wickersham et al., 2007; Lima et al., 2008), soon one could even manipulate neuronal activity in-vivo (Boyden et al., 2005; Tan et al., 2006; Han and Boyden, 2007;

Zhang et al., 2007; Lima et al., 2008) in a cell-type or pathway specific manner.

I am interested in studying how attention modulates information processing in the auditory cortex. I first focused on intermodal selective attention and proceeded to develop a task that requires the rats to switch its attention between audition and another sensory modality. The choice of using olfaction as the second modality came naturally as rats are extremely good at sensing odors. And the 2AC behavior paradigm I used to train rats was originally adapted from an olfaction task (Uchida and Mainen, 2003). I first tested whether the rats can successfully switch their attention between audition and olfaction in blocks of trials. For the next few rats I increased the difficulty of the olfaction task to engage more attentional resources. I implanted tetrodes in the auditory cortex of all five rats and recorded neural activities while the rats performed the intermodal selective attention task. I found neural correlates of intermodal selective attention in a small population of neurons. In most cases, neural responses to the stimuli were enhanced in the auditory block.

5.1 Rats can perform intermodal selective attention task

I trained five rats to perform an intermodal selective attention task with alternating auditory and olfactory blocks. In the early version of the task (asymmetric task), three rats performed a two-tone discrimination task without odor during the auditory block. In the olfactory blocks, these rats performed the odor discrimination task with two pure odors (table 5.1 & figure 5.1a).

Later on, in the more challenging version of the task (pseudo-symmetric task), two rats performed a two-tone discrimination task in the auditory block with a single non-meaningful null odor (table 5.1 & figure 5.1b). The logic of using a null odor is to control for odor induced non-attentional effect on the auditory responses. The possibility that the rats will use the null odor as a cue for the auditory block does not concern me since it may actually help the rats to pay attention to the auditory stimuli. I also increased the difficulty of the olfaction task to engage more attentional resources. In the olfactory blocks, these rats performed odor discrimination task with odor mixtures of two pure odorants at different ratio (30/70 vs. 70/30).

The performance for both versions of the task was similar and can reach the criteria of above 80% correct trials in both auditory and olfactory blocks. The rats were then trained for an additional one to two weeks before electrode implantation and physiological recording. During the recording sessions, I typically observed a slight performance drop in the auditory blocks and to a lesser extend in the olfactory blocks (figure 5.2). The performance in discordant trials is slightly lower than in concordant trials, but still higher than in auditory trials. This shows that the task is challenging enough that when the auditory stimulus contradicts the olfactory stimulus, the rat has to make an effort to ignore the auditory stimulus in an olfactory block. In accordance with this interpretation, the reaction time of correct discordant trials is also longer than the reaction time of correct concordant trials (figure 5.3B&C). The reaction time of olfactory trials, both concordant and discordant, is consistently longer than the reaction time of auditory trials, indicating the rats were indeed performing olfactory discrimination in the olfactory trials, even in concordant trials (figure 5.3B&C).

I have also tried and succeeded in training rats to perform in “symmetric” inter-modal selective attention task in which the odor distracters in the auditory block are the same odors presented in the olfactory block. The initial performance is around 75% correct trials; however, the rats' performance drops after the first session. I suspect the rats initially can switch attention between modalities by virtue of counting block lengths or durations they used to get from earlier "pseudo-symmetric" sessions. But the rats fail to realize the block change even though each block change is preceded with 10 trials of pure auditory or olfactory trials without distracters. Better cueing strategies will be needed to signal rats to switch attention between modalities.

5.2 Tetrode recording

I used tetrodes to record single unit activity from left auditory cortex in 5 rats performing intermodal selective attention task. Due to the geometry and distance between the neurons and the tetrode wires, the action potential signals from nearby neurons can be picked up by each of these 4 leads with different strength. This allows us to record multiple nearby neurons at the same time. An example of unit isolation is shown in figure 5.4.

Among a total of 802 units I have recorded, 205 units (25.6%) showed significant onset responses for at least one pure tone stimulus. And the firing rate during the onset responses or spontaneous activity was greater than 3 Hz. These 205 units were further analyzed in the following way: I looked for attentional modulation effects during two behavior epochs: (1) spontaneous activity during stimulus anticipation and (2) tone evoked activity (figure 5.1C).

5.3 Anticipatory spontaneous activity

I found in 20 units slow ramping of firing rate right after the animal made a center poke to trigger the stimulus that lasted throughout the whole stimulus sampling duration (anticipation & stimulus duration). Figure 5.5 shows an example of such unit. Even though this stimulus anticipating ramping activity is not strongly modulated by attention, it could reflect the animals' intrinsic expectancy of the stimulus presentation in a similar manner to the reward timing effect Shuler and Bear reported (Shuler and Bear, 2006)

5.4 Neural correlates of intermodal selective attention

5.4.1 Attentional modulation of stimulus evoked response

In some units I found that manipulating the animals' attention dramatically affects the units' responses. In figure 5.6, the left column shows the tone-evoked response of one such unit to the low frequency stimulus. During the auditory block (shown in red) the unit responded to the stimulus with a short transient onset response. However, in the olfactory block (shown in green) the response was completely suppressed. The tone-evoked response of the same unit to the high frequency stimulus (right column) is unaffected.

Another example in figure 5.7 (left column) shows a similar short transient onset response to the stimulus in the auditory block. Surprisingly, in the olfactory block, not only was the onset response not observed, I also found long lasting inhibition of neural activity throughout the duration of the stimulus. This unit also exhibited modulation of spontaneous firing rate by switching the animal's attention between modalities during the stimulus anticipation period

Not all attentional modulation has the same sign. I also observed opposite type of attentional modulation. In figure 5.8, two example single units showed suppressed stimulus evoked response by attention.

5.4.2 Attentional modulation of spontaneous firing rate

Attention not only can modulate stimulus evoked responses, it can also modulate base line spontaneous firing rate of a neuron (Luck et al., 1997). As mentioned earlier in figure 5.7 and another example in figure 5.9, manipulating the animals' attention can modulate spontaneous firing rate of auditory cortical neurons during the stimulus anticipation period.

5.4.3 Group analysis

In each behavior session, two pure tone stimuli were presented (high frequency and low frequency) to the rats and therefore two unique stimulus-evoked onset responses (10~50 ms after tone onset) were obtained for each unit. I found 255 unique onset responses that were significantly different compared to the spontaneous activity after center poke and before stimulus onset. 23 unique onset responses (9%) showed enhancement of firing rate in the auditory block compared to in the olfactory block while 14 unique onset responses (5.5%) were modulated in the opposite direction. In total, 37 onset responsive units (14.5%) showed modulation of onset responses by shifting the animals' attention between the auditory and olfactory modalities (table 5.2). Although I clearly demonstrated that the effect of attentional modulation can be very strong, the percentage of units showing modulation is small. This is probably an underestimation since I only probed the neurons with limited set of auditory stimuli.

During the stimulus anticipation period, I found that slightly more units (38 units, 18.5%) showed weaker but significant modulation of spontaneous firing rate by attention. To further examine the direction of modulation, 18 units (8.8%) showed enhancement of spontaneous firing rate in the auditory block compared to in the olfactory block while 20 units (9.8%) showed the opposite effect (table 5.2).

To further quantify the relationship between attentional modulation of anticipatory spontaneous activity and stimulus-evoked responses, I calculated an attentional Modulation Index (MI): the difference in firing rate between auditory block and olfactory block divided by the sum of the firing rate in both blocks. Modulation Index ranges from -1 to 1. A positive MI means enhancement of firing rate in the auditory blocks and/or suppression of firing rate in the olfactory blocks. Figure 5.10 shows the distribution of MI for onset responses and anticipatory spontaneous activities. The effect of attentional modulation tends to be stronger for the onset responses than the anticipatory spontaneous activities. There is no obvious correlation between MI for onset responses and anticipatory spontaneous activities (correlation coefficient=-0.01).

5.5 Intermodal selective attention modulates choice probability

To determine whether an animal's performance can be predicted by its neurons' activity in the auditory cortex, I calculated choice probability, the probability of correctly predicting the rat's choice given the neuronal firing rate in each trial, for each unique onset response (Britten et al., 1996). This measure is based on ROC analysis and compares the left going and right going trials for a given stimulus. In the auditory block for a given pure tone, choice probability compares neuronal activity in correct versus error trials. In the olfactory block, however, choice probability compares neuronal activity in correct concordant and error discordant trials versus correct discordant and error concordant trials. Choice probability ranges from 0 to 1, with 0.5 being chance level. A choice probability larger than 0.5 means an increase in the unit's firing rate is correlated with animal's choice of response corresponding to the preferred stimulus. An example unit's onset response that predicts the animal's choice in the auditory block but not in the olfactory block is shown in figure 5.11.

Choice probability for the population of unique onset responses in the auditory block is slightly larger than 0.5 but highly significant (0.512 ± 0.0037, $p<0.001$). There are also more units with significant choice probability larger than 0.5 (figure 5.12). This indicates that, in the auditory block, the trial-to-trial variability in the neural responses to the stimulus is correlated with the animal's choice. To put it plainly, the animal could potentially solved the task by "listening" to its neurons in the auditory cortex to make its decision.

However, population choice probability in the olfactory block is not different from 0.5 (0.499 ± 0.003, $p>0.05$). This is probably due to the fact that the animal is performing an olfactory discrimination task and has little use of its auditory neurons and therefore the trial-to-trial variability in the neural responses to the stimulus is not correlated with the animal's choice.

Table 5.1

Task category	Null odor (auditory block)	Task Odors (olfactory block)	Number of rats
Asymmetric	-	Caproic acid / Hexanol	N=2
	-	R(-)-2-octanol / S(+)-2-octanol	N=1
Pseudo-symmetric	Caproic acid	R(-)-2-octanol / S(+)-2-octanol mixture (70/30 vs. 30/70)	N=2

Table 5.1 Odorants presented in the auditory and olfactory blocks in the asymmetric and pseudo-symmetric version of the intermodal selective attention task.

Figure 5.1

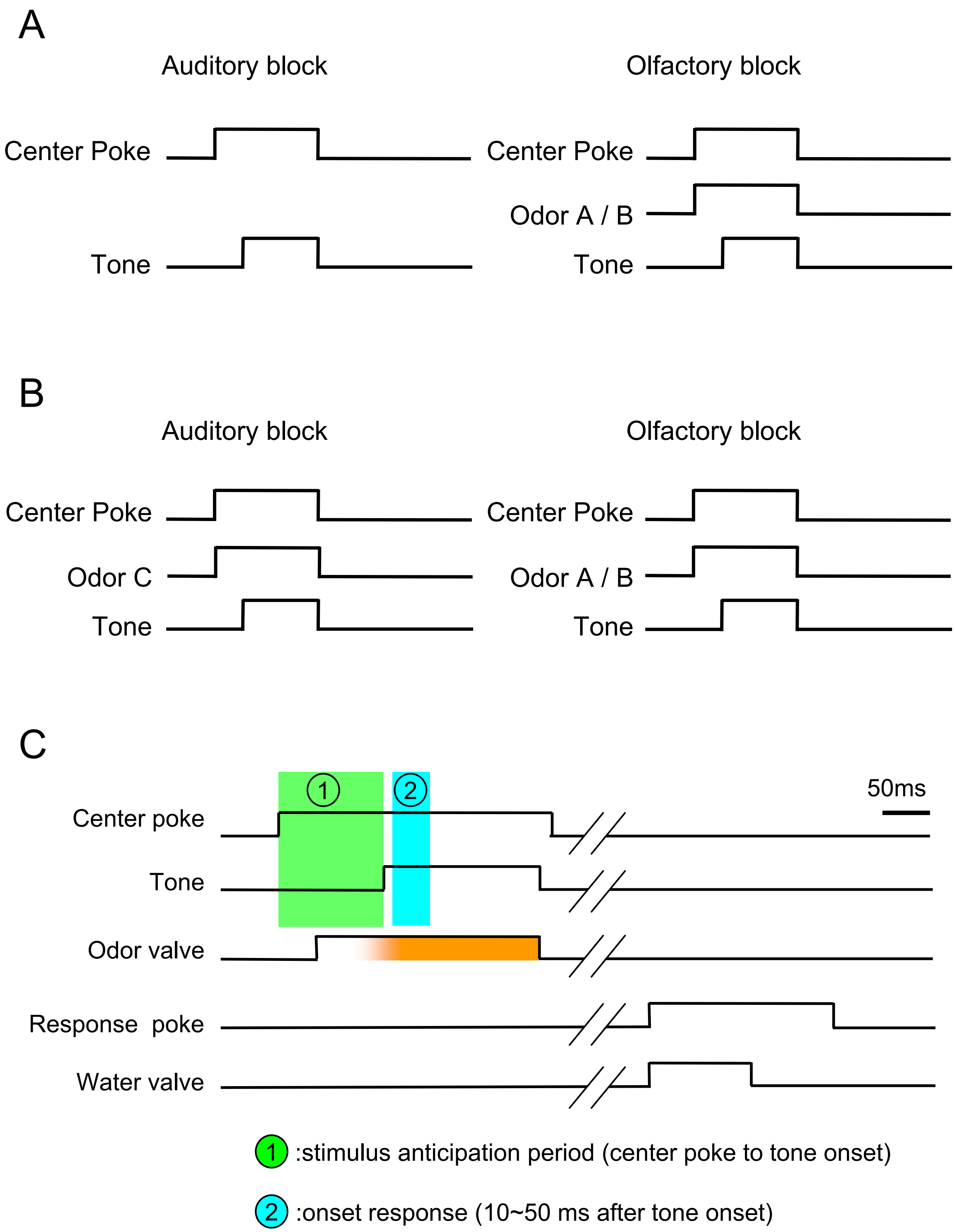
A
Auditory block
Olfactory block
Center Poke
Tone
Center Poke
Odor A / B
Tone
B
Auditory block
Olfactory block
Center Poke
Odor C
Tone
Center Poke
Odor A / B
Tone
C
①
②
50ms
Center poke
Tone
Odor valve
Response poke
Water valve
① :stimulus anticipation period (center poke to tone onset)
② :onset response (10~50 ms after tone onset)

Figure 5.1 **A**. Event timing diagram in an earlier version of the task (asymmetric task, 3 animals). Odor A and odor B are pure odorants. **B**. Event timing diagram in second version of the task (pseudo-symmetric task, 2 animals). Odor A and odor B are mixture of pure odorants in 70/30 and 30/70 ratio. Odor C is delivered in the auditory block as “null odor”. **C**. Timing diagram of two behavioral epochs analyzed in the intermodal selective attention task. Note that odor valves are turned on prior to tone onset. This is to account for the odor delivery latency of the system (see appendix 1).

Figure 5.2

A

		Auditory block		Olfactory block	
		no odor / odor C		odor A	odor B
Tone	Low	Left	Left	Left	**Right**
	High	Right	Right	**Left**	Right

▨ : discordant trials

B

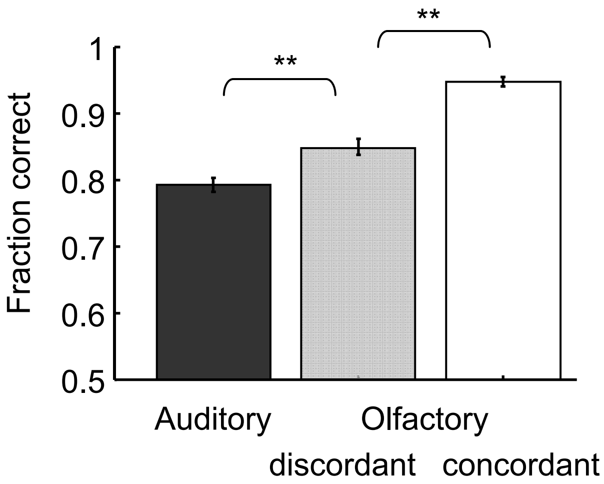

Figure 5.2 **A**. The task contingency in the auditory and olfactory blocks. **B**. Average performance of five rats in the intermodal selective attention task. Olfactory performance is higher than auditory performance ($p<0.001$). Performance in concordant trials is better than in discordant trials ($p<0.001$).

Figure 5.3

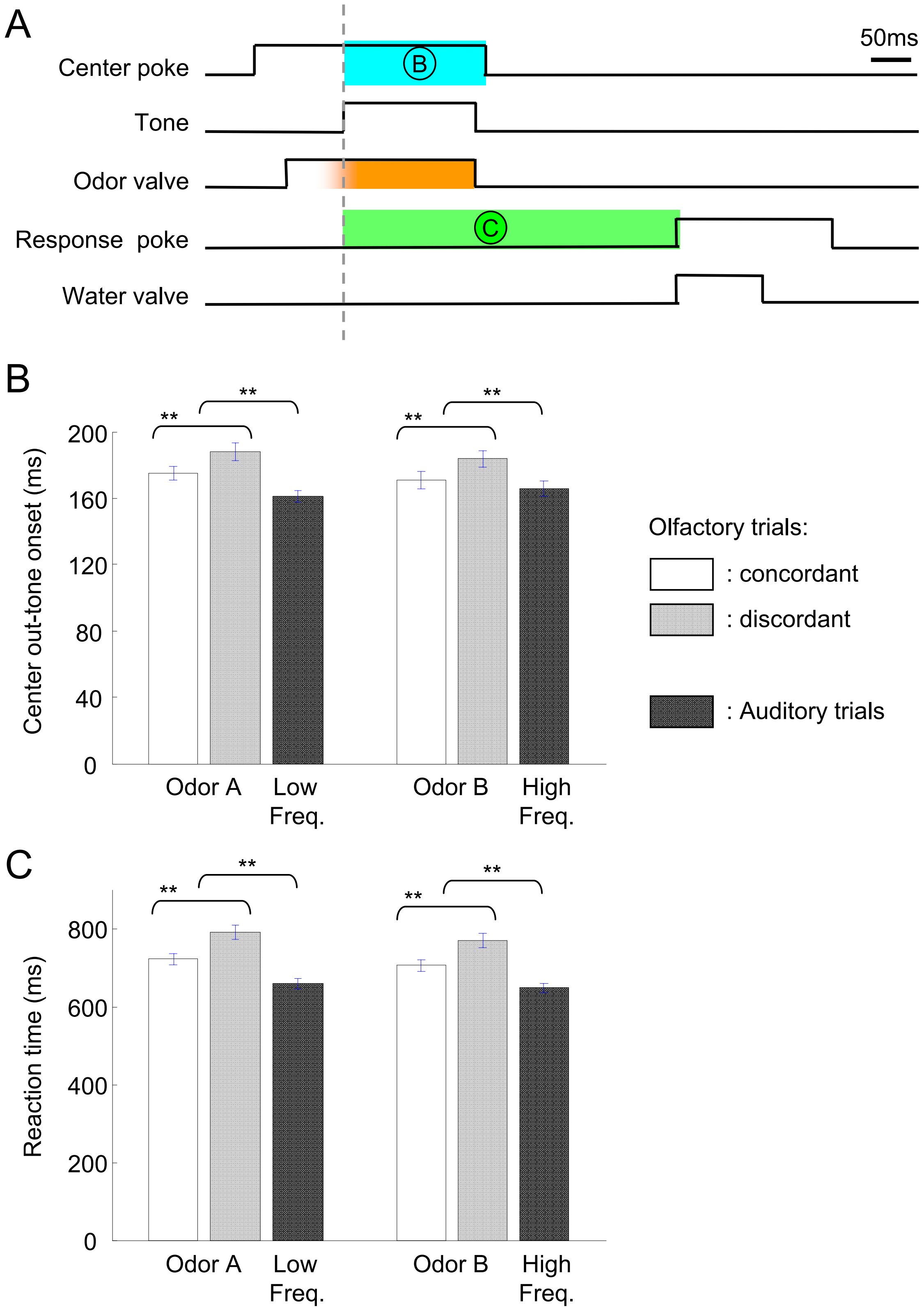
A
Center poke
Tone
Odor valve
Response poke
Water valve
B
C
50ms
B
**
**
**
**
200
160
120
80
40
0
Center out-tone onset (ms)
Odor A
Low Freq.
Odor B
High Freq.
Olfactory trials:
: concordant
: discordant
: Auditory trials
C
**
**
**
**
800
600
400
200
0
Reaction time (ms)
Odor A
Low Freq.
Odor B
High Freq.

Figure 5.3 **A**. Event timing diagram showing two measure of reaction time: (b) from tone onset to center poke out, (c) from tone onset to response port in, same as reaction time defined before. **B**. Time of tone onset to center-poke-out for correct olfactory (concordant and discordant) and auditory trials. Odor A and low frequency tone correspond to left response port. Odor B and high frequency tone correspond to right response port. The reaction time of the discordant trials is always longer than in concordant trials ($p<0.001$). The reaction time of the olfactory trials is consistently longer than auditory trials ($p<0.001$).

Figure 5.4

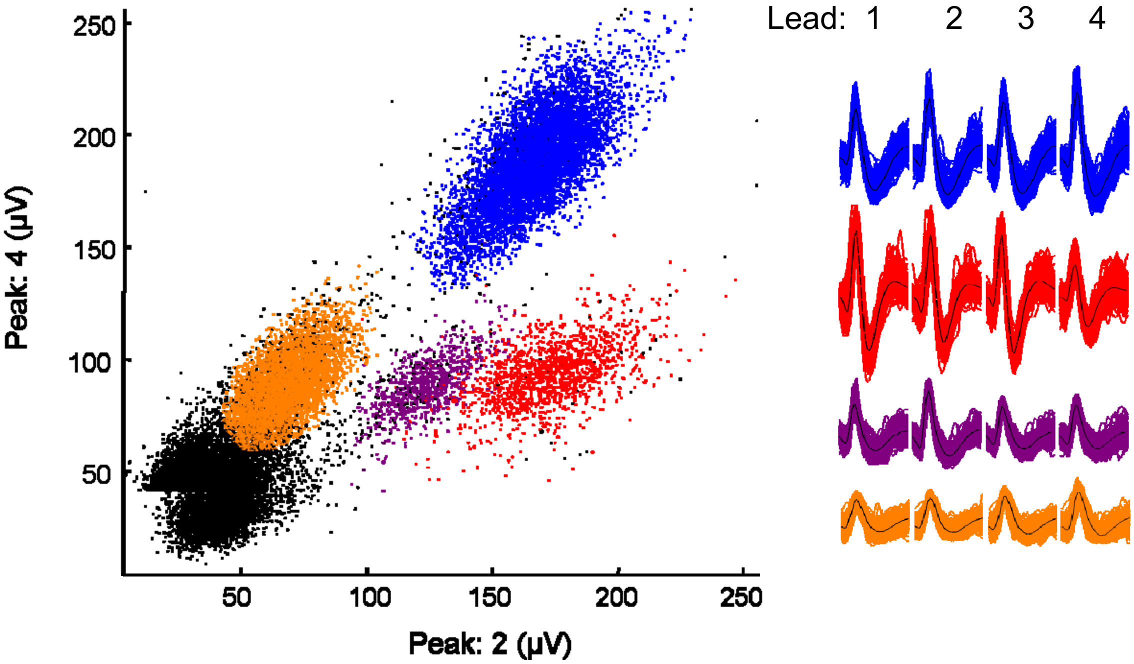

Figure 5.4 A example of unit isolation from tetrode recording data. **Left:** the peak of action potential signal picked up by lead 2 versus lead 4. **Right:** the spiking waveforms of four isolated units.

Figure 5.5

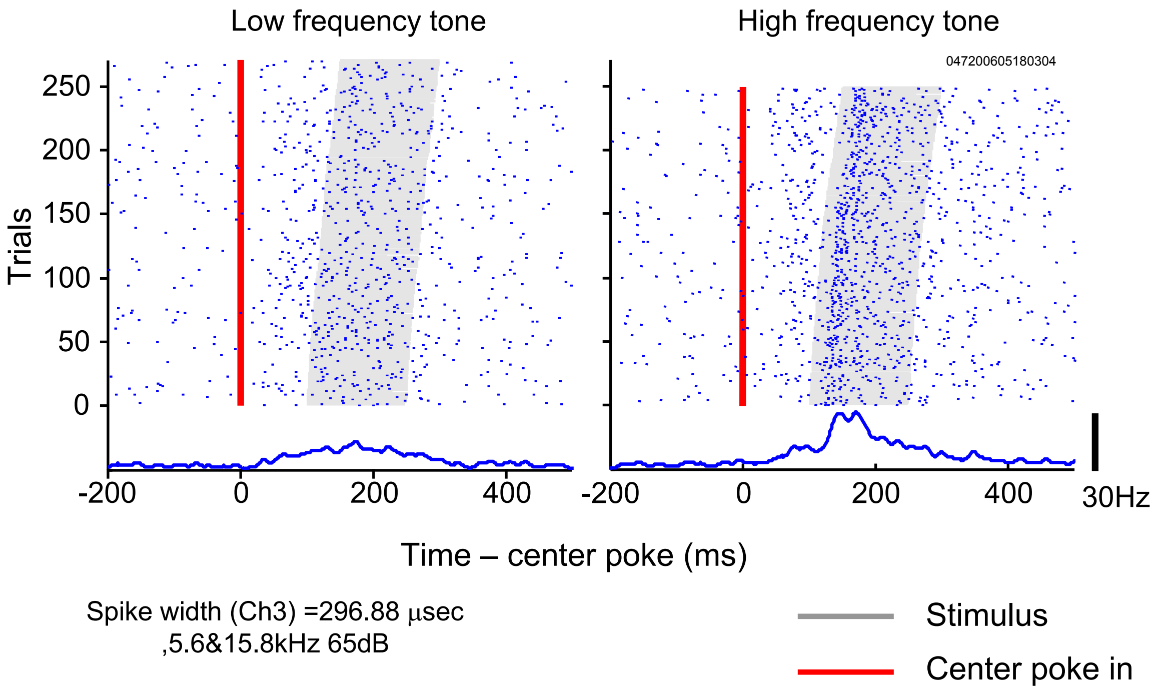

Figure 5.5 An example of a unit showing slow and long lasting ramping of firing rate after center poke. The enhanced firing lasted throughout the stimulus duration. Trials are pooled from both auditory and olfactory blocks. The spike raster are aligned to center poke in and sorted by stimulus anticipatory duration. Stimuli: 5.6 & 15.8 kHz 65dB pure tone.

Figure 5.6

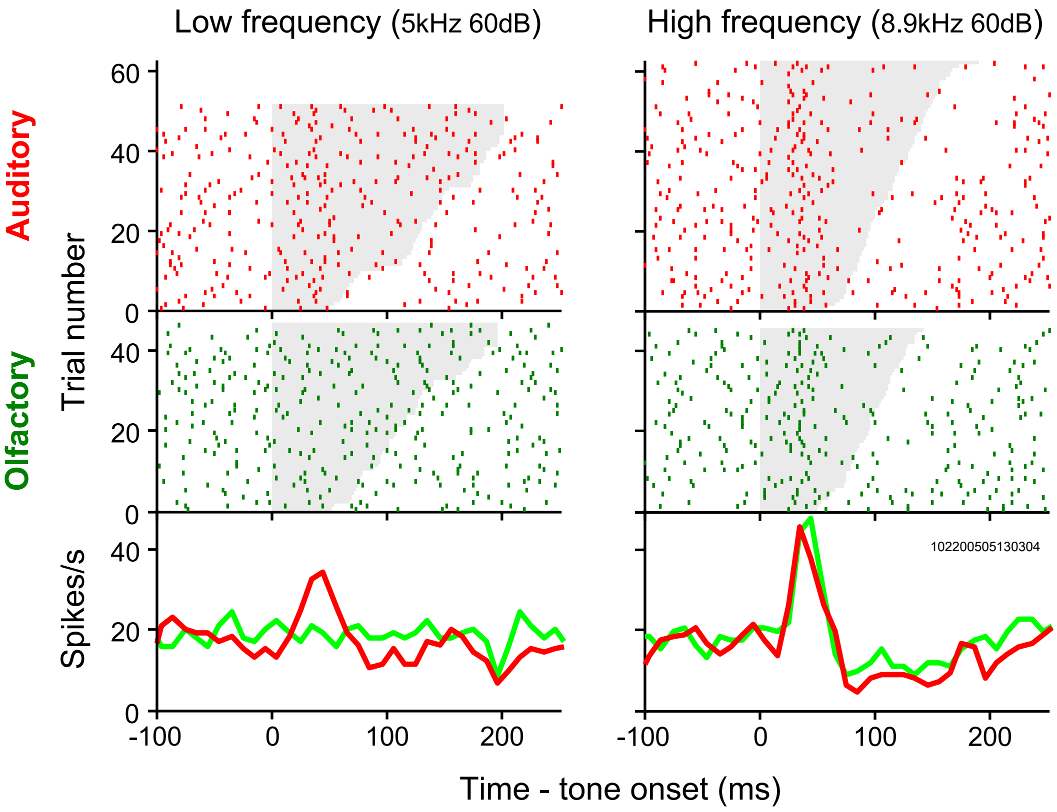

Figure 5.6 Enhanced stimulus evoked response by attention in the auditory block. **Left:** example of a unit's onset response modulated by attention. Red ticks: spikes in the auditory block. Green ticks: spikes in the olfactory block. Trials are sorted by stimulus duration (grey lines). Stimulus: 5 kHz 60dB pure tone. Red line: auditory peristimulus time histogram (PSTH). Green line: olfactory PSTH. **Right:** onset response of the same unit to 8.9 kHz 60dB pure tone.

Figure 5.7

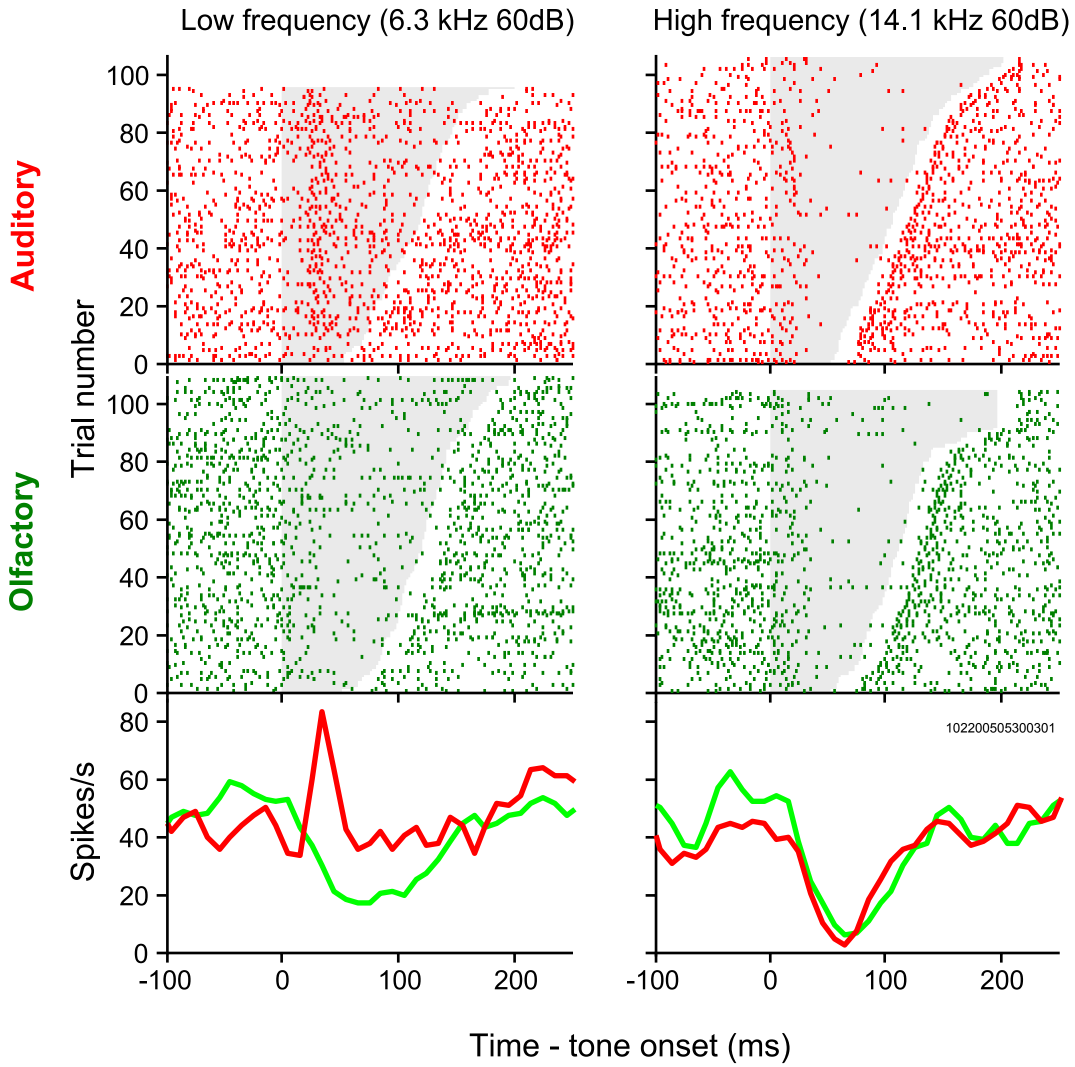

Figure 5.7 Another example of enhanced stimulus evoked response by attention in the auditory block. **Left:** example of a unit's onset response modulated by attention. Note the strong suppression of the response by the stimulus in the olfactory block. Stimulus: 6.3 kHz 60dB pure tone. **Right:** onset response of the same unit to 14.1 kHz 60dB pure tone.

Figure 5.8

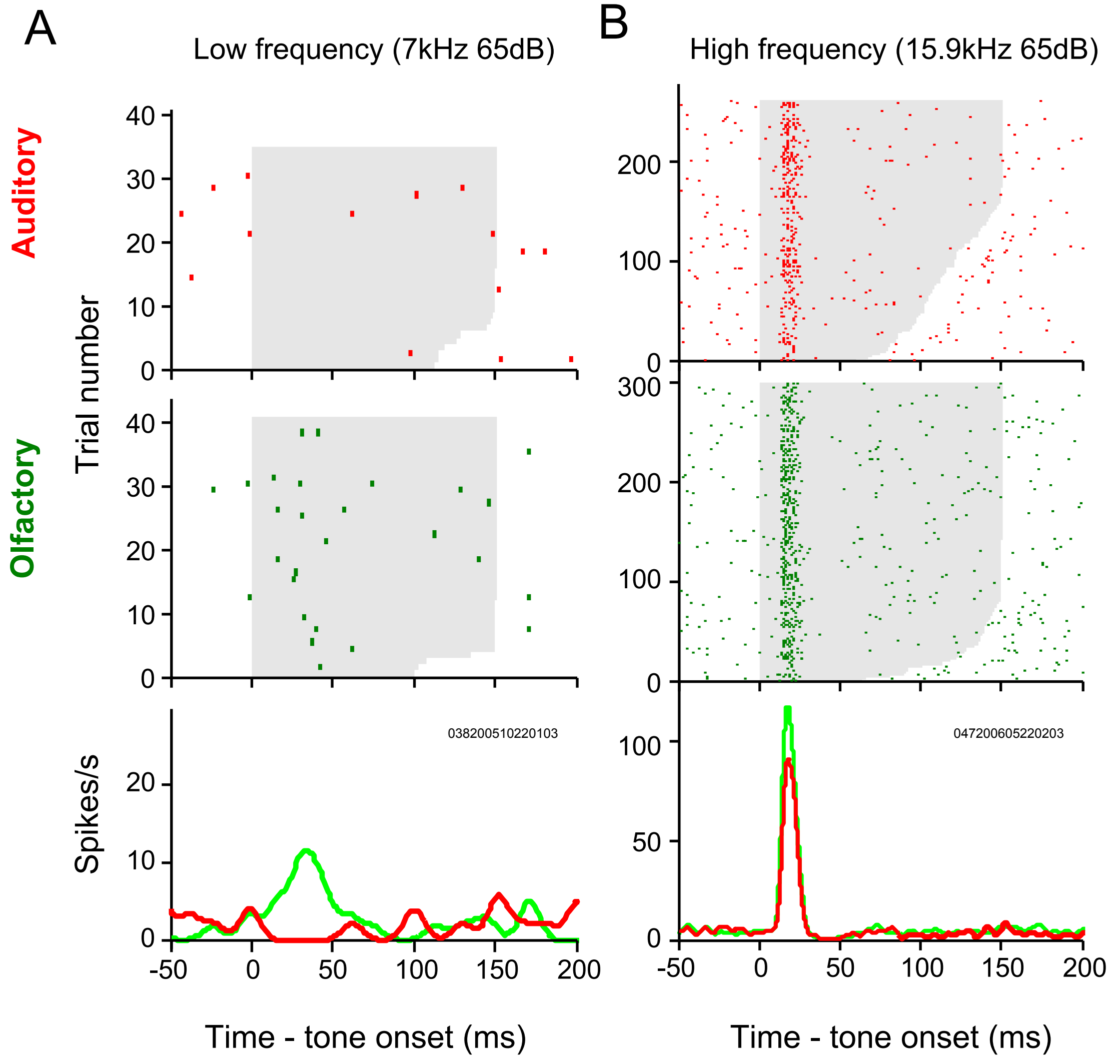

Figure 5.8 Examples of suppressed stimulus evoked response by attention in the auditory block. **A**. Example of a unit's onset response modulated by attention. Stimulus: 7 kHz 65dB pure tone. **B**. Example of another unit's onset response modulated by attention. Stimulus: 15 kHz 65dB pure tone.

Table 5.2

		Stimulus anticipation*	Tone evoked responses
Auditory block	enhanced:	8.8 %	9.0% **
	suppressed:	9.8 %	5.5 % **
	total (unit-tone):	-	14.5 % **
	total (units):*	18.5 % *	11.2% [a]*
Combined	total (units):*	15.1% [b]*	

*: out of 205 responsive units
**: out of 255 unique onset responses (unit-tone)

Corrected for multiple comparison assuming all events are independent to get combined $p<0.05$, Wilcoxon rank sum test :
[a] : $p<0.0253$ for two events
[b] : $p<0.017$ for three events

Table 5.2 Summary of attentional modulation in stimulus anticipation period and stimulus evoked response as proportion of unique onset responses (unit-tone) and units.

Figure 5.9

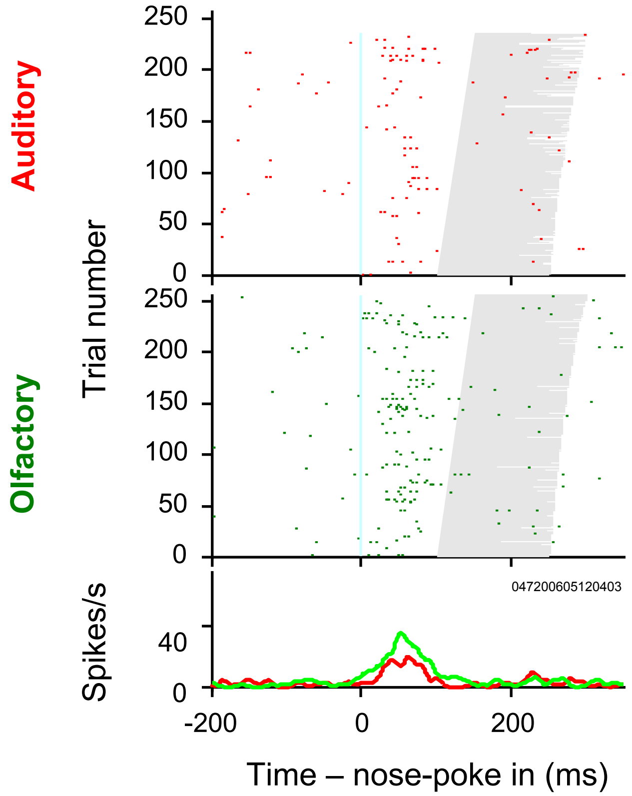

Figure 5.9 Examples of suppressed spontaneous firing rate during stimulus anticipation period by attention in the auditory block. Trials are aligned to center port in event and sorted by the duration of stimulus anticipation period.

Figure 5.10

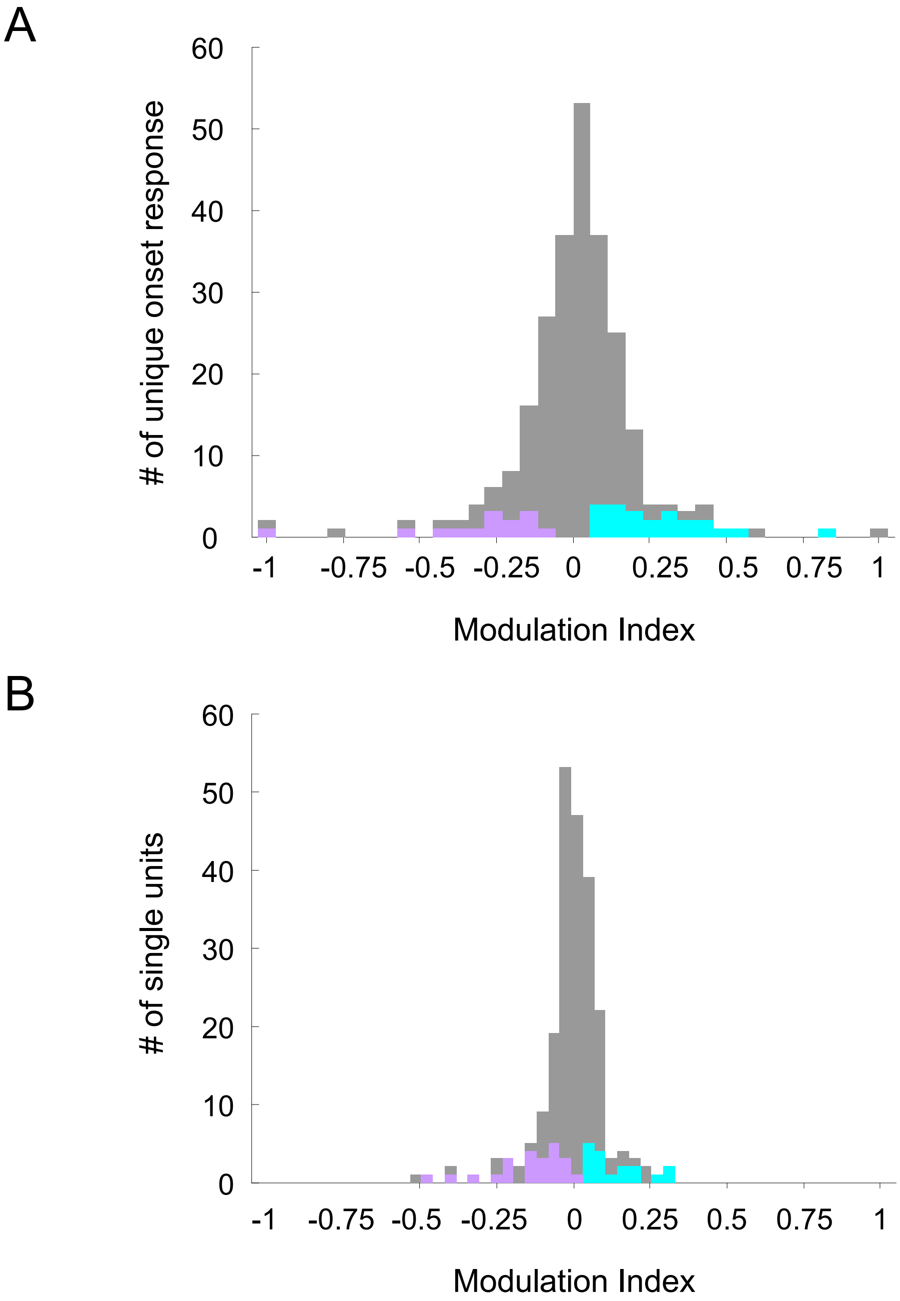

Figure 5.10 **A**. Distribution of modulation index for unique onset responses. Cyan & purple: modulation index for significantly modulated unique onset responses. **B**. Distribution of modulation index for anticipatory spontaneous activity. Cyan & purple: modulation index for significantly modulated single units.

Figure 5.11

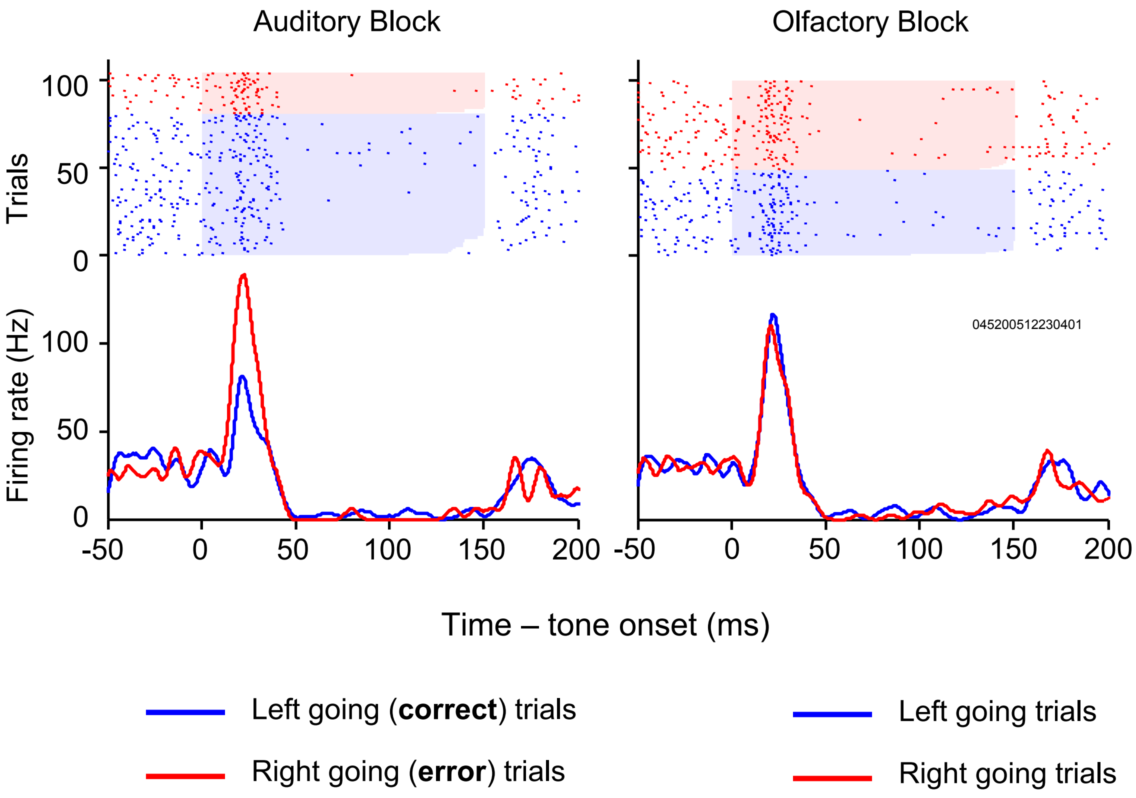

Figure 5.11 Stimulus evoked onset response grouped by the animal's choice from an example unit showed significant choice probability in the auditory block.

Figure 5.12

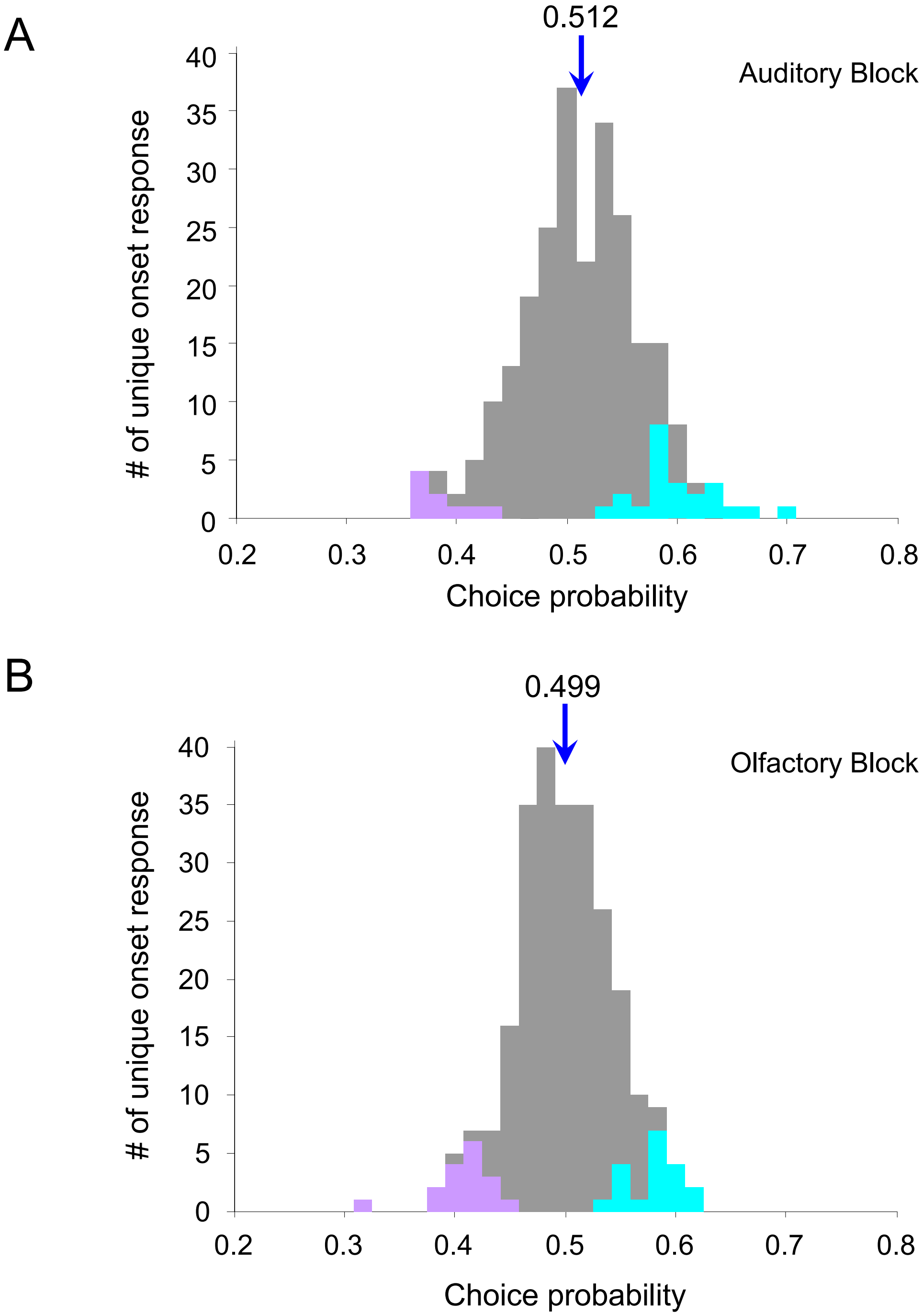

Figure 5.12 **A**. Distribution of choice probability for unique onset responses in auditory block. **B**. Distribution of choice probability for unique onset responses in olfactory block. Cyan & purple: choice probability that significantly differs from 0.5.

Chapter 6

Conclusion and perspective

I have developed and trained rodents to perform a 2AC auditory discrimination task. I have shown that the auditory cortex is involved in two-tone discrimination. I have also developed and trained rodents to perform an auditory-olfactory intermodal selective attention task. I have found neural correlates of intermodal selective attention in the rat auditory cortex. I have also found significant choice probability in auditory cortical neurons while the animal is performing an auditory discrimination task.

6.1 Manipulating task difficulty

I first set out to design a task that embeds the signal in the background white noise so we can easily manipulate the task difficulty by changing the signal to noise ratio (chapter 3). I found the discrimination performance can be systematically perturbed by changing the stimulus parameters. I would suggest using this strategy (varying SNR) when one needs to measure behavior variables like performance or reaction time in relationship to task difficulty. However, if one would like to record neural activities from the auditory cortex, responses to broadband white noise may mask the responses one may potentially be interested in. In this case, other means of varying task difficulty is recommended.

6.2 Measuring frequency discrimination threshold

For the purpose of testing the rats' ability to discriminate frequencies, it is natural to present the stimuli that contain only pure tones. I decided that it is not necessary to embed the pure tones in background white noise for the stimuli when testing rat's frequency discrimination threshold. In order to make sure the rats only use frequency information, I carefully calibrated my setup. During the process of training rats to do the frequency discrimination task, I encountered a few rats that showed irregular psychometric curve with extremely good performance at some frequencies. It turns out early on during training, in addition to / other than use the frequency information, these rats learned to use sound intensity information. Further testing by varying sound intensity confirmed the suspicion and these rats were discarded from further training.

The rats have different auditory sensitivity at different tone frequencies measured by detection threshold (Hack, 1960; Kelly and Masterton, 1977; Syka et al., 1996). Evan though the sound intensity (60 dB SPL) in the frequency discrimination task I used is well above the threshold around 8 kHz (<10 dB SPL), considering the fact that auditory intensity discrimination threshold of the rats is only 1~3 dB SPL at 8 kHz 40~50dB SPL above threshold (Hack, 1971; Syka et al., 1996), it is possible that by changing only stimulus frequency, the perceived intensity will also change with it. Especially in paradigms that present multiple stimuli of the same frequency, a small deviant in frequency (oddball) can escape the effect of stimulus specific adaptation and unleash stronger responses (Ulanovsky et al., 2003). Therefore, one can never be sure whether a rat only uses frequency information when discriminating pure tones. Unable to rule out this possibility, I refrained from referring to the task as "frequency discrimination" and used a more precise term “two-tone discrimination” in most of the thesis. Nevertheless, the frequency discrimination Weber ratio measured in my task is comparable to previous studies (Syka et al., 1996; Talwar and Gerstein, 1998).

To better control for the confound of possible changes in perceived sound intensity in a frequency discrimination task, I would suggest using a roving intensity paradigm during behavioral training to constantly change the sound intensity so that the only information a rat can use to perform the task is the tone frequency.

6.3 Cortical inactivation and frequency discrimination performance

Using the dual modality 2AC paradigm and cortical inactivation of primary auditory cortex (chapter 4), I confirmed a previous study (Talwar et al., 2001) showing that the primary auditory cortex is involved in frequency / two-tone discrimination in rats. However, I found that the animals still can perform the discrimination at above chance level after cortical inactivation. The novel finding in my study is that tone detection is not a prerequisite for frequency / two-tone discrimination. The animals may not be able to detect tones after cortical inactivation but when forced still can discriminate two tones with broad separation in frequency space. Human patients with bilateral auditory cortex lesion also display similar capability sometimes called “deaf hearing’ (Engelien et al., 2000; Garde and Cowey, 2000). When the patients were instructed to attend to auditory modality or perform auditory forced-choice tasks, the ability to detect and perceive auditory stimuli was temporally restored.

What possible mechanisms could account for the residual performance observed in the cortical inactivation experiments? One possibility is that the secondary auditory areas around the primary cortex could still provide some information about the stimuli. Even though the amount of muscimol applied is enough to inactivate the primary auditory cortex as described in chapter 4, the surrounding secondary auditory areas TE2 and TE3 (Roger and Arnault, 1989; Romanski and LeDoux, 1993; Kimura et al., 2003) may not be inactivated completely and might still perform some minimum discrimination. TE2 and TE3 receive extralemniscal ascending auditory inputs from suprageniculate nucleus (SG) and the dorsal division of the MGN (Kimura et al., 2003; Donishi et al., 2006). Other than callosal connections to contralateral homotopic cortex, TE2 and TE3 also projects to the dorsal and external cortices of the inferior colliculus (IC), central gray, deep layers of the superior colliculus (SC), reticular thalamic nucleus (RT), and caudate putamen (CPU) (McGeorge and Faull, 1989; Arnault and Roger, 1990). Through the connections to medial striatum, information from TE2 and TE3 may enter the cortico-basal ganglia loops (Graybiel, 2000, 2005) and reach other cortical and subcortical structures.

Another more likely possibility is that the stimulus information may be passed to other cortical areas through subcortical pathways. There are two possible subcortical pathways for auditory information to reach other non-auditory cortical areas. The first is thalamostriatal pathway. The medial division of the MGN and SG project to caudalmost portions of the CPU and the amygdala (LeDoux et al., 1985). The second pathway is through the superior colliculus. Despite the visual nature of the superficial layers in SC, intermediate and deep layers of SC receive multimodal sensory inputs (Meredith and Stein, 1983; Middlebrooks and Knudsen, 1984; McHaffie et al., 1989; Meredith et al., 1992; Gaese and Johnen, 2000). Intermediate and deep layers of the SC neurons in rats receive auditory information from bilateral dorsal nucleus of the lateral lemniscus, ipsilateral external nucleus of the IC and dorsomedial part of IC (Druga and Syka, 1984; Skaliora et al., 2004). Intermediate and deep layers of the SC neurons also projects to the midline and intralaminar thalamic nuclei (Carstens et al., 1990; Krout et al., 2001). Each of these thalamic nuclei receiving SC input projects to specific regions of the cortex and striatum (Berendse and Groenewegen, 1990, 1991). Overall these thalamic nuclei project to the widespread, predominantly frontal, cortical areas with some also project to the motor cortex or parietal cortex (Berendse and Groenewegen, 1991). The subcortical targets of these thalamic nuclei include different parts of nucleus accumbens and CPU some also project to and the olfactory tubercle (Berendse and Groenewegen, 1990). Information in the ascending auditory pathway may modulate activity in the basal ganglia, amygdala, and cerebral cortex through the SC's projections to these midline and intralaminar thalamic nuclei.

6.4 Neural correlates of intermodal selective attention

I have trained rats to perform an auditory-olfactory intermodal selective attention task (chapter 5). Due to the limited stimulus ensemble (low and high frequency pure tones) I used, I found only 26% (205 units) of the total 802 recorded units responding to the stimuli. Most of the unresponsive units have very low firing rate. Since I under-sampled the frequency space, it is possible that a low firing rate neuron could fire vigorously to stimuli of other frequencies (appendix 4). However, for a typical broadly tuned neuron, both stimuli I tested would give us strong responses (appendix 5).

It is proposed that given a reasonable sized stimulus ensemble and enough time for active searching in the stimulus space, it's possible more neurons will become responsive (Wang et al., 2005). However, because I simultaneously recorded many units from multiple tetrodes and the time constrain, I did not optimize the stimuli for a particular unit. Even if I tried a larger stimulus set, data from our lab suggests that it does not improve the yield of responsive neurons (Hromadka et al., 2008).

Among the responsive units, only a small portion (15.1%) of them showed attentional modulation in either the stimulus anticipatory activity or onset response. For the onset response, I only analyzed the response 10~50 ms after the tone onset. This is due to variable stimulus duration controlled by the rats in the task design. Since I can only be sure of the animal's head position when the animal is in the center port, I terminated the stimulus as soon as the animal withdraw it nose from the center port. With the earphone holder design by Gonzalo Otazu, now I can deliver constant stimulus disregard of the animal's position (appendix 4, 5&6). This would allow us to measure the full dynamics of the responses such as off-responses (appendix 6) that were discarded in the present study.

Using a modified 2AC paradigm with go signal and earphone holder implants, it would be possible to deliver more stimuli per trial and therefore achieving higher yield of responsive units as well as measurement the receptive field. It would be interesting to see whether with prolonged stimulus presentation time one could find stronger attentional modulation effect.

One could argue that since rats are extremely good at sensing odor, the olfactory task doesn’t really draw attention resources away from the auditory cortex. Based on the performance and reaction time of concordant versus discordant trials, we believe that the rats do need to focus their attention on the olfactory task in order to ignore the auditory distracters. If the argument that olfaction is a special modality for rats were true, one would predict that a visual-auditory intermodal selective attention task would engage more attention resources and have stronger modulation on the neural activity. This is one of the future directions of our lab.

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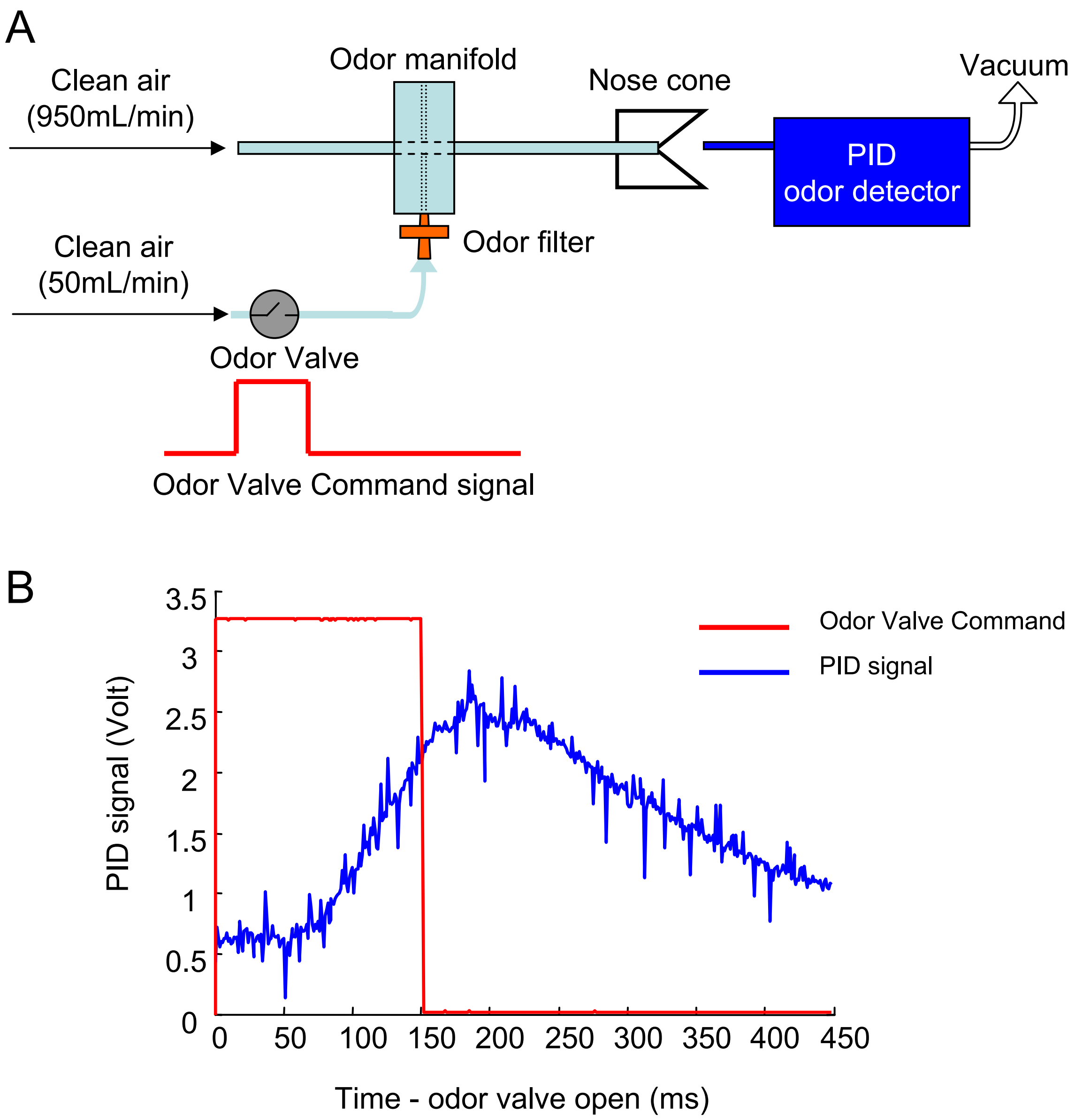
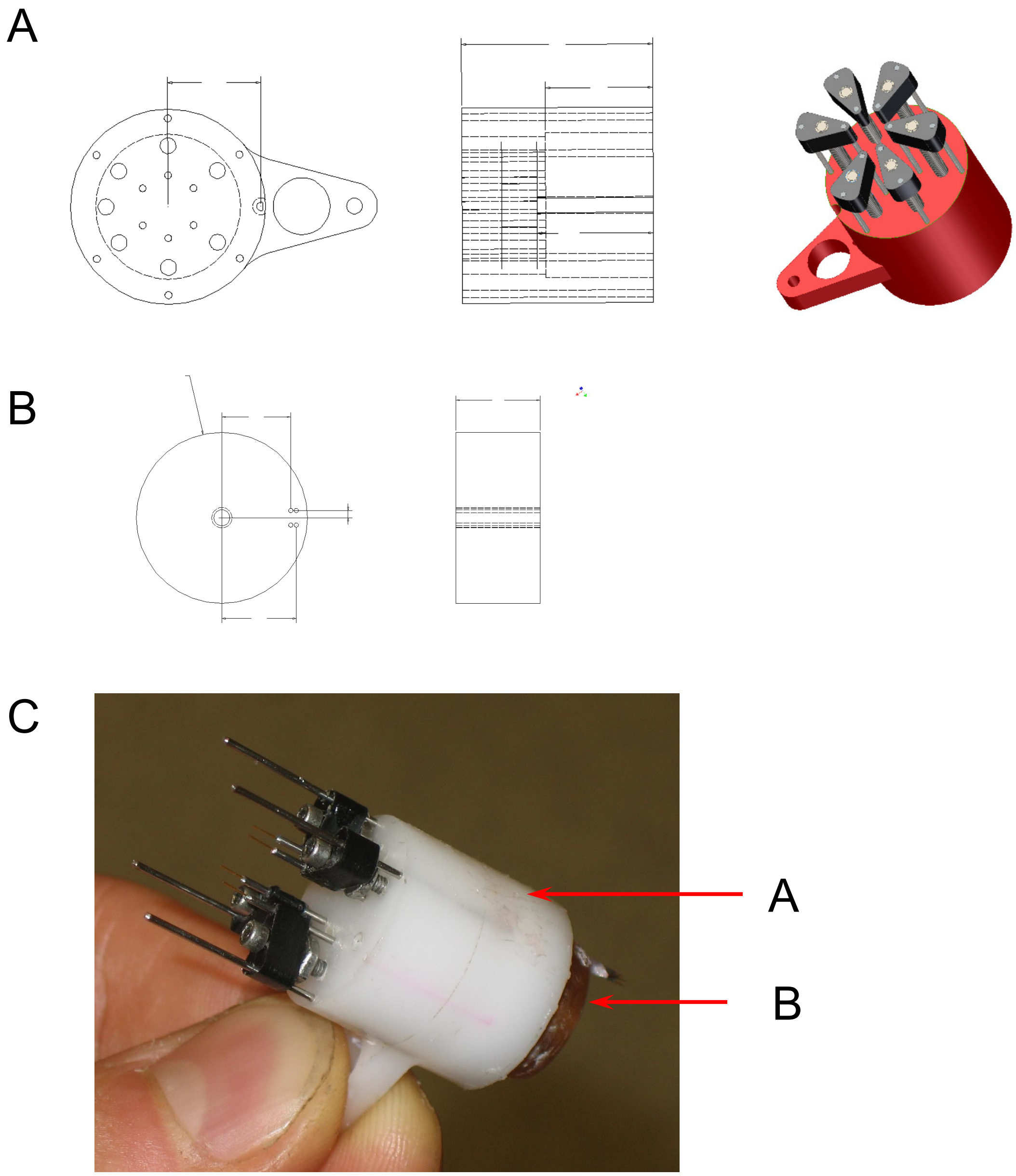
Appendix 1



Appendix 1. Odorant delivery setup and delivery time course

A. Schematics of odorant delivery setup. Odorant is loaded in the odor filter. When the odor valve is opened, 5% of the total air flow passes through the odor filter. A photo-ionization detectors (PID) monitor is used to measure the time course of odorant delivery. **B**. PID signal following the opening of odor valve. There is a latency of 80~90ms before the odorant was detectable by the PID.

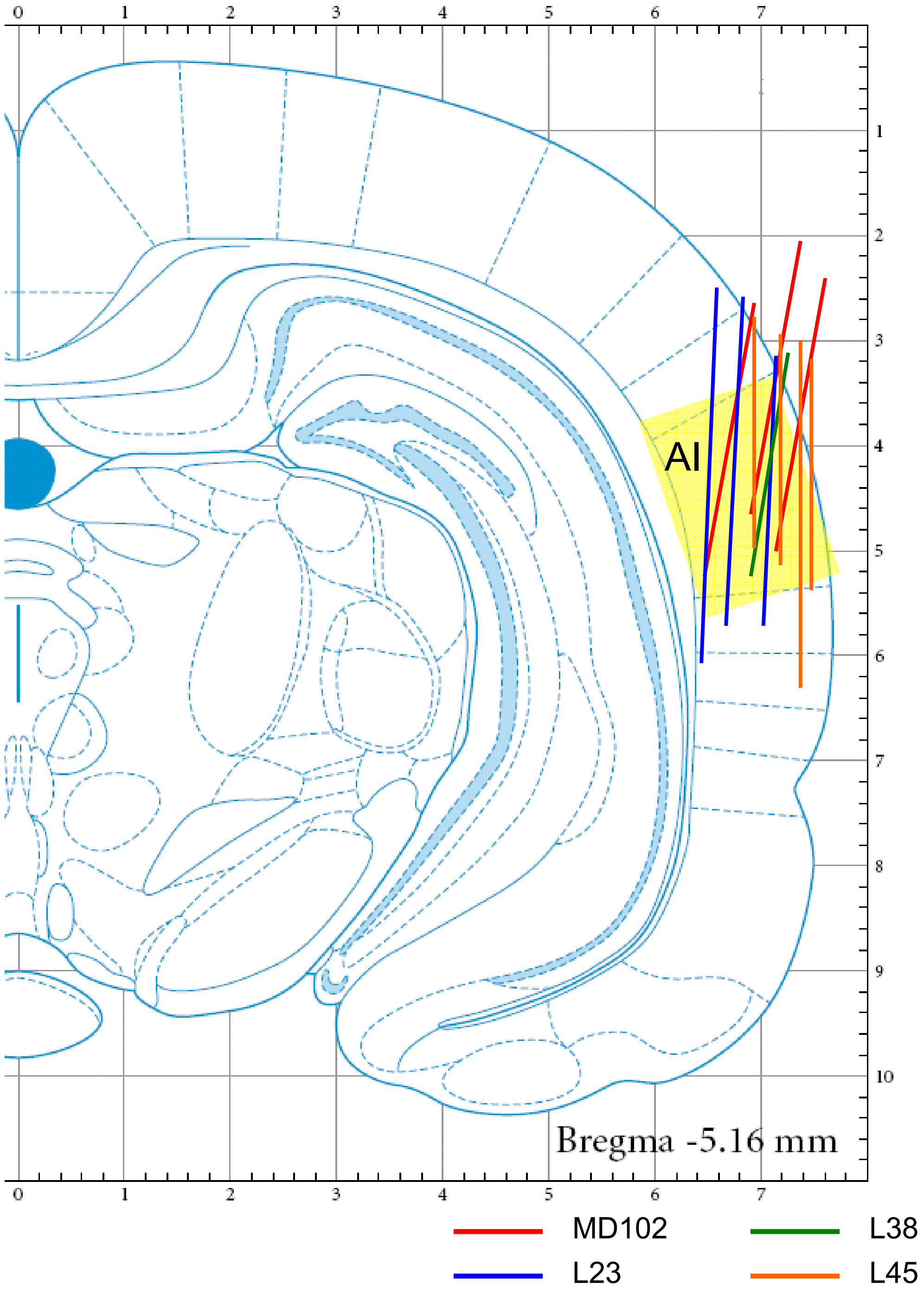
Appendix 2



Appendix 2. Schematic diagrams of the custom made tetrode drive

A. Main turret that holds 6 independent movable tetrodes. **B**. Guide tube positioning disk. The pattern of holes on the disk determines the spacing and location of the tetrodes. **C**. The assembled tetrode drive will fit inside a protective cap, not shown here.

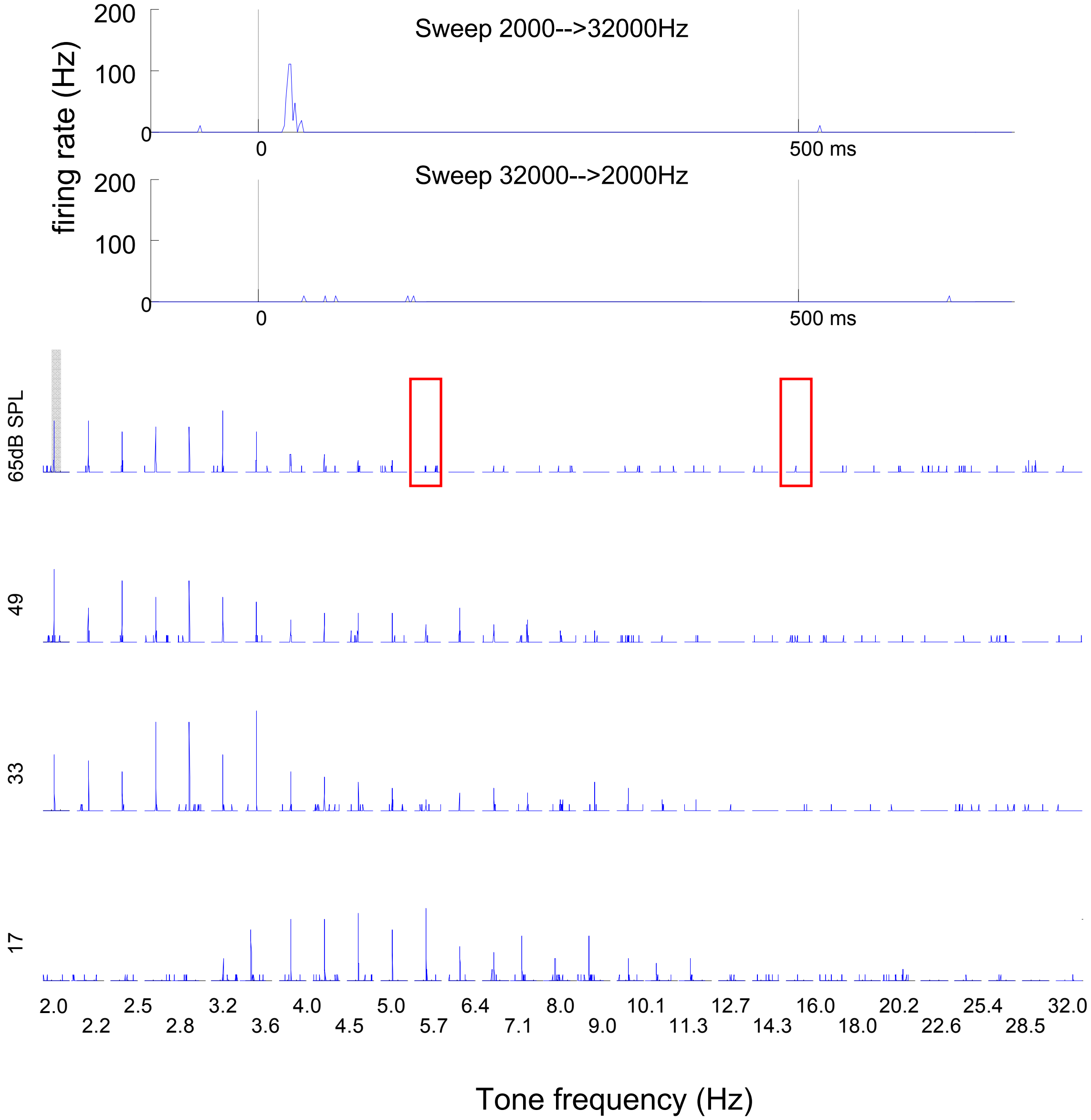
Appendix 3



Appendix 3. Reconstructed electrode tracks

Tetrode track was reconstructed by small lesions at the end of the recording from four animals. Highlighted area: left primary auditory cortex.

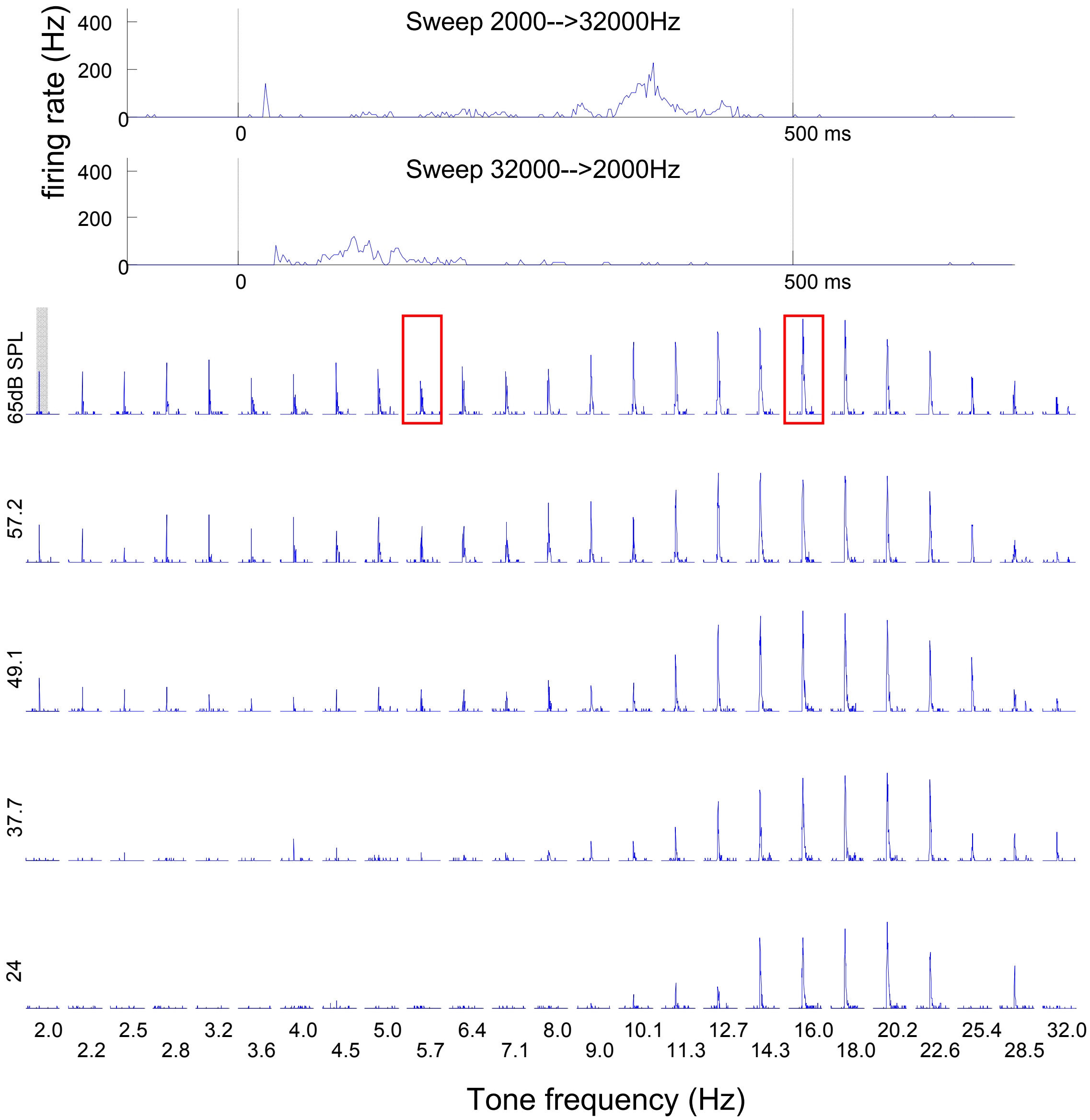
Appendix 4



Appendix 4. An example unit tuning curve from a freely moving animal

This unit would be classified as an unresponsive unit if presented stimuli in the red rectangles. Top two rows: response to tone sweeps. Bottom: response to pure tones. Grey bar: 100 ms pure tone. Responses were recorded while the animal is freely moving but not performing a task. Unit not included in this study.

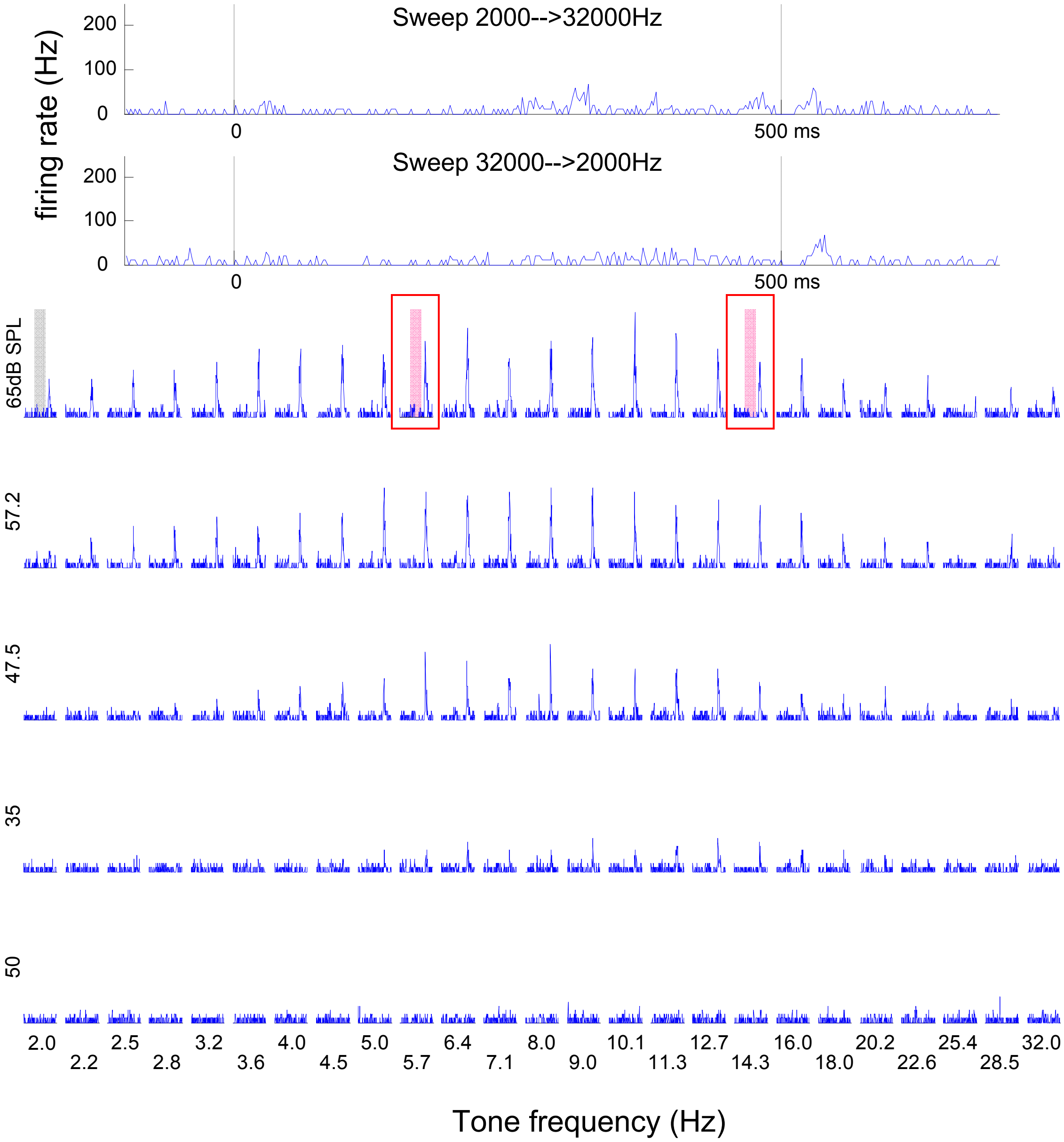
Appendix 5



Appendix 5. Another example unit tuning curve that's broadly tuned

This unit would be classified as an **responsive unit** when presented stimuli in the red rectangles. Top two rows: response to tone sweeps. Bottom: response to pure tones. Grey bar: 100 ms pure tone. Responses were recorded while the animal is freely moving but not performing a task. Unit not included in this study.

Appendix 6



Appendix 6. An example unit tuning curve with strong off-responses

This unit would be classified as an unresponsive unit when presented stimuli in the red rectangles due to weak onset response. Top two rows: response to tone sweeps. Bottom: response to pure tones. Grey & pink bars: 100 ms pure tone. Responses were recorded while the animal is freely moving but not performing a task. Unit not included in this study.