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NEURAL CORRELATES OF DETECTION AND DECISION IN AUDITORY CORTEX DURING FREQUENCY DISCRIMINATION

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOENGINEERING

BY

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Dedication

To my wife, Katie, who puts up with me.

To my parents, who recognized my need to continue school.

And to Drew, who convinced me to go to grad school, then convinced me to graduate.

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Abstract

This dissertation presents the results of a series of experiments designed to expand our understanding of the role that auditory cortex plays in threshold-level frequency discrimination. The unifying hypothesis of this dissertation is that auditory cortex is involved in discrimination of fine-grained frequency differences. Recording neural responses to auditory stimuli during performance of a difficult frequency discrimination task opens the door to investigating possible neural mechanisms which may encode frequency change detection. Additionally, simultaneous recording of neural and psychophysical data allows for insight into the rats' perception of or lack of perception of a frequency change (as inferred from the behavioral response to the stimuli).

Briefly, the results of these studies show that rats are capable of performing a frequency discrimination task and that their thresholds are comparable to those found in other mammals, including humans, making them a suitable candidate for studying how fine grain frequency differences are processed. Post-surgery psychophysical results show that implantation of a chronic recording device in the rat's auditory cortex did not have a large effect on frequency discrimination thresholds or other behavioral measures. Recordings from rat auditory cortex revealed a neural correlate of the frequency discrimination ability in the onset response of cortical neurons to the target tone. Additionally, the onset latency of auditory cortex responses was shown to be correlated with behavioral reaction times. The results document an increase in firing rate (facilitation) relative to the reference tone based on whether the rat made a decision to respond or not to respond to the presentation of a target tone. This facilitation was not stimulus specific, as was shown to be correlated with behavior, but not the frequency or frequency shift. Finally, the response strength of auditory cortex to frequency changes was shown to be modulated according to the relevance to task performance. Facilitation to the frequency change was not seen when the frequency change was irrelevant to the task the despite correct performance of the task.

The results of this series of experiments implicate auditory cortex as an important component in the resolution of small frequency differences and suggest that signals from auditory cortex may play a significant role in the perception of frequency changes as well as in the decision to respond to those frequency changes. Future studies in this field could help to elucidate how neural codes from sensory cortex relate to perception and how they contribute towards the formation of decisions while performing a detection task such as the one presented here.

Chapter 1

1. Introduction

Helen Keller expressed the importance of hearing in modern society, saying *"*Blindness separates us from things; deafness separates us from people*."* For humans, hearing has developed an important role as the basis for verbal communication. However, even before the advent of speech, humans were heavily reliant on auditory information. A fine sense of hearing may have enabled early humans to survive. While ancestral humans were likely aided by visual information in tasks necessary to thrive such as gathering food, locating shelter, finding companionship, or navigating obstacles (Rodman 2004), subtle sound differences could provide more immediate and necessary information by alerting would-be prey to the advances of predators. Unlike vision, hearing is not limited by lighting conditions or obstacles that may block lines of sight.

One component of sound information is frequency. Auditory frequency is defined as the rate of oscillation in a sound wave and is the primary determining factor in what humans perceive as pitch. One key component of human verbal communication is the transmission of subtle differences in frequency. While timing differences play a role in verbal communication, especially in Latin-based languages (Shannon *et al*., 1995), the ability to interact as a community is partially dependent on our ability to resolve subtle differences in spectral information (Zeng *et al*., 2005). Additionally, auditory information can alert us to the approach of modern dangers, such as speeding cars or trains, where small differences in frequency can inform a listener whether a sound source is quickly advancing or traveling away from them.

1.1. Functions of the Auditory Pathway

In humans, sound waves first enter the body at the ear canal. The pinna, the flesh-covered cartilage surrounding the ear canal, helps to funnel sound waves into the ear canal, especially from forward facing sources. Pressure from sound waves vibrates the tympanic membrane located at the innermost point of the ear canal. The tympanic membrane pushes on and creates movement in a chain of three tiny bones, the ossicles, which constitute the middle ear: the malleus, the incus, and the stapes. Motion from the ossicles serves to create pressure on the cochlea, which displaces the virtually incompressible cochlear fluid within. Movement of the cochlear fluid is transferred into oscillations of the basilar membrane, causing it to stimulate inner and outer hair cells by sliding across them. The hair cells are arranged tonotopically, and transform the previously mechanical energy into electrical signals containing frequency information along the central auditory pathway.

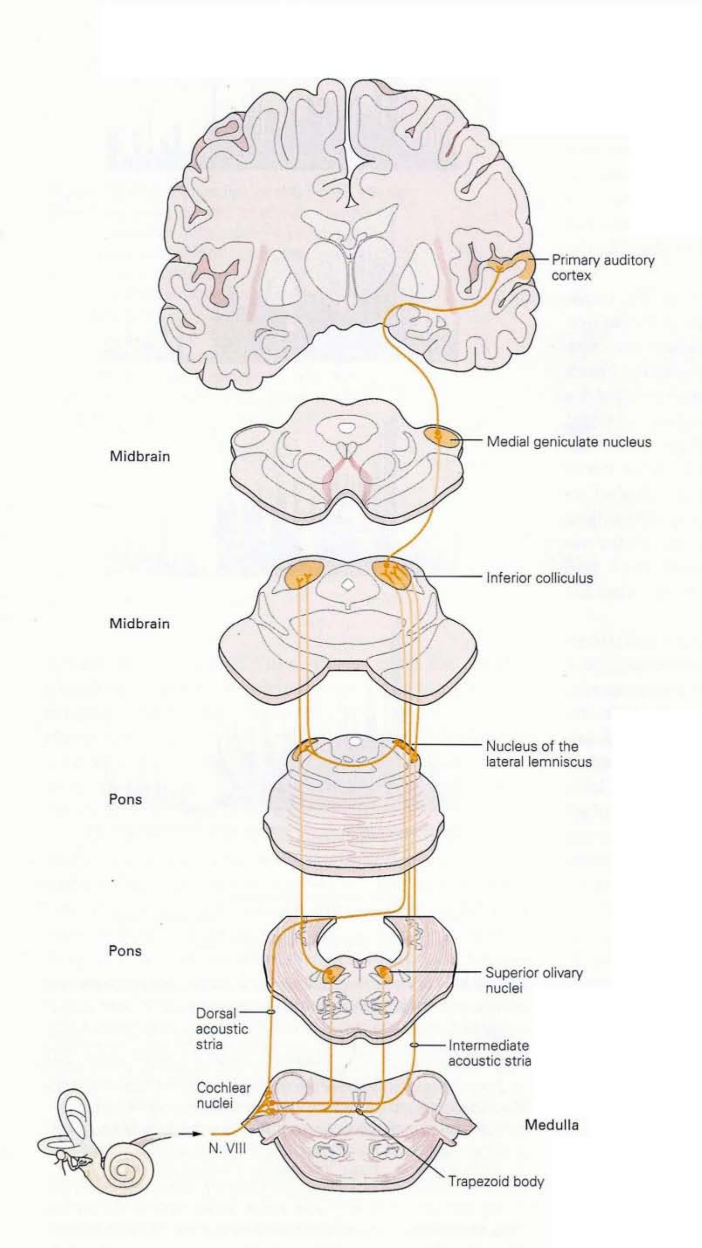


Figure 1.1. The auditory neural pathway. From Kandel *et al*., 2000 (adapted from Brodal 1981).

From the inner ear, auditory information travels along auditory nerve fibers and enters the brain stem through the 8th cranial nerve. From the 8th cranial nerve it enters the medulla and is divided into 3 pathways at the cochlear nucleus. The trapezoidal body pathway travels to the pons where sound localization is performed at the superior olivary nuclei complex by comparing differences in certain features of the sound information received from either ear. The medial superior olive nucleus compares temporal delays, while the lateral superior olive nucleus uses differences in intensity to identify the special origin of the sound source. The 3 pathways converge in the midbrain where sound localization information from the pons is processed in the inferior colliculus. From the midbrain the sound information is delivered to the diencephalon (thalamus) at the medial geniculate nucleus, where it is relayed to the primary auditory cortex.

In humans, the primary auditory cortex is ventral to the lateral sulcus. It is arranged tonotopically, with lower frequencies represented in the rostral regions and higher frequencies in the caudal regions (Bendor & Wang, 2005). Layers I, II, III, and IV of the primary auditory cortex receive input from the thalamus. Layer V projects back to the thalamus, and Layer VI sends signals to the thalamus as well as to other cortical areas, notably the secondary auditory areas (Read *et al*., 2002). Secondary "belt" auditory areas surround the primary auditory cortex and are thought to perform higher-order auditory processing (Penfield & Perot, 1963).

1.2. Role of Auditory Cortex in Frequency Discrimination

Many species of mammals have an auditory cortex region functionally and organizationally similar to that of humans (Kaas & Hackett, 2000; Hackett *et al*., 2001; Wallace *et al*., 1997; Bizley *et al*., 2005; Winer & Lee, 2007). The similarities in auditory systems allow for data to be compared across species, and perhaps most importantly applied to human systems.

Reports on performance from early experiments in auditory cortex ablation studies failed to consistently show any significant impairment in frequency discrimination ability. Cats with bilateral lesions of the auditory cortices displayed a "temporary amnesia" in their ability to discriminate changes in frequency but that ability eventually returned (Butler *et al*., 1957; Goldberg & Neff, 1961), while slightly different stimulus parameters such as testing at different reference and target frequencies or ablation of additional cortical areas resulted in a permanent inability to relearn frequency discrimination tasks post-op in cats (Meyer & Woolsey, 1952) and dogs (Allen, 1946). These varied results led some investigators to conclude that the auditory cortex is not necessary for auditory perception (Masterton & Berkley, 1974).

More recent investigations concerning the role of primary auditory cortex in frequency discrimination have led to a more clear understanding. Mongolian gerbils did not show any deficit in their ability to discriminate large changes (3 octaves difference) in pure tones 2-5 days after bilateral ablation of primary auditory cortex (Ohl *et al.*, 1999). Rats were still able to significantly perform frequency discrimination tasks of at least half octave changes within 3 days of bilateral ablation of primary auditory cortex (Ono *et al*., 2006). Only one lab found an impaired ability in rats to discriminate between broad falling and rising tone sequences after bilateral ablation of primary auditory cortex, though their report did not disclose the length of time the rats were allowed to recover post-op (Rybalko *et al*., 2006). These reports suggest that the auditory cortex may not be essential for perception or discrimination of broad frequency changes if the subject is allowed ample time to recuperate following surgery.

Additional clues to the role of primary auditory cortex in frequency discrimination are revealed when testing with a stimulus set that includes changes in frequency difference that are smaller than half-octave changes. Fine-grained frequency perception testing has resulted in higher-threshold frequency difference limens (Tramo *et al.,* 2002) and impaired discrimination abilities (Mendez & Geehan, 1988) in some human patients with bilateral primary auditory cortex lesions. Investigators found similar results when testing smaller frequency change discrimination ability in macaques with bilateral auditory cortex lesions, concluding that the monkeys were unable to determine whether or not the sound stimulus they were presented with contained a change in frequency (Harrington *et al.*, 2001). The results of these studies seem to imply that though primary auditory cortex may not be necessary for broader, coarse frequency discrimination, it is necessary for the detection of smaller, fine-grained frequency changes.

Investigations utilizing immediate and reversible disabling effects through administration of drugs have helped to further clarify the role of primary auditory cortex in normal frequency discrimination. The Doppler-shifted constant-frequency (DSCF) area in bats, a disproportionately large area of the bat primary auditory cortex, is dedicated to processing critical fine-grained frequency components of bat sonar within a narrow frequency range

(Suga *et al*., 1987). Neurons in DSCF feature very narrow frequency response areas (Suga & Manabe, 1982). Application of muscimol, a GABA$_A$ receptor agonist, to DSCF had no effect on coarse frequency discrimination, but significantly impaired ability to discriminate small differences for up to 3.2 hours (Riquimaroux *et al.*, 1991). Similar results were found in rats when bilateral muscimol administration to primary auditory cortex initially blocked all tone detection, with coarse frequency discrimination ability returning several hours before small frequency differences were able to be resolved (Talwar *et al*., 2001). These studies support the hypothesis that primary auditory cortex is necessary for detection of threshold-level frequency changes.

1.3. Importance of Fine Frequency Resolution in Humans

It is possible that the ability to resolve small frequency differences is instrumental in the comprehension and retention of instructions or is an underlying factor in learning or attention disorders. Frequency discrimination ability has been correlated with SAT math scores (Watson, 1991) and reading ability (Ahissar *et al*., 2000). Additionally, impaired frequency discrimination has been associated with Attention Deficit Hyperactivity Disorder in children (Sutcliffe *et al*., 2005), language impairment (Mengler *et al*., 2005), language learning disorders (Elliot *et al*., 1989), and certain forms of dyslexia (Banai & Ahissar, 2004). A better understanding of how we discriminate subtle frequency differences could lead to a more complete understanding of these disorders and eventually result in treatments though pharmacological manipulation or electrical stimulation.

1.4. Neural Mechanisms of Detection and Link to Decision Making

Linking neural activity to behavioral abilities and sensory perception makes a powerful case for establishing the role of cortical areas and is a central goal for contemporary neuroscience. Neural correlates of detection ability have been shown in both firing rate and spike timing of response in various sensory systems.

One of the earlier and more notable contributions towards linking neural activity to perception comes from Britten *et al*. (1992), who showed that weakly tuned individual cells of monkey middle temporal visual area increased in firing rate in correlation with the detectability of motion in a visual direction discrimination task. Later work from this lab theorized how input from multiple directionally-tuned middle temporal visual area cells may be combined in higher areas to determine motion direction of the stimulus (Shadlen *et at*., 1996). Further work on the middle temporal visual area in monkeys revealed that responses there were not only correlated with detection, but were also predictive of the monkeys' responses on individual trials, whether the trial was performed correctly or incorrectly (Britten *et al*., 1996). These groundbreaking discoveries paved the way for future investigations seeking detection and decision correlates in other sensory systems.

A handful of studies have reported finding detection correlates in the auditory system. Responses correlated with behavioral detection thresholds have been shown for tone detection (Niwa *et al.,* 2012a; Niwa *et al.,* 2012b; Zhang *et al.*, 2012), click-train speed discrimination (Dong *et al.*, 2011), acoustic flutter discrimination (Lemus *et al.*, 2009) and speech sound discrimination (Engineer *et al*., 2008; Shetake *et al*., 2011; Tsunada *et al.*, 2011; Ranasinghe *et al*., 2012). However, no one has shown a neural mechanism for discrimination of frequency, which is thought to be one of the more fundamental components of sound. In this study I will show that the onset response to tones containing a change in frequency contains information suitable to encode the detection of differences in frequency.

1.5. Dissertation Outline

The starting hypothesis of this dissertation was that auditory cortex contributes to the resolution of fine-grain frequency differences. The introductory chapter reviews previous auditory cortex ablation studies and justifies why I am looking at auditory cortex for further evidence linking that area to frequency discrimination. The next chapter reviews the general experimental apparatus and procedures used throughout this report.

Chapter 3 reports on the psychophysical thresholds of a previously unreported strain and gender of rat during a repeating standard frequency discrimination task. This chapter serves to establish that the species used in subsequent chapters has comparable thresholds to other mammalian species and thus is a suitable candidate for modeling of the auditory system. Additionally, this chapter shows that implantation surgery does not change behavioral trends in the frequency discrimination task, validating the comparison of neural and psychophysical data presented in subsequent chapters.

Chapter 4 investigates possible connections between AC responses to frequency differences and the behavioral detection ability of rats. Increases in onset firing rate to deviant frequencies are shown to be correlated with the hit rate. Onset latencies are likewise correlated to behavioral reaction times. Both of these pieces of evidence further support the case for AC as an important area in discrimination of frequencies.

Chapter 5 delves further into the correlation between neural responses and behavioral measures and investigates whether the neural mechanism found in the onset response to auditory stimuli is predictive of the subjects' behavioral decision regarding detection of the change in frequency. The results demonstrate that increases in onset firing rate to deviant tones at different frequencies can predict the behavioral decision of the rat while performing a frequency discrimination task. Additionally, this chapter shows that neural responses in auditory cortex to frequency changes are modulated by relevance.

Chapter 6 reviews the main findings of this dissertation. I form general conclusions about the results of the studies presented. I also suggest directions for future research to further understanding of the role of auditory cortex in frequency discrimination.

Chapter 2

2. Apparatus and General Procedures

Testing methods common to each chapter of this dissertation are reported here. Additional procedures specific to each chapter are described in the Methods section of that chapter.

Briefly, male Long-Evans hooded rats were trained on a go/no-go task. Subjects were housed on a 12:12 light-dark cycle with training and testing being conducted during the light part of the cycle. Animals were maintained at or above 85% of pre-training weight (~200 grams). Animals were allowed access to food if they were unable to earn enough food during testing to maintain 85% of their pre-training weight. The care and use of animals in this study conformed to the NIH guidelines and the University of Oklahoma Laboratory Animal Resources and Institutional Animal Care and Use Committee (IACUC) regulations.

2.1. Apparatus

Subjects were trained and tested in an acoustically transparent acrylic cage inside a double-walled, anechoic acoustic chamber (~-30dB attenuation). A nosepoke with an infrared beam was mounted in the back wall of the cage and a piezoelectric loudspeaker (CTS Powerline KSN-1165) was mounted directly above the rat’s head (~35cm) when engaged in the poke.

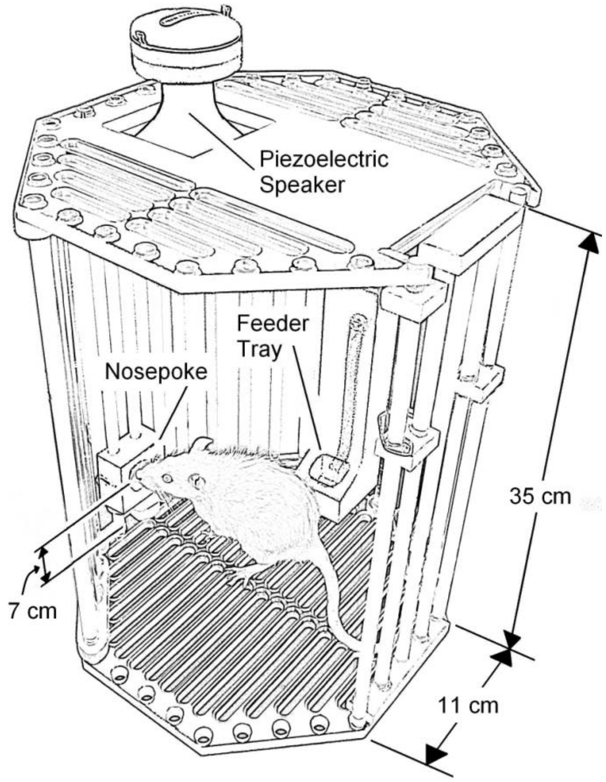


Figure 2.1. Diagram showing typical testing booth set up and dimensions.

2.2. Calibration

Tone amplitude for all stimuli in this study was set to 60 dB sound pressure level (SPL), relative to a 20 µPa (RMS) standard, using a voltage–intensity calibration function specific to the loud-speaker that was ascertained using a 1/4-inch ACO Pacific condenser microphone placed in the approximate center of the stereotypical head position. Calibration functions were determined for 533 frequencies, spanning a range from 1 to 40 kHz with 0.01 octave spacing, by playing a pure tone at each frequency at set amplitude of 1 volt and recording the resultant intensity. Calibrations coefficients were calculated assuming sound intensity varies linearly with voltage amplitude within the intensity range tested and that a voltage amplitude of zero would produce an intensity equivalent to the 20 µPa (RMS) background noise standard. Linear interpolation was used to set voltage amplitudes for frequencies not explicitly tested during calibration. Adjusting for the rat audiogram, 60 dB SPL should be approximately between 40 and 60 dB above hearing threshold (Kelly & Masterton, 1977; Borg, 1982) within the tested frequency range of 2 to 32 kHz, and the effective sound level at those endpoints should be ~40 and ~55 dB, respectively.

Acoustic stimuli were presented to behaving animals using the calibrated, free-field speaker in a double-walled acoustic chamber. A custom-made head-stage amplifier (Tucker Davis Technology) was directly attached to the electrode connector. Neural data were digitized at 25 kHz and band-pass filtered from 825 to 4500 Hz, 6 dB/oct.

2.3. Behavioral Paradigm

Rats initiated a trial by engaging a central infrared-monitored nosepoke. Following a 50-ms delay, stimuli were delivered as a discrete tone train composed of a random number of reference tones seamlessly followed by two discrete target tones. The target tones were shifted from the reference frequency by ±0 to 1 octave (±0–67%). Frequency change percentages were calculated using the direction-insensitive equation: $\Delta f = 2 \cdot |(f_{\text{reference}} - f_{\text{target}})| / (f_{\text{reference}} + f_{\text{target}})$. Reference and target tones were 200-ms in duration with 5-ms onset and offset cosine ramps, with a 200-ms inter-tone interval (ITI). The frequency change occurred in the ITI, and the number of reference tones was randomly set between 1 and 18, generating discrete nosepoke hold times in multiples of the 400-ms tone/ITI period. A trial was scored as a hit if the subject withdrew from the nosepoke within 600 ms of the onset of the first target tone. Two target tones were presented so rats could not cue on silence. Hit trials were rewarded with a 45-mg food pellet. Failure to withdraw within 600-ms of the onset of the first target tone was scored as a miss and resulted in a 5-second “time out”. Catch trials (0% Δf) were presented in order to estimate false alarm rate. 8.57% of trials were catch trials. Withdrawal from the nosepoke within 600 ms of the 0% frequency “change” in a catch trial was scored as a false alarm. Remaining in the nosepoke through a catch trial was scored as a correct rejection. Trials in which the rats withdrew before the onset of the target tones were scored as an abort and were discarded from analysis.

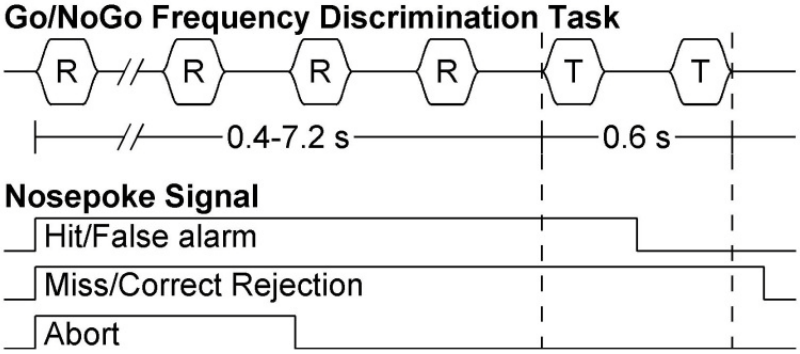


Figure 2.2. Behavioral paradigm for the discrete tone frequency discrimination task. Rats initiated trials by engaging a nosepoke which triggered a series of 1 to 18 reference tones (”R”) followed by a pair of target tones (“T”) from a speaker overhead. Withdrawal from the nosepoke within 600 ms of the onset of the first target tone was scored as a “hit” or “false alarm.” Withdrawal after 600 ms was scored as a “miss” or “correct rejection.” All tones were 200 ms in duration and were separated by 200-ms silent inter-tone intervals.

Reference frequencies in training were varied over 18 frequencies ranging from 2.31 to 27.66 kHz in 0.2105 octave steps. Subjects were initially trained with target tones of ±5–20% Δfs while required nosepoke hold times were incrementally increased up to 20 seconds. Subjects graduated to testing when they showed detection for all |Δf| ≥ 10% with a d' index of ≥1.96 (Green & Swets, 1966) and could hold for as long as 20 seconds before target tone presentation.

The size of the stimulus set was decreased during testing in order to ensure repeated presentations of individual stimuli by limiting reference frequencies to 3 frequency values: 4.15, 8.61, and 17.85 kHz. The total stimulus set used in testing consisted of 105 combinations of the 3 reference frequencies with upward and downward Δfs of ±1–9% in 1% steps, ±10–20% in 2% steps, ±half and ±full octave changes, and 0% change catch trials. Stimuli from abort trials were added to the end of the stimulus set for retesting. Hold times were randomly set between 0.4 and 7.2 s and were not reset by aborts.

2.4. Surgery and Recording

Prior to testing, rats were implanted in auditory cortex (AC) with micro-wire recording arrays under ketamine, xylazine and acepromazine anesthesia as previously reported (Sloan *et al*., 2009). Following a midline incision, removal of a portion of the right temporalis muscle and placement of 6 bone screws, a craniotomy was performed over auditory cortex. The dura was resected and cortical probes were implanted to a depth of 500-800 µm in the primary auditory cortex. Kwikcast silicone rubber was used to close the craniectomy. Acrylic was used to seal and attach the electrode connector to the skull.

2.5. Analysis

Neural recordings were obtained with an array of 16 independent microelectrodes. To determine whether or not a channel was recording activity from a tone-driven multi-unit cluster (MUC), peri-stimulus time histograms (PSTHs) were constructed from responses to all target tones $|\Delta f| \geq 10\%$ within a recording session. An MUC was determined to be "tone-driven" if the mean onset response to the target tones exceeded 3 standard deviations from the spontaneous rate. The spontaneous rate was calculated over the 50 ms prior to tone onset during the silent inter-stimulus interval period. The mean onset response was defined as the average firing rate from 10-30 ms after target tone onset. Recordings from MUCs which did not show tone-driven activity were excluded from the study.

Spike activity was analyzed by first binning spike times into PSTHs with 1-ms time bins and converted to spike rate. PSTHs from MUCs were smoothed using a 5-ms moving window. Temporal based spike sorting was attempted on all channels, but action potentials were irresolvable to single units at the majority of sites, and all signals are henceforth assumed to originate from multi-unit clusters. Single recording electrodes were temporally separated into up to three individual MUCs.

Chapter 3

3. Psychophysical Results of Rats Performing a Frequency Discrimination Task

3.1. Introduction

Frequency discrimination studies in rats that have traditionally used two behavioral paradigms to measure frequency discrimination abilities: two alternative forced choice (2AFC) and repeating standard. 2AFC paradigms, in which rats are presented with a pair of tones and must decide if their frequencies were the same or different, tend to report higher thresholds (Kelly, 1973; Syka *et al.*, 1996; Talwar & Gerstein, 1999). Repeating standard Go/No-Go paradigms, in which rats are presented with a sequence of reference tones at one frequency followed by a sequence of target tones at another frequency, tend to report lower frequency detection thresholds (Talwar & Gerstein, 1998, Sloan *et al.*, 2009). Talwar and Gerstein argued that a “repeating standard” paradigm presents a “detection” problem (*when* a frequency change occurs), and that two-tone, go/no-go trial paradigms present an “identification” problem (*if* a frequency change occurred). Animals performing a 2AFC frequency discrimination task are presented with all stimuli before being asked whether the stimuli are the same or are different. In this way they are asked to identify whether or not a frequency change occurred. A repeating standard frequency discrimination task requires subjects to listen to a series of stimuli and try to detect when the stimulus frequency changes. Reference sequence durations are typically randomized so that the subject cannot predict when targets will be presented and must continually compare current stimuli to previous stimuli. There is evidence supporting the use of different strategies for different types of frequency discrimination tasks in the discovery of different cortical processing pathways for different frequency discrimination tasks. Cats with bilateral lesions of the primary auditory cortices, suprasylvian and lateral gyri were unable to relearn a two-tone frequency discrimination task at Δf = 40% (Thompson, 1960), while cats with bilateral lesions of the same cortical areas were able to relearn a repeating standard frequency discrimination task at Δf = 18% (Cranford, 1978). In Sloan *et al*. (2009) we hypothesized that frequency difference limens (FDLs; the smallest difference in frequency that can be detected more accurately than at chance levels) measured with repeating standard paradigms are smaller due to the priming of the auditory system with preceding iso-frequency sequences.

Cortical priming, in a general sense, is any process by which neural responses to preceding stimuli affect the responses to following stimuli. Some types of auditory priming include forward masking, in which responses to preceding stimuli inhibits responses to following stimuli (Brosch & Schreiner, 1997), and repetition suppression (sometimes referred to as “habituation”; Wehr & Zador, 2005; Qin & Sato, 2004; Weinberger, 1991), in which responses to stimuli are inhibited by repeated exposure. Auditory stream biasing is another priming phenomenon in which forward facilitation/suppression builds up over the course of a sequence of discrete tones, leading to the percept of the sequence as a continuous stream instead of individual, discrete sounds (Rogers & Bregman, 1993; Beauvois & Meddis, 1997). Auditory stream formations are dependent on parameters such as tone duration and inter-stimulus intervals (Bregman *et al*., 2000), and under the proper conditions can occur in under a second (Micheyl *et al*., 2005). If auditory stream formation is occurring during a repeating standard paradigm then discrete tone sequences may be perceived as a single, unified sound object in the same way that a continuous tone of the same duration as the sequence would be perceived as a single sound object. Our previous study on the discrimination of tone-step frequency shifts within continuous tones revealed lower FDLs in rats than when performing a similar task using discrete tone sequences (Sloan *et al*., 2009). However, FDLs for the discrete tone task appeared to decrease as the overall tone sequence duration was increased, which may also have increased the likelihood that sequences were perceived as streams. It is possible that auditory streaming increases the likelihood of detecting a difference in stream tones.

In our previous study (Sloan *et al*., 2009), all frequency differences were preceded by at least 2.1 seconds of reference tones, which may have induced a bias towards perception of the reference frequency sequence as an auditory stream and the presentation of the target as the beginning of another. This study proposes that increasing the number of repeating tones preceding a target frequency change will result in improved detection ability. To test this hypothesis we changed parameters of the frequency discrimination task. In the revised paradigm presented here the minimum number of reference tones preceding a target frequency change was lowered to 1 and the maximum was set at 18. All parameters of the discrete tone task from the present study are the same as from Sloan *et al*. (2009), except that inter-stimulus interval was increased from 100 ms to 200 ms. This should not impact behavioral measures, as variance of inter-stimulus interval has been shown to have no effect on FDLs (Talwar & Gerstein, 1998). By randomizing the number of reference tones and including trials with fewer reference tones it should be possible to measure the effect that the number of reference tones has on FDLs of rats.

Another objective of this report is to measure the effect that reference frequency has on FDLs of rats. All animal species have ranges of sound frequency that are more crucial to survival and communication. As an example, bats have overrepresented frequency representation in primary auditory cortex of cells tuned to detect changes in the Doppler shift constant frequency (~64 kHz) used in the echo locating of their prey (Suga *et al.*, 1987). Early audiometric tests estimated that rats have extremely high-frequency hearing ranges and reported that albino rats are capable of discriminating frequencies up to approximately 48 kHz (Gourvitch & Hack, 1966). Later tests, however, reported that the preferred hearing range of albino rats is from 4 kHz to 38 kHz (Kelly & Masterton, 1977). Assuming that the preferred hearing range of albino rats is similar to that of the strain used in this experiment, the present study investigates how target frequency changes outside of the rat's preferred range might affect detection ability. This report also uses the opportunity to test another strain and gender of rat. Logistical and practical concerns made this strain of rat easily available for these experiments, and had the additional benefit of allowing us to add to the overall body of literature on rat frequency discrimination.

The final objective of this study is to ensure that the rats are capable of performing fine-grained frequency discrimination both before and after implantation surgery. The ability to link neural responses to behavioral measures is fundamental in modern neuroscience studies. The ultimate goal of this series of experiments is to study the neural responses in AC during discrimination of fine-grained frequency differences. Data from the subjects used in this study will be invalidated if they incapable of performing this task. Non-invasive means of obtaining neural signals, such as fMRIs, are typically cost-prohibitive and can restrict movement of subjects. Implantation of chronic, multi-channel recording devices allows for neural data to be recorded simultaneously from numerous cells while permitting the subject to move about relatively unhindered, but at the cost of some damage to the implanted area. Temporal lobe damage (including auditory cortex) have been shown to have varying effects on the localization and recognition of auditory stimuli (Penfield & Evans, 1934; Bocca *et al*., 1955; Sanchez-Longo *et al*., 1957; Jerger, 1960; Kimura, 1974), as well as perception and discrimination of tonal patterns (Milner, 1958). It is important to know if neural responses being gathered from implanted subjects represent normal behavior or if damage to cortical areas from implantation changes the strategies implemented by those subjects in performance of tasks. By comparing pre-surgery behavioral measures to post-surgery behavioral measures we hope to ascertain whether or not behavioral responses in a frequency discrimination task change due to implantation surgery.

3.2. Methods

Data from 9 un-implanted male Long-Evans hooded rats were obtained in this study. Six of the rats from the un-implanted group were implanted with a chronic, multi- electrode recording device implanted onto their AC. Behavioral measures of 6 implanted rats were included in the post-surgery group. Additional details concerning the Methods of this study can be found in Chapter 2.4. The care and use of animals in this study conformed to NIH guidelines and the University of Oklahoma Laboratory Animal Resources and Institutional Animal Care and Use Committee (IACUC) regulations.

3.3. Results

3.3.1. Pre-surgery

Behavioral testing was conducted over 348 individual testing sessions (median 44 sessions per animal, n = 9 animals). Animals were previously familiarized with a “tone step” discrimination task as described in the methods section for ~3-7 daily sessions before beginning training on the discrete tone discrimination task. The learning curve of the rats (Figure 3.1) shows a steady increase in false alarm corrected hit rate (ANOVA; $F_{(14, 132)} = 2.559$, $p = 0.003$) as function of training session number. False-alarm corrected hit rate is used as the measure of learning in this analysis to show that learning of this task is a combination of both increasing hit rate and decreasing false-alarm rate. The learning curve of the Long-Evans rats shown in this study resembles the learning curve of the Sprague-Dawley rats reported by Sloan *et al*. (2009).

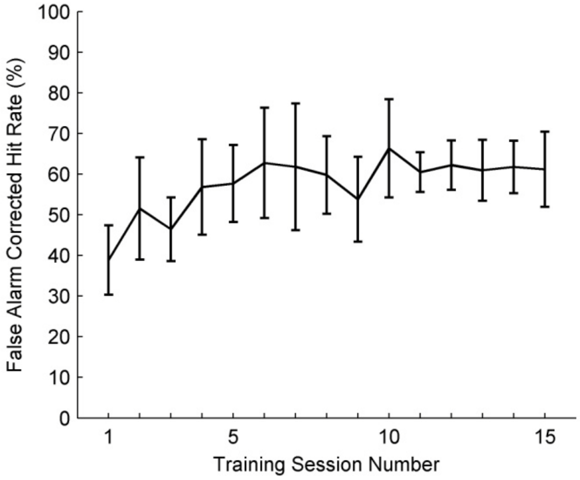


Figure 3.1. Mean false alarm-corrected hit rate from all animals (n = 9) as a function of training time. Error bars show 95% confidence intervals. All mean false-alarm corrected hit-rates were above "chance" (8.33% correct) during these training sessions.

Data from 47 sessions (median 3 sessions per animal) was discarded due to sub-criterion performance (<75% false alarm-corrected hit rate) to half- and full-octave shifts expected to be easily detected, supra-threshold frequency changes. Figure 3.1 includes data from trials with frequency differences below half-octave changes and thus does not represent 75% false-alarm corrected hit rate being achieved in the mean of any session number. The number of included pre-implant testing sessions per animal ranged from a minimum of 12 to a maximum of 137. Overall thresholds were not significantly correlated with the number of testing sessions (*r* = 0.59, *p* = 0.098).

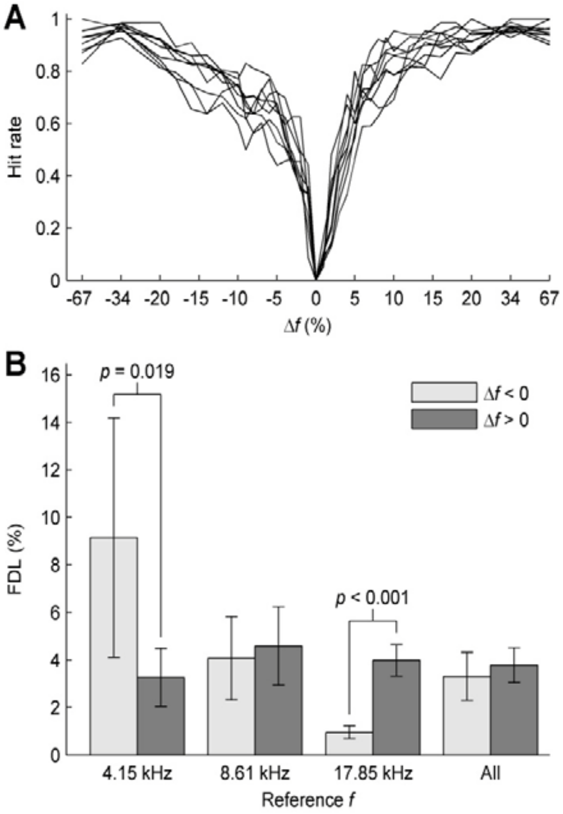


Figure 3.2. A) Psychometric functions of pre- surgery hit rate as a function of Δf for all animals (n = 9). Hit rates were combined across all reference frequencies. B) Comparison of frequency difference limens (FDLs) of upward and downward frequency change directions as a function of reference frequency. Error bars show 95% confidence interval.

Uncorrected hit rates for each animal (n = 9) are shown as a function of Δf in Figure 3.2A. These psychometric curves resemble curves from similar frequency discrimination paradigms used by Sloan *et al*. (2009). The FDLs of upward and downward frequency changes are plotted for each reference frequency to investigate the effects that frequency change direction has on detection ability (Figure 3.2B). FDLs for the lowest reference frequency (4.15 kHz) were significantly higher for decreasing frequency changes (t-test, *p* = 0.019). Conversely, FDLs for the highest reference frequency (17.8 kHz) were significantly higher for increasing frequency changes (t-test, $p < 0.001$). FDLs did not show a significant difference for frequency change direction at the middle reference frequency (8.6 kHz; t-test, $p = 0.63$).

The data suggest that there is an effect of frequency change direction at the lowest (4 kHz) and highest (17.8 kHz) reference frequencies. However, these edge effects are likely due to the small number of reference frequencies used in this study. Previous frequency discrimination studies which used a larger number of reference frequencies found slightly lower FDLs to downward frequency changes (Sloan *et al*., 2009), although this effect was inconsistent with trends found in lower and higher reference frequency FDLs from the present study. The edge effects seen in the FDLs at the lower reference frequency could also be partially due to the downward target frequency changes from the 4.14 kHz reference frequency being located outside of the rat's preferred hearing range (Kelly & Masterton, 1977), and thus being difficult to detect. Walker *et al.* also showed that in cases of frequency discrimination tasks that used two or fewer reference frequencies, ferrets attempted to at least partially employ a perfect pitch strategy, essentially discounting reference tones, which biased their hit rates towards targets in the middle of the frequency range (2009). Although directional effects were significant, the effect size was small compared to overall FDLs and both upwards and downwards FDLs were within a range of FDLs consistent with previous reports of frequency discrimination in rats (Kelly, 1970; Syka *et al.*, 1996; Talwar & Gerstein, 1998; Talwar & Gerstein 1999; Sloan *et al.*, 2009). Because frequency change direction showed no consistent effects across reference frequencies and no significant effect on FDLs for all reference frequencies combined (t-test, p = 0.396), frequency changes will be reported as directionless |Δf|%.

Average hit rates showed significant increase (ANOVA; $F_{(17,\ 161)}$ = 12.13, *p* < 0.0001) as a function of increasing |Δf| (Figure 3.3A). The pre-surgery average 50% hit rate FDL was interpolated at |Δf| = 3.58%. Mean hit rates were significantly correlated with increasing |Δf| (Spearman's rank test; *r* = 0.99, *p* < 0.0001). It should be noted that mean hit rates to octave changes (67% |Δf|; 94% ± 2.8%) were smaller than hit rates to half octave changes (34% |Δf|; 97% ± 1.1%), although the difference between these two target ranges was not significant (t-test; *p* = 0.12).

Average reaction times to targets on "go" trials ("false alarm" response to Δf = 0% catch trials and "hit" response to trials with |Δf| > 0% targets) are shown in Figure 3.3B as a function of |Δf|. Mean reaction times ranged from 315 ms ± 24 ms at Δf = 0% to 153 ms ± 22 ms at one octave changes. Larger frequency differences resulted in faster response times (Spearman's rank test; *r* = -0.98, *p* < 0.0001; ANOVA; $F_{(17,\ 161)}$ = 39.25, *p* < 0.0001) as a function of increasing |Δf|.

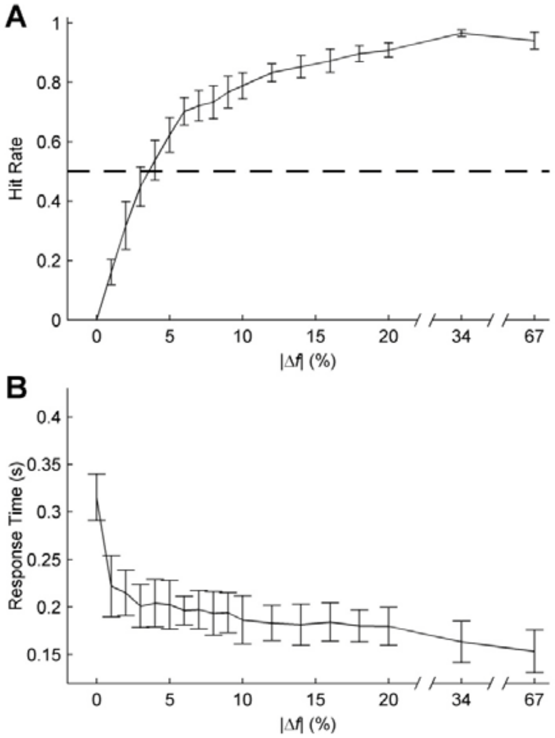


Figure 3.3. Psychometric functions (pre-surgery) of **A)** mean hit rate and **B)** response time to 'go' response trials and from all animals (n = 9) as a function of absolute frequency difference. The dashed line in the top plot shows to the 50% correct threshold, corresponding to |Δf| = 3.58%. Error bars show 95% confidence intervals.

To investigate the effects of trial hold time on frequency discrimination ability, a plot of FDL as a function of the number of reference tones is shown in Figure 3.4. Detection thresholds were inversely correlated with number of reference tones (Spearman's rank test; $r = -0.74$, $p < 0.001$) and were shown to decrease (ANOVA; $F_{(17,\ 155)} = 3.91$, $p < 0.0001$) as a function of increasing number of reference tones. Response times were likewise inversely correlated with number of reference tones (Spearman's rank test; $r = -0.94$, $p < 0.0001$) and were shown to decrease (ANOVA; $F_{(17,\ 155)} = 6.72$, $p < 0.0001$) as a function of increasing number of reference tones. Hit rates were divided into 5 |Δf|% range bins representing different ranges of detectability and are shown in Figure 3.4C. All |Δf|% bins showed increases in hit rate that were correlated with increasing number of reference tones (see Appendix A).

These results support the hypothesis that the number of reference tones has a significant effect on the behavioral responses of the rat. However, when looking at trials that only include 3+ reference tones, neither detection thresholds (ANOVA; $F_{(15, 137)} = 0.89$, $p = 0.578$), response time (ANOVA; $F_{(15, 143)} = 0.408$, $p = 0.975$), nor hit rates in any |Δf| bin (see Appendix A) retain a significant increase as an effect of number of reference tones. These results show that rats achieve asymptotic performance measures after exposure to 3 reference tones.

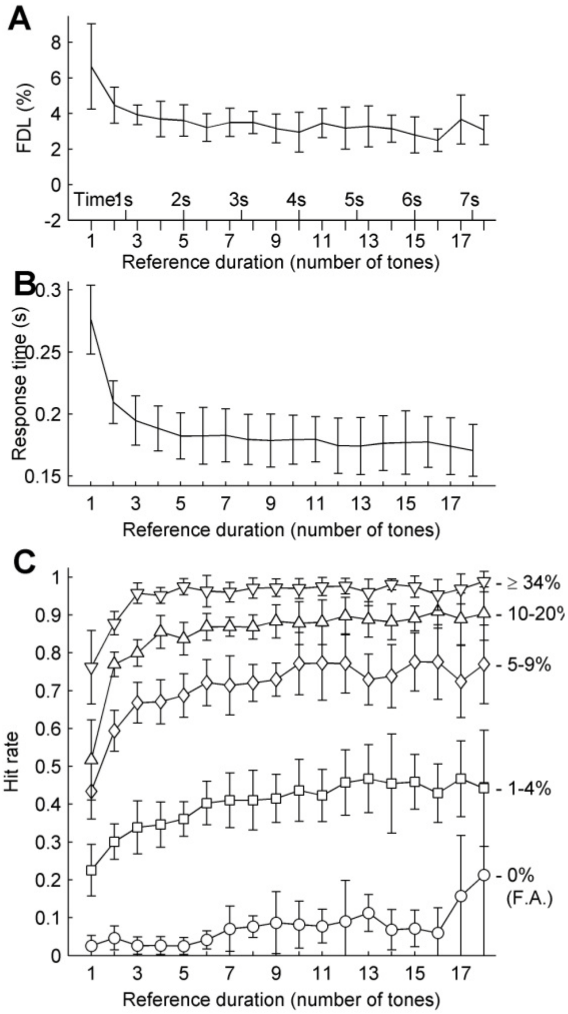


Figure 3.4. Psychometric functions (pre-surgery) of **A)** mean frequency difference limen (FDL) **B)** mean response time to non-catch trials resulting in a "hit" and **C)** mean hit rate are shown as a function of number of reference tones from all animals (n = 9). Hit rates are divided into 5 |Δf|% range bins. Error bars show 95% confidence intervals.

3.3.2. Post-surgery

All 9 rats reached performance criteria (achieving a significant d-prime rating of ≥ 1.96 for trials within a session and were able to hold for a 20 s reference duration) within 5 sessions of discrete tone discrimination training at the most difficult testing level. Total time spent training from tone-step familiarization to surgery was generally 15-30 days. Rats became eligible for electrode implantation after reaching performance criteria. One rat died during the implantation surgery. Data from another two of the rats were excluded from further analysis because the stimulus set used for testing them was slightly different from the stimulus set used to test the remaining 6 rats.

Rats were given 3 days to recuperate after implantation surgery before testing was resumed. The session immediately following surgery of each rat was omitted from all analysis to allow the animal to become reacquainted with the task. Additionally, this extra “practice” session was intended to give the animal more time to recover from the effects of surgery.

Figure 3.5 shows FDLs and hit rates for sessions before and after surgery. Analyses of pre- and post-implantation sessions reveal an apparent effect of surgery, especially for discrimination at the lowest reference frequency (4.14 kHz). Several data points for discrimination thresholds for the lowest reference frequency lie well above the unity line, indicating that surgery may have had a pronounced effect on animals’ ability to discriminate tones around that frequency. The 6 rats averaged a significant (t-test; $p = 0.001$) threshold increase of 4.75% $|\Delta f|$ at the 4.14 kHz reference frequency after implantation surgery. FDLs between pre- and post-surgery measures at the 2 highest reference frequencies (8.61 and 17.85 kHz) increased only 0.31% and 0.70%, respectively. A majority of the points (8 of 12) for the higher two reference frequencies lie above but still near the unity line, showing that although discrimination thresholds were better before surgery they were less affected by the procedure. These increases, though small, were shown to be significant (t-test; $p = 0.002$ for 8.61 kHz and $p < 0.001$ for 17.85 kHz).

The sample size and number of sessions included in each of these groups were the same. The data therefore suggest that these deficits in FDL were an effect of implantation surgery. It should be noted, however, that even after surgery the rats were still displaying the ability to discriminate fine-grained frequency differences.

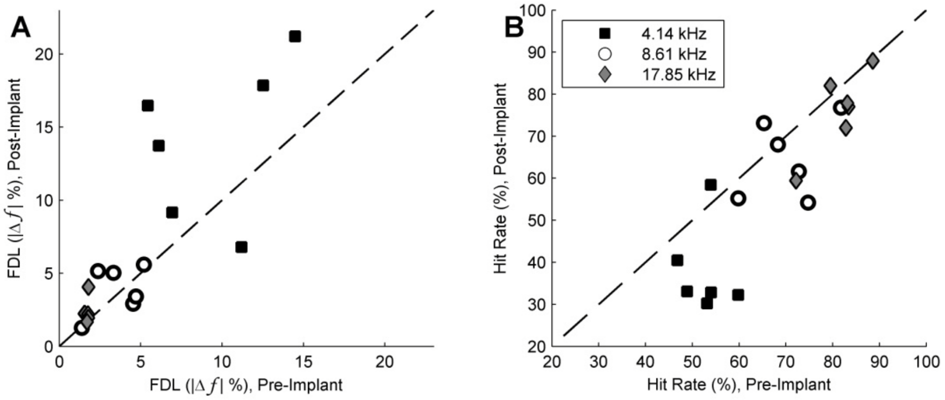


Figure 3.5. Effects of surgery on frequency discrimination as measured by **(A)** 50% correct performance threshold (|Δf| %) and **(B)** hit rate. Each scatter point represents one animals (n = 6) that provided psychophysical data after implantation surgery. Data from each rat were calculated from results of the 3 pre-surgery sessions against the 2nd-4th post-surgery sessions. Dashed lines show the unity lines for each figure.

Plotting the pre- and post surgery false alarm-corrected hit rates (Figure 3.5B) mirrors effects seen in the threshold plot (Figure 3.5A). Performance for the two highest reference frequencies decreased only 5.60% and 5.55% for the 8.61 and 17.85 kHz reference frequencies, respectively. However, paired t-test analysis shows that the differences at both of these reference frequencies were significant ($p < 0.001$ for both reference frequencies).

The 6 rats averaged a 14.90% drop in hit rate for trials at the 4.14 kHz reference frequency after implantation surgery, which was also shown to be a significant change ($p < 0.001$). Figure 3.5 represents mean measures collected from 3 pre-surgery and the 2nd-4th post-surgery sessions, however, extending the analysis to include all pre- and post-surgery sessions confirms that discrimination ability remains impaired after surgery, with the greatest effect being seen at the 4.14 kHz reference frequency.

Data from 12 sessions (median 2.5 sessions per animal) were discarded due to sub-criterion performance (<75% false alarm-corrected hit rate) to half- and full-octave shifts which should be supra-threshold. The number of included post-implant testing sessions per animal ranged from a minimum of 7 to a maximum of 57. Overall thresholds were not significantly correlated with the number of testing sessions ($r = 0.552$, $p = 0.244$).

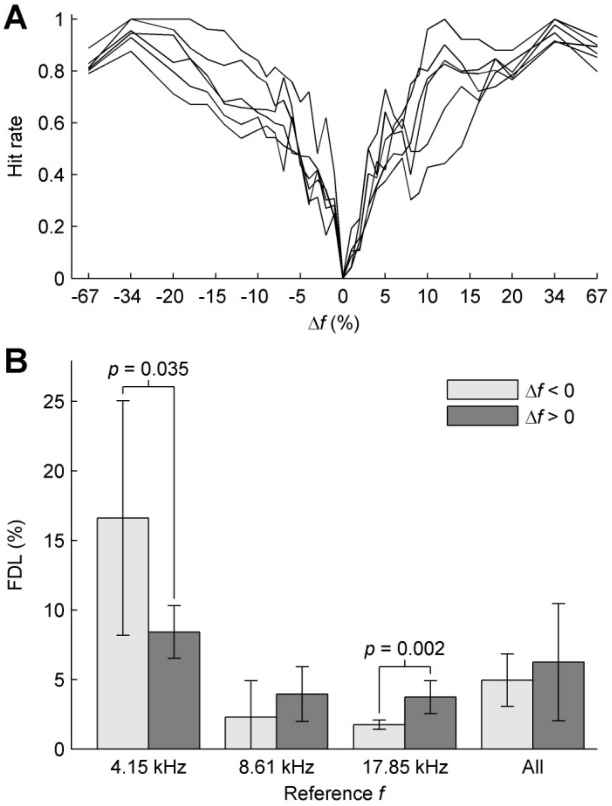


Figure 3.6. A) Psychometric functions of post- surgery hit rate as a function of Δf for all animals (n = 6). Hit rates were combined across all reference frequencies. **B)** Comparison of frequency difference limens (FDLs) of upward and downward frequency change directions as a function of reference frequency. Error bars show 95% confidence interval.

Uncorrected hit rates for each animal (n = 6) are shown as a function of Δf in Figure 3.6A. FDLs of upward and downward frequency changes are plotted for each reference frequency to investigate the effects that frequency change direction has on detection ability (Figure 3.6B). FDLs for the lowest reference frequency (4.15 kHz) were significantly higher for decreasing frequency changes (t-test, p = 0.035). FDLs for the highest reference frequency (17.8 kHz) were significantly higher for increasing frequency changes (t-test, p = 0.002). FDLs did not show a significant difference for frequency change directions of the middle frequency (8.6 kHz; t-test, p = 0. 223). Because post-op frequency change direction still showed no effect on FDLs for all reference frequencies combined (t-test, p = 0. 223), frequency changes will continue to be reported as directionless |Δf|% (see p. 26 for a discussion of these effects).

Post-surgery trends in behavioral data as functions of |Δf| were similar to trends seen in the pre-surgery data. Average hit rates showed general increases and a significant difference (ANOVA; $F_{(17,\ 107)} = 26.54$, $p < 0.0001$) as an effect of increasing |Δf| (Figure 3.7A). The post-surgery average 50% hit rate frequency difference limen was interpolated at |Δf| = 4.85%, increasing 1.27% from pre-surgery measures. Mean hit rates were significantly correlated with increasing |Δf| (Spearman's rank test; $r = 0.99$, $p < 0.0001$). As with the pre-surgery data, hit rates to octave changes (67% |Δf|; 85% ± 3.9%) were again smaller than hit rates to half octave changes (34% |Δf|; 96% ± 4.0%), this time showing a significant difference between the two (t-test; $p = 0.0015$).

Average reaction times of "go" trials ("false alarm" response to Δf = 0% catch trials and "hit" response to trials with |Δf| > 0% targets) are shown in Figure 3.7B as a function of |Δf|. Mean reaction times ranged from 298 ms ± 100 ms at Δf = 0% to 179 ms ± 30 ms for one-octave changes. Larger frequency differences resulted in faster response times (Spearman's rank test; $r = -0.83$, $p < 0.0001$; ANOVA; $F_{(17,\ 107)} = 14.61$, $p < 0.0001$), indicating that target |Δf| has a significant effect on response latency.

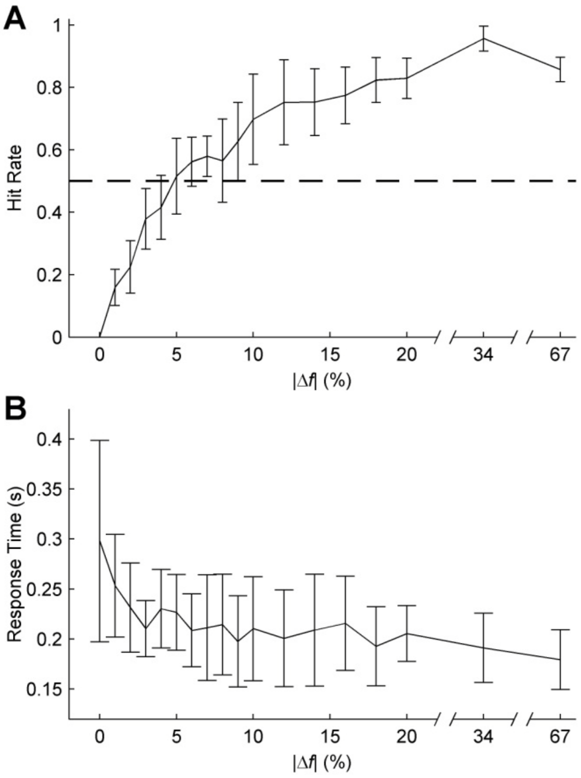


Figure 3.7. Psychometric functions (post-surgery) of **A)** mean hit rate and **B)** response time to 'go' response trials and from all animals (n = 6) as a function of absolute frequency difference. The dashed line in the top panel shows to the 50% correct threshold, corresponding to |Δf| = 3.58%. Error bars show 95% confidence intervals.

The effect of number of reference tones on post-surgery rat psychometrics is investigated in Figure 3.8. As with pre-surgery thresholds, the mean post-surgery detection thresholds of all test subjects were inversely correlated with number of reference tones (Spearman's rank test; $r = -0.73$, $p < 0.001$). However, unlike effects seen in the pre-surgery group, post-surgery thresholds from all test subjects were not significantly variant (ANOVA; $F_{(17, 94)} = 0.74$, $p = 0.752$) as a function of number of reference tones. This lack of variation in post-surgery detection thresholds is likely due to the wide range of thresholds from subjects at trials with one reference tone (5.57% ± 5.50%). Trends in post-surgery response times remained similar to pre-surgery trends, as response times decreased for trials with 1 to 3 reference tones before leveling out. However, neither the inverse correlation (Spearman's rank test; $r = -0.21$, $p = 0.41$) nor the decrease as a function of increasing reference tone (ANOVA; $F_{(17,\ 94)} = 0.74$, $p = 0.75$) were significantly different after implantation. Post-surgery hit rates in all ranges of detectability showed a correlation with increasing number of reference tones (see see Appendix A). Additionally, the middle 3 |Δf| hit rate bins (1-4%, 5-9% and 10-20% |Δf|) showed an increase in mean hit rate as a function of increasing reference tone

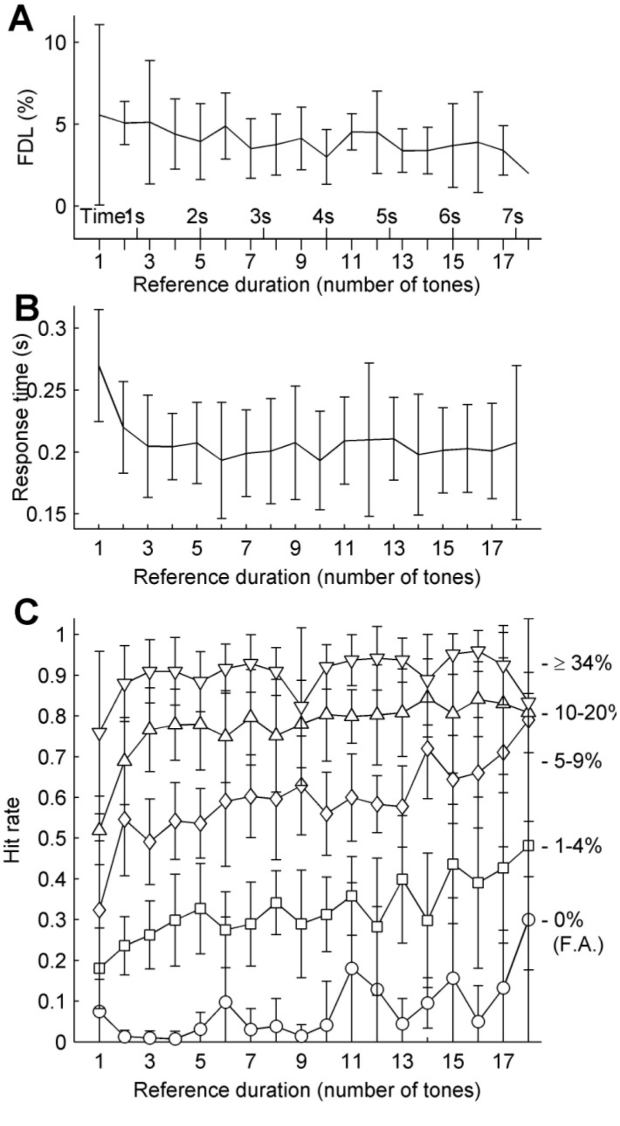


Figure 3.8. Psychometric functions (post-surgery) of **A)** mean frequency difference limen (FDL) **B)** mean response time to non-catch trials resulting in a "hit" and **C)** mean hit rate are shown as a function of number of reference tones from all animals (n = 6) prior to implantation surgery. Hit rates are divided into 5 |Δf|% range bins. Error bars show 95% confidence intervals.

number (see Appendix A), again showing that the number of reference tones has a significant effect on the behavioral responses of the rat. Of the 3 |Δf| hit-rate bins which increased as a function of number of reference tones, only 2 were not significantly changed for trials with 3+ reference tones (see Appendix A). These results suggest that, unlike un-implanted rats, implanted rats continue to benefit from further reference tone exposures. It is possible that this is an effect of practice, as the implanted rat group represents data from six of the same, albeit more-experienced, rats from the un-implanted group.

3.4. Discussion

This chapter presents behavioral measures of a previously undocumented strain and gender of rat performing a repeating standard go/no-go frequency discrimination task. Analysis was performed to investigate what potential effects reference frequency and the number of reference tones might have on FDLs of rats. Finally, this chapter shows that rats retain the ability to discriminate fine-grained frequency differences after implantation surgery on AC, which justifies the use of these subjects in subsequent chapters as an auditory systems model for studying the neural mechanisms of fine-grained frequency discrimination.

3.4.1. Effects of streaming on frequency discrimination

Previous reports from our lab have suggested that rats would show improved performance and decreasing response latency with increasing numbers of preceding reference tones in frequency discrimination tasks (Sloan *et al*., 2009). However, due to the relatively long minimum reference tone duration (2.1 s) of trials in the paradigm used for that previous report, no significant effect was found. It was hypothesized that significant effects might be seen if the minimum reference duration were decreased below 2.1 seconds.

The foundation for this hypothesis is found in other studies measuring rat performance during frequency discrimination tasks. Rats performed better in a repeating standard task with 5-35 seconds of exposure to 250-ms discrete reference tones (separated by 350-ms inter-stimulus intervals) than to a two-tone discrimination task with only a single 250 ms discrete reference tone preceding the target (Talwar & Gerstein, 1998). The average threshold for detection (defined as $A' = 0.85$) for the repeating standard task was $\Delta f = 3.26\%$, while the two-tone discrimination task averaged a larger detection threshold of $\Delta f = 6.25\%$. Another similar two-tone discrimination study using longer, 1-s pure tones separated by a 300-ms inter-stimulus interval yielded a relatively high detection threshold (defined as false alarm corrected hit rate = 50%) of $\Delta f = 5.7\%$ (Syka *et al*., 1996). The findings of these studies indicate that one brief reference tone may not be enough to achieve maximum frequency discrimination ability.

It is possible that frequency discrimination ability is enhanced when deviant frequency tones break a stream that has been established from exposure to the reference frequency. In this scenario frequency discrimination ability enhancement would be a function of number of individual tones over time. Single, continuous tones have been shown to require longer exposure to build up a streaming effect than repeated discrete tones (Bregman & Dannenbring, 1973; Tougas & Bregman, 1990). Alternatively, these results could indicate that repeating standard and 2AFC tasks are processed differently, possibly due to the different tactics (“detection” vs. “identification”) utilized to perform the tasks.

The current data show that at least 2 reference tones (800-ms hold time, a total of 400-ms tone presentation) are required for near-threshold |Δf| targets (5-9%) to reach 50% false alarm corrected hit rate. Additionally, larger |Δf| target ranges (10-20% and full octave) showed lower hit rates to trials with only a single reference tone and continual improvement to trials with at least 4 reference tones. Response latency to “hit” trials resemble effects seen in hit rate, with longer reaction times being seen in trials with 1 or 2 reference tones for all |Δf| targets. Thus it is apparent that longer exposure to reference tones enables better performance and faster reaction times in rats performing discrete tone “repeating standard” frequency discrimination tasks.

It is interesting that half-octave |Δf|s had a higher hit rate in both the un-implanted and implanted groups, although this effect was only significant in the implanted group. One possible explanation for the deficit in octave hit rates is the shared harmonic between the reference and target tone. A target tone that is one octave away from the reference, while being well above detection threshold, would share harmonic frequencies with the preceding reference tone. It is possible that rats use harmonic differences as a partial cue in detecting frequency differences, and thus are less capable of discriminating between tones that have differences of one octave than between tones with differences that are not one octave. However, it has been suggested that harmonic cues are not utilized when performing frequency discrimination or frequency modulation direction identification tasks, but that they do appear to be utilized in simple frequency modulation detection (Shackleton & Carlyon, 1994). While the role of harmonics has been explored using complex frequency modulation stimuli (Moore & Glasberg, 1990; Plack & Carlyon, 1995), the role that harmonic similarities play in simple pure-tone frequency discrimination warrants further investigation in future studies. It would be interesting to see if hit rates to 2, 3, or 4 octave changes might be less than hit rates to 1.5, 2.5 or 3.5 octaves.

3.4.2. Effects of frequency change direction

The poor performance on downward-going low frequency shifts and upward-going high frequency shifts shows a bias that could suggest the rats at least partly employed a “perfect pitch” strategy similar to that seen in ferrets by Walker *et al.* (2009). Walker *et al.* trained ferrets in a 2AFC frequency discrimination paradigm thought to require comparison of a target frequency to a reference frequency, but tests with multiple reference frequencies showed that ferrets ignored the reference frequency and attempted to classify target frequencies irrelative to reference frequencies. Since there are only 3 reference frequencies used in the present study, it could be that the rats attempt to employ a strategy akin to a bank of 4 bandpass filters, essentially trying to identify frequencies that are not one of the references, as opposed to notch filters, which one would expect to perform best when the summed distance to each notch is greatest, i.e. when the target frequency is at the edges. Presumably, based on the 18-reference frequency results reported by Sloan *et al*. (2009), this directional bias can be somewhat countered by employing multiple references within a session, but this would decrease the repetitions of each unique stimulus and lessen the strength of statistical analysis.

The high FDL to downward shifts at the 4.15 kHz reference frequency might be expected, as audiometric testing on intensity difference limens of albino rats showed that ~4 kHz was the lower border of the rats' preferred frequency range, which was shown to have an upper border at ~38 kHz (Kelly & Masterton, 1977). A downward target change from 4.15 kHz would fall outside the rats' preferred hearing range. However, the difference in frequency change direction at the highest frequency is surprising. Upward target changes from 17.85 kHz, even as large as one octave, would still fall well within the rats' preferred hearing range.

Consistent effects of frequency change direction have been difficult to pinpoint. Syka *et al*. (1996) found no significant effect of frequency change direction in pigmented rats, but with a small sample size of 3 rats, while Sloan *et al*. (2009) found lower thresholds for downward changes when combining across 18 reference frequencies and with a sample size of 24 rats. Talwar & Gerstein (1998) only tested with upward-going shifts. Cats (Brown *et al*., 2004), Cercopithecus monkeys, and one Rhesus monkey (Sinnott *et al*., 1987) showed lower FDLs for downward shifts, similar to Sloan *et al*. (2009), but Japanese macaques and humans have shown lower FDLs for upward shifts (Sinnott *et al*., 1987).

The relatively small number of reference frequencies used in this study, and the significant differences between upward and downward frequency difference limens, suggest that there may be significant directional effects with physiological underpinnings. However, other studies that have tested with larger numbers of reference frequencies have not found consistent directional effects despite observing similar significant comparisons. Sinnot *et al.* tested passerine birds with as many as 7 reference frequencies, and noted similar directional effects, better for upwards shifts at lower reference frequencies and better for downward shifts at higher reference frequencies (1980). However, the directional effects were not consistent between the endpoints, i.e. there was no inflection point where the directional effect flipped, but rather alternating directional preference that would be difficult to explain physiologically. Sloan *et al.* (2009) observed the same effect in rats when testing with 18 different reference frequencies. Significant directional effects were seen at some reference frequencies, but the effects varied across the spectrum without any clear trend. The directional effects reported here do show significance, but this might more reflect an influence of standard statistical tests that aren't conservative with regards to psychophysical data comprised of thousands of data points.

While these studies do not conclusively demonstrate that directional effects exist, it is likely that if they exist they are small enough that they are often missed with small experimental sample sizes. The presence of such directional effects might be an evolutionary tool, designed to make animals more tuned to sound sources that are moving towards or away from them. Doppler effects of sound sources moving closer to the listener create higher pitched frequencies, and it would stand to reason that species typically hunted as prey might be more attuned to upward frequency changes to give an early warning of advancing predators. Predatory fish species have been shown to respond differently than fish species typically hunted as prey to wave patterns simulating still water, surface winds, and approaching boats (Gabel *et al*., 2010). This suggests that animals may be more attuned to stimuli warning them of dangers, with different interactions based on their location on the food chain. However, to date no study has been conducted to support the hypothesis that mammalian predator and prey species have Doppler shift specific preferences.

3.4.3. Comparison to previous studies

While the discrete tone frequency discrimination paradigm presented in this paper is novel for measuring FDLs in hooded rats, the results are still comparable to other repeating standard paradigms used in previous studies.

Figure 3.9 shows the results of this study as compared to previous studies measuring FDLs in rats.

There are numerous differences in behavioral paradigm which may account for the slight differences in FDLs reported in this and other rat frequency discrimination studies. Syka *et al.* (1996) only utilized a 2AFC paradigm. Talwar & Gerstien (1998) used a repeating standard paradigm which resulted in FDLs of 3.06% for all frequencies combined. However, rats in Talwar & Gerstein's study had a minimum of 5 s of reference tone before presentation of the target and were given a relatively long window (2 s) in which to indicate perception of the target, suggesting that this task may have been less difficult than that reported here. The repeating standard frequency detection paradigm used in this study is derived from that used in Sloan *et al.* (2009). The behavioral paradigm used in this paper only differs from that used by Sloan *et al.* (2009) in that this paper used fewer reference frequencies (3 as compared to 18, spread over roughly the same frequency range) and that this paper included shorter hold times (0.4 s compared to 2.1 s in Sloan *et al.*, 2009). Sloan *et al* reported an FDL of 3.06% for all frequencies combined. The un-implanted group data from the present study are comparable to a 50% correct frequency change threshold of 3.58% for all frequencies combined.

Further differences between this study and those previous are in the strain of animals tested. Syka *et al.* (1996) performed experiments using female Long-Evans. Talwar & Gerstien (1998) used female Wistar. Sloan *et al*. (2009) used female Sprague-Dawleys. The present data represents thresholds of the male Long-Evans strain. It is unlikely however that gender or strain variance account for differences in measured FDLs, which are more likely due to differences in behavioral paradigm.

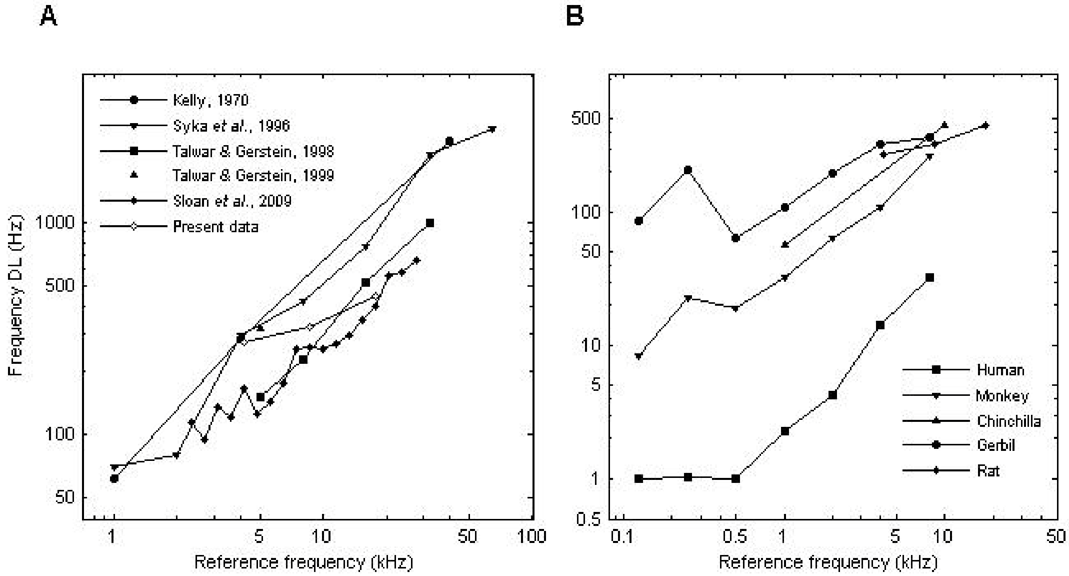


Figure 3.9. A) A comparison of measured FDLs of this study against FDLs measured in rats of previously published studies. **B)** A comparison of FDLs measured for five mammalian species using "repeating standard" frequency discrimination tasks: Human, Monkey, Gerbil (Sinnott *et al.*, 1992), Chinchilla (Prosen *et al.*, 1989), and Rat (present data).

3.4.4. Effects of surgery of performance

Overall behavioral trends between un-implanted and implanted rats were similar in every behavioral measure. Hit rates, FDLs, and response times of both groups showed improvement to larger |Δf| targets and trials with a greater number of reference tones. Implanted rats appeared to continue to improve behavioral frequency change detection with increasing number of reference tones, while frequency change detection in un-implanted rats approached an asymptote at 3 reference tones (1.2 s). This could be an effect of implantation surgery. Damage to AC has been shown to have various effects on auditory task performance (Meyer & Woolsey, 1952; Butler *et al.*, 1957; Goldberg & Neff, 1961; Kelly, 1973; Ohl *et al.*, 2001). However, it could also be an effect of training. All implanted animals (n = 6) had previously learned the frequency discrimination task and provided un-implanted data. Previous studies have shown improved discrimination thresholds with repeated practice in humans (Ari-Even Roth *et al*., 2003), monkeys (Recanzone *et al*., 1993), and rats (Syka *et al*., 1996; Talwar & Gerstien, 1998). It is also possible that the lack of asymptotic behavioral measures in the implanted rats is due to the smaller sample size of that group compared to the un-implanted group. Several data points showed a wide range of values during 1 and 2 reference tone trials, which may have washed out any significant effect that might have been seen in shorter duration trials with a larger sample size.

Chapter 4

4. Neural Correlates of Frequency Discrimination in Behaving Rats

4.1. Introduction

Neural correlates of behavior have been extensively studied in the visual (Tolhurst *et al*., 1983; Britten *et al*., 1992; Shadlen *et al*., 1996; Liu and Newsome, 2005; Purusgothaman & Bradley, 2005) and somatosensory systems (Hernandez *et al*., 2000; Romo *et al.*, 2002; Luna *et al.*, 2005; Vazquez *et al.*, 2012). Similarly, different types of sound stimuli detection have been linked to neural response activity in primary auditory cortex (A1). Tone detection (Niwa *et al*. 2012a; Niwa *et al*. 2012b; Zhang *et al.*, 2012), click-train speed discrimination (Dong *et al.*, 2011), acoustic flutter discrimination (Lemus *et al.*, 2009) and speech sound representation (Engineer at al., 2008; Shetake *et al.,* 2011; Tsunada *et al.*, 2011; Ranasinghe *et al*., 2012) have all been shown to have neural correlates in responses of auditory cortex (AC).

A previous publication from our lab has demonstrated that rats performing a discrete tone discrimination task can reliably detect frequency differences as low as 0.03 octaves, or ~ 3% (Sloan *et al.*, 2009). Similar 50% correct frequency discrimination thresholds have been found in previous rat studies (Syka *et al.*, 1996; Talwar & Gerstein, 1998; Talwar & Gerstein, 1999).

However it has not been shown that auditory cortex is capable of resolving threshold level frequency differences. Supra-threshold frequency differences (~17% Δf) have been documented in spectro-temporal receptive fields of ferret A1 cells. These cells displayed enhanced responsiveness to target and suppressed responsiveness to reference tones (Fritz *et al*., 2005). Learning a frequency discrimination task has been shown to increase target tone onset response in A1 of owl monkeys (Blake *et al*., 2002). Both of these reports reveal neural mechanisms within AC which, by increasing contrast between reference and target tone responses, could facilitate discrimination of frequency differences. However, neither study described the neural correlates of threshold-level frequency discrimination in behaving animals.

If A1 is involved in threshold-level frequency discrimination then behavioral detection ability should be correlated with neural activity in AC. To test this hypothesis multi-unit neural activity in AC of rats was recorded during a discrete tone frequency discrimination task. The purpose of this study was to determine if AC encodes small frequency differences on par with behavioral thresholds. The results of this study demonstrate that increases in the firing rate during the onset responses of AC cells to deviant frequencies (target tones) are correlated with detection ability. Additionally, the data demonstrate that the onset latency of AC cells to deviant frequencies is correlated with the behavioral response time (nose poke withdrawal). These results demonstrate that AC contains sufficient information for performance of threshold-level frequency discrimination.

4.2. Methods

A total of 5 male Long-Evans hooded rats were used in this study. Neural recordings were taken from AC of each subject via chronic, multi-electrode implants. Additional details concerning the Methods of this study can be found in Section 1.4. The care and use of animals in this study conformed to NIH guidelines and were in accordance with the University of Oklahoma Laboratory Animal Resources and Institutional Animal Care and Use Committee (IACUC) regulations.

4.3. Results

Tone-driven neural activity was recorded from a total of 85 electrodes implanted chronically in AC of 5 rats while they performed a discrete tone frequency discrimination task. Spike sorting analysis revealed that 12 of the 85 channels were recording activity from two different multi-unit clusters (MUCs), so that activity from a total of 97 MUCs was recorded. These recordings were used to determine the correlation between neural activity and two behavioral measures: hit rate and response time.

The rats were tested on equivalent upward (+Δf) and downward (-Δf) frequency changes. In concordance with previous studies (Syka *et al*., 1996, Sloan *et al*. 2009) the rats in the present study did not show a significant difference in their ability to discriminate ascending and descending frequency changes (ANOVA; $p = 0.811$) for all reference frequencies combined, thus frequency differences in this study are reported in absolute magnitude.

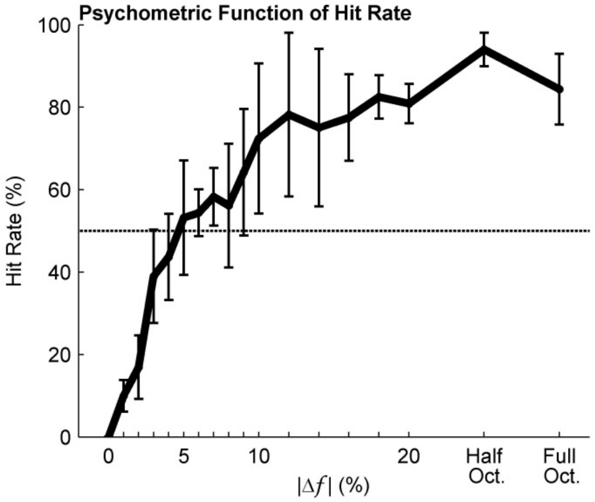


Figure 4.1. The psychometric function of mean hit rate is shown from all rats (n = 5) as a function of absolute frequency difference. The dotted line shows 50% correct threshold, corresponding to |Δf| = 4.66%. Reference frequencies used were 4.15, 8.61, and 17.85 kHz. Error bars show 95% confidence intervals.

Analysis of the behavioral data reveals that discrimination performance improved with increasing frequency difference. Spearman's rank test showed that average hit rates were significantly correlated with frequency difference (*r* = 0.99, *p* < 0.001). The average 50% hit rate frequency difference limen was interpolated at |Δf| = 4.66%.

The predominant neural responses to tone presentations were onset and offset responses. Behavioral reaction times, measured as the time from tone onset to nosepoke withdrawal, to more salient target tones (|Δf| ≥ 10%) averaged 199.7 ms from tone onset while performing frequency discrimination tasks, indicating that rats responded to large Δf targets before offset response to tone presentation is seen in the AC responses (~210 ms). This timing difference suggests that the offset response of tones may not be playing a role in frequency discrimination.

Some MUCs showed increased firing rates during presentation of target tones for the duration of the tone. It is possible that this increased spike rate during target tone presentation could be a factor in the detection of a difference in frequency. The mean population spike activity diagram (Figure 4.4A) shows an increased spike rate during target tone presentation (seen beginning at ~550 ms) to larger |Δf| targets. However, an analysis of response times indicates that increases in spike rate prior to target tone cessation could be motion artifact generated by the sound the rat makes as it is withdrawing from the nosepoke. This hypthesis is supported when dividing the neural responses into trials in which the rats did and did not respond to the target tone (discussed further in Chapter 4; Figure 4.3). Trials in which the rats did not respond to target tones do not show an increase in spike rate during presentation of the target tone.

Reaction times indicate that neural activity after target tone onset response may be motion artifact and/or irrelevant to the process of frequency discrimination. Therefore, I hypothesize that behavior in the frequency discrimination task may be most clearly represented by the onset response to the target tone. The onset response to reference tones typically occurred at ~10 ms and ended ~30 ms after tone onset. The difference in firing rate between target and reference tones within this window will be the neural mechanism focused on in this and the following chapters.

MUCs from this study showed a diversity of response types and bandwidth tuning. Neural recordings were analyzed from single MUCs from multiple sessions pooled together to create a spectro-temporal spike activity diagram (STSAD) (n = 97). Multiple sessions were pooled in this way, as responses recorded from single trials or even single sessions did not typically contain enough spikes to achieve statistical significance. Differences in mean onset spike rate between final reference tone and first target tone were calculated as a function of |Δf| for each MUC. The correlation between the difference in onset response spike rate to frequency changes and the behavioral hit rate detection were individually calculated for each MUC. Increases in firing rate to target tones from 46 of the 97 MUCs (47.4%) from this study were correlated with behavioral performance. Mapping of the location of the behaviorally correlated MUCs in relation to each other within each rat showed that behaviorally correlated MUCs were scattered over the entire cortical area covered by the 16 channel electrode array.

An example MUC with neural responses that are significantly correlated to hit rate (Spearman's rank test; $r = 0.73$, $p = 0.0008$) is shown in Figure 4.2. Onset responses to the target tone for Δf = 0% catch trials closely resemble the onset responses to the reference tone, as would be expected. However, as the frequency difference between reference and target tone increases, the onset firing rate difference between reference and target tone increases. This increase in onset firing rate is closely correlated with the hit rate of this rat during sessions from which these neural data were recorded.

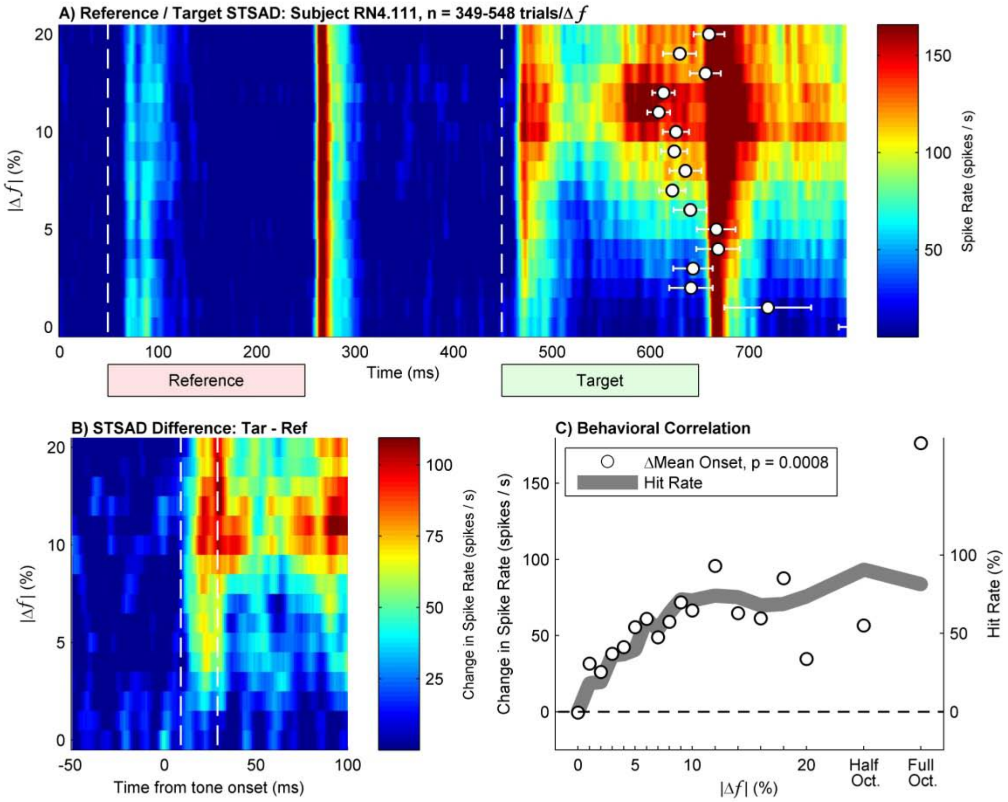


Figure 4.2. **A)** The mean STSAD of final reference tone and the first target tone of an example MUC with an increase in mean onset firing rate that is correlated with detection ability (n = 46 of 97 MUCs) is shown as a color map for |Δf| ranging from 0% to 20%. Mean response times to target tones at each |Δf| are shown by white markers with error bars indicating 95% confidence interval. The color bar shows the spike rate (spikes / s) scale. The dashed white lines at 50 ms and 450 ms represent tone onsets for the reference and target tones. The pink and green boxes show reference and target presentation times (50-250 ms and 450-650 ms, respectively). **B)** The mean difference STSAD between the final reference tone and the first target tone is shown for |Δf| ranging from 0% to 20%. The dashed white lines represent the mean onset window (10-30 ms) used for analysis. **C)** Mean population changes in onset spike rate between target tone and final reference tone (circles) and psychometric function of correlated mean hit rate (gray curve; Spearman's rank test; *r* = 0.73, *p* = 0.0008) are shown as a function of target |Δf|. The dashed black line represents 0% change in spike rate and 0% hit rate. STSADs are shown smoothed with a 5 ms/3 |Δf| box smooth. Reference frequencies used were 4.15, 8.61, and 17.85 kHz.

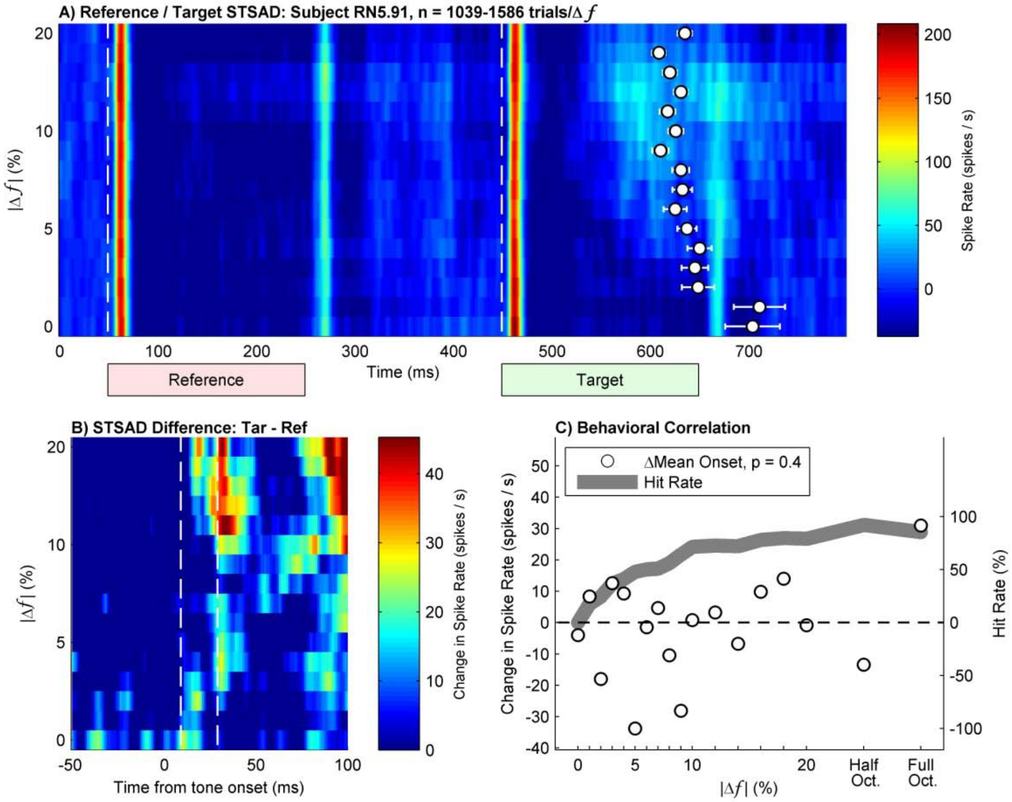


Figure 4.3. **A)** The mean STSAD of final reference tone and the first target tone of an example MUC with an increase in mean onset firing rate that is not correlated with detection ability (n = 51 of 97 MUCs) is shown as a color map for |Δf| ranging from 0% to 20%. Mean response times to target tones at each |Δf| are shown by white markers with error bars indicating 95% confidence interval. The color bar shows the spike rate (spikes / s) scale. The dashed white lines at 50 ms and 450 ms represent tone onsets for the reference and target tones. The pink and green boxes show reference and target presentation times (50-250 ms and 450-650 ms, respectively). **B)** The mean difference STSAD between the final reference tone and the first target tone is shown for |Δf| ranging from 0% to 20%. The dashed white lines represent the mean onset window (10-30 ms) used for analysis. **C)** Mean population changes in onset spike rate between target tone and final reference tone (circles) and psychometric function of un-correlated mean hit rate (gray curve; Spearman's rank test; *r* = 0.19, *p* = 0.44) are shown as a function of target |Δf|. The dashed black line represents 0% change in spike rate and 0% hit rate. STSADs are shown smoothed with a 5 ms/3 |Δf| box smooth. Reference frequencies used were 4.15, 8.61, and 17.85 kHz.

Figure 4.3 shows an example MUC with an invariable onset response to all target tones across all frequency differences |Δf| ≤ 20%. The onset to target tones in this cell resembles the onset to preceding reference tones.

Analysis of the difference in spike rate between reference and target tones over the 10-30 ms onset window shows that target tones ranged from -33.8 to 31.0 spikes / s. These differences were not correlated with hit rate detection of this rat during sessions from which these neural data were recorded (Spearman's rank test; $r = 0.19$, $p = 0.44$). Non-behaviorally correlated increases in onset spike rate to target tones were seen in 51 of 97 tone-driven MUCs.

The mean normalized population response of all behaviorally correlated MUCs (n = 46 of 97) is shown in Figure 4.4A. Responses were normalized to the onset of the reference tone. Responses to 0% Δf target tone catch trials show similar onset response rates and inhibitory periods as responses to identical preceding reference tones. However, onset responses to |Δf | > 0% target tones differed from those of reference tones, featuring stronger and longer-lasting onset responses as the |Δf| increased.

Figure 4.4B shows the difference in onset responses between target and reference tones as a function of frequency difference for the population. In general, larger frequency differences result in larger increases in firing rate. There is a ~25% increase in the peak firing rate near the detection threshold of 4.66%. Responses to 0% Δf target catch trials show no difference compared to responses to the identical preceding reference tones. However, the data show a ~20% increase in peak firing rate for sub-threshold (2-4% |Δf|) changes, with ever larger increases for increasing frequency differences. It is clear that AC onset responses increase to even sub-threshold frequency differences during behavior.

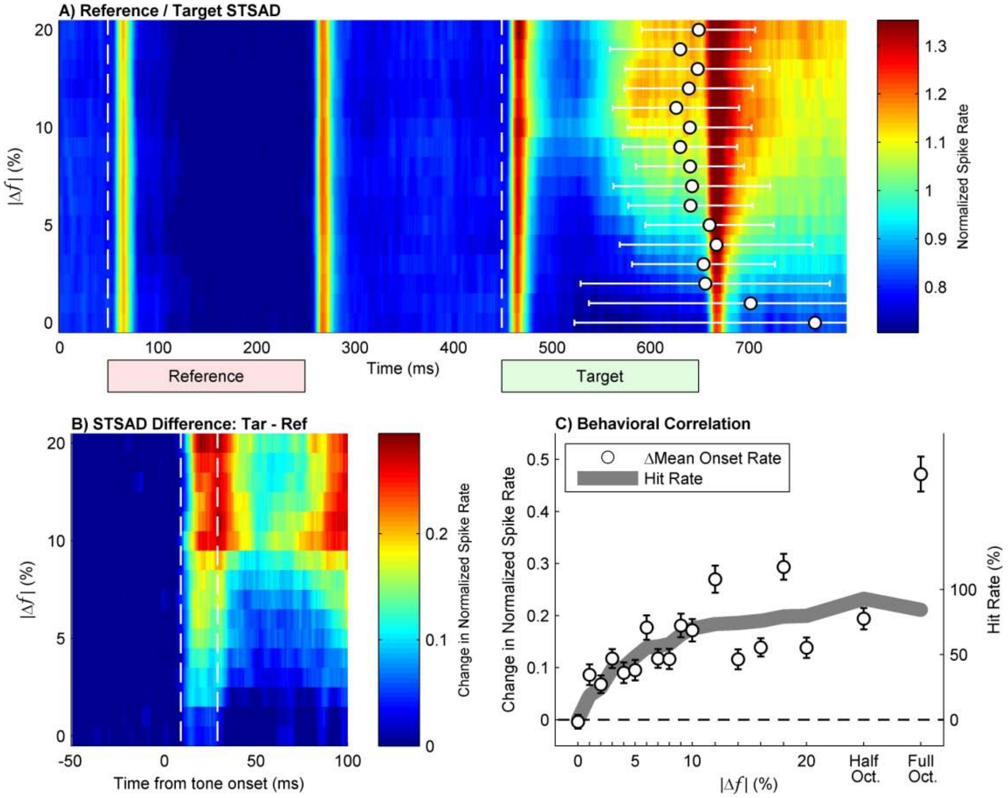


Figure 4.4. A) Mean population STSAD of final reference tone and the first target tone are shown combined for all behaviorally correlated multi-unit clusters (n = 46 from 665 sessions) as a color map for |Δf| ranging from 0% to 20%. Multi-unit clusters were normalized by the average onset amplitude of the final reference tone. Mean response times to target tones at each |Δf| are shown by white markers with error bars indicating 95% confidence interval. The color bar shows the normalized spike rate (%) scale. The dashed white lines at 50 ms and 450 ms represent tone onsets for the reference and target tones. The pink and green boxes show reference and target presentation times (50-250 ms and 450-650 ms, respectively). **B)** Mean difference in STSADs between the final reference tone and the first target tone is shown for all behaviorally correlated multi-unit clusters for |Δf| ranging from 0% to 20%. The dashed white lines represent the mean onset window (10-30 ms) used for analysis. **C)** Mean population changes in onset spike rate between target tone and final reference tone (circles) and psychometric function of correlated mean hit rate (gray curve; Spearman's rank test; *r* = 0.79, *p* = 0.0001) are shown as a function of target |Δf|. The dashed black line represents 0% change in spike rate and 0% hit rate. Error bars show 95% confidence interval. STSADs are shown smoothed with a 5 ms/3 |Δf| box smooth. Reference frequencies used were 4.15, 8.61, and 17.85 kHz.

The difference in mean onset firing rate, averaged over 10-30 ms following tone onset, between target and reference tones from the 46 behaviorally correlated MUCs (gathered from a total of 665 sessions) is shown as a function of |Δf| in Figure 4.4C. In general, the mean onset firing rate difference increases with increasing |Δf| targets, ranging from -0.40% ± 1.32% for 0% Δf catch trials to 47.16% ± 3.36% at full octave changes. Also shown is the mean hit-rate as a function of |Δf| weighted to the number of MUCs recorded from each session, which shows similar trends and is significantly correlated to the changes in the driven rate of AC responses (Spearman's rank test; $r = 0.79$, $p = 0.0001$). Onset responses in AC gradually increase with increasing frequency differences between the reference and target tones.

It should be noted that while normalized increases in onset response to octave frequency changes (47.1 ± 3.36%) were substantially larger than increases to half-octave frequency changes (19.4 ± 2.06%) the hit rate between these two frequency difference magnitudes was shown (in the previous chapter) to be significantly higher for half octave changes. The larger increase in neural response seen in AC to octave frequency changes paired with the lower detection rates would seem to suggest that signals from AC are not playing a key role in the detection of those frequency changes. It is possible that other processing areas are sending conflicting information about the presence of an octave frequency change (possibly confounded by harmonic similarities), which when integrated with information from AC results in decreased detection ability.

The activity of the remaining 51 MUCs (gathered from a total of 398 sessions) that were not behaviorally correlated individually was also not significantly correlated with behavior (Spearman's rank test; $r = 0.41$, $p = 0.09$) when combined into a mean normalized population response. However, when all recordings were combined across all 97 tone-driven MUCs (from 1063 sessions), regardless of significant individual correlation with behavior, the correlation of mean onset response to hit rate was also significant (Spearman's rank test; $r = 0.66$, $p = 0.003$), though less representative of behavioral measures.

The behavioral analysis shows significant directional effects at the lower (4.14 kHz) and higher (17.8 kHz) reference frequencies for the post-surgery group (Figure 3.6B). However, with only one reference frequency tested in the middle range, it's difficult to argue that the directional effects definitely reflect some physiological cause. Other studies that used more reference frequencies did not see consistent directional effects that might be suggested by this data (Sinnott *et al*., 1980; Sloan *et al*., 2009). If a psychophysical frequency change direction bias does exist, then the neural mechanism which is shown to correlate with hit rate with respect to directionless frequency changes in trials combining all reference frequencies should also be representative of hit rate with respect to frequency change direction in trials at individual reference frequencies. The preceding analysis groups reference frequencies to preserve sample sizes for statistical analysis, but a separate analysis for each reference frequency actually suggests that there may be neurophysiological correlates to the directional effects. Spearman's rank test correlations show that the increase in onset response to target tones as a function of Δf with respect to frequency change direction were significantly correlated to hit rate for trials at 4.14 kHz (r = 0.51, p = 0.002) and 17.8 kHz (r = 0.82, p < 0.0001) reference frequencies, showing that changes in onset firing rate have the same biases towards upward and downward frequency changes. These results add validity to the proposed neural mechanism of detection by showing that effects which may cause changes in detection ability are also affecting the neural responses associated with that detection.

Another common measure of behavioral response is reaction time. B.F. Skinner's law of latency states that reaction time is inversely related to the salience of the stimulus (Skinner, 1938), and larger frequency differences should result in faster response times. Behavioral reaction time was measured during testing as the time it took the rats to withdraw from the nosepoke following the onset of the target tone.

Onset latency was defined as the first point on the PSTH for the target tone response at which the spike rate exceeded 2 standard deviations from the spontaneous rate. The spontaneous rate was calculated over the 50 ms prior to tone onset during the silent inter-stimulus interval period. Figure 4.5 shows the onset latency and the mean reaction time to "go" trials (trials resulting in a hit or false alarm) as a function of the difference in frequency of the target from the reference. The data show decreasing reaction times and shorter onset latency to increasing frequency differences. Spearman's rank test shows a significant correlation between response time and onset latency in AC ($r = 0.87$, $p < 0.0001$). This close relationship between neural response onset latency and behavioral reaction times is consistent with the hypothesis that activity in AC contains information sufficient to drive threshold-level frequency discrimination.

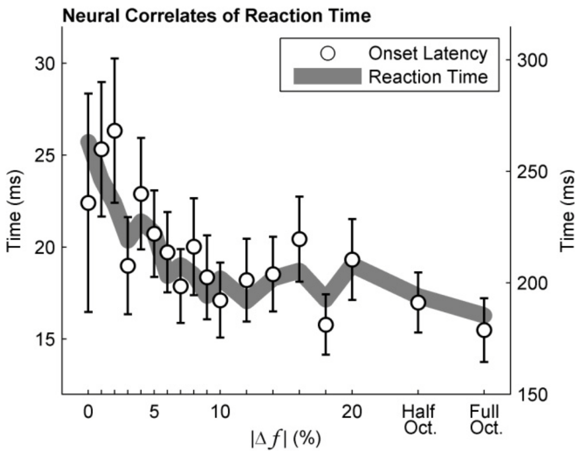


Figure 4.5. Mean population changes in target tone onset response latency, measured as the first point in the target response PSTH which exceeded 2 standard deviations of the spontaneous rate (white points), and the psychometric function of response time (gray line; Spearman's rank test; $r = 0.87$, $p < 0.0001$) to trials which resulted in a "Go" response are shown as a function of target |Δf|. Error bars show 95% confidence interval of tone-driven MUCs (n = 97 from 1063 sessions). Data was collected in 5 ms moving-window bins. Reference frequencies used were 4.15, 8.61, and 17.85 kHz.

4.4. Discussion

Action potentials were recorded from AC neurons in chronically implanted rats performing a frequency discrimination task and two neural correlates of behavior were identified. First, increases in firing rate to the onset of the target tone relative to the reference were shown to be correlated with frequency discrimination performance. This observation is consistent with reports in primary somatosensory (Hernandez *et al.*, 2000) and visual (Tolhurst *et al*., 1983; Britten *et al.*, 1998; Liu & Newsome, 2005) cortex that neural responses are correlated with behavioral discrimination. Second, the onset latencies of AC responses to target tones were shown to be correlated with behavioral response time.

The role of AC in frequency discrimination has historically been difficult to establish. Frequency specific information is first resolved at the cochlea before being passed through sub-cortical auditory areas. Several studies have shown that the sub-cortical processing is sufficient for gross frequency discrimination, as rats with intact sub-cortical nuclei but bilaterally ablated AC have been able to discriminate sounds with large frequency differences (Meyer & Woolsey, 1952; Butler *et al.*, 1957; Goldberg & Neff, 1961; Kelly, 1973; Ohl *et al.*, 2001). However, acute bilateral deactivation of A1 with the $GABA_A$ agonist muscimol has been shown to temporarily eliminate the ability of rats to detect tones or perform frequency discrimination (Talwar *et al*., 2001). As the drug effect fades, the ability to detect tones returns first, followed by the ability to discriminate large frequency differences and finally the ability to discriminate smaller frequency differences. Similar results were found in the specialized Doppler-shifted constant-frequency A1 area of the mustached bat when fine grained frequency resolution was impaired for up to 3.2 hours following muscimol deactivation (Riquimaroux *et al*., 1991). Bilateral lesion studies in rats showed that AC is required for discrimination of subtle differences in complex speech sounds (Porter *et al*., 2010) and amplitude modulated sounds (Cooke *et al*., 2007), but not for detection of large differences in frequency or intensity. Similar deficits in detection of subtle sound differences have been shown in human patients with bilaterally destroyed A1 (via stroke or seizure; Mendez & Geehan, 1988, Tramo *et al*., 2002). The results of these studies suggest that A1 is most likely normally involved in resolving fine-grain frequency differences in auditory stimuli. The correlation between AC responses at near-threshold level and behavior presented here supports this hypothesis.

The number of cells needed to accurately represent behavioral measures is an often-debated subject. A vision study on the detection of motion signals found that responses of single middle temporal visual area (MT) cells of monkeys were typically adequate to encode psychophysical measures (Britten *et al.*, 1992). However, the responses of single MT cells during a visual speed discrimination task was found to be less representative of behavior than population MT responses when using stimuli at near perceptual threshold levels (Liu & Newsome, 2005). MUCs, but not single units, in A1 were correlated with speech sound discrimination (Engineer *et al*., 2008). Even pooling responses of weakly correlated MT cells (which may be optimized to respond to different aspects of visual stimuli) produced better correlations with behavior than single cell responses (Shadlen *et at*., 1996).

While population coding may represent behavioral measures more accurately than single cells, it does not necessarily preclude contributions of specialized cells. A study in MT of monkeys found that population responses of finely tuned cells closely correlated with perceptual decisions even though population responses of all active MT cells showed poor correlation with behavior (Purushothaman & Bradley, 2005). The data presented here are consistent with these findings and show that the correlation between behavior and increases in firing rate onset responses between final reference and first target tone averaged over the entire population of tone-driven MUCs (Spearman's rank test; $r = 0.66$, $p = 0.003$) was less than the correlation for a restricted population only including individually behaviorally correlated MUCs (Spearman's rank test; $r = 0.79$, $p = 0.0001$). This finding, along with the individually significant correlation found in the nearly half of tone-driven MUCs (47.4%), suggests that frequency difference information may be processed by a specialized subset of cells and not integrated broadly from AC.

Recently it has been shown that both humans (Bitterman *et al*., 2008) and non-human primates (Bartlett *et al*., 2011) have a subset of A1 cells with narrow frequency tuning on the order of 0.08 octave bandwidths, or an ~11% frequency difference, in humans and 0.05 octaves, or an ~7% frequency difference, in monkeys, yet both species have displayed the ability to resolve frequency differences far smaller (Sinnott *et al*., 1985; Sinnott & Brown, 1993; Wienicke *et al*., 2001). There are many models that can explain how these narrowly tuned cells could be used to perform finer resolution frequency discrimination. However, the discrepancy between behavioral thresholds and frequency selectivity of these fine-tuned cells suggests that single cell model hypotheses are not adequate. Bartlett *et al*. (2011) proposed that these finely tuned neurons serve to sharpen frequency responses in A1, but that population coding is still required for resolution of near threshold changes. While sorting of the MUCs in this study was attempted, very few were resolvable to single units. Therefore, this study cannot determine if single units encode these behavioral measures. However, the population of AC units (Figure 4.3) clearly shows that even sub-threshold frequency differences are being encoded by combined responses of AC cells.

It is important to note that both of these studies, which report finding ultra-fine frequency resolution A1 cells, were conducted on passively listening human subjects (Bitterman *et al.*, 2008) and non-human primates (Bartlett *et al.*, 2011). It is well-documented that behavioral context alters neural response properties in AC (Fritz *et al.*, 2003; Atiani *et al*., 2009; Otazu *et al*., 2009). A study with anesthetized cats has shown that preceding auditory stimuli can alter the response of neurons to following stimuli if they are within 430 ms (Brosch & Schreiner, 1997), effectively masking the latter tone making it indistinguishable from the first. However, both monkeys and humans are able to discriminate complex, closely-spaced (<430 ms) auditory stimuli (monkey howl vocalizations) when attending in a behavioral task (Fugate *et al*., 2008). It is likely that the ability of the auditory system to resolve small frequency differences might similarly only be seen while rats are attending to stimuli. This hypothesis is supported by the findings of Recanzone *et al*. (1993), who showed that attending to auditory stimuli decreased the frequency response area of A1 cells in owl monkeys. Despite the clear representation of threshold-level behavior in population responses, the possibility of single cell models also representing behavioral thresholds cannot be discounted. It is possible that the finely-tuned frequency response areas of single cells during task engagement could also account for behavioral frequency resolution abilities.

In addition to behavioral discrimination of frequency differences being encoded in the mean firing rates of AC cells, neural response latency was shown to be correlated with reaction times of rats performing a frequency discrimination task. This study demonstrates that more salient frequency differences resulted in faster behavioral response times. Likewise, more salient frequency differences achieved significantly higher-than-spontaneous firing rates more quickly. The correlation between psychophysical reaction time and AC firing latency supports the hypothesis that frequency difference information is present within activity of AC.

The study presented in this chapter shows that information necessary to encode threshold-level frequency differences is present in AC. The information from this study, however, cannot definitively lead to the conclusion that discrimination of frequency differences is occurring there. Auditory information ascends from the thalamus to AC. AC has been shown to be necessary for threshold-level frequency discrimination, however the present study cannot determine if AC is the first location within the neural auditory pathway to encode this information, or if it is just a necessary relay point.

Future studies pairing recordings from AC and sub-cortical auditory nuclei in behaving animals could enhance understanding of the role of subcortical auditory nuclei in frequency discrimination.

Chapter 5

5. Neural Correlates of Decision Making in Rats Performing a Frequency Discrimination Task

5.1. Introduction

Recent studies have shown neural correlates in auditory cortex (AC) related to discrimination of such auditory stimuli as acoustic flutter (Lemus *et al*., 2009), click-train speed (Dong *et al*., 2011), speech sounds (Engineer at al., 2008; Shetake et al., 2011; Tsunada *et al.*, 2011; Ranasinghe *et al*., 2012), amplitude modulation (Niwa *et al*., 2012a, Niwa *et al*., 2012b) and simple tone detection (Zhang *et al*., 2012). The previous chapter of this dissertation describes neural responses which are highly correlated with detection ability of threshold-level differences during performance of a frequency discrimination task. The extent to which this neural mechanism may also encode information underlying subsequent behavioral decisions remains to be answered.

Neural correlates of discrimination often contain responses from both correct and incorrect judgments about the same stimuli. Neural responses can be correlated with actual stimulus dimensions and/or what the subject judges those dimensions to be. Neural correlates of discrimination in the visual system have been shown to also be predictive of the subject's response. Weakly tuned individual cells in the medial temporal gyrus of monkeys were shown to not only correlate with discrimination thresholds

(Britten *et al*., 1992, Shadlen *et al.*, 1996), but to also predict behavioral decisions in a motion direction discrimination task (Britten *et al*., 1996). Similar results have been shown in the somatosensory system as well. Increases in firing rate of primary somatosensory cells were shown to correlate with the detection ability of monkeys performing a vibrotactile discrimination task (Hernandez *et al.*, 2000). Later studies in primary (Luna *et al*., 2005) and secondary somatosensory cortices (Romo *et al.*, 2002) showed that this detection mechanism was predictive of behavioral reports.

It is uncertain, however, whether the neural responses in AC simply reflect stimulus features, or also reflect the animal's behavioral decisions regarding the stimulus. The correlation between neural responses from AC and corresponding behavioral decisions has met with conflicting reports. Recordings from A1 were shown to represent discrimination thresholds, but did not represent behavioral decisions of subjects performing acoustic flutter discrimination (Lemus *et al.*, 2009), click train discrimination (Dong *et al.*, 2011), or tone-detection (Zhang *et al*., 2012). However, A1 responses from monkeys performing a more difficult amplitude modulation discrimination task were shown to be correlated with the animal's behavioral decision (Niwa *et al.*, 2012b). Higher firing rates were seen in responses of A1 during trials in which monkeys reported detection of an amplitude modulated tone versus trials with the same stimulus for which monkeys reported no detection.

The purpose of this study is to investigate whether or not there is a correlation between neural responses in AC and behavioral decisions during performance of a threshold-level frequency discrimination task. Additionally, responses in AC were monitored during a task when the subjects are trained to ignore the same frequency changes to determine if differences in neural responses to identical stimuli are modulated by task relevance.

5.2. Methods

A total of 5 male Long-Evans hooded rats were used in this study. Neural recordings were taken from AC of each subject via chronic, multi-electrode implants. Additional details concerning the Methods of this study can be found in Chapter 2.4. The care and use of animals in this study conformed to NIH guidelines and University of Oklahoma Laboratory Animal Resources and Institutional Animal Care and Use Committee (IACUC) regulations.

5.2.1. Noise Detection Task

In addition to the frequency discrimination task which has been described in previous chapters, this study reports on neural responses during testing of a broadband noise detection task. Four of the rats which participated in the frequency discrimination task from this report also participated in the noise detection task. The noise detection task was similar to the frequency discrimination task in several ways. The rats had no indication at the beginning of the sessions which stimulus set they would be tested on that day. However, they typically only required 1 or 2 trials before beginning to perform the task correctly.

Rats initiated a trial by engaging a central nosepoke. Following a 50 ms delay, stimuli were delivered as two discrete 200 ms, 60 dB reference tone trains (R1 and R2) composed of a random number of tones followed by a pair of broadband noise targets. R1 contained between 1-18 tones, followed by R2 which also contained between 1-18 tones, but the number of tones in the combined reference sequences never exceeded 20 tones. The number of tones in each reference train was independent of each other. Reference tones and reference trains were separated with a 200 ms inter-tone interval. The frequency of R1 was randomly varied between 3 frequency values: 4.15, 8.61, and 17.85 kHz. R2 was at a second frequency different from R1 (except during $\Delta f = 0\%$). The frequency of R2 was shifted either upward or downward from R1 by a frequency step of $\Delta f = \pm 0$–9% in 1% steps, or ±10–20% in 2% steps. Frequency change percentages were calculated using the direction-insensitive equation: $\Delta f = 2 \cdot |(f_{R1} - f_{R2})| / (f_{R1} + f_{R2})$. The final tone of R2 was followed by a pair of 200 ms, 60 dB broadband noise targets. Hits, misses, false alarms, and correct rejection trial outcomes were dependent on the rat’s response to the broadband noise targets following R2.

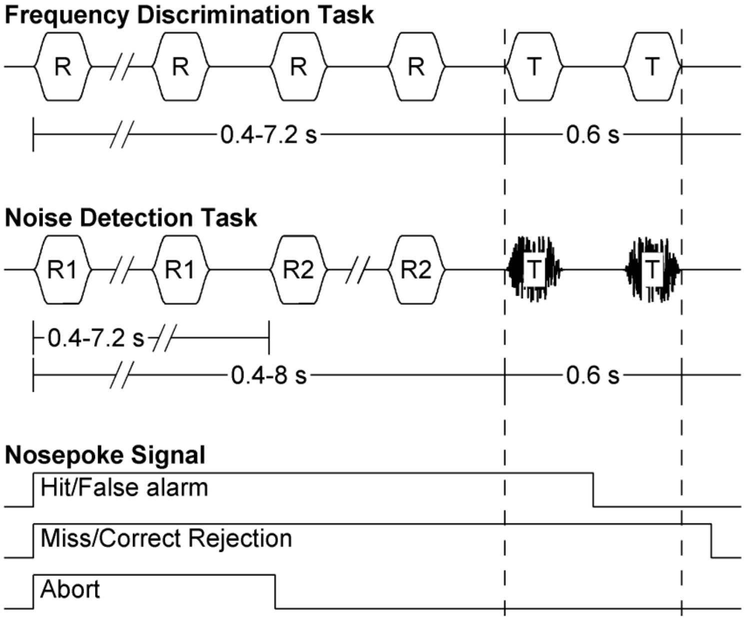


Figure 5.1. Behavioral paradigms for the discrete tone frequency discrimination and noise detection tasks. For the frequency discrimination task rats initiated trials by engaging a nosepoke, which triggered presentation of a series of between 1 to 18 reference tones (”R”) followed by a pair of target tones (“T”). For the noise detection task subjects would elicit 2 reference tone sequences (“R1” and “R2”) which would each contain between 1 and 18 reference tones each, followed by a pair of broadband noise targets (“T”). Withdrawal from the nosepoke within 600 ms of the onset of the first target was scored as a “hit” or “false alarm.” Withdrawal after 600 ms was scored as a “miss” or “correct rejection.” All tones and noise bursts were 200 ms in duration and were separated by 200 ms silent inter-tone-intervals.

A trial was scored as a hit if the subject withdrew from the nosepoke within 600 ms of the onset of the first target noise burst. Two target noise bursts were presented so rats could not cue on silence. Hit trials were rewarded with a 45 mg food pellet. Failure to withdraw within 600 ms of the onset of the first target noise burst was scored as a miss and resulted in a 5 second “time out” during which the cage lights were extinguished and nosepoke signals were ignored. Catch trials (trials in which no noise burst followed R2) were presented in order to estimate false alarm rate. Withdrawal from the nosepoke within 600 ms prior to the end of the final tone of R2 during a target-less catch trial was scored as a false alarm. Remaining in the nosepoke through a catch trial was scored as a correct rejection. Trials in which the rats withdrew before the onset of the target tones were scored as an abort and were discarded from analysis.

5.3. Results

Recordings were taken from 66 electrodes with tone driven neural activity implanted chronically in 5 rats. Although recordings were stable during sessions, day-to-day variability suggested that recordings might not consistently include the same set of neurons, so each multi-unit cluster was analyzed independently for each recording session. In total, recordings from 240 multi-unit cluster samples were collected over a 6 month period from 5 animals (66 electrodes) while performing a discrete tone frequency discrimination task. The previous chapter of this dissertation showed that the difference in peak driven onset rate in rat AC between reference and target tones was correlated with behavioral detection. The present study investigates whether or not neural responses in auditory cortex reflect behavioral decisions during a frequency discrimination task.

Examples of different neural responses from trials resulting in Go and No-go behavioral decisions to identical $|\Delta f|$ targets are shown for an example MUC as a peri-stimulus time histograms (PSTHs) in Figure 5.2. Figure 5.2A-B shows final reference and first target tone responses of a unit when the target tone is shifted by 10%. There is no significant difference in mean onset response rate to the reference tones (2-sample t-test; $p = 0.37$) between behavioral decisions, but the onset response to the target tone is significantly larger preceding a Go (hit) decision than a No-go (miss) decision (2-sample t-test; $p = 0.015$). The increased onset response to targets for Go decisions seen in this MUC was typical of those recorded in this study.

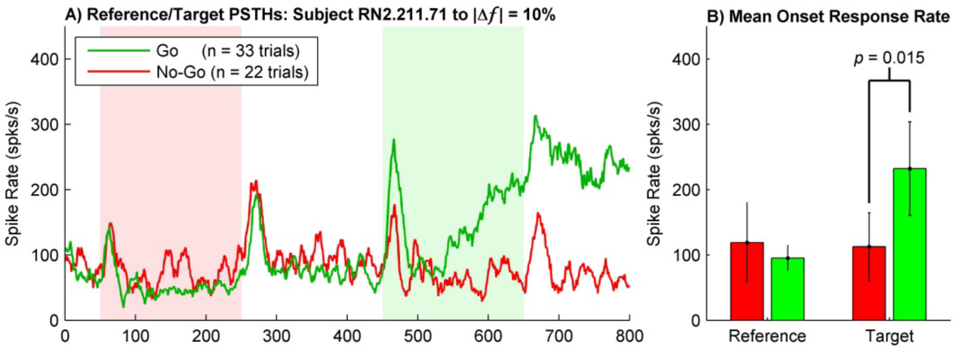
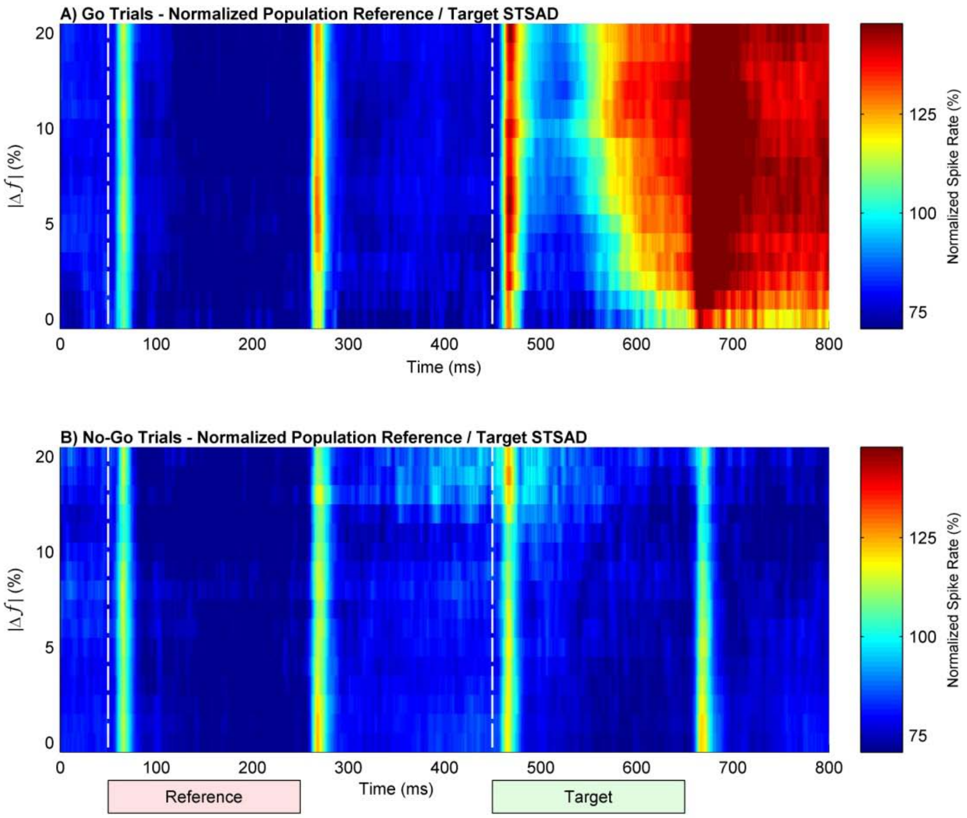


Figure 5.2. The mean response PSTHs of an individual MUC is shown for "go" and "no-go" decisions to final reference and target tones. **A)** This MUC shows similar responses to the reference tone, but increased onset response to "go" (hit) decisions at |Δf| = 10%. **B)** Mean onset spike rates (measured from 10-30 ms after tone onsets) for the MUC shown in Figure 5.2A were not different between behavioral decisions for reference tones (t-test; $p = 0.37$), but were for targets (t-test; $p = 0.015$). The red and green highlighted areas show reference (50-250 ms) and target (450-650 ms) presentation times, respectively. Data were collected in 10 ms moving-window bins. Reference frequencies used were 4.15, 8.61, and 17.85 kHz.

Significant differences between mean onset responses of Go and No-go decisions to Δf = 0% catch trials were rarely found. It is possible that this is because AC does not encode the perception of frequency differences when none are present. It is also possible that the rarity of significant effects of this type for catch trials is a statistical effect due to subjects' low false alarm rates. However, when combing the neural responses from false alarm and correct rejection trials of all MUCs from all sessions (n = 299), a significant difference can be seen in the neural data between these two behavioral outcomes (2-sample t-test; *p* = 0.01). This difference can be seen in Figure 5.6. Overall, the responses from these example MUCs suggest that auditory cortex does not simply encode stimulus features, but may also contain information sufficient to predict behavioral decisions during a frequency discrimination task.

The mean normalized population responses of all MUCs to the final reference and first target tones are shown in Figure 5.3 as spectro-temporal spike activity diagrams (STSADs). Neural responses preceding Go (Figure 5.3A) and No-go (Figure 5.3B) decisions were normalized to the mean onset response to the final reference tone for that MUC. Responses to reference tones in either decision outcome typically featured an onset response to the reference tones occurring ~10-30 ms after the beginning of the tone, followed by marked inhibition during tone presentation, and an offset response beginning ~10 ms after tone cessation. Responses to target tones also featured an onset response from ~10-30 ms after the beginning of the tone and an offset response beginning ~10 ms after tone cessation. The increased response during target presentation beginning ~550 ms into the trials resulting in a Go decision is likely a result of motion artifact, defined as a neural response to the sound produced by the rat while withdrawing from the nosepoke, as indicated by the reaction time data shown in Figure 3.4. The lack of spike rate increase during No-go trials in which the rat did not generate sound during withdrawal from the nosepoke (because in this instance withdrawal did not occur) supports this.


A) Go Trials - Normalized Population Reference / Target STSAD

B) No-Go Trials - Normalized Population Reference / Target STSAD

Figure 5.3. Mean population STSAD for the final reference tone and the first target tone are shown for all tone-driven MUCs (n = 299 sessions) as a color map for |Δf| ranging from 0% to 20% for trials which resulted in A) a "Go" behavioral response and B) a "No-go" behavioral response. The color bar indicates normalized spike rate (spikes/s). The dashed white lines at 50 ms and 450 ms represent tone onsets. The green boxes show reference and target presentation times (50-250 ms and 450-650 ms, respectively). STSADs are shown smoothed with a 5 ms/3 |Δf| box smooth. Reference frequencies used were 4.15, 8.61, and 17.85 kHz. Isolated multi-unit clusters for both responses were normalized to the average onset firing rate for the final reference tone for the "Go" trials of that multi-unit cluster for that |Δf|.

To more clearly see the difference in the population response preceding Go versus No-go decisions, the responses preceding No-go decisions were subtracted from the responses preceding Go decisions (Figure 5.4). There is no difference in mean reference tone onset response across |Δf| target trials

($F_{(15,\ 9404)} = 1.13$, $p = 0.32$), nor between Go and No-go outcomes ($F_{(1,\ 9404)} = 0.12$, $p = 0.71$), indicating consistent responses to reference tones regardless of proceeding target frequency change or behavioral decision. The onset responses to target tones, however, are stronger in trials resulting in a Go decision than in trials resulting in a No-go decision ($F_{(1,\ 9404)} = 306.9$, $p < 0.0001$). Even mean onset responses to sub-threshold |Δf| targets were significantly different between trials resulting in Go and No-go decisions ($F_{(1,\ 2868)} = 79.4$, $p < 0.0001$). Additionally, the differences in target onset response rates are shown to be different across |Δf| ($F_{(15,\ 9404)} = 6.31$, $p < 0.0001$).

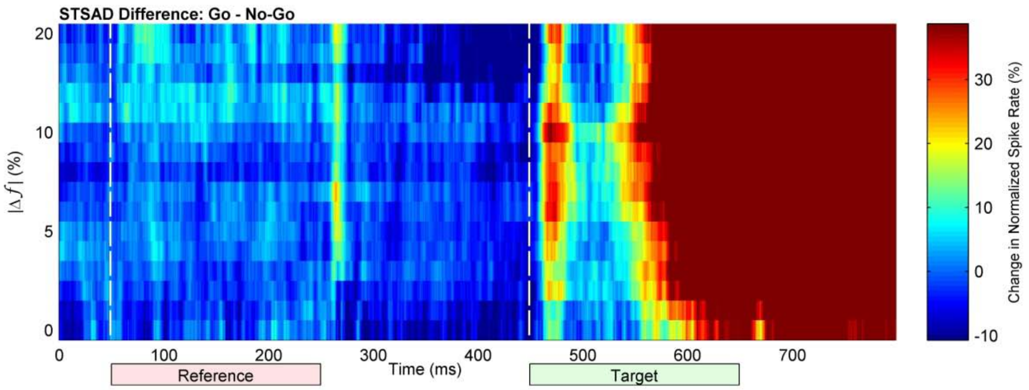


Figure 5.4. Mean population (n = 299 sessions) difference STSAD between "Go" and "No-go" responses of the onsets of the final reference and the first target tones are shown for all tone-driven MUCs. The color bar indicates change in normalized spike rate. The dashed white lines at 50 ms and 450 ms represent tone onsets. The pink and green boxes show reference and target presentation times (50-250 ms and 450-650 ms, respectively). STSADs are shown smoothed with a 5 ms/3 |Δf| box smooth. Reference frequencies used were 4.15, 8.61, and 17.85 kHz. Recordings from each isolated multi-unit cluster for both behavioral outcomes were normalized to the average onset firing rate for the final reference tone to trials preceding a Go decision.

The previous chapter of this dissertation showed that larger frequency differences between reference and target tones resulted in higher hit rates, and that the increase in hit rate was correlated with increases in the mean onset response difference between reference and target tones for all trials regardless of outcome. To investigate the relation between this mechanism and the perception of a frequency difference (as inferred from the rats' behavioral indication) the population onset response to the preceding reference tone was subtracted from the response to the target for trials resulting in Go (Figure 5.5A) and No-go decisions (Figure 5.5B). Responses to target tones in all non-catch trials preceding Go decisions were significantly increased relative to the responses to preceding reference tones (multiple comparisons t-test, Bonferroni correction, $\alpha = 0.0016$; see Appendix B for aggregate p-values). Conversely, for most supra-threshold trials ($|\Delta f| \geq 5\%$, excluding $|\Delta f| = 14$ and 20%) resulting in No-go decisions, the responses to target tones were not significantly different from responses to preceding reference tones (multiple comparisons t-test, Bonferroni correction, $\alpha = 0.0016$; see Appendix B). Interestingly, however, responses to most non-catch sub-threshold targets ($1\% \leq |\Delta f| \leq 4\%$, excluding $|\Delta f| = 3\%$) in trials resulting in No-go decisions were in fact significantly greater than responses to the preceding reference tones (multiple comparisons t-test, Bonferroni correction, $\alpha = 0.0016$; see Appendix B). This could indicate that response differences, while significant, did not exceed some threshold required for detection, and/or that misses for supra-threshold frequency changes were more due to procedural errors than actual non-detection. A “procedural error” in this case may be due to the rat not paying attention to the stimuli, or otherwise being distracted from performance of the task, which may cause the rat to miss a large, normally detectable frequency change.

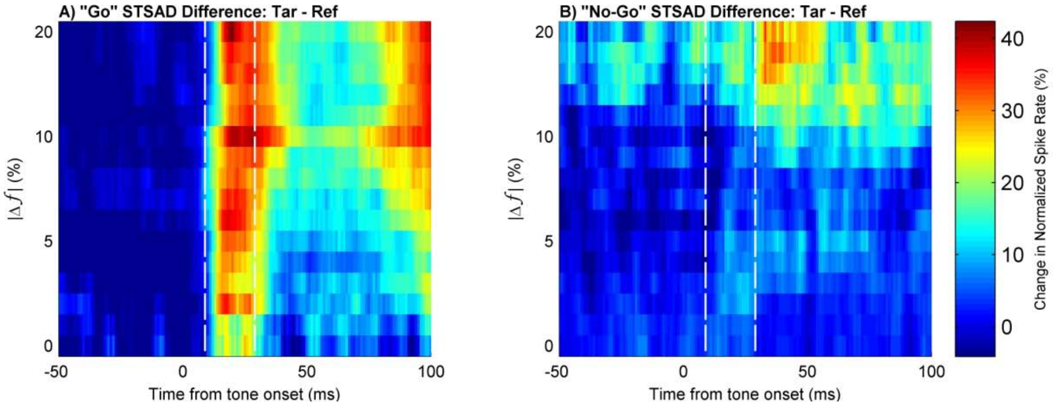


Figure 5.5. The mean population (n = 240) difference STSAD between the onsets of the final reference tone and the first target tone are shown for all tone-driven MUCs as a color map for |Δf| ranging from 0% to 20% for trials which resulted in **A)** a “Go” behavioral response and **B)** a “No-go” behavioral response. The color bar indicates change in normalized spike rate (spikes/s). The dashed white lines indicate tone onset windows from 10-40 ms after tone onsets. STSADs are shown smoothed with a 5 ms/3 |Δf| box smooth. Reference frequencies used were 4.15, 8.61, and 17.85 kHz. Recordings from each isolated multi-unit cluster for both behavioral outcomes were normalized to the average onset firing rate for the final reference tone to trials preceding a Go decision.

The difference in mean onset firing rate (measured 10-30 ms after tone onsets) between reference and target tones of each behavioral decision is shown as a function of |Δf| in Figure 5.6. Trials resulting in a Go decision showed an increase in mean onset response ranging from 10.4% ± 7.6% to 41.5% ± 5.6% for |Δf| ≥ 20%. Responses to target tones preceding No-go decisions varied little from reference tones, ranging from -2.25% ± 4.3% to 16.1% ± 11.6% of normalized mean reference tone onset spike rate. The mean firing rate differences to trials resulting in Go decisions were significantly different from No-go trials (ANOVA; $F_{(1,\ 9404)} = 252.54$, $p < 0.0001$). Especially interesting is that even during 0% Δf target catch trials the mean firing rate differences were significantly higher in trials resulting in a Go (false alarm) than in trials resulting in a No-go (correct rejection; 2-sample t-test; $p = 0.01$). The mean onset firing rate differences were not correlated with |Δf| from either trials resulting in Go ($r = 0.23$, $p = 0.37$) or trials resulting in No-go decision ($r = 0.24$, $p = 0.36$). This could indicate that the differences in neural onset responses that were correlated to hit rate seen in the previous chapter may have been due to an averaging effect that gives more weight to neural signals from No-go trials (with smaller onset response increases) to smaller, sub-threshold |Δf| targets or to neural signals from Go trials with higher hit rates, and larger onset response differences, for larger |Δf| targets. Finally, the mean onset firing rate differences from both behavioral decisions were likewise not correlated with each other ($r = -0.32$, $p = 0.22$).

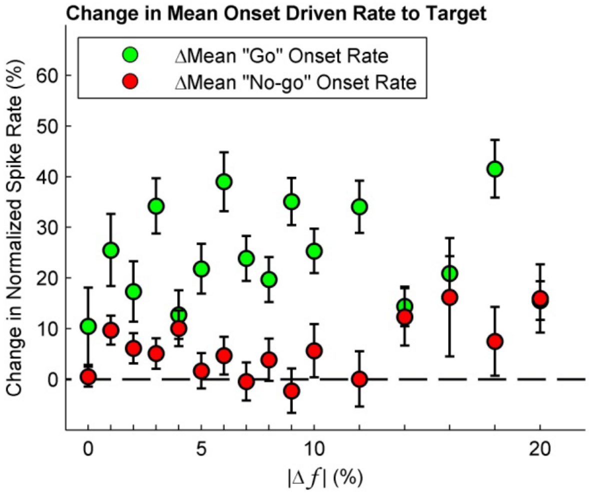


Figure 5.6. Mean normalized population (n = 299 sessions) changes in onset amplitude from final reference tone to first target tone are shown for all tone-driven MUCs for trials which resulted in a “go” response (green) and trials which did not (red) as a function of target |Δf|. The dashed black line represents a 0 spikes/s change in onset amplitude spike rate. Error bars show 95% confidence interval. Data were collected in 5 ms moving-window bins. Reference frequencies used were 4.15, 8.61, and 17.85 kHz. Recordings from each isolated multi-unit cluster were normalized to the average onset firing rate for the final reference tone.

The neural responses seen during the frequency discrimination task suggest that AC increases firing rate to frequency changes when detected and does not increase firing rate when frequency changes are not detected. To test this further, AC responses to frequency differences were measured in a test in which the rats were not required to detect frequency changes. A broad-band noise detection task was designed in which the detection target was a noise burst, which followed a sequence of reference tones which contained an irrelevant frequency shift.

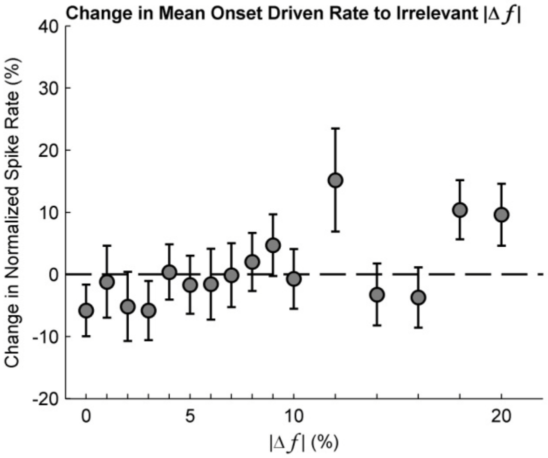


Figure 5.7. Mean normalized population (n = 176) changes in onset spike rate between final R_1 and first R_2 tones are shown as a function of target |Δf| for trials in which the frequency change was irrelevant to the task. Dashed black line represents 0% change in mean onset amplitude spike rate (measured 10-30 ms after tone onsets). Error bars show 95% confidence interval. Data were collected in 5 ms moving-window bins. Reference frequencies used were 4.15, 8.61, and 17.85 kHz. Recordings from each isolated multi-unit cluster were normalized to the average onset firing rate for the final reference tone.

Differences in mean onset driven rate to the irrelevant frequency differences (between the final reference tone of R_1 and the first reference of R_2) are shown in Figure 5.7. The size of response differences to irrelevant |Δf| are much smaller than what is seen during Go trials of the frequency discrimination task. Irrelevant response differences in mean onset spike rate ranged from -05.8% ± 4.8% to 15.2% ± 8.2% of normalized mean reference tone onset spike rate. Differences between mean onset rate between reference and target tones were not statistically different (ANOVA; $F_{(1,\ 5695)} = 0.85$, $p = 0.35$). However, analysis of the mean onset spike rate changes show that mean response differences were correlated with increasing |Δf| (Spearman's rank test; $r = 0.57$, $p = 0.02$).

It is also possible that the smaller increases in onset response to irrelevant frequency changes are due to ease of the noise detection task compared to the frequency discrimination task. Increasing task difficulty has been shown to increase effects designed to improve discrimination and enhance responses to attended stimuli (LaBerge *et al*., 1991; Lavie & Cox, 1997; Spitzer *et al*., 1988; Spitzer & Richmond, 1991; Boudreau *et al*., 2006; Chen *et al*., 2008; Atiani *et al*., 2009; Niwa *et al*., 2012a) in the visual and auditory systems. To investigate the possibility that task difficulty may be responsible for the extreme modulation, or possibly complete lack of modulation, of the firing rates to the irrelevant frequency change during the noise detection task the rats in this study were tested on a second, easier frequency discrimination task which only contained supra-threshold frequency change targets of |Δf| ≥ 10%. As with the standard frequency discrimination task, onset response differences to frequency changes resulting in a Go decision during this less difficult frequency discrimination task were found to be larger than onset response differences from trials resulting in No-go decisions (Figure 5.8; ANOVA; $F_{(1,\ 4365)} = 249.86$, $p < 0.0001$). Thus, it is unlikely that the decreased responses to irrelevant frequency changes in the noise detection task were due to task difficulty and were more likely an effect of modulation due to task relevance.

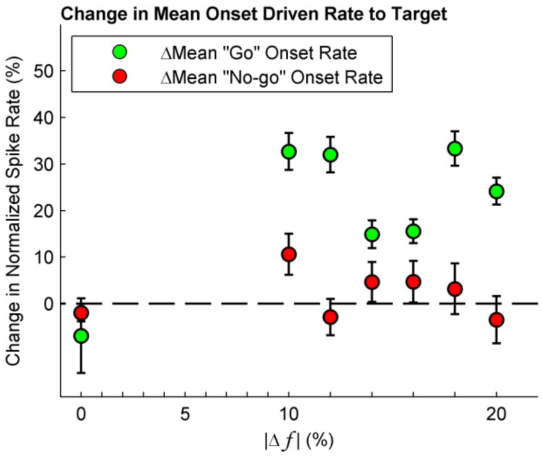


Figure 5.8. Mean normalized population from “easy” frequency discrimination task (n = 309 sessions) changes in onset amplitude from final reference tone to first target tone are shown for all tone-driven MUCs for trials which resulted in a “go” response (green) and trials which did not (red) as a function of target |Δf|. The dashed black line represents a 0 spikes/s change in onset amplitude spike rate. Error bars show 95% confidence interval. Data were collected in 5 ms moving-window bins. Reference frequencies used were 4.15, 8.61, and 17.85 kHz. Recordings from each isolated multi-unit cluster were normalized to the average onset firing rate for the final reference tone.

5.4. Discussion

The previous chapter of this dissertation describes neural responses in rat AC that are highly correlated with frequency discrimination ability. These results suggested that AC neurons might contain information sufficient for making behavioral decisions in a frequency discrimination task for even near-threshold differences. To investigate the hypothesis that AC responses contain not only stimulus information but also additional information that affects subsequent behavioral decisions, the neural responses recorded in rat AC were grouped into the two behavioral outcomes available in this task:

nosepoke withdrawal (a Go decision, indicating a perceived frequency change) and non-withdrawal (a No-go decision, indicating no perceived change in frequency). This chapter shows that responses in AC were predictive of the subject's decision to respond to a frequency change in a threshold-level frequency discrimination task.

Signals predicting behavioral decisions (and presumably indicating perceived changes in frequency) in a frequency discrimination task were shown to be represented in the increased difference in onset response firing rates between reference and target tones. Target tones in trials resulting in a Go decision generated higher increases in firing rates between the mean onset response to reference and target tones than in trials resulting in a No-go decision, for all $|\Delta f|$ targets tested. In addition, neural responses in AC to $\Delta f = 0\%$ catch trials that resulted in Go decisions (false alarms) were higher than in trials that resulted in No-go decisions (correct rejections), indicating a possible *perception* of a frequency difference when none existed. Sub-threshold frequency changes showed significant but small increases in firing rate to target tones even in No-go decisions, suggesting that firing rates of AC cells may be required to increase beyond a threshold to trigger a Go decision. There was no increase in onset response firing rate between reference and supra-threshold target tones in trials which resulted in a No-go decision, further supporting the hypothesis that differences in AC onset firing rate are required for generating a Go response. It is possible that these supra-threshold frequency differences did not elicit small, but significantly increased onset responses similar to near-threshold frequency differences due to effects of inattention to a demonstrably salient stimulus.

The contribution AC makes towards decisions in various auditory tasks is unclear. Neural correlates of acoustic flutter discrimination (Lemus *et al.*, 2009), click train discrimination (Dong *et al.*, 2011), and tone-detection (Zhang *et al*., 2012) all reported that increases in firing rate of cells in A1 were representative of detection thresholds but were not predictive of behavioral decisions. A study on amplitude modulated tone discrimination reported that responses in A1 were both representative of thresholds and predictive of behavioral decisions (Niwa *et al.*, 2012b); however, they were unable to find significant neural correlates of decision during the onset of their target stimulus. To the best of the author's knowledge this report is the first to show decision correlates in AC occurring specifically at the onset of the stimulus.

Niwa *et al*. (2012b) reported that firing rate of multi-unit clusters of single units in A1 significantly increased during presentation of target tones in trials in which monkeys reported hearing an amplitude modulated tone versus trials in which they did not. The nature of the amplitude modulation task requires neural responses to phase lock onto temporal patterns created by the stimuli. This phase locking effect is typically not instantaneous, and thus Niwa *et al*. (2012b) correlated behavioral decisions with neural responses averaged over the entire 800 ms stimuli. This long time window could allow for feedback from other areas to influence responses in A1. Because the monkeys had to wait until after completion of the target presentation to report detection, it is possible that the increased firing rate in A1 that they correlated with a Go decision may be more a depiction of general A1 activity anticipating the opportunity to respond. However, whether or not this activity is an anticipatory response, it is still correlated to the animal's perception of the stimuli as inferred from the behavioral decision. The response window that was used to correlate neural responses with behavioral decision in this report is much smaller (20 ms) and ends 30 ms after tone onset. This early onset time window indicates that the information predicting decisions is either already in AC by the time the stimulus reaches AC, or that these responses are modulated by some area that can affect AC responses on a very short time scale.

Results from the frequency discrimination task lead me to believe that increases in A1 response to a deviant frequency tone resulted in a Go behavioral decision. Attending to different aspects of stimuli has been shown to modify responses of A1 cells in monkeys (Hocherman *et al.*, 1976). If the rats were simply not paying attention to the stimuli they may have had larger responses to the preceding reference tones, which would decrease the difference in response between reference and target tones. The data, however, shows no difference in response to reference tones between trials resulting in Go and No-go decisions, suggesting that the decision to Go is based on the response to the frequency difference. It is possible that there exists some mechanism which enhances responses to frequency differences while attending to a task. Evidence of this mechanism has been seen in monkey A1 when onset response to target tones increased only after the subject learned a frequency discrimination task (Blake *et al.*, 2002), suggesting that exposure to frequency changes prior to learning the task were undetected and/or non-attended. For the present study, such a mechanism could enhance responses to target tones during trials which resulted in a Go decision, but perhaps not enhance responses to target tones during trials in which the rat was not attending to the stimuli (No-go decisions).

To test the hypothesis that AC actively increases responses to attended changes in frequency, responses were recorded during a task in which rats were trained to ignore a frequency shift, but had to detect a broad-band noise following the frequency shift. As a result, it was possible to record responses in AC to frequency changes during trials in which the animal withheld behavioral response to the frequency shift. Onset responses to irrelevant frequency changes were not significantly larger than responses to preceding reference tones, supporting the hypothesis that enhanced frequency change responses in AC may be specific to behaviorally relevant stimuli. Additionally, because the rats did not respond to the irrelevant frequency change, but waited to respond to a broad-band noise target, it may be inferred that increases in AC response to the frequency changes in the original frequency discrimination task are specific to the decision to go. Other studies in the auditory system also support the idea that task relevance plays a role in modulation of AC. Adaptation of ferret A1 stereo-temporal receptive fields (STRFs) is greater during behavior than during passive listening (Fritz *et al.*, 2003; Fritz *et al*., 2005b; Elhilali *et al*., 2007). Additionally, ferret A1 has been shown to rapidly modulate STRF frequency representation in order to adapt to the present task, and can quickly revert to pre-testing representation after testing (David *et al*., 2012). These results support the idea that the same AC cells that report detection of a frequency change by increasing firing rate may not increase firing rate to frequency changes when they are irrelevant to the task being performed.

Establishing the origin of these responses that appear to predict behavioral decisions would increase understanding of how frequency differences are processed and relayed in the auditory system. The fact that increases in onset response to frequency changes were significant during the frequency discrimination task, in which frequency changes were relevant, but not significant during the noise burst detection task, in which frequency changes were irrelevant, suggests that frequency change detection is not an entirely pre-attentive process. However, it is not possible to conclude from the present study that auditory cortex is the locus of the behavioral decisions. Given that the neural correlate of behavior is during the typical onset response for the stimuli, wherever the variability in the neural response originates, it is able to modulate auditory cortex within milliseconds of the tone onset. It is possible that increased onset response strength to the target tone in auditory cortex is a primary contributor to the behavioral response in decision centers of the brain. Additionally, it could be that the same predictive response mechanism can be seen in lower auditory processing centers. Future studies will need to be conducted to see if behavioral correlates can be found in upstream auditory areas. By working backwards through the sub-cortical areas it could be possible to locate the origin of this neural mechanism and enhance present understanding of the role of AC in frequency discrimination.

Determining the contribution of AC responses to the formation of decisions will further help to define the role of AC in frequency discrimination and auditory processing in general. Decision correlates in a visual discrimination task have been found in higher order processing centers such as the parietal lobe (Shadlen & Newsome, 1996) and prefrontal cortex (Kim & Shadlen, 1999) of monkeys, and it is similarly likely that perceptual information from the auditory cortices is turned into a behavioral decision at one of these higher cortical areas. Responses in the pre-frontal cortex of ferrets have been shown to contain behaviorally relevant information for performance of an auditory discrimination task (Fritz *et al*., 2010), but no direct link between activity in AC and activity in higher processing areas has been shown. Future studies with simultaneous recordings from AC and higher decision centers may help to determine how signals from individual AC cells contribute to determining behavioral decisions in a frequency discrimination task and where a final decision may be generated.

The results of this study show that AC neurons contain the signals sufficient to drive behavioral decisions in a frequency discrimination task. Going even further, these results suggest that responses in primary sensory areas might reflect the subjects' perception of the stimulus more than they reflect the actual stimulus features. This report also shows that these signals are modulated by some process associated with task relevance. It is likely that these findings will play an integral part in establishing the role that AC plays in discrimination of complex and/or near-threshold frequency differences, as well as helping to distinguish between stimulus features and perception of those stimulus features.

Chapter 6

6. General Conclusions

6.1. Review of Main Findings

In preceding chapters I have presented the results of a series of studies designed to investigate possible ways in which auditory cortex might resolve threshold-level frequency differences. Previous ablation studies have implicated auditory cortex as a fundamental component in the resolution of very small frequency differences. This dissertation reports on a neural response mechanism present during the onset response of tones that is both representative of detection thresholds and predictive of decisions in performance of a frequency discrimination task.

The psychophysical experiment in Chapter 3 shows that behavioral measures both before and after implantation surgery on a new strain and gender of rat performing a difficult frequency discrimination task are comparable to those shown by other mammals and therefore validates the use of this strain of rat as a model for testing in the auditory system. I also show evidence that suggests the rats may have been adopting a frequency identification strategy rather than a frequency discrimination strategy towards performing this task due to the high frequency difference limens for target frequencies outside of the reference frequency range. A third finding is that rats require 3 reference tones (1.2 s) for auditory information, possibly streaming effects, to build up before rats can perform at asymptotic behavioral measures. A fourth finding suggests that rats are less capable of discriminating between octave frequency differences than they are half octave frequency differences, and I hypothesized that discrimination of harmonics may play a role in the strategy adopted by the rats during performance of this frequency discrimination task.

Neural recordings from auditory cortex of rats performing a frequency discrimination task in Chapter 4 show that increases in population onset response to frequency changes relative to onset responses to preceding reference tones are highly correlated with detection ability. Additionally, onset latencies of population responses to frequency changes are shown to be correlated with reaction times of the rat. The relation between behavioral measures and neural responses in this chapter suggests that auditory cortex is involved in the discrimination of threshold-level frequency differences.

Chapter 5 elaborates on the findings of the previous chapter by showing that the neural mechanism which was shown to describe threshold-level detection ability is not an automatic, pre-attentive mechanism of detection. Increases in population onset response were shown to be predictive of the rat’s behavioral decision in a trial, regardless of whether the trial was performed correctly or incorrectly. Finally, the neural mechanism previously associated with frequency discrimination was shown to be modulated by behavioral relevance. Changes in onset response to irrelevant frequency changes were shown to be much lower than onset response to relevant, detected frequency changes and were similar in dynamic range to relevant but undetected frequency changes.

The results presented here support the hypothesis that auditory cortex is fundamental in the discrimination of threshold-level frequency differences. Auditory cortex was shown to contain signals that are present when rats indicate detection of frequency differences and are not present when frequency differences are apparently not detected. Additionally, these signals are present when the rat reacts to a frequency change when none is actually presented, showing that the signals encode perception as inferred by behavioral responses.

6.2. Directions for Future Research

Identification of the strategies used by the rats for performance of the frequency discrimination task might lead to a better understanding of the behavioral thresholds. Psychophysical data might not represent the truest measure of pure frequency discrimination ability if the rats are confounding the task by using harmonic differences as a cue or if they are applying additional bounds to the task by attempting to detect frequency change targets that lie only within certain range. Adding several frequency changes in multiples of octaves to the stimulus set could provide a large enough sample to determine if rats are using harmonic differences as cues to detect frequency changes. This amended stimulus set should also include several very large frequency changes which are not octave multiples so that rats do not adjust their strategy to wait for the frequent, very easy to detect large octave multiple frequency changes.

One possible solution to correcting for rats performing a frequency identification strategy would be to set reference frequency to within a 6 kHz range (for example 4-10 kHz) and hold it for ~20 trials. After ~20 trials slide the reference frequencies range up 3 kHz, so that the new range of reference frequencies partially overlaps the reference frequency range from the previous block of trials. Adapting the reference frequencies in this way should cause poor performance to any rat adopting a frequency identification strategy and force them to adopt a new strategy.

The results of this study show that auditory cortex contains signals predicting behavior in a frequency discrimination task. However, based on the current results it cannot be conclusively determined which area of the auditory system these signals originate from. It is possible that these signals are generated in auditory cortex. It is also possible that they are generated earlier in the auditory pathway and that auditory cortex serves only as a necessary way-point for this information to pass through. By recording from earlier points in the auditory neural pathway it could be possible to determine the origin of these signals and further elaborate auditory cortex’s role in frequency discrimination.

This study is likewise unable to conclude how signals present in auditory cortex contribute towards perception of frequency differences. It is possible that auditory cortex is the sole contributor towards decisions concerning frequency discrimination. However, it is also possible that other areas submit information for performance of this task. A study involving microstimulation applied to auditory cortex during performance of a frequency discrimination task could help to determine if responses there are driving behavior. Additionally, simultaneous recordings from auditory cortex and higher decision centers could result in a better understanding of where decisions are made concerning frequency discrimination and what weight signals from auditory cortex carry in determining those choices.

An exciting new avenue in the world of prosthetics could be opened if future studies determine that auditory cortex is the locus of frequency discrimination in the cortex. If application of microstimulation to the auditory cortex results in the perception of frequency differences, it could be possible to convert signals (verbal communication, e.g.) from one source into digital signals that are delivered directly to auditory cortex. This type of prosthesis would be instrumental in returning hearing to deaf people who are not candidates for cochlear implants, possibly due to damaged auditory pathways.

The introductory chapter of this dissertation links deficits in frequency discrimination in humans to attention and learning disorders. Thus, it might be possible to improve attention and learning ability in humans by improving their frequency discrimination ability. The results of this study show that the neural mechanism of frequency discrimination is modulated by attention to relevant auditory stimuli. Repeated performance of a threshold-level frequency discrimination task during developmental periods may result in improved frequency discrimination ability, and could lead to decreased deficits in disorders linked to frequency discrimination.

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Appendix A

Statistical citations of ANOVA and Spearman’s Rank Correlation for the mean hit rate as a function of number of reference tones into 5 |Δf|% range bins detailed in Chapter 3 (Figures 3.4C and 3.8C).

Table A1: ANOVA and Spearman's Rank Correlation for Figures 3.4C and 3.8C

		0% Δf (catch)	1-4% |Δf|	5-9% |Δf|	10-20% |Δf|	≥34% |Δf|
Pre-surgery (statistics on Figure 3.4C)						
Hit Rate (ANOVA)	1+ ref tones	$F_{(17, 161)} = 1.72$, $p = 0.045$	$F_{(17, 161)} = 3.234$, $p < 0.0001$	$F_{(17, 161)} = 6.61$, $p < 0.0001$	$F_{(17, 161)} = 16.08$, $p < 0.0001$	$F_{(17, 161)} = 11.41$, $p < 0.0001$
	3+ ref tones	$F_{(15, 143)} = 1.59$, $p = 0.085$	$F_{(15, 143)} = 1.217$, $p = 0.2673$	$F_{(15, 143)} = 1.255$, $p = 0.24$	$F_{(15, 143)} = 1.711$, $p = 0.056$	$F_{(15, 143)} = 0.68$, $p = 0.80$
Correlation (Spearman)	1+ ref tones	$r = 0.75$, $p = 0.0005$	$r = 0.92$, $p < 0.0001$	$r = 0.83$, $p < 0.0001$	$r = 0.94$, $p < 0.0001$	$r = 0.58$, $p = 0.014$
	3+ ref tones	$r = 0.69$, $p = 0.0044$	$r = 0.88$, $p < 0.0001$	$r = 0.76$, $p = 0.001$	$r = 0.91$, $p < 0.0001$	$r = 0.40$, $p = 0.13$
Post-surgery (statistics on Figure 3.8C)						
Hit Rate (ANOVA)	1+ ref tones	$F_{(17, 106)} = 1.53$, $p = 0.10$	$F_{(17, 107)} = 1.83$, $p = 0.036$	$F_{(17, 107)} = 3.44$, $p < 0.0001$	$F_{(17, 107)} = 2.81$, $p = 0.0008$	$F_{(17, 107)} = 0.8982$, $p = 0.58$
	3+ ref tones	$F_{(15, 94)} = 1.50$, $p = 0.12$	$F_{(15, 95)} = 1.276$, $p = 0.24$	$F_{(15, 95)} = 2.07$, $p = 0.020$	$F_{(15, 95)} = 0.40$, $p = 0.98$	$F_{(15, 95)} = 0.5252$, $p = 0.92$
Correlation (Spearman)	1+ ref tones	$r = 0.69453$, $p = 0.0019$	$r = 0.81$, $p < 0.0001$	$r = 0.84727$, $p < 0.0001$	$r = 0.92$, $p < 0.0001$	$r = 0.47$, $p = 0.049$
	3+ ref tones	$r = 0.77$, $p = 0.00074$	$r = 0.72$, $p = 0.0022$	$r = 0.80$, $p = 0.0003$	$r = 0.88$, $p < 0.0001$	$r = 0.31$, $p = 0.23$

Appendix B

Statistical citations of Bonferroni corrected t-tests ($\alpha = 0.0016$) comparing the mean population onset firing rates of the target response from each behavioral decision /|Δf|% to the mean population onset firing rate of the preceding reference tone. Behavioral decision /|Δf|s with a significant effect are bolded. These results are depicted in Figures 5.5 and 5.6.

Table B1: Comparison of onset responses from reference and target tones

|Δf|	Behavioral Decision	t-test (Bonferroni correction, $\alpha = 0.0016$)
0%	Go	$p = 0.0097$
	No-Go	$p = 0.67$
1%	Go	**$p > 0.0001$**
	No-Go	**$p > 0.0001$**
2%	Go	**$p = 0.0014$**
	No-Go	**$p = 0.00018$**
3%	Go	**$p > 0.0001$**
	No-Go	$p = 0.0022$
4%	Go	**$p > 0.0001$**
	No-Go	**$p > 0.0001$**
5%	Go	**$p > 0.0001$**
	No-Go	$p = 0.53$
6%	Go	**$p > 0.0001$**
	No-Go	$p = 0.013$
7%	Go	**$p > 0.0001$**
	No-Go	$p = 0.76$
8%	Go	**$p > 0.0001$**
	No-Go	$p = 0.072$
9%	Go	**$p > 0.0001$**
	No-Go	$p = 0.19$
10%	Go	**$p > 0.0001$**
	No-Go	$p = 0.033$
12%	Go	**$p > 0.0001$**
	No-Go	$p = 0.94$
14%	Go	**$p > 0.0001$**
	No-Go	**$p > 0.0001$**
16%	Go	**$p > 0.0001$**
	No-Go	$p = 0.0036$
18%	Go	**$p > 0.0001$**
	No-Go	$p = 0.028$
20%	Go	**$p > 0.0001$**
	No-Go	**$p > 0.0001$**