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SPECTROTEMPORAL DYNAMICS OF NEURAL RESPONSES IN AUDITORY CORTEX DURING FREQUENCY DISCRIMINATION

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SPECTROTEMPORAL DYNAMICS OF NEURAL RESPONSES IN AUDITORY CORTEX DURING FREQUENCY DISCRIMINATION

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOENGINEERING

BY

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Dedication

I dedicate this dissertation to my parents, who worried I wouldn't go to college and then worried I'd never stop going.

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Abstract

Small differences in the frequencies of a sound provide a significant amount of information about the identity, position, and motion of the source. Humans' auditory frequency discrimination ability also allows us to understand the subtle modulations that comprise spoken language. It should not be surprising, then, that deficits in auditory frequency discrimination ability have been often implicated in various language learning disorders. Understanding the neural basis for such deficits is difficult because the processes underlying auditory frequency discrimination haven't been fully identified. Recent evidence suggests that primary auditory cortex may be the ultimate arbiter of behavioral frequency resolution, but the frequency- and intensity-dependent properties of phasic-type responses most often seen in primary auditory cortex neurons do not correspond well with psychophysical trends. Tonic-type response components that occur after the phasic component in primary auditory cortex neurons may display frequency-selectivity more consistent with observed psychophysics, but are not yet well characterize in most animals. Studying the contribution of tonic responses to frequency discrimination behavior requires an approach that can examine both neural activity in primary auditory cortex and psychophysics in the same animals.

This dissertation presents the results of three experiments designed around the unifying hypothesis that tonic neural responses in primary auditory cortex are crucial for fine-grained frequency perception. The experiments involve both psychophysical measurements from behaving rats and *in vivo*,

unanaesthetized neural recordings from primary auditory cortex. These techniques are ultimately combined to connect frequency discrimination behavior with the directly associated cortical neural activity.

In general, the psychophysical results show that rats have behavioral frequency discrimination acuity comparable to other mammals and are a suitable model for frequency discrimination research. Neural recordings from passively-listening animals show that rat primary auditory cortex neurons respond with both phasic and tonic components to pure tone stimuli as have been seen in other animals. When neural responses are recorded from primary auditory cortex of rats performing a frequency discrimination task, attention to the task enhances suppression during the beginning of the tonic response component, presumably to better sharpen frequency-selectivity. Furthermore, changes in spikerate during this post-phasic response component are significantly correlated with the corresponding psychophysical performance on the frequency discrimination task. The post-phasic component of neural responses in primary auditory cortex, the initial part of the tonic response, may directly encode behavioral resolution for frequency discrimination.

The results of these studies suggest that attention-modulated mechanisms of inhibition in the cortex are crucially involved in auditory frequency discrimination. Intriguing avenues of research radiate from this conclusion, from the possibility of correcting auditory deficits with inhibition-targeting interventions to the possibility of using frequency discrimination as a diagnostic method to identify more global inhibitory deficits.

Chapter 1

1. Introduction and General Background

In purely physical terms sound is a transmitted vibration, a single dimension of pressure varying in time. When detected, transduced, and integrated over time by the human auditory system, sound is deconstructed into four basic perceptual dimensions: frequency, amplitude, time, and space. Each perceptual dimension informs us about certain aspects of the sound, such as the identity, distance, immediacy, and direction of the source. That sort of knowledge undoubtedly saved many of our common ancestors from noisy, toothsome predators and still keeps modern man from stepping in front of speeding buses. Between then and now, however, human cognition of these basic dimensions of sound has evolved well beyond threat detection, allowing us to decode spoken language and to find pleasure in weaving sound's perceptual dimensions into the artificial patterns of symphonies.

Our expanded perception of the four basic dimensions of sound relative to other animals has given us advantages in sensitivity, particularly for frequency. Yet, as with any detection system, there is still a limit to the precision of our resolution along each dimension, and there is naturally variation between the resolving power of individuals. For the neuroscientist, these observations raise intriguing questions. What component of the auditory

system defines our resolution for a particular dimension of sound? How does this component differ between humans and other animals? How does this component differ between individuals? What are the consequences of individual differences?

Examination of the physiological processes underlying even any one of the four perceptual dimensions of sound is beyond the scope of a single thesis, so in this research I've chosen to investigate a hypothesis concerning only frequency resolution. Deficits in frequency resolution, defined by the frequency differences subjects can discriminate between successive stimuli, have been implicated in certain forms of dyslexia (Banai & Ahissar, 2004), and in children with specific language impairment (Mengler *et al*., 2005). Pitch discrimination ability is a reliable predictor of language learning disorders (Elliot *et al*., 1989). Those effects might be expected due to the dependence of spoken language on hearing, but recent studies have found surprising correlations with seemingly non-auditory abilities. In studies comparing learning disabled and normal subjects, frequency discrimination ability was significantly correlated with SAT math scores (Watson, 1991) and reading ability (Ahissar *et al*., 2000) for both the learning disabled *and* normal subjects, a relationship that may be particular only to the auditory sensory modality (Hulslander *et al*., 2004). Either auditory frequency discrimination contributes to these auditory related and non-auditory abilities, or the neural mechanisms underlying each share some common process. A better understanding of the neural substrate for frequency resolution might then also lead to the development of better treatment

strategies for learning disorders and perhaps better overall strategies for learning in general.

1.1. A brief review of the auditory system

The first place to look for factors affecting frequency discrimination ability would naturally be in the precision of the transducer that converts sound energy into neural impulses, the basilar membrane of the cochlea. Briefly, sound vibrations at the tympanic membrane are transmitted to the oval window of the cochlea through the ossicles of the middle ear, causing fluid waves within the joined compartments of the scala tympani and the scala vestibuli (Robles & Ruggero, 2001). Standing waves are created at points on the tapered basilar membrane corresponding to the resonating frequency, with high frequencies resonating at the thick base while low frequencies resonate at the thin apical end, such that it acts like a biomechanical Fourier transform. Mechanical energy is then transduced into neural impulses when inner hair cells are excited by sliding shear forces from the tectorial membrane at the points of resonance.

Humans have ~3500 inner hair cells arrayed linearly along the ~35 mm length of the basilar membrane (Nadol, 1988), responding to frequencies over a range of ~10 octaves from 20 Hz to 20 kHz (Sivian & White, 1933). If inner hair cells behaved like simple binary detectors, like the keys of a piano, then the precision of the transduction system would be equal to an average frequency spacing of 0.003 octaves, or a Weber fraction of ~0.2%. However, while the action potential output of inner hair cells is binary at any one instant, integrated

over time the firing rate reflects a quantitative measure of the amplitude of the resonance at each point (Russell & Sellick, 1978). Frequencies corresponding to points between discrete inner hair cell spacings could be determined through interpolation by neurons further up in the subsequent auditory pathway, increasing frequency resolution.

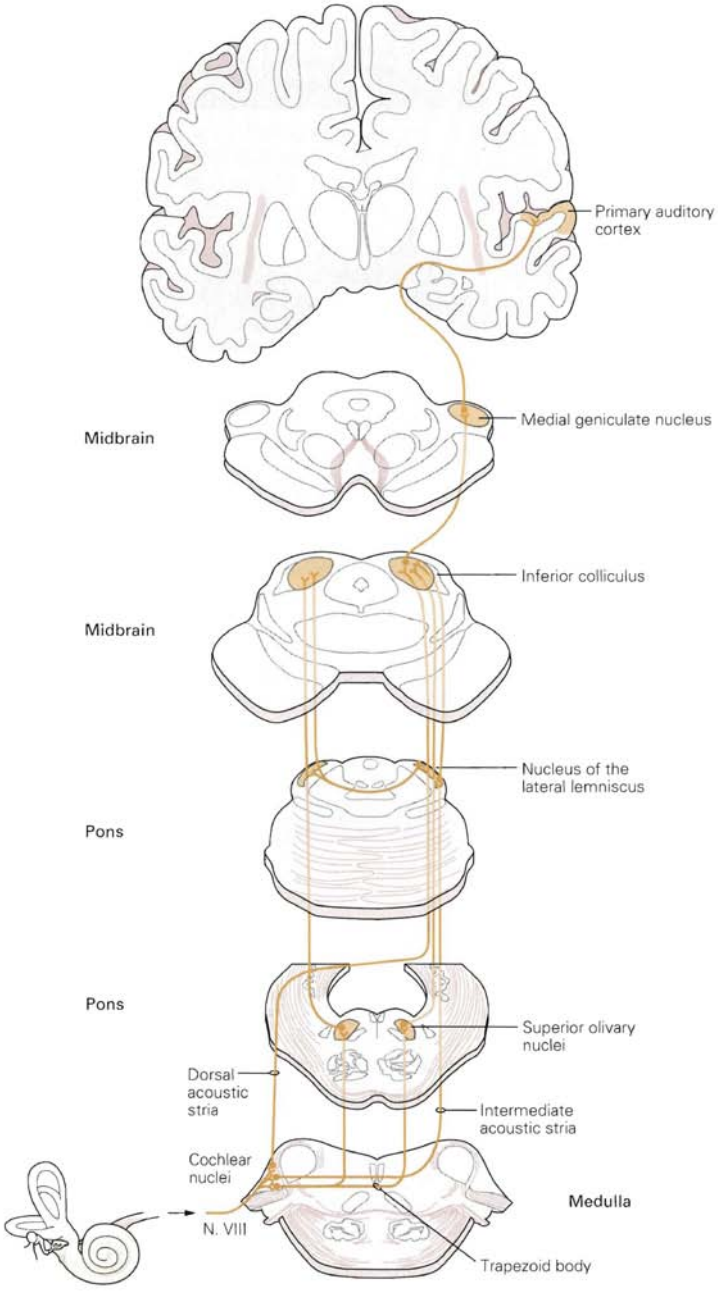

Figure 1.1. The auditory neural pathway. From Kandel *et al.*, 2000.

The tonotopic organization of the cochlea is preserved in neuronal frequency-selectivity throughout the auditory pathway up to primary auditory cortex (A1), such that at all major points auditory frequency information is represented as a spatial code (for review see Smith & Spirou, 2002 or Malmierca, 2001). The frequency tuning of neurons at each level is largely similar in shape to the tuning curves of inner hair cells, such that the tonotopic arrangement of neurons represents a contiguous filter bank. At each level, the resolution of frequency information input can potentially be enhanced by interpolation, worsened by integration, or simply relayed to the next level. However, there must be at least one component along the auditory pathway that encodes frequency information at a resolution equivalent to frequency discrimination ability from which decision centers, such as the frontal cortex or sensory integration areas, ultimately receive information.

One difficulty in identifying auditory centers relevant to frequency discrimination is that there are so many candidates. Auditory information from the cochlea must pass through synapses at a minimum of six subcortical nuclei before reaching the auditory cortex. Auditory information is first passed from the cochlea to the cochlear nucleus (CN) of the brainstem by the auditory nerve. The CN projects within the brainstem to the superior olivary complex (SOC), known to be involved mainly in sound localization, which in turn projects to the lateral lemniscus (LL), which also participates in sound localization. Information is then passed up to the inferior colliculus (IC) in the midbrain and then passed again up to the medial geniculate body (MGB) of the thalamus.

Finally, the primary auditory cortex (A1) receives the input from the ventral subdivision of the MGB ~8-15 ms after the sound is received by the ear (Smith & Spirou, 2002; Malmierca, 2003). This is, of course, a grossly simplified outline of an auditory pathway in which information is modified by both ascending and descending projections and by input from attentional and motivational systems. However, recent studies provide a good justification for focusing an investigation into frequency discrimination squarely at the top of the pathway, primary auditory cortex (A1).

1.2. Auditory Cortex's Role in Frequency Discrimination

Until the late 1980's, A1 had effectively been ruled out as site of interest in frequency discrimination. Subjects with bilateral A1 lesions were generally capable of basic auditory frequency discrimination, and would typically recover substantial hearing (for review see Pickles, 1988). These reports led some to suggest that primary auditory cortex isn't necessary for the perception of pure tones (Masterton & Berkley, 1974; Neff *et al*., 1975). However, more recent studies showed that typical clinical frequency discrimination tests administered to bilateral A1 lesion patients tested only with "generous" frequency differences with Weber fractions of >10%. Studies that tested with *fine-grained* frequency differences (<10%) did show significant impairment in bilateral A1 lesion patients (Mendez & Geehan, 1988; Tramo *et al*., 2002) and some non-aphasic cerebrovascular accident patients (Divenyi & Robinson, 1989) compared to normal controls. Without the auditory cortex, patients apparently can recover

the ability to discriminate large frequency differences, but they will show significantly elevated thresholds of detection, called frequency difference limens (FDLs).

Animal studies show the same dichotomy of results dependent on tested frequency differences. In three rodent studies that tested frequency discrimination performance after bilateral ablation with differences of ½ octave or greater (Ohl *et al*., 1999; Ono *et al*., 2006; Rybalko *et al*., 2006), only one found significant impairment (Rybalko *et al*., 2006). Conversely, another study on bilaterally-lesioned macaques that explicitly tested small frequency difference limens found thresholds were more than double those of comparison animals (Harrington *et al*., 2001). More dramatic evidence of the involvement of auditory cortex in frequency discrimination comes from studies in which ablations were simulated during behavior by reversibly inactivating auditory cortex with local infusions of the $GABA_A$ agonist muscimol. Riquimaroux *et al*. (1991) showed that focal application of muscimol to the DSCF area of bat auditory cortex disrupted the bats' ability to discrimination fine frequency differences, but coarse frequency discrimination was unimpaired. Rats' discrimination performance was impaired at both fine and coarse frequency differences following local muscimol infusion over A1, but the ability to make course discrimination returned hours before the ability to make fine discriminations (Talwar *et al*., 2001). Overall, these reports suggest that A1 may be involved in, but isn't necessary for, coarse frequency discrimination, but that A1 is necessary for fine-grained frequency discrimination of pure tones.

Note that individual differences in FDLs that correlate well with language disorders (Mengler *et al.*, 2005), reading ability (Ahissar *et al*., 2000), and math scores (Watson, 1991) are all on the order of fine-grained frequency differences (<10%).

1.3. Possible Neural Mechanisms

Attempts to correlate cortical neural frequency-selectivity with psychophysical performance have also befuddled questions into of the role of A1 in frequency discrimination. Pure tone FDLs, which are typically ~0.01-0.05 octaves for humans and other mammals (for review see Fay, 1974), differ greatly from the spectral bandwidths of typical A1 neurons' receptive fields to pure tones, reported to be as wide as 3-5 octaves for some intensities (Sally & Kelly, 1988; Gaese & Ostwald, 2003; Kaur *et al*., 2004; Moshitch *et al.*, 2006). Paradoxically, these typical spectral bandwidths also systematically increase with increasing stimulus intensity (Kilgard & Merzenich, 1999; Schreiner *et al*., 2000), whereas psychophysical FDLs decrease with increasing intensity (Harris, 1952; Wier *et al*., 1977; Syka *et al*., 1996). According to Tramo *et al*. (2005), candidate theories of neural frequency and pitch encoding attempting to resolve these discrepancies can be divided into two types: temporal and spectral pattern.

Possible temporal codes for fine-grained frequency perception in A1 generally suppose a contribution of precision spike timing to provide near-behavioral resolution. Metherate *et al*. (2005) suggest that direct

thalamocortical projections carrying high-resolution frequency information first arrive at narrow target areas in layer IV/V of A1, followed by relatively low resolution frequency information relayed by intracortical connections. Fine-grained frequency information may therefore not be contained in overall integrated spike-rates, but rather in the first short-latency spikes. Under this model, inactivation of intracortical connections should isolate short-latency, narrow-bandwidth responses, and indeed the focal application of muscimol, a $GABA_A$ agonist, appears to fully suppress longer-latency components (Kaur *et al*., 2004). However, it's difficult to explain under this model the psychophysical effects of stimulus duration on frequency discrimination, in which FDLs increase sharply with decreases in tone durations below ~30-50 ms in humans (Moore, 1973; Hall and Wood, 1984; Hartmann *et al*., 1985; Freyman & Nelson, 1986) and rats (Talwar & Gerstein, 1998).

Spectral pattern models of fine-grained frequency perception suffer similar difficulties in explaining psychophysics, due in some part to the high prevalence of phasic "ON" responses of A1 neurons to pure tones. Spectral pattern models presume the existence of highly frequency-selective inputs which can represent high resolution frequency information in overall spiking activity (Tramo *et al*., 2005), but integration of spike-rate across the ~20-30 ms length of phasic responses cannot account for duration-dependent psychophysics. However, the reported prevalence of purely phasic responses could be inflated due to a preponderance of anesthetized preparations in the literature. Neural responses recorded from A1 of awake subjects show

considerably more complex spectrotemporal activity (Gaese & Ostwald, 2003; Sutter *et al*., 1999). Recent reports suggest that the majority of A1 neurons show both broadly-tuned phasic and narrowly-tuned tonic or sustained responses to pure tones (Chimoto *et al*., 2002; Qin *et al*., 2003; Qin & Sato, 2004; Wang *et al*., 2005). Qin and Sato (2004) have theorized that the phasic components of the response encode temporal information about a sound while subsequent tonic components, characterized by steady continuous firing throughout the remaining stimulus duration, encode spectral information.

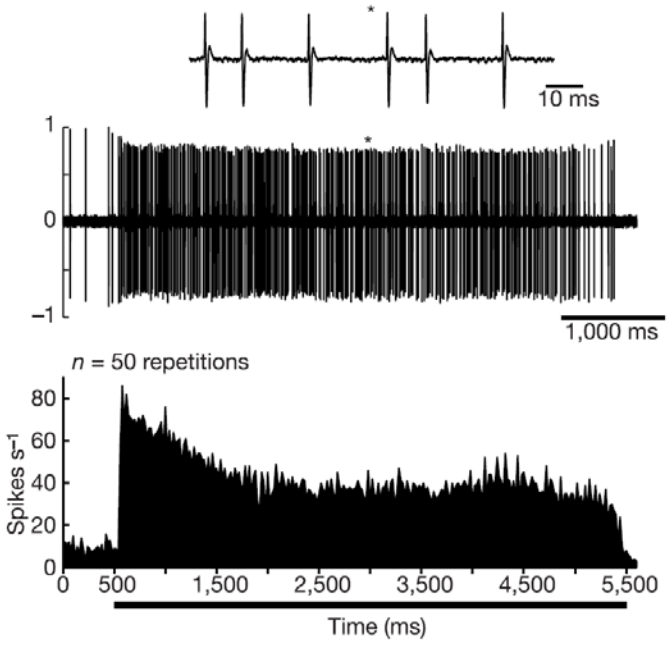

Figure 1.2. An example trace and peri-stimulus time histogram from a single neuron in A1 displaying tonic firing throughout a 5-second long 9.3 kHz frequency pure tone. From Wang *et al.*, 2004.

1.4. Tonic Responses in A1

Qin and Sato's theory about the involvement of tonic responses in frequency resolution is partly supported by favorable comparisons between the reported spectrotemporal dynamics of tonic responses (Qin *et al*., 2003; Qin & Sato, 2004; Wang *et al*., 2005) and the psychophysical effects of tone duration

on frequency discrimination ability. FDLs increase sharply with decreases in tone durations below ~30-50 ms in humans (Moore, 1973; Hartmann *et al*., 1985; Freyman & Nelson, 1986) and rats (Talwar & Gerstein, 1998). Similarly, phasic-tonic neurons in A1 show relatively non-frequency-selective phasic responses to the first ~30 ms of a tone before a transition to a more frequency-selective tonic response (Chimoto *et al*., 2002; Qin *et al*., 2003; Qin & Sato, 2004; Wang *et al*., 2005). Tones shorter than phasic onset responses likely do not elicit tonic responses, and this may be related to dramatic decreases in frequency discrimination ability. As tone durations increase, the appearance of tonic responses seemingly correspond with an asymptotic "leveling-off" of frequency discrimination ability between ~50-100 ms (Moore, 1973; Hall & Wood, 1984; Freyman & Nelson, 1986).

A more concrete appraisal of the importance of A1 tonic responses to frequency perception might be inferred from estimates of their prevalence, but reported proportions of neurons with tonic responses vary greatly between studies. Reported prevalence of tonic responders has been as low as 12-25% in the anesthetized (Talwar & Gerstein, 2001) or awake (Gaese & Ostwald, 2001; Gaese & Ostwald, 2003) rat and monkey (Durif *et al*., 2003), but as high as 60-80% in awake cats (Chimoto *et al*., 2002; Qin *et al*., 2003; Qin & Sato, 2004) or macaques (Recanzone, 2000). In one intracortical recording study in awake humans, 19 of 26 (~73%) recorded neurons were found to have tonic responses (Howard *et al*., 1996). Comparison of experimental methods between studies suggests that there are two likely causes of tonic response

under-representation that should favor acceptance of higher estimates. First, common anesthetics such as sodium pentobarbital or ketamine used in acute preparations have been shown to be $GABA_A$ agonists and NMDA antagonists, respectively (for review see Richards, 1995). Tonic excitation and inhibition are believed to be mediated by NMDA and $GABA_A$ receptors, respectively, and therefore common anesthetics may block tonic excitation (Gaese & Ostwald, 2001). In a study of A1 repetition rate coding 40% of multi-unit clusters showed significant nonsynchronous tonic responses in awake rats, but under ketamine anesthesia none of those units had significant tonic responses (Rennaker *et al*., 2007a). Second, the narrow frequency-selectivity of tonic responses may fall into "gaps" in low resolution tuning curves, such that tonic excitation is missed because of the use of low-resolution stimulus sets. Reported proportions of tonic responders are typically lower in studies using large test frequency spacing (Gaese & Ostwald, 2003) and higher in studies using high resolution tuning curves (Howard *et al*., 1996) or a series of progressively narrower tuning curves (Chimoto *et al*., 2002).

In general, compared to phasic responses to pure tones, tonic responses have much narrower frequency receptive fields. Whereas broadly-tuned phasic responses in A1 are likely excitatory responses to thalamocortical input, the sharpening of neuronal receptive fields that occurs during the transition from the initial phasic "ON" response to the subsequent tonic response might be explained by intracortical mediation (Sutter *et al*., 1999; Qin & Sato, 2004; Wang *et al*., 2005; Hoshino, 2007). In an optical imaging study of anesthetized

gerbil A1, Horikawa *et al*. (1996) found that pure tones first elicited fast excitation across a large cortical area, followed by inhibition which reduced excitation to a narrow area or band such that a small excitatory cortical area (~500-700 μm width at 75 dB SPL) was surrounded by areas of inhibition. At the single neuron level, phasic excitatory "ON" responses are believed to be mediated by non-NMDA receptors receiving thalamocortical input. The subsequent "late-phase" response is believed to be mediated by either excitatory NMDA receptors if the stimulus frequency is a preferred frequency or by inhibitory $GABA_A$ receptors if it is not. Therefore the sharpening of tonic response receptive fields is likely due to GABAergic inhibition (Horikawa *et al*., 1996; Wang *et al*, 2002).

Evidence for the non-NMDA→NMDA/$GABA_A$ model of tonic responses comes from pharmacological manipulations in gerbils (Foeller *et al*., 2001; Kurt *et al*., 2006), guinea pigs (Horikawa *et al*., 1996), rats (Kaur *et al*., 2004), and chinchillas (Wang *et al*., 2002). Focal application to A1 of the non-NMDA receptor antagonist CNQX blocks broad, phasic "ON" responses, but not tonic excitation or inhibition (Horikawa *et al*., 1996). Application of APV, an NMDA receptor antagonist, blocks tonic excitation but not phasic excitation or tonic inhibition (Horikawa *et al*., 1996). Application of bicuculline, a $GABA_A$ antagonist, blocks tonic inhibition and typically results in widened receptive fields and higher firing rates (Horikawa *et al*., 1996; Foeller *et al*., 2001; Wang *et al*., 2002; Kurt *et al*., 2006). Conversely, application of the $GABA_A$ agonist muscimol narrows receptive field bandwidth and decreases firing rates to pure

tones, which are reversible by the application of picrotoxin, a $GABA_A$ antagonist (Kaur *et al*., 2004).

Attempts to correlate the effects of these pharmacological manipulations with behavioral effects are hindered largely by technical difficulties. Most agonist/antagonist compounds must be applied by local infusion, because of an inability to cross the blood-brain barrier or because of confounding effects when administered systemically. To date, only two studies that I am aware of have succeeded in testing the effect of local agonist/antagonist infusion into auditory cortex on awake, behaving subjects (Riquimaroux *et al*. 1991; Talwar *et al*., 2001).

Behaviorally, the importance of tonic responses in frequency discrimination might be inferred from receptive field plasticity during and following frequency discrimination training. FDLs decrease with training in humans (Ari-Even Roth *et al*., 2003), monkeys (Recanzone *et al*., 1993), and rats (Syka *et al*., 1996; Talwar & Gerstein, 1998). In monkey A1, training improvements are accompanied by a corresponding narrowing of neural frequency receptive field bandwidth for tones 10 dB above detection threshold compared to untrained control animals (Recanzone *et al*., 1993), although this effect has not been tested specifically for tonic responses. In shorter-term plasticity, spectrotemporal activity of some A1 neurons show significant changes dependent on attentional state during frequency discrimination behavior in ferrets (Fritz *et al.*, 2005), macaque monkeys (Durif *et al*., 2003), and baboons (Gottlieb *et al*., 1989), typically seen as an overall increase in

response strength during active attention. Similar response-enhancement effects are seen by fMRI in humans passively or actively listening to vowels and speech sounds (Grady *et al*., 1997; Jäncke *et al*., 1999). Furthermore, when attention is focused on a task involving the discrimination of tones super-imposed on band-limited noise, significant sharpening is seen in the population-level frequency tuning of magnetoencephalographic recordings from auditory cortex (Okamoto *et al.*, 2007). If tonic responses are specifically involved in fine-grained frequency discrimination, tonic frequency receptive fields might be expected to narrow with long-term training or to increase in strength and frequency-selectivity during discrimination performance relative to passive listening.

Predictions of the effect of attention on A1 tonic responses can also be drawn from tonic response correlates in the visual cortex, in which attention and behavioral states have been consistently shown to alter neural response properties (Reynolds *et al*, 2000; Haenny & Schiller, 1988; Smallman *et al*., 1996; Uka *et al*., 2005; Roberts *et al*., 2005). While there is still a debate whether attention results in an enhancement of responses (McAdams & Maunsell, 1999) to attended visual stimuli or increased contrast (Haenny & Schiller, 1998; Spitzer, 1998) it is clear that attention preferentially alters responses to relevant stimuli (for a review see Treue, 2001). Furthermore much of the attentional enhancement seen in visual cortex during discrimination tasks occurs during the tonic portion of the response (McAdams & Maunsell, 1999). Altogether, if attention were to have the same effect in auditory cortex,

we might expect to see enhancement of the tonic response in animals performing a frequency discrimination task.

1.5. Thesis Outline

This introductory chapter has hopefully concisely detailed the background for what will be the unifying hypothesis throughout this thesis, that tonic-responding neurons in primary auditory cortex are crucial for fine-grained frequency perception. This hypothesis is ideally suited for a comprehensive investigation that combines neural recording with behavioral paradigms to study the activity of A1 neurons as subjects perform frequency discrimination. The subjects chosen for all the following investigations were female albino Sprague-Dawley rats, a choice that allows for direct comparison of results with similar rat model studies of A1 tonic responses (Gaese & Ostwald, 2001; Gaese & Ostwald, 2003) and auditory frequency discrimination (Talwar & Gerstein, 1998; Syka *et al*., 1996).

The following three chapters present the results of three select experiments I have carried out in my graduate career. In general chapters are largely adapted from articles submitted for publication, so they are organized in the classical introduction, methods, results, discussion, and conclusions format. The introduction presents background material specifically relevant to each experiment. Methods particular to each study are then detailed, but common procedures are only described once and the referred back to. The discussion in each chapter is specific to each experiment, and the impact of the results as

they pertain to the overall hypothesis of tonic response involvement in frequency discrimination are addressed finally in the final chapter.

Chapter 2 begins by examining A1 neural responses in awake, non-behaving rats. The experiment is primarily designed to examine A1 neuronal adaptation to repeating isofrequency sequences in passively-listening animals. In subsequent behavioral tasks, subjects will have to discriminate frequency changes within repeating sequences of tones. These results enable later comparisons that can highlight the effects of attention in frequency discrimination. However, these results also will demonstrate that a large proportion of A1 neurons in these subjects display tonic firing throughout the duration of pure tone stimuli.

Chapter 3 examines the psychophysical abilities of our subjects without behavioral recordings. Prior to implantation with neural recording electrodes, subjects must be trained on frequency discrimination task. The addition of a testing phase between training and implantation added little extra work, but returned results that considerably add to the literature on frequency discrimination in the rat. The experiment contrasts two behavioral paradigms that were considered for use with concurrent neural recording, and detail the reasons for ultimately selecting the more difficult paradigm.

Chapter 4 combines the electrophysiological methods from Chapter 2 with the behavioral paradigm from Chapter 3 and examines the effect of attention and the correspondence of neural response properties to concurrent psychophysical results. The results demonstrate neural correlates of frequency

discrimination that match psychophysical acuity, and provide some evidence that this acuity arises from intracortical connections.

Chapter 5 presents the main findings and general conclusions of the thesis, and suggests directions for future research based on the results.

Chapter 2

2. A1 Responses during Passive Listening

2.1. Introduction

Previous studies that have estimated the proportion of tonically-responding neurons in rat primary auditory cortex (A1) have presented stimuli to either anesthetized (Talwar & Gerstein, 2001) or passive-listening, awake subjects (Gaese & Ostwald, 2001; Gaese & Ostwald, 2003). Based on results from other mammals (Gottlieb *et al.*, 1989; Durif *et al.*, Fritz *et al.*, 2005), we might expect attentional frequency discrimination behavior to dynamically modify A1 responses, with increases in response strength or changes in frequency tuning. Prior to any behavioral testing, it would be beneficial to establish passive-listening "baseline" estimates of tonic response prevalence and characteristics in our subjects, so final results can be compared to both passive-listening and behaving paradigms. Passive-listening testing with stimulus parameters similar to planned behavioral test stimuli will also help to identify attentional modulation in subsequent behavioral recordings.

One conspicuous feature of the behavioral test stimuli we will later use (detailed in the next chapter) are isofrequency tone sequences that create a background "context" for frequency discrimination, which may result in stimulus-specific adaptation to reference frequencies. In natural settings, stimulus-

specific adaptation to recent auditory stimulus history is essential for extracting important sound information from a chaotic acoustic milieu. By adapting to selectively suppress responses to common stimuli, the auditory cortex becomes better prepared to identify novel deviations from the background context, deviations that may demand attention or action. Several recent studies have illuminated neural correlates in auditory cortex for such complex context-dependent phenomenon as mismatch negativity (Ulanovsky *et al*., 2003; 2004) and auditory stream perception (Fishman *et al*., 2004; Micheyl *et al*., 2005).

Neural correlates of more basic preattentive learning processes, such as sensitization or habituation, can be elicited with repetition of a single stimulus. Condon and Weinberger (1991) showed that repetition of an isofrequency tone will selectively suppress auditory cortex responses to that tone during and following repetition, but to our knowledge, their auditory habituation paradigm has not been revisited since. In that time there have been reports that context-dependent adaptation in A1 is largely presentation rate-dependent (Ulanovsky *et al*., 2003; Fishman *et al*., 2004). Rate-dependent habituation has been noted in somatosensory (Melzer *et al*., 2006) and visual cortices (Motter 2006), and habituation of the auditory evoked response is rate-dependent in cats (Cook *et al*., 1968) and humans (Fruhstorfer *et al*., 1975), but none have yet identified this rate dependence with intracortical recordings in a classical auditory habituation paradigm.

Another reason for revisiting habituation in auditory cortex is to identify possible differential adaptation between expected phasic and tonic responses.

Phasic and tonic responses are believed to be mediated by separate mechanisms (Ulanovsky *et al*., 2004; Wang *et al*., 2005), perhaps thalamocortical and intracortical connections, respectively, and these separate mechanisms may be concerned with temporal and spectral information, respectively (Qin *et al*., 2003; Qin & Sato, 2004; Hoshino, 2007). If such is the case, then an analysis of auditory habituation with an emphasis on distinctions between phasic and tonic response components may help reveal contributions by these separate mechanisms.

This chapter revisits the classical auditory habituation paradigm to study the short-term effects of single-stimulus habituation in auditory cortex, with a primary goal of determining the effects of stimulus presentation rate. The analysis will also pay particular attention to the prevalence and characteristics of phasic and tonic responses, and will examine possible spectrotemporal complexity during adaptation.

2.2. Methods

2.2.1. Surgery

Surgery and experiments were performed at the University of Oklahoma in accordance with the University of Oklahoma Laboratory Animal Resources and Institutional Animal Care and Use Committee (IACUC) regulations. Subjects were 12 adult female Sprague-Dawley rats, 3-6 months old, weighing 250-350 g (Charles Rivers Labs; Wilmington, MA). Anesthesia was induced

with a mixture of ketamine, xylazine, and acepromazine (50, 20, and 5 mg/kg, respectively). Atropine and dexamethazone were administered subcutaneously prior to and following surgery. Surgical protocol has been previously reported in detail (Rennaker *et al*., 2005a).

Briefly, a midline incision was made to expose the top of the skull. The right temporalis muscle was partially dissected and a #55 stainless steel bit was used to drill holes for skull-cap fixation bone screws; one in each frontal bone and two in each parietal bone. A 6 mm^2 rectangle craniotomy was made in the right temporal bone ~3-6 mm caudal to bregma and immediately ventral to the temporal ridge. The dura was resected to expose the pia, and primary auditory cortex (A1) was located using surface vasculature, which has been shown to have a generally consistent orientation to A1 across albino rats (Sally & Kelly, 1988). Multi-electrode arrays were rapidly inserted into layer IV/V (500-600 μm depth) using a custom mechanical inserter (Rennaker *et al*., 2005b). A silicone elastomer, Kwik-Cast (World Precision Inc.; Sarasota, FL), was used to seal and cover the craniotomy, and acrylic was used to form a skullcap securing the electrodes in place. Sutures were sewn at the front and back of the incision. The prophylactic antibiotic minocycline was administered with water (50 mg/L) for 2 days prior to and 5 days following surgery to lessen the inflammatory immune response (Rennaker *et al*., 2007).

2.2.2. Electrophysiology

Multi-channel arrays consisted of 15 polyimide-insulated tungsten micro-wires (50 μm diameter) arranged in three rows of five spaced at 500 μm intervals in the rostral-caudal and ventral-dorsal directions. Two additional Teflon-insulated tungsten micro-wires were placed 500 μm dorsal to the array and stripped of insulation prior to insertion to act as low-impedance references. Electrodes had an average impedance of 60 kΩ at 1 kHz in saline. Details of electrode construction have been previously reported (Rennaker *et al*., 2005a).

A head-stage amplifier (Tucker Davis Technologies; Alachua, FL) was directly attached to the electrode connector. Neural signals were sampled at 25 kHz, amplified, and band-pass filtered between 500 Hz and 5 kHz using Tucker-Davis Technologies (TDT) System 3 hardware. Brainware software (TDT) was used for recording and auditory stimulus control. Spike waveforms were detected by threshold crossings, and stored for offline sorting and analysis.

Spike-sorting was first performed with a unsupervised superparamagnetic clustering program adapted from Quiroga *et al*. (2004). The results of the unsupervised clustering were manually verified and waveform templates were created from separated spike-shapes. Waveform templates for each channel were then reapplied to all recordings within the same session, but not across days, for consistent sorting between runs. Many multi-unit clusters (MUCs) were irresolvable to single units, evidenced by inter-spike intervals of less than an absolute refractory period of 1 ms and high spikerates. For the purposes of this analysis, all units are hereafter assumed to be MUCs.

2.2.3. Acoustic stimuli

Stimuli were generated digitally (RP2; TDT) at a sampling rate of 100 kHz, converted to analog voltage, attenuated (PA5; TDT), and played through a calibrated piezo-electric speaker. A series of broadband clicks (~1 ms duration, 3 dB points at 1.6 and 31.6 kHz) were used to elicit auditory-driven action potentials for threshold setting. Iso-intensity tuning curves (IsoTCs) consisted of a set of 20 pure tones, with logarithmically-spaced frequency from 2 to 32 kHz (0.21 octaves apart), of 100 ms duration (5 ms cosine ramp rise/fall) and 55 dB intensity, repeated 30 times at a stimulus presentation rate (SPR) of 2 Hz. Inter-tone interval was not randomized. The 55 dB intensity was chosen as a standard for all experiments since it was suprathreshold for nearly all auditory-driven channels and 20-30 dB suprathreshold for typical channels. Intensity thresholds were verified with a similar multi-intensity tuning curve. Iso-frequency repeating tones used for inducement of habituation were single pure tones of 100 ms duration and 55 dB intensity, with frequencies chosen directly from the IsoTC. Habituation tones were presented at an SPR of 2, 1, 0.5, 0.2, or 0.1 Hz. Spectral properties of stimuli were confirmed using a ¼" condenser microphone (ACO Pacific, Inc.; Belmont, CA) and a digital oscilloscope.

2.2.4. Experimental Procedure

Subjects were housed separately within a vivarium on a 12:12 light-dark cycle, and experiments were run during the light part of the cycle. On the day of recording, subjects were placed in a custom restraint harness and

suspended 6 inches below a calibrated, free-field speaker in a double-walled acoustic chamber. The custom restraint allowed for ~25 degrees of head movement to either side, although subjects would typically sit still and quietly for the duration of testing, since they had been accustomed to restraint prior to implantation. Speaker calibration was performed with a microphone in a position approximate to the center of subjects' heads.

Passively-listening subjects were connected to the multi-channel recording system and were first presented with broadband click stimuli, during which action-potential thresholds were dynamically set and then locked-in for each channel at twice the RMS value of the channel signal. Dynamic threshold setting was not used during pure-tone recordings because thresholds could drop to a noise level during sweeps that elicited no action potentials.

Experiments began with recordings taken during one IsoTC set (30 repetitions) to initially assess receptive fields (RFs) across channels. From the RFs of active channels a habituation tone frequency was chosen such that it corresponded to or was near the best frequency (BF) on at least one channel. The habituation tone frequency (HF) was never the lowest two frequencies (2 kHz and 2.31 kHz) or the two highest frequencies (27.65 kHz and 32 kHz) of the IsoTC set, such that effects on neighboring frequency responses could be investigated. An experimental schedule was then constructed as a block design consisting first of a pre-habituation IsoTC set (PRE), followed by 100 repetitions of the repeating habituation tone (HAB) presented at an SPR chosen at random from the five tested, and then followed by a post-repetition IsoTC set

(POST). The scheduling program allowed for near-instant transitions between stimulus sets.

Preliminary data suggested that habituation-induced adaptation recovered within minutes, so it was possible to run multiple experiments within a session. Subjects underwent 1 to 5 habituation runs per session, 1 session per day, with at least 15 minutes of silence or exposure to lab noise separating the end of one run and the beginning of the next. Subjects were not allowed to sleep while in the custom restraint; alertness was verified by direct observation between runs and by observation through a closed-circuit monitor during runs.

2.2.5. Data Analysis and Statistics

For all data analysis, the spontaneous spikerate was calculated over the 35 ms prior to the stimulus-onset, and then was subtracted from stimulus-driven responses. Spontaneous activity was pooled for each MUC within PRE, REP, and POST blocks, but was not pooled between blocks. Response latency for each MUC was determined from individual peri-stimulus-time-histograms (PSTHs) pooled from responses to all frequencies in the PRE stimulus set. Spiking activity was collected into 1 ms time bins, and latency was defined as the time of the first bin with a spikerate greater than the upper 95% confidence interval of spontaneous spikerate.

For all statistical comparisons, a relatively conservative α of 0.01 was used to avoid type I error, particularly because of the large sample sizes of the population data. Analysis of individual MUCs was carried out using non-

parametric statistics. Puri-Sen 2-way ANOVAs, with rows of tone frequency and columns of time (5 ms bins), were used to identify significant interaction between stimulus-driven activity and frequency selectivity ($\alpha = 0.01$). Significant interaction proved to be a reliable, objective indicator of pure-tone-driven activity. Within a fixed time window, a Kruskal-Wallis one-way ANOVA, with columns of tone frequency, was used to identify significant frequency selectivity ($\alpha = 0.01$). Note that this test returns significance for both frequency-selective excitation and suppression. Finally, a Wilcoxon's Matched-Pairs Signed-Rank (MPSR) test was used for comparisons between particular frequency responses within set time windows and spontaneous rate, or between the same frequency response in different blocks ($\alpha = 0.01$).

Standard parametric statistics (1- and 2-way ANOVA, t-test; $\alpha = 0.01$) were used to analyze population effects. To study population-level effects, individual MUC frequency responses were normalized by the PRE response, such that normalized values represent the percent change in response from PRE spikerate for each particular frequency. For suppressive responses, this means that negative normalized values represent decreased suppression and increased spikerate above the PRE suppressed spikerate in response to that particular tone frequency. This convention allows for the combining of excitation and suppression data in the case that habituation induces a decrease in contrast, which will later be shown.

Tuning properties of MUCs were determined with respect to BF, which was determined within a fixed time window by first calculating 99% confidence

intervals for the spikerate elicited by each PRE frequency. The BF was defined as the frequency for which the spikerate had the highest confidence interval lower bound. BF was therefore a function of both spikerate and spikerate variability, such that a frequency that elicited a more consistent, lower spikerate could be defined as the BF compared to a frequency that elicited larger, but more erratic, responses.

The stability of HF responses prior to habituation was quantified by comparing the HF response in a given run's PRE block against the same frequency response in the preceding PRE block. Note that by this method, the stability of the HF response prior to the first run was not determined. The stability of the tuning properties of individual MUCs was also estimated by similar comparisons of BF and excitatory and suppression bandwidth from consecutive PRE blocks prior to habituation.

To visualize the effects of repetition on the HF responses, the plots presented here use a combination of moving-window smoothing, for the PRE and POST blocks, and an expanding-window smoothing for the HAB block, in which the HF response at each repetition is averaged with all the response to all preceding repetitions, similar to that used by Cook *et al*. (1968) for plotting habituation effects. This graphing method more clearly highlights SPR-dependent trends. Results visualized with expanding-window analyses were verified with concurrent moving-window analyses.

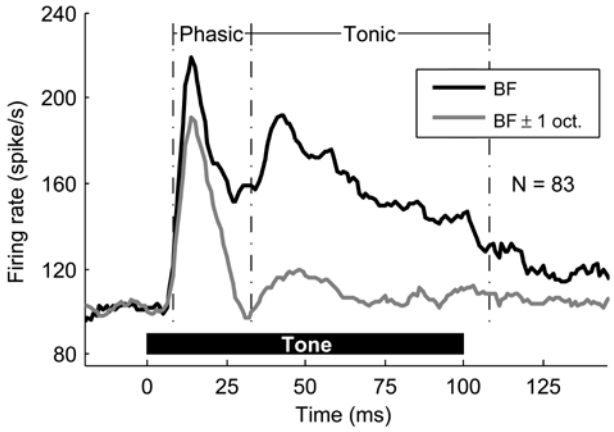

Figure 2.1. Two mean PSTHs showing the difference between On-BF (black) and Off-BF (gray) responses in a population of MUCs with both phasic and tonic response components. The boundaries of the phasic (8-33 ms post-stimulus-onset) and tonic (33-108 ms) analysis windows used for calculating spikerate in all subsequent analysis are shown as the dashed vertical lines.

2.3. Results

2.3.1. Phasic and Tonic Responses

132 Multi-unit clusters (MUCs) from 12 subjects exhibited significantly driven, frequency-selective responses to 55 dB pure tones prior to habituation runs (Puri-Sen 2-Way ANOVA, interaction $\alpha = 0.01$). Of these, 130 had latencies of less than 15 ms calculated from peri-stimulus-time-histograms (PSTHs). The two MUCs with latencies greater than 15 ms were excluded from analysis on suspicion of being in non-primary auditory cortex. Of the remaining 130 MUCs, a majority showed both significant phasic (onset) and tonic (sustained) responses. Two mean PSTHs pooled from 83 MUCs with both phasic and tonic excitatory best frequency (BF) responses are shown in Figure 2.1. In these MUCs, BF tones elicited phasic excitation followed by sustained tonic excitation, whereas off-BF tones elicit only phasic excitation and often tonic suppression. In general, phasic responses were excitatory, responding to a wide bandwidth of tone frequencies. Tonic responses were a mix of

excitation and suppression, with excitation confined to a narrow bandwidth near the BF. The post-stimulus-onset time windows defining phasic and tonic responses for this analysis are 8-33 ms and 33-108 ms, respectively, shown as the dashed boxes in Figure 2.1.

For off-BF frequencies, phasic excitation was frequently followed by tonic suppression, such that when activity was averaged over a long time window, significant excitation and significant suppression had a combined firing rate not significantly different from spontaneous firing rate. Out of all PRE recordings, there were 593 cases of significant phasic excitation (measured over 8-33 ms post-stimulus-onset) followed by significant tonic suppression (measured over 33-108 ms) in response to single tones (MPSR, $\alpha = 0.01$). When spikerate was calculated within a 100 ms window combining phasic and tonic responses (from 8-108 ms), only 113 (~19%) of the 593 cases showed a response significantly different from spontaneous. To avoid overlooking significant phasic and tonic responses due to cancelling summation, and to identify possible differences in habituation effects, phasic and tonic responses were analyzed separately for the remainder of the analysis.

The presence of driven phasic or tonic responses was identified by testing for significant frequency-selective spikerates within the phasic and tonic time windows (Kruskal-Wallis ANOVA, $\alpha = 0.01$). Of the 130 MUCs, 9 showed only phasic responses, 5 showed only tonic responses, and 116 had both phasic and tonic responses. It's important to note that this method of analysis does not differentiate between frequency-selective excitation and frequency-

selective suppression. The 83 MUCs included in Figure 2.1 are a subset of the 116 phasic-tonic MUCs that show significant excitatory tonic BF responses. In most MUCs, phasic excitation occurred for a wide bandwidth of test frequencies, typically spanning ~1-2 octaves. Suppressive responses were very rarely seen within the phasic response window. The frequency selectivity of tonic excitation, by contrast, was narrower, usually spanning ~0.5 octaves or less, and often flanked by sidebands of suppression. The BF tuning of phasic and tonic excitation was largely similar, although phasic BFs tended to be slightly higher in frequency than the subsequent tonic BF in phasic-tonic responders by about 1 frequency step on average.

2.3.2. Stability of Responses Prior to Habituation

The stability of RFs prior to habituation runs was tested by comparing BF and habituation frequency (HF) response spikerates between consecutive PRE IsoTCs within a session. In 326 comparisons of phasic BF determined from consecutive PRE IsoTCs, 229 (70%) showed changes of only one frequency step (0.21 octaves) or less. In 310 comparisons of tonic BF, 178 (57%) showed changes of only one frequency step or less. While these numbers do show that tonic response BF was less consistent from run to run, distributions of change in BF were approximately normal in shape for both phasic and tonic BFs. MUCs' phasic and tonic response spikerates to a HF tone prior to repetition were also tested for significant changes between two consecutive PRE IsoTCs prior to habituation runs (MPSR, $\alpha = 0.01$). Of 220 comparisons of consecutive

PRE recordings for which there was a significant phasic HF response prior to habituation, 30 (14%) showed significant differences in HF response between recordings. Of 123 comparisons for which there was a significant tonic HF response, 20 (16%) showed significant differences. Despite the relatively frequent occurrence of significant HF response differences prior to habituation runs, there was no directional trend in those significant shifts. The mean significant phasic HF spikerate shift was -13.68 spikes/s, which was not significantly different from no shift (t-test, p = 0.122). Likewise the mean significant tonic HF spikerate shift was -5.25 spikes/s, which was not significantly different from no shift (p = 0.375).

2.3.3. Habituation Effects on the Phasic Response

Wide-bandwidth phasic excitation was the predominant response seen for pure tones. Significant suppressive phasic responses were too rare (N≤ 8 for any SPR) to analyze robustly and are generally excluded from this analysis. Overall, excitatory phasic HF responses decreased during habituation runs dependent on the stimulus presentation rate (SPR). An example of one MUC undergoing repetition suppression at the fastest (2 Hz) and slowest (0.1 Hz) SPR in separate sessions is shown in Figure 2.2A. In one run during the 3rd session for this subject, an 8.6 kHz repeating tone was presented at a 2 Hz SPR, causing the phasic HF response of MUC06031 to decrease dramatically in the ~3 seconds of repetition, after which the response remained decreased until the end of the HAB block. The phasic HF response recovers almost

immediately with the beginning of the POST IsoTC. Two days later, in another run during the 5th session for this subject, the same 8.6 kHz repeating tone was presented at a 0.1 Hz SPR, for which MUC06031 shows no phasic response decrease. Following the 0.1 Hz SPR habituation run, the POST response recovery is similar to that seen for 2 Hz SPR habituation. The PRE and POST RFs from both runs are shown in Figure 2.2B; note the slight drift in frequency-selectivity between sessions, which was not uncommon, but infrequently of large magnitude.

Habituation effects on phasic excitatory responses across all runs were SPR-dependent similar to the example in Figure 2.2. Population means for 2 Hz and 0.1 Hz SPR runs through the PRE, HAB, and POST blocks are shown

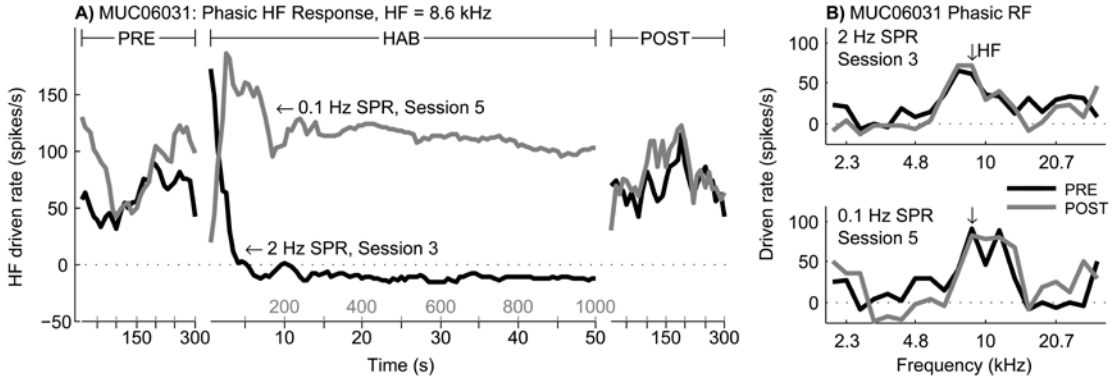

Figure 2.2. A) An example of the effect of habituation on phasic spikerate for one MUC undergoing two separate habituation runs, one with repeating tones presented at a 2 Hz SPR (black line) and one presented at a 0.1 Hz SPR (grey line), during two separate sessions. Spikerates recorded during RF testing prior to (PRE) and following (POST) the repeating tone block (HAB) are smoothed with a 100 second (10 repetition) moving-window. To better visualize the SPR-dependent effects, spikerate recorded during the HAB block is smoothed with an expanding-window average, in which the HF response at each repetition is averaged with all preceding repetitions. HAB blocks contained the same number of tones and therefore have different overall timescales at the different SPRs. In the 2 Hz SPR HAB block tones were presented every ½ second for 50 seconds (bottom timescale, black) and in the 0.1 Hz SPR HAB block tones were presented every 10 seconds for 1000 seconds (top timescale, gray). **B)** The PRE (black) and POST (grey) phasic RFs recorded prior to and following the 2 Hz SPR run (upper panel) and the 0.1 Hz SPR run (lower panel) shown in **A**.

in Figure 2.3A. Qualitatively, of the 86 runs on phasic HF responses at a 2 Hz SPR, 72 (84%) of runs showed decreases in HF response during the last 30 (71-100) repetitions of the HAB block, 33 (38%) of which were significant decreases (MPSR, $\alpha = 0.01$). Of 64 runs on phasic HF responses at a 0.1 Hz SPR, 31 showed increases in response, 9 of which were significant (Fig. 2.3B). Population mean change in the phasic HF response for each SPR during the last 30 repetitions of the HAB block is shown in Figure 2.3C. Generally, phasic HF responses adapted during HAB blocks dependent on SPR, and an ANOVA run across SPRs for the mean phasic HF spikerate change shows significant effects of SPR in the last 30 repetitions of the HAB block ($F_{4,349} = 18.04$, $p < 0.001$). The two fastest SPRs of 2 Hz and 1 Hz SPRs elicited significant normalized spikerate decreases of ~40%, the 0.5 Hz SPR elicited a smaller decrease of ~15%, while 0.2 Hz and 0.1 Hz SPRs elicited small, nonsignificant increases of ~10%.

Interestingly, significant SPR-dependent effects on phasic HF responses did not extend into the POST recording. In a 10-repetition moving-window analysis of the POST phasic HF response, there were no significant differences between SPR groups at any point (not shown; ANOVA, $\alpha = 0.01$). Overall percent change across all 30 repetitions of the POST block for excitatory phasic HF responses for each SPR tested is shown in Figure 2.3D. While there is an overall significant POST decrease for 1 Hz SPR habituation, there is no corresponding significant decrease for the 2 Hz SPR as might be expected, and an ANOVA indicated no significant difference between SPRs ($F_{4,349} = 1.26$, $p =$

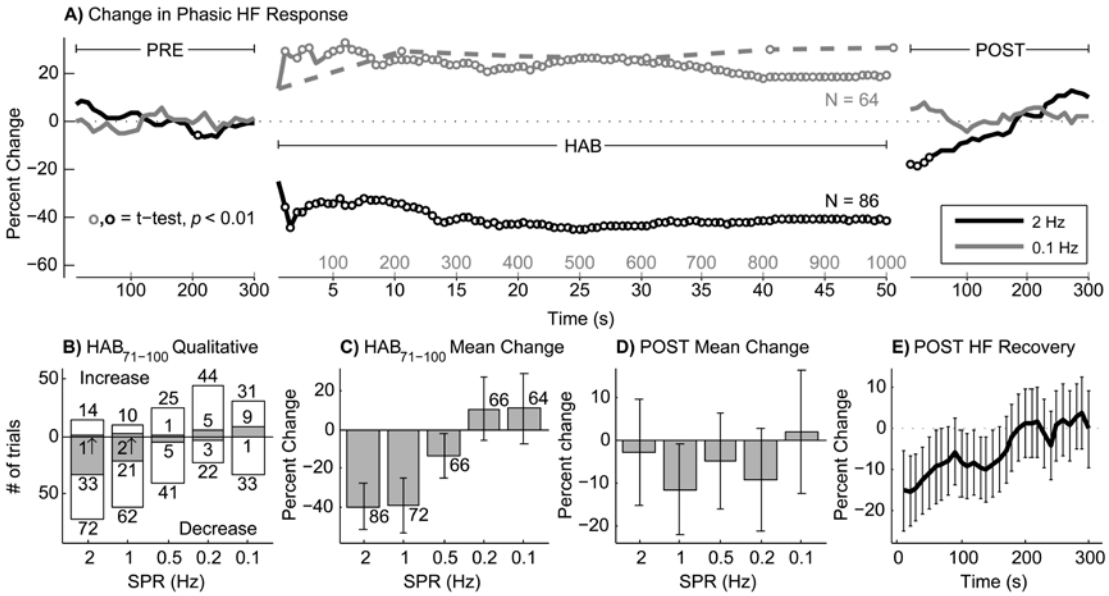

Figure 2.3. A) The population mean change in normalized phasic HF responses is shown for the fastest (2 Hz, black line) and slowest (0.1 Hz, gray line) tested SPR through the PRE, HAB, and POST blocks. HAB blocks contained the same number of tones regardless of SPR and therefore have different overall timescales for each. In 2 Hz SPR HAB blocks tones were presented every ½ second for 50 seconds (bottom timescale, black) and in 0.1 Hz SPR HAB blocks tones were presented every 10 seconds for 1000 seconds (top timescale, gray). For comparison with an equal timescale, the first six repetitions for the 0.1 Hz SPR mean change are also plotted on the same timescale as the 2 Hz SPR mean change (dashed gray line). For both SPRs, time points with significant population-level changes are indicated with open circles (t-test, $\alpha = 0.01$). **B)** The qualitative effects of habituation on phasic HF responses, measured during the last 30 repetitions of the HAB recording, for all tested SPRs are summarized as histograms of the number of MUCs showing increases in spikerate (upward-going histogram) or decreases in spikerate (downward-going histogram). The solid gray portion of each bar indicates the proportion of those changes that are significant (MPSR, $\alpha = 0.01$). **C)** The mean change in phasic HF response between the PRE block and the last 30 repetitions of the HAB block is shown for each SPR. The total sample size for each SPR, shown on the plot, includes spikerates from all MUCs, regardless of whether MUCs showed habituation-induced increases or decreases or if changes were significant. **D)** Mean percent change in phasic HF response between the PRE and POST recording blocks. Sample size for each SPR is the same as in C. **E)** Normalized phasic HF response recovery in the POST recording block following HAB blocks, combined for all runs irrespective of SPR. Each POST repetition of all IsoTC stimuli took 10 seconds, but stimuli were randomly ordered in presentation, so HF presentation time points are only approximate. All mean values in this figure are shown with 99% confidence intervals.

0.289). If SPR-dependent effects on the phasic HF response persisted into the POST recording, they were irresolvable with the temporal resolution of the POST block. Since there were no evident SPR-dependent directional effects in the POST recording, phasic responses were pooled across all runs, combining across SPRs, to examine the time-course of recovery and the frequency-specificity of habituation effects. Overall, HF responses were significantly decreased during the first ~100 seconds, after which the HF response recovered to PRE levels (Fig. 2.3E).

3.3.4. Habituation Effects on the Tonic Response

Excitation and suppression were equally prevalent in the tonic component of the pure tone response. Habituation with any SPR tended to decrease the contrast in tonic responses, decreasing excitation and decreasing suppression, such that both excitatory and suppressive responses approached the spontaneous spikerate. An example of decreasing contrast in excitatory and suppressive tonic responses seen in one habituation run in one subject is shown in Figure 2.4. Habituation with a 20.7 kHz repeating tone at a 1 Hz SPR decreased the excitatory tonic HF response of MUC12161 to that tone. In the same run, recorded on a different electrode, the suppressive tonic HF response of MUC12031 to the 20.7 kHz tone increased towards the spontaneous rate. In the POST recording, MUC12161's excitatory tonic HF response recovered steadily, whereas MUC12031's response recovered towards suppression only initially. The PRE and POST RFs for both MUCs are also shown in Figure

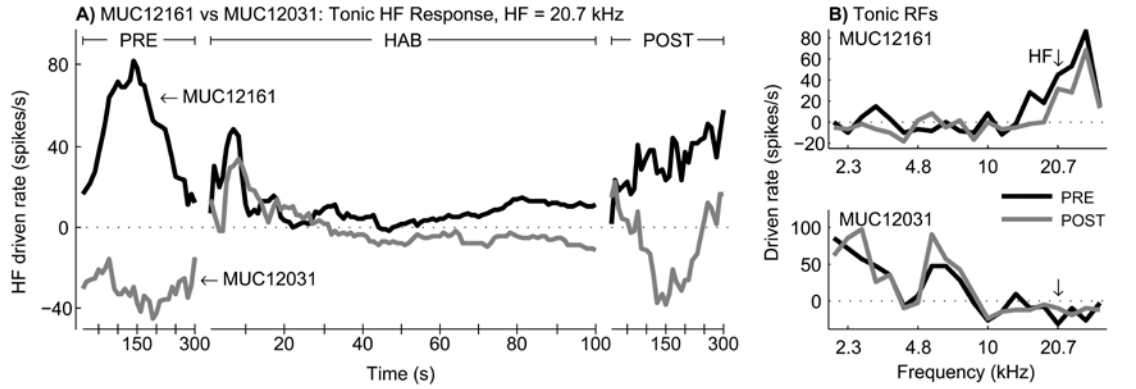

Figure 2.4. A) An example of a decrease in overall contrast of tonic spikerate (33-108 ms post-stimulus-onset) from two MUCs recorded concurrently from the same subject in one habituation run with a 1 Hz SPR. MUC11091 (black line) shows significant tonic excitation to the HF in the PRE recording (MPSR, $p < 0.001$), while MUC11021 (grey line) shows significant tonic suppression to the same HF in the same PRE recording (MPSR, $p = 0.001$). Habituation with a 1 Hz SPR causes the spikerate from both MUCs to approach the spontaneous spikerate baseline. This plot is shown with the combination of moving-window and expanding-window averages shown and explained in Figure 2.2. **B)** The PRE (black line) and POST (grey line) tonic RFs for MUC11091 (upper panel) and MUC 11021 (lower panel) from the habituation run shown in **A**. The HF is indicated by the arrow.

2.4B, and both show some small apparent lasting effects of habituation on the HF response.

The decrease in contrast of tonic responses induced by habituation was observed to be common across runs and subjects, with both excitatory and suppressive tonic spikerates adapting towards spontaneous rate. A 2-way ANOVA with groups of SPR and tonic excitation/suppression was carried out on the normalized change in tonic HF response during the last 30 repetitions (71-100) of the habituation block to determine if excitation and suppression data could be combined. There was no effect of tonic excitation vs. suppression ($F_{1,193} = 1.49$, $p = 0.223$), so excitatory and suppressive tonic responses are combined hereafter for population analysis and normalized such that changes

in spikerate towards spontaneous count as decreases, i.e. an increase in an originally suppressive response spikerate would be considered a decrease in response. This normalization method places an emphasis on loss of contrast, rather than on absolute increases or decreases in spikerate.

The 2-way ANOVA also showed no significant effect of SPR during the last 30 repetitions of the HAB block for tonic HF responses (F4, 193 = 3.01, p = 0.019). Population means for tonic HF response changes during 2 Hz and 0.1 Hz SPR habituation runs are shown in Figure 2.5A. Unlike with phasic HF responses, tonic HF responses decrease significantly for both the fastest and slowest tested SPRs, and remain depressed throughout the POST block. Histograms of the qualitative effects of repetition on tonic HF responses for all tested SPRs are shown in Figure 2.5B, and the mean change in tonic HF responses is shown in Figure 2.5C. While not all SPRs elicited mean decreases in tonic HF response, the near equivalent decreases seen following 2 Hz and 0.1 Hz SPR habituation show a stark difference with the results for phasic HF response habituation.

Just as there was no significant effect of excitation versus suppression for tonic HF responses in the HAB block, neither was there a significant difference in the POST block (2-way ANOVA, $F_{1,193}$ = 0.57, p = 0.453). There was, however, a significant effect of SPR ($F_{4,193}$ = 4.87, p = 0.001) following habituation, although all SPRs induced decreases in the tonic HF response, which was significant for the 2 Hz, 1 Hz, and 0.1 Hz SPRS (t-test, α = 0.01). To compare recovery of tonic HF responses to that of phasic HF responses, tonic

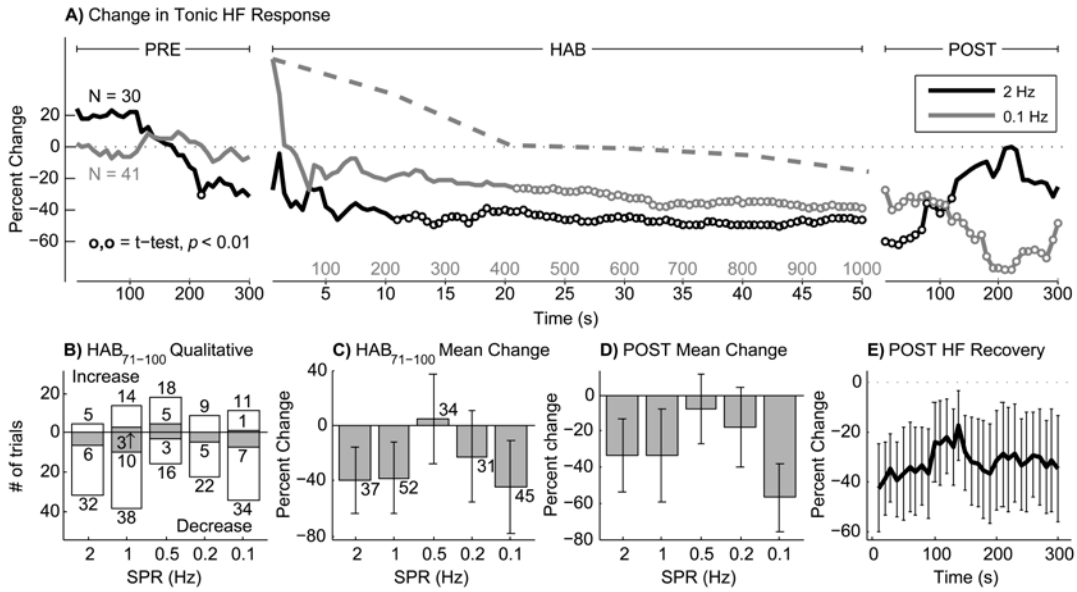

Figure 2.5. Excitatory and suppressive tonic responses have been combined and normalized such that decreases indicate a decrease in contrast in which spikerate changes towards spontaneous rate. For initially suppressive responses, increased spikerate would then be classified as a negative normalized change. **A)** The population mean change in normalized tonic HF responses is shown for the fastest (2 Hz, black line) and slowest (0.1 Hz, gray line) tested SPR through the PRE, HAB, and POST blocks. For comparison with an equal timescale, the first six repetitions for the 0.1 Hz SPR mean change are also plotted on the same timescale as the 2 Hz SPR mean change (dashed gray line). For both SPRs, time points with significant population-level changes are indicated with open circles (t-test, α = 0.01). **B)** The qualitative effects of repetition tonic HF responses for all tested SPRs, measured during the last 30 repetitions of the HAB recording, are summarized as histograms of the number of MUCs showing increases in response (upward-going histogram) or decreases in response (downward-going histogram). The solid gray portion of each bar indicates which proportion of those changes are significant (MPSR, α = 0.01). **C)** The mean change in tonic HF response between the PRE block and the last 30 repetitions of the HAB block is shown for each SPR. The total sample size for each SPR, shown on the plot, includes responses from all MUCs, regardless of whether MUCs showed habituation-induced increases or decreases or if changes were significant. **D)** Mean percent change in tonic HF response between the PRE and POST recording block. Sample size for each SPR is the same as in C. **E)** Normalized tonic HF responses, combined for all runs irrespective of SPR, did not recover during the 5 minute POST recording. All mean values in this figure are shown with 99% confidence intervals.

HF responses in the POST recording were pooled across SPRs (Fig. 2.5E), since the significant ANOVA result reflects only differences in the magnitude of response decrease and not the direction of change. The pooled population

mean shows that the tonic HF response is significantly decreased following habituation, and while the response does appear to recover partially, it remains significantly decreased throughout the 5 minute length of the POST recording.

3.3.5. Lasting Effects on the Habituation Frequency Response

The slow recovery of tonic HF responses following habituation was not noticed in pilot studies, and the experiment design wasn't optimized to study lasting effects. Multiple runs were frequently carried out within a recording session and it's possible that lingering habituation effects could combine over consecutive runs. However, the same HF was never used twice in a session, so HF-specific effects could not compound across runs. To identify possible lasting effects of habituation, phasic and tonic responses to the HF of the first run in multi-run sessions were tracked across all subsequent PRE and POST blocks. Figure 2.6A shows the mean normalized responses to the run #1 HF over run #1 and two subsequent runs. While phasic HF responses appear to be relatively consistent and only show minor decreases, tonic HF responses never recover from the ~35% decrease seen in run #1. However, this persistent decrease in tonic responses is not an HF-specific effect, but rather a general depression of tonic contrast. Tonic responses to frequencies that are not used as HFs in any run of the session show the same decreases, while phasic non-HF responses are again only slightly decreased (Fig. 2.6B). Furthermore, plots of normalized spontaneous rate in Figure 2.6A-B show that the tonic decreases are not caused by any general decrease in excitability;

spontaneous rates actually increase on average by about 10% over the three runs shown.

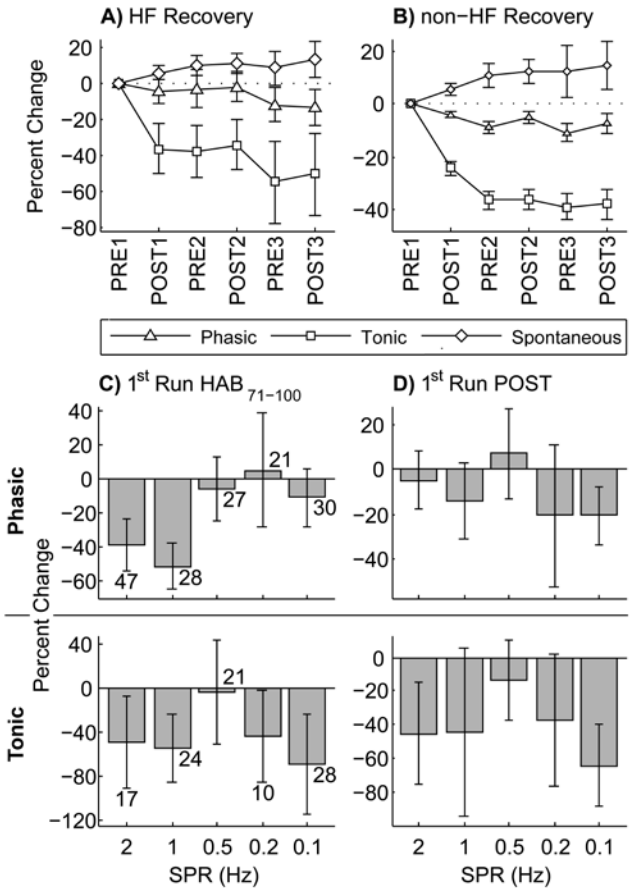

Figure 2.6. **A)** For multi-run sessions, recovery of the HF response from the first habituation run could be tracked across subsequent. The plot shows the mean change in HF response for phasic (Δ) and tonic (□) responses, as well as the mean change in spontaneous rate (◊), between the PRE and POST recording blocks for the HF used in the first run (PRE1, POST1) and the mean change in HF response continuing into the second (PRE2, POST2) and third (PRE3, POST3) run, with 99% confidence intervals. HF-specific effects could not compound within a session because the same HF was never used twice in the same day. **B)** For comparison, the mean phasic (Δ), tonic (□), and spontaneous (◊) change in response is plotted for all frequencies not used as an HF in any run of the session. **C)** To examine the influence of multi-run adaptation on the results, mean change in phasic (upper panel) and tonic (lower panel) HF response between the PRE recording and the last 30 repetitions of the HAB block is shown for each SPR excluding all but the first run of each session, with 99% confidence intervals and sample size shown. **D)** The mean change in phasic (upper panel) and tonic (lower panel) HF responses between the PRE and POST recording block, excluding all but the first run of each session, with 99% confidence intervals. Sample size is the same as in C.

It's possible that the long-lasting decrease in stimulus-driven tonic contrast or the slight decrease in phasic excitability reduced the dynamic range of HF responses in the later runs of multi-run sessions, so that the habituation-induced effect sizes seen with the full dataset might be biased. To test whether general within-session responsiveness decreases influenced the preceding results, we re-analyzed the data using only the first run of each session. Overall, habituation effect magnitudes and SPR-dependence for phasic and tonic responses were similar to those seen in Figures 2.3 and 2.5, respectively. An analysis of the last 30 (71-100) HF repetitions of the HAB block for the first run alone (Fig. 2.6C) showed similar significant SPR-dependence for phasic responses (ANOVA, $F_{4,148} = 10.96$, $p < 0.001$), but not for tonic responses ($F_{4,95} = 2.94$, $p = 0.025$). The differences between PRE and POST phasic and tonic HF responses for the first run alone were also similar to the previous analyses (Fig. 2.6D), showing no SPR-dependence for phasic responses ($F_{4,148} = 3.03$, $p = 0.019$) or tonic responses ($F_{4,95} = 2.43$, $p = 0.053$). Non-specific decreases in tonic contrast and phasic excitation may have lowered the dynamic range for adaptation of HF responses, but the relative magnitudes and patterns of adaptation were consistent.

2.3.6. Frequency-Specificity of Habituation

Non-specific decreases in tonic responses were also apparent in the tonic RFs from the POST recording. RF changes for phasic and tonic responses were examined for frequency selectivity by normalizing each

frequency response by the change in response of that frequency from the PRE to the POST. There is a small, significant decrease of less than 10% at the HF for phasic responses (t-test, p = 0.010), which is not seen at the immediately surrounding frequencies, but this significance may more reflect the large sample size of the combined SPRs (N = 354) than a considerable habituation effect (Fig. 2.7A, top). The significant mean change in tonic RF at the HF ($p < 0.001$) is much larger by comparison, but the tonic RF is also significantly decreased at many frequencies near and away from the HF (Fig. 2.7A, bottom), as might be expected from the generalized decrease in tonic activity across runs shown in Figure 2.6B.

The lack of a lasting, frequency-specific habituation effect may seem in conflict with the results of the classic auditory habituation study of Condon and Weinberger (1991), but the disagreement may stem more from the normalization method used in their analysis. The RF changes for both phasic and tonic responses shown in Figure 2.7A reflect absolute percent change from the PRE RF. Condon and Weinberger's analysis emphasized HF response change relative to the maximum absolute change seen at any frequency in the RF, thereby quantifying the tendency of the HF response to be the most changed frequency response in the RF. Using the same relative RF change normalization method, both phasic and tonic RFs from this data show preferential decreases at the HF response (Fig. 2.7B). Taken together, the absolute and relative RF changes might be evidence of two simultaneous processes of adaptation: a stimulus-specific decrease induced by habituation

and a general decrease in excitability reflecting either a change in behavioral state or longer-term stimulus history.

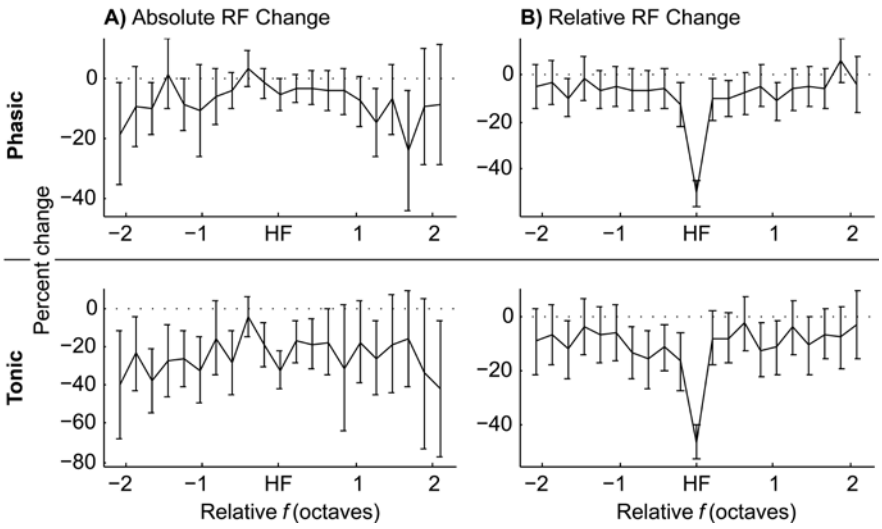

Figure 2.7. A) The mean percent change in phasic (upper panel) and tonic (lower panel) receptive fields between the PRE and POST recording blocks, combined across all SPRs, as a function of frequency-distance from the HF. The mean HF response is significantly decreased for both phasic (t-test, $p = 0.010$) and tonic ($p < 0.001$) responses, but so are responses to tones away from the HF, particularly for tonic receptive fields. **B)** Changes in phasic (upper panel) and tonic (lower panel) receptive fields between the PRE and POST recording blocks appear more HF-specific when response changes are normalized relative to the maximum change seen in the RF using the method of Condon and Weinberger (1991). Change in spikerate at each frequency is divided by the change in spikerate for the frequency showing the largest absolute change, such that population means tend to show which frequency, relative to the HF, often or more consistently showed the largest change.

2.3.7. Effect of BF-HF Distance

The preceding analysis assumes that habituation effects are independent of tuning, meaning, for example, that significant off-BF excitation was grouped for analysis with significant on-BF excitation. To test this assumption, phasic and tonic HF responses were classified according to spikerate relative to the BF spikerate and by the position of where the HF fell on

the RF, higher or lower than the BF of the MUC. Excitatory responses were classified as either near-BF, if significant HF response spikerates were 75-100% of the BF response spikerate, or lower- or upper-off-BF, if significant HF response spikerates were 0-75% of the BF response for HFs either lower than or above the BF, respectively. Significant suppressive tonic HF responses were classified as either lower- or upper-off-BF, but suppressive phasic responses were too rare to analyze robustly. 2-Way ANOVAs ($\alpha = 0.01$), with columns of on-BF/off-BF groups and rows of SPR, were carried out on normalized change values averaged over the last 30 (71-100) repetitions of the HAB block and for the entire POST block. The only significant effect seen in all tests was a phasic response dependence on SPR during the HAB block ($F_{4,339} = 18.12$, $p < 0.001$), as previously shown. There was no significant effect of RF position for either phasic or tonic responses during the HAB or POST blocks, nor any significant interaction. Tuning relative to the HF apparently had little influence on habituation effects; both phasic and tonic responses seemed to undergo habituation similarly regardless of whether the HF was preferentially driven.

2.4. Discussion

This classical habituation paradigm used short, repetitive tonal sequences to investigate the dependence of short-term habituation in auditory cortex on SPR and to reexamine the lingering effect of habituation on RFs. Phasic and tonic components of neural responses were analyzed separately, partly to avoid cancelling summation of phasic excitation and tonic suppression,

but also to identify possible differences in the magnitude or SPR-dependence of habituation. There were three main results. 1) The phasic response to a repeating tone decreases only for SPRs faster than ~0.5 Hz, whereas tonic responses decreased at the slowest SPRs. 2) Immediately following habituation, both the phasic and tonic responses show decreases at the frequency of the repeated tone, but these post-habituation decreases are independent of SPR and not entirely frequency-specific. 3) Phasic responses generally recovered from habituation within ~2 minutes, but tonic responses were not seen to recover within the entire POST block.

2.4.1. SPR-Dependence During Habituation

The largest decreases in phasic responses during habituation with the 2 Hz and 1 Hz SPRs came between the first and second tone, suggesting that the SPR-dependence of decreases in phasic responses during short-term habituation is likely due in large part to the refractory process of forward masking, in which responses to a tone are suppressed by responses to the preceding tone. Given that habituation was weak for repetition at a 0.5 Hz SPR and altogether absent for the 0.2 Hz SPR in this data, this forward masking refractory process must have a temporal integration window of about 2-5 seconds. This estimated integration window of forward masking in auditory cortex neurons is substantially longer than previous estimates found in rats (Wehr & Zador, 2005) and cats (Brosch & Schreiner, 1997), but those estimates may be low due to confounding effects of anesthesia. Recent two-stimuli

forward masking studies in awake primates report significant cortical suppression lasting as long as 1-5 seconds (Bartlett & Wang, 2005; Werner-Reiss *et al*., 2005). Furthermore, a 2-5 second temporal integration window is similar to estimates from habituation studies of auditory ERPs (Fruhstorfer *et al*., 1970; Budd *et al*., 1998) and MEGs (Sams *et al*., 1993) in humans. An important semantic point to note here is that the rate-dependent, refractory-like decreases seen here may not actually fit a strict psychological definition of habituation (Budd *et al*., 1998). A better term for describing rate-dependent decreased responsiveness to repetition may be the more inclusive "repetition suppression" (Grill-Spector *et al*., 2006).

An interesting comparison can be made between the SPR-dependent reduction of the phasic response seen here and cortical response decreases seen in other studies during the formation of auditory stream percepts (Fishman *et al*., 2004; Micheyl *et al*., 2005). In those studies, responses to one tone of a two-tone alternating sequence decreased with the same SPR-dependence and timescale as human psychophysical reports of hearing similar sequences as two segregated streams. In this study, the temporal integration window may represent the boundary between hearing an isofrequency tone sequence as a series of distinct events or as a stream percept. Macken *et al*. found such a SPR-dependence with isofrequency tone sequences in psychophysical tests, showing that sounds have less distracting power when perceived as continuous streams (2003). If perceptual streaming of repetitive stimuli is dependent on consecutive stimuli falling within the 2-5 second temporal integration window

shown by these cortical MUCs, it may help explain anecdotal phenomenon such as the inability to ignore the sound of a slowly dripping faucet.

Contrary to the suppression of phasic responses seen for faster SPRs, the two slowest SPRs tested appear to cause enhancement of the response to the habituating tone, which is probably due to release from some mode of suppression occurring during the PRE recording. There are two candidate mechanisms for such suppression. First, a study of neuromagnetic auditory evoked fields in humans has shown that the auditory cortex can adapt to "expect" successive stimuli to vary in frequency, and will show elevated responses when then presented with a repeated tone frequency (Rosburg, 2004). Repeated tones in the HAB block may be classified as "deviant" relative to the randomized-frequency PRE block. Second, with stimulus sets containing multiple frequency tones used to evaluate RFs, randomized presentation will result in occasional forward masking by similar-frequency tones being played in succession, which occurs often enough with a 20-frequency stimulus set as to cause a significant bias in the determined RF (Ulanovsky *et al*., 2004). If such is the case, then the small enhancements seen in phasic response at slower SPRs may be artifacts stemming from the comparison of responses between HAB blocks with 0.2 and 0.1 Hz SPRs and PRE blocks with a 2 Hz SPR. The selection of the SPR for PRE and POST iso-intensity tuning curves involved a tradeoff between matching the SPR of PRE block to the HAB block and working within the timeframe that subjects would cooperatively endure the restraint hammock, ~60-90 minutes. A PRE block played at the slowest SPR, 0.1 Hz,

would take ~100 minutes, and a full 0.1 Hz SPR PRE-HAB-POST run would take ~4 hours.

Unlike phasic responses during habituation, tonic responses showed less substantial decreases during the first several repeated tones, and there was no apparent SPR-dependent trend, which argues against the contribution of a similar refractory process reflecting primarily the influence of the immediately preceding tones. Rather, tonic response adaptation is more likely related to stimulus history integrated over a longer timescale than phasic responses, which has been shown in a related study on probability-dependent adaptation in A1 (Ulanovsky *et al*., 2004). Similar differential adaptation between phasic and tonic responses during repetition is also seen in the visual cortex (Motter *et al*., 2006).

2.4.2. Post-Habituation Adaptation

Immediately following habituation, decreases were seen in both phasic and tonic response components regardless of SPR. This may be easily explained for tonic responses, in that the SPR-independent decreases seen during habituation carried over into the POST recording. SPR-independent decreases in the POST phasic response, however, indicate a transition from a primarily refractory adaptation to some longer timescale adaptation which was masked for slower SPRs during habituation. Given the rough equivalence of POST phasic decreases across SPRs, the relevant temporal integration window for this adaptation must be at least as long as the longest habituation block, ~16

minutes. It's also possible that longer-term phasic adaptation is more related to an atemporal count of stimulus events, such that the 100 habituation events would cause the same POST adaptation regardless of timing, but this theory would be difficult to test in a way that could concretely separate temporal effects, especially in the relatively short sessions awake subjects will cooperatively endure.

It was not only surprising to find that lasting habituation of both phasic and tonic responses was SPR-independent, but also somewhat non-specific. Decreases in both phasic and tonic responses are more consistently seen at the habituation frequency, but significant decreases also occur at points across the entire RF. In their study of habituation in auditory cortex, Condon and Weinberger (1991) noted similar low-magnitude non-specific POST decreases, but the non-specific effects seen in this study may seem more severe because of a difference in normalization methods for population averaging. The overall POST effect of deeper frequency-specific decreases and shallower non-specific decreases is likely due to two coincidental mechanisms. The habituation frequency-specific decrease likely represents suppression of specific synapses, which occur at least in part in thalamocortical connections given the frequency-specific decrease seen in the short-latency phasic responses. Added to this specific decrease is a general decrease in excitability, particularly in the tonic response, which may be due to some "fatigue" of intracortical connections reflected in the lack of tonic POST recovery and may account for the "incubation" effect seen by Condon and Weinberger (1991). Similar general

decreases in responsiveness across a session have been seen in anesthetized cats (Ulanovsky *et al*., 2004; Gourévitch & Eggermont, 2008), suggesting that it is not due to behavioral state changes such as drowsiness or inattention in our awake subjects. If generalized decreases reflect an adaptation to common stimulus features independent of frequency, this dataset is ill-suited to pinpoint the cause.

2.4.3. Limitations of this Study

In this study there are three main procedural choices that may have interacted with results. First, studies focusing on distinctions between phasic and tonic responses typically use relatively long stimuli, on the order of 200-1000 ms (Qin *et al*., 2004; Wang *et al*., 2005). The short 100 ms tones used here may not have provided enough time for full development of tonic effects. However, the time-course of phasic responses seen in these MUCs shows the phasic-tonic transition occurring at about 30 ms post-stimulus-onset, leaving about 75 ms of tonic response. Second, because the attentional state of the subjects was not explicitly controlled, there may be unidentified underlying behavioral effects. Although subjects were frequently monitored for alertness, there can be no estimating attendance to the test stimuli. Some studies show that attention can modulate A1 neural responses, possibly sharpening tuning during active listening (Fritz *et al*., 2005; Durif *et al*., 2003). Auditory habituation, however, is thought to be largely preattentive, and attentional state has been shown to have no effect on supposed preattentive types of adaptation

such as habituation of auditory ERPs in humans (Frushstorfer *et al*., 1970; Maclean *et al*., 1975), auditory stream segregation in monkeys (Micheyl *et al*., 2005), or forward masking in monkeys (Werner-Reiss *et al*., 2005). Finally, this study only looked at neural responses in auditory cortex, and therefore cannot speculate on the originating locus of the adaptation seen. Units in subcortical nuclei have shown habituation in response to repetition similar to that used in this experiment (Nuding *et al*., 1999; Perez-Gonzalez *et al*., 2005), so cortical habituation might only be inherited, particularly for phasic responses thought to be fed by thalamocortical excitation.

2.5. Conclusions

Other studies in rats have estimated the prevalence of tonically-responding neurons in A1 to be ~12-30% (Gaese & Ostwald, 2001; Talwar & Gerstein, 2001; Gaese & Ostwald, 2003), whereas 89% of multi-unit clusters in this study had significant, frequency-selective tonic responses, with 64% showing tonic excitation at the best frequency. Our relatively high estimate for rats might differ from previous reports due to anesthesia (Talwar & Gerstein, 2001) or perhaps due to a greater number of stimulus repetitions in contributing to significance tests (Gaese & Ostwald, 2001; Gaese & Ostwald, 2003). The high prevalence for the rat reported here, however, is in agreement with ~60-80% tonic response prevalence reported in awake cats (Chimoto *et al*., 2002; Qin *et al*., 2003; Qin & Sato, 2004), macaques (Recanzone, 2000), and humans (Howard *et al*., 1996). While it is possible that large differences exist in the

distributions of firing patterns of A1 neurons between lower-order mammals such as the rat and higher-order mammals such as the cat, monkey, and human, our data would suggest that differences are more likely due to experimental procedure or acoustic stimulus parameters. Given similar stimuli and recording procedures, we would expect to see similar proportions of tonically-responding neurons in A1 in the subsequent experiments with these subjects.

All behavioral tests detailed in the next two chapters will require subjects to identify frequency changes against a repeating, isofrequency reference background. Tones in those reference sequences will be played at presentation rates of about ~3 Hz. Based on these results, we might expect to see phasic responses in A1 quickly habituate after just a few tones, while tonic responses more slowly decrease. However, we might also expect increases in response strength due to the heightened attentional state during frequency discrimination behavior (Gottlieb *et al.*, 1989; Durif *et al.*, 2003; Fritz *et al.*, 2005a, 2005b). These contrasting modes of adaptation present two intriguing possibilities that need to be tested in behavioral recordings. First, if phasic and tonic responses show habitation to tone repetition during attentional behavior, that may represent incorporation of pre-attentive “priming” of the auditory system to better detect frequency deviants (Ulanovsky *et al.*, 2003, 2004). Second, if phasic and tonic responses do not decrease with tone repetition, then attention must override the habituation processes. We might also expect

attention to the task to prevent the general decreases in tonic responsiveness seen in this throughout the course of passive-listening sessions.

To summarize, a majority of A1 neurons in these subjects show tonic as well as phasic responses to pure tones. Based on previous reports of attentional enhancement of responses, we expect to see stronger and perhaps more prevalent tonic responses during frequency discrimination.

Chapter 3

3. Rat Frequency Discrimination Psychophysics

3.1. Introduction

Rats are a commonly used animal model in neurophysiological studies of the auditory system, but they are relatively underrepresented in behavioral studies of frequency discrimination. This disparity may stem from Fay's 1974 review that showed they had relatively poor acuity, based on Kelly's early data (1970), compared to other mammals and might therefore be a poor choice for related research. More recent studies in the rat have reported rats' frequency discrimination ability to be comparable to other mammals (Syka *et al.*, 1996; Talwar and Gerstein, 1998; Talwar and Gerstein, 1999). To complement neurophysiological studies it would be desirable to continue accumulating a large body of literature on rat frequency discrimination, but behavioral studies can often be prohibitively time-consuming. Of the many paradigms that have been developed which minimize training time in animals by simplifying task requirements, the 'repeating standard' paradigm has been shown to work well for testing frequency discrimination in rodents (Prosen *et al.*, 1989; Sinnott *et al.*, 1992; Talwar and Gerstein, 1998). In a 'repeating standard' task subjects must detect a change in frequency within a sequence of repeating discrete tones. While this type of task has demonstrated advantages in efficiency of

training, would it be possible to simplify the task, and further reduce training time and perhaps increase the proportion of subjects able to successfully acquire the task, while continuing to reliably measure frequency discrimination?

Detection of a linear frequency modulation sweeps called a “glide,” between two constant frequency tones has previously been used as a measure of frequency discrimination in humans (Shower and Biddulph, 1931; Lyzenga *et al.*, 2004). As glide durations decrease and overall stimulus duration increases, lengthening the constant frequency components, least detectable frequency measured frequency difference limens (FDLs) approach frequency discrimination thresholds (Nabelek and Hirsh, 1969; Arlinger, 1977; Sek and Moore, 1999; Lyzenga *et al.*, 2004). For very rapid glides, listeners apparently rely mainly on detection of the difference between the constant frequencies of the bounding tones and not detection of the glide itself (Arlinger, 1977). Therefore, a task that requires detection of a fast frequency transition connecting two tones of different frequency may measure frequency discrimination comparable to tasks that require comparison of two distinct tones separated in time. In a study on human temporal acuity, Wier and Green found that discrimination of the frequency change direction within two-frequency tones containing an instantaneous, phase-matched frequency shift also approximately measured frequency discrimination (1975). Despite this seemingly close correspondence, the only study, to our knowledge, that has used detection of instantaneous, phase-matched frequency transitions as a measure of frequency

discrimination was Roverud's study in the lesser bulldog bat (1999), which found comparable results with other bat studies.

This study investigates the use of two 'repeating standard' paradigms for measuring frequency discrimination ability in rats. In the first task subjects must detect an abrupt change in frequency within a continuous reference tone. We will borrow terminology from Roverud and refer to this type of stimulus as a 'tone-step' (1999). The second task uses the more traditional paradigm in which subjects must detect a change in the frequency of a reference sequence of tones separated in time, which we will refer to as 'discrete' tones. Pilot studies showed that these two task represent strikingly different levels of difficulty for rats. Only roughly half of subjects were able to learn the discrete tone task to criterion, while all subjects were able to learn the tone-step task, and quickly. If the tone-step task provides a reliable measurement of frequency discrimination ability comparable to the discrete tone task, then use of the tone-step paradigm may make for more efficient and conscientious use of animals. In this study we compare FDLs measured with a tone-step detection task against FDLs measured in a more traditional discrete tone task to determine if the tone-step task provides a measurement of frequency discrimination, and in testing with both tasks we attempt to add to the body of data on rat frequency discrimination ability.

3.2. Methods

3.2.1. Subjects

A total of 24 female Sprague-Dawley albino rats were used as subjects in this study over a period of 2 years. Subjects began training at ages of 3-6 months and weighed 200-300 g at start. Subjects were housed singly or in pairs, depending on size, within a vivarium on a 12:12 light-dark cycle. Subjects had free access to water, but were food restricted. Food was removed 24 hours before training or testing began, and subjects were then maintained at or above 85% of pre-training weight. Subjects were trained to detect a difference in tone frequency to receive a 40 mg food chocolate-flavored reward (BioServ). Subjects that did not obtain enough food during training or testing were given supplemental rat chow to maintain body weight. Training and testing lasted for 2-4 months for each subject with sessions 5-7 days per week. On days when subjects were neither tested nor trained, they were given supplemental food. 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.

3.2.2. Apparatus

Subjects were trained and tested in an acrylic cage inside a double-walled, anechoic acoustic chamber. The acrylic cage had a machined

octagonal top and bottom, with walls constructed of round acrylic rods, designed to minimize acoustic reflection and electrical noise for subsequent neurophysiological studies. Integrated in the back wallpiece was an infrared sensor-monitored nosepoke, also made from machined acrylic, centered ~7 cm above the cage floor. Two acrylic feeder trays were integrated into the orthogonal wallpieces on each side of the nosepoke, just above the cage floor. A piezoelectric loudspeaker (CTS Powerline KSN-1165) was attached to the cage top, ~35 cm above the cage floor, and approximately centered above the body position of a rat engaging the nosepoke. The behavioral chamber was lighted by two small, direct-current light bulbs above the cage top that could be turned on or off to indicate session initiation or negative reinforcement "time-outs."

Sessions were controlled by custom MatLab software on a PC computer interfacing with an external real-time digital-to-analog processor (RP2.1; Tucker-Davis Technologies). The real-time processor continuously monitored the nosepoke sensor and controlled the cage lights. Pure tone stimuli were generated digitally in MatLab at a sampling rate of 100 kHz for each trial as sine waves of desired frequency and duration with 5 ms onset and offset cosine ramps. Stimuli were uploaded to the real-time processor between trials for conversion to analog voltage and playback at the initiation of the next trial.

3.2.3. 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 loudspeaker. Subjects were required engage and hold in the nosepoke during trials, so calibration was carrried out with a 1/4 in. ACO Pacific condenser microphone placed in the approximate center of the stereotyped head position. Calibration functions were derived for discrete frequencies by measuring SPL for the middle segments of ramped sine-wave tones generated digitally in MatLab, uploaded to the real-time processor, and played from the loudspeaker. Test tone voltage amplitudes ranged logarithmically from 0.01 to 2 V or until SPL exceeded 80 dB, with measurements repeated 3-5 times. For each frequency the calibration function was determined by a logarithmic regression curve iteratively fit to the measured SPL values, rejecting outliers with residuals greater than twice the standard deviation. Calibration functions were determined for 533 frequencies spanning a range from 1 to 40 kHz with 0.01 octave spacing, a resolution finer than any frequency difference used in any subsequent behavioral test. The calibration showed that the loudspeaker had a flat frequency response between 2 and 32 kHz. For any arbitrary stimulus used in behavioral training or testing that had a frequency not explicitly calibrated for, the voltage amplitude for 60 dB SPL was linearly interpolated from voltage-intensity functions of the two nearest calibration frequencies.

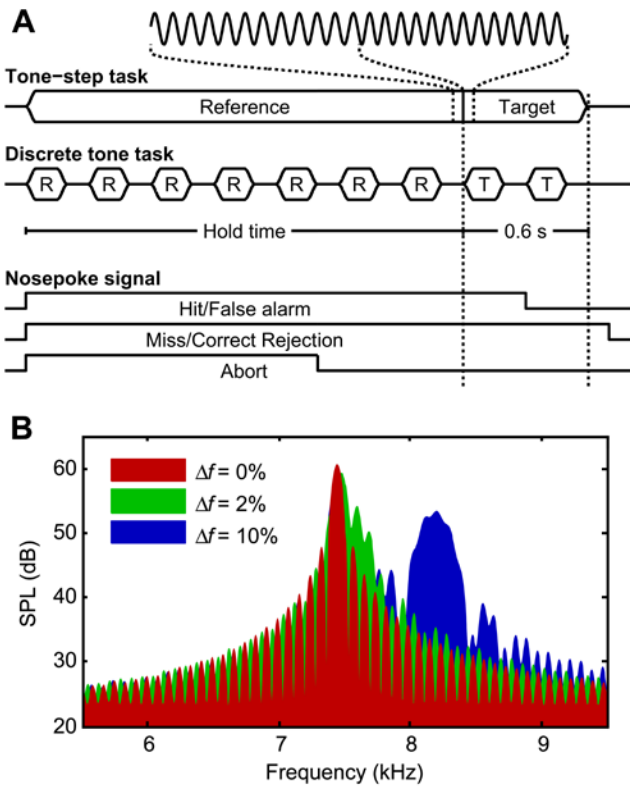

Figure 3.1. **A)** A schematic representation of the tone-step and discrete tone tasks showing scoring for possible nosepoke withdrawal scenarios. The waveform cut-out at top shows an example of phase-matching at the reference-target transition in the tone-step task for a 15% Δf with a reference frequency of 2.31 kHz. **B)** Examples of acoustic spectra from three 12 ms signal snippets centered around the tone-step frequency transition for a 7.44 kHz reference tone with Δfs of 0% (catch trial stimulus), 2% (f_{TAR} = 7.58 kHz), and 10% (f_{TAR} = 8.20 kHz).

3.2.4. Tone-Step Task

In their first session food-deprived subjects received a 40 mg food pellet for any engagement of the nosepoke. Starting with the second session, subjects were required to detect changes in the frequency of a continuous reference tone in order to receive the food reward. A time diagram of the tone-step task is shown Figure 3.1A. Trials began when subjects nosed and held in the central nosepoke, immediately triggering the presentation of the continuous reference. After a randomly-preset hold time, the frequency of the continuous tone would be abruptly changed to a target frequency, and subjects would indicate detection by withdrawing from the nosepoke. The transition was phase-matched to minimize transients, and any differences in the calibrated voltage amplitude of the reference and target sinusoids was smoothed with a 5

ms cosine ramp on the higher-voltage side of the transition. Despite the instant change in frequency at the transition, this method of joining reference and target tones resulted in relatively little spectral splatter compared to control "transitions" with no frequency change (0%), as seen in discrete Fourier transforms of signal snippets, centered around the transition, recorded at a 100 kHz sampling rate with a 1/4 in. ACO Pacific condenser microphone (Fig. 3.1B).

If subjects withdrew from the nosepoke within 600 ms of a frequency change the trial was scored as a hit. Failure to withdrawal within 600 ms was scored as a miss. Catch trials in which continuous tone frequency did not change at the transition were included to estimate false alarm rate. A nosepoke withdrawal during a catch trial within 600 ms following the 'transition' was scored as a false alarm. A catch trial was scored as a correct rejection when a subject held in the nosepoke longer than 600 ms after the 'transition.' If a subject withdrew from the nosepoke at any time before the frequency transition, the trial was scored as an abort. Hits were rewarded with 40 mg food pellets. Misses, false alarms, and aborts resulted in 5-10 second time-outs in which the cage lights were turned off and the program was paused. Correct rejections were neither rewarded nor punished.

In all tone-step training and testing sessions, reference frequency was randomly varied between trials over 18 frequencies ranging from 2.31 and 27.66 kHz in 0.2105 octave steps. The difference between reference and target frequency was randomly varied, both with upward and downward frequency changes (Δfs), between a range of 0 and 0.281 octaves in 0.0140 octave

steps, which approximately correspond to Weber fractions of 1% when calculated with the direction-insensitive equation: $\Delta f = 2 \cdot |(f_{reference} - f_{target})| / (f_{reference} + f_{target})$. In initial training sessions Δfs were ±10-20% while subjects were trained to hold in the nosepoke for increasing hold times. Subjects graduated to testing when they showed detection of >|5|% Δfs with a *d′* signal detection index of >1.96 (Green & Swets, 1966) and could hold for as long as 20 seconds before a frequency transition, although hold times were shorter during testing.

Tone-step frequency difference limens (FDLs) were tested by the method of constant stimuli. The stimulus set contained all 378 combinations of the 18 reference frequencies noted above with upward Δfs of 1 to 10% or downward Δfs of -1 to -10% and catch trials with 0% change. The stimulus set was randomized at the beginning of testing and was re-randomized after each stimulus had been presented in a completed trial. Completed trials were defined as a trial scored as a hit, miss, false alarm, or correct rejection. Stimuli from aborted trials were added to the end of the stimulus set for retesting. Subjects completed 6-12 repetitions of the stimulus set. Hold time was randomly set for between 2-7 seconds during testing and was not reset with the stimulus following aborts, so that subjects were required to complete trials with the full range of hold times.

3.2.5. Discrete Tone Task

All subjects were first trained on the tone-step task before beginning training on the discrete tone task. For discrete tone training the reference and target tones were 200 ms in duration with 5 ms onset and offset cosine ramps, with a 100 ms inter-tone interval (ITI). The discrete task is similar to the tone-step task in nearly all aspects, except that it uses discrete tones, the frequency change occurs in the ITI between tones, and the number of reference tones is randomly set between 7 and 23, generating discrete nosepoke hold times in multiples of the 300 ms tone/ITI period. The 600 ms response period begins at the onset of the first of two tones at the target frequency. Definitions of a hit, miss, false alarm, correct rejection, or abort are the same as in the tone-step task (Fig. 3.1).

Reference frequency in training and testing was varied between the same 18 frequency values used in the tone-step task and Δfs also varied within the same octave range with the same 0.0140 octave (~1%) steps. Subjects were again first trained with ±10-20% Δfs while required nosepoke hold times were incrementally increased. For the discrete tone task, subjects graduated to testing when they showed detection of >|10|% Δfs with a *d'* index of >1.96 and could again hold for as long as 20 seconds before presentation of the target frequency, although again hold times were shorter during testing.

Discrete tone task testing consisted of two phases, each testing by the method of constant stimuli. In the first phase all tones were 200 ms in duration with a 100 ms ITI. The stimulus set consisted of all 558 combinations of the 18

reference frequencies with upward and downward Δfs of ±1-15% and 0% change catch trials. Preliminary results showed that discrete tone FDLs might be larger than tone-step FDLs, so the tested Δf range was increased from ±10% to ±15%. In the second phase of testing, tone duration was varied, with values of 10, 20, 50, 100, and 200 ms, and ITI was increased accordingly to maintain a tone+ITI period of 300 ms. For this phase only three of the standard reference frequencies were used: 4.15, 8.61, and 17.85 kHz. The stimulus set for this phase consisted of 465 combinations of duration, reference frequency, and Δf. Both phases were otherwise similar to tone-step testing. Aborted trials were added to the end of the stimulus set for retesting. Hold times were randomly set between 2-7 seconds and were not reset by aborts. Subjects completed 10-15 repetitions of the stimulus set.

3.2.6. Analysis

Catch trials were inserted into the stimulus set to estimate the false alarm rate during sessions, but *true* false alarm rate was used in all final analyses. In this 'repeating standard' paradigm, the long reference tone or tone sequence can be divided into contiguous response windows, within which an abort should technically be classified as a false alarm and a sustained nosepoke hold throughout should be classified as a correct rejection. *True* false alarm rate (F) can be calculated by summing the number of estimated false alarms and the number of aborts, and dividing by the number of actual and 'virtual' catch trials. Hit rate (H) was calculated for all non-catch trial stimuli

by dividing the number of hits by the number of completed trials for that stimulus. Hit rates were corrected for false alarm rate prior to FDL measurements using Heffner and Heffner's (1988) commonly-used correction formula $H_c = H \cdot (1-F)$, in which H_c is corrected hit rate. For each reference frequency (and tone duration for the discrete tone task) FDLs were then determined from performance curves plotting H_c versus Δf, defined as the linearly-interpolated smallest Δf value for which subjects show 50% performance. Note here that the 0.0140 octave spacing for Δf used is actually equivalent to 0.97% spacing, whereas a 0.0145 octave spacing would have been more precisely equivalent to 1% spacing. The 0.0140 octave spacing was chosen to agree with stimuli from other concurrent experiments, and the true 0.97% Δf spacing is used in all FDL calculations.

Since both negative-going (downward) and positive-going (upward) Δfs were tested, there are two respective FDLs measured from each v-shaped performance curve for each reference frequency (and tone duration for the discrete tone task). In some parts of the analysis, FDLs are calculated without respect to reference frequency or upward versus downward direction.

This study uses false alarm-corrected hit rate to determine FDLs similar to most previous studies to allow easy comparison. More recent studies have used signal detection theory to control for response bias (Talwar and Gerstein, 1998; Talwar and Gerstein, 1999). For this study, a concurrent signal detection analysis was carried out using the nonparametric index *A'*. Signal detection

FDLs (see Appendix B) and resultant trends closely corresponded to corrected hit rate results, so only the corrected hit rate results are presented here.

3.3. Results

3.3.1. Tone-Step Task

All 24 subjects were first trained on the tone-step task following one session of nosepoke training. Mean hit rates and false alarm rates for the first 10 tone-step training sessions are shown in Figure 3.1. Subjects quickly associated nosepoke withdrawals in response to frequency changes with feeding, and hit rates were >80% even in the first session. Training improved performance mainly by decreasing false alarm rate. All but 3 subjects reached the performance criterion required to graduate to tone-step testing, a d' of 1.96 for $|\Delta f|$s > 5%, in 10 sessions or less (median = 6 sessions). The remaining 3 subjects graduated to testing after 12, 14, and 15 tone-step training sessions, respectively.

FDLs for the tone-step task were determined from false alarm-corrected hit rate for 22 subjects at 18 reference frequencies and for upward and downward frequency changes. Subjects completed a minimum of 6 repetitions of the stimulus set (median = 10 repetitions) for a minimum total of 2268 tested stimuli. Data was combined across sessions, with subjects completing trials for a median of 276 stimuli per session, over the course of a median of 14.5 tone-step testing sessions. Uncorrected hit rate curves for each subject, combined

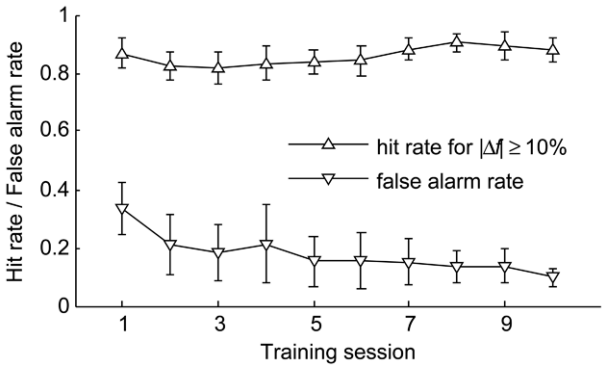

Figure 3.2. Mean hit rate and false alarm rate from all subjects (N = 24) during training on the tone-step task as a function of training session. Error bars show 95% confidence intervals.

across all reference frequencies, are shown in Figure 3.3A. Overall false alarm rates in the tone-step were between 0.05 and 0.2 (median = 0.084). Hit rate curves were approximately v-shaped, but did not appear symmetrical for upward and downward Δfs, with noticeably poorer performance for upward Δfs.

Tone-step FDLs are shown in Figure 3.3B as a function of reference frequency and frequency change direction. A 3-way ANOVA on FDLs showed significant main effects of subject ($F_{21,786} = 18.00$, $p < 0.0001$), reference frequency ($F_{17,786} = 35.18$, $p < 0.0001$), and frequency change direction ($F_{1,786} = 75.68$, $p < 0.0001$). There was also significant interaction between all groups (subject and reference frequency: $F_{357,786} = 1.54$, $p < 0.0001$; subject and frequency change direction: $F_{21,786} = 3.55$, $p < 0.0001$; reference frequency and frequency change direction: $F_{17,786} = 2.84$, $p = 0.0002$). Differences in tone-step FDLs between reference frequencies were quite large compared to differences between upward and downward frequency changes at each reference frequency. In general, tone-step FDLs decreased with increasing reference frequency from ~4% to ~2%. Tone-step FDLs for upward frequency changes

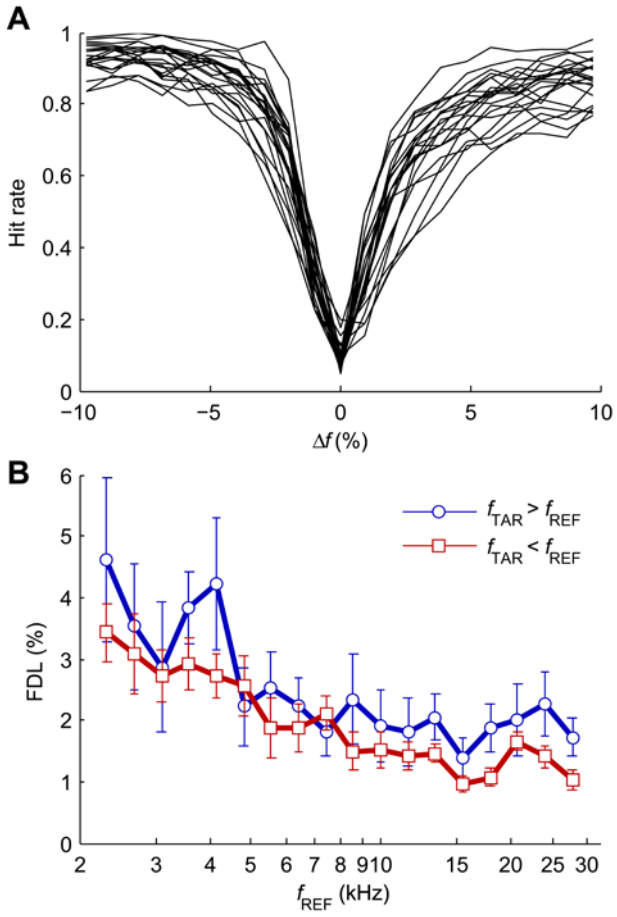

Figure 3.3. A) Psychometric functions of hit rate for the tone-step task as a function of Δf (expressed as Weber fractions in percent) for all subjects (N = 22) that underwent tone-step testing, with hit rate combined across all reference frequencies. **B)** Population mean tone-step FDLs for all upward ($f_{TAR} > f_{REF}$, blue circles) and downward ($f_{TAR} < f_{REF}$, red squares) Δfs at each reference frequency. Error bars show 95% confidence intervals.

were larger than those for downward at most reference frequencies by 0.55% on average. Directional difference trends were also most consistent for reference frequencies above 8 kHz. Overall mean FDLs, irrespective of reference frequency and frequency change direction, for the tone-step task were 1.93 ±0.27%.

Since subjects typically only completed testing on a fraction of the stimulus set in one session, it is only possible to determine FDLs within a single session by ignoring reference frequency or frequency change direction. To determine whether tone-step FDLs showed improvement between sessions during testing, session FDLs for each subject were tested for a decreasing

trend with a Spearman rank correlation ($\alpha = 0.05$). Only 5 subjects showed significant negative rank correlation between tone-step FDL and session number. Presumably most subjects' thresholds had reached an asymptotic level during training prior to tone-step testing.

3.3.2. Discrete Tone Task

Of an initial group of 20 subjects that completed tone-step testing, 11 were able to successfully transition to the discrete tone task. To see if tone-step testing resulted in over-training that inhibited learning of the discrete tone task, the final 4 subjects were transitioned directly from tone-step training to discrete tone training without undergoing tone-step testing. Only 2 of the 4 subjects were able to learn the discrete tone task to criterion. Those 2 subjects of the 4 that couldn't learn the discrete tone task were transitioned back to the tone-step task, which they successfully complete testing on. Successful learning of the discrete tone task appears to be more dependent on subjects' individual abilities than on the training order. For the 13 subjects that did successfully transition to the discrete tone task, most reached performance criterion, a d' of 1.96 for $|\Delta f| > 10\%$, in less than 10 discrete tone training sessions (median = 8 sessions).

In the first phase of discrete tone testing, tone duration was held to 200 ms while reference frequency and frequency change direction were varied. Data was again combined across sessions, with subjects completing testing in a median of 9.5 sessions. Uncorrected hit rate curves for each subject in the

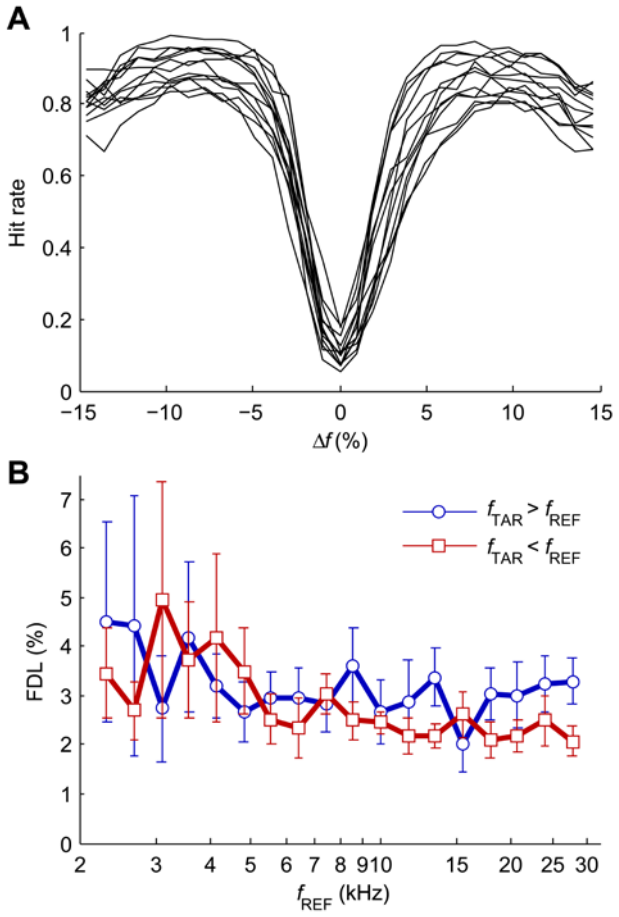

Figure 3.4. A) Psychometric functions of hit rate for the discrete tone task as a function of Δf (expressed as Weber fractions) for all subjects (N = 13) that underwent discrete tone testing, with hit rate combined across all reference frequencies. **B)** Population mean discrete tone FDLs for all upward ($f_{TAR} > f_{REF}$, black circles) and downward ($f_{TAR} < f_{REF}$, gray squares) Δfs at each reference frequency. Error bars show 95% confidence intervals.

discrete tone task, for 200 ms tones combined across all reference frequencies, are shown in Figure 3.4A. Overall false alarm rates were similar to the tone-step task, ranging between 0.05 and 2 (median = 0.101). Hit rate curves were less sharply v-shaped, rounded by lower hit rates for -1% and 1% Δfs. The asymmetry for frequency change direction was also less pronounced than in the tone-step task. Interestingly, all subjects showed better performance for nearer-threshold 8-10% $|\Delta f|$s than for larger 13-15% $|\Delta f|$s. After correcting for false-alarm rate, mean hit rate for 8-10% $|\Delta f|$s were significantly greater than mean hit rate for 13-15% $|\Delta f|$s (paired t-test, $p < 0.0001$), although for both $|\Delta f|$ ranges performance was certainly suprathreshold. Intriguingly, this suggests

that underlying frequency discrimination processes may more successfully detect near-threshold differences rather than large, “obvious” differences.

Discrete tone task FDLs for 200 ms duration tones are shown in Figure 3.4B (and listed in supplementary Table S1) as a function of reference frequency and frequency change direction. A 3-way ANOVA showed significant main effects of subject ($F_{12,461}$ = 15.87, p < 0.0001), reference frequency ($F_{17,461}$ = 6.36, p < 0.0001), and frequency change direction ($F_{1,461}$ = 11.81, p = 0.0007), again with significant interaction between all groups (subject and reference frequency: $F_{204,461}$ = 1.51, p = 0.0019; subject and frequency change direction: $F_{12,461}$ = 2.32, p = 0.0084; reference frequency and frequency change direction: $F_{17,461}$ = 4.70, p < 0.0001). Differences in FDL dependent on reference frequency were less pronounced than in the tone-step task, decreasing from ~4% to ~3% as reference frequency increased. The greater variability in FDL for frequencies below ~5 kHz is largely attributable to 2 subjects that showed much larger FDLs at those frequencies than did other subjects. Differences between upward and downward Δfs were somewhat smaller than in the tone-step task, with upward FDLs about 0.35% larger than downward FDLs on average. Again, the directional difference trends were most consistent for the higher reference frequencies. Overall mean FDLs for the discrete tone task were 2.78 ±0.29%, disregarding reference frequency and frequency change direction. Individual subjects discrete tone task FDLs for each reference frequency are presented in Appendix A, Table A.1.

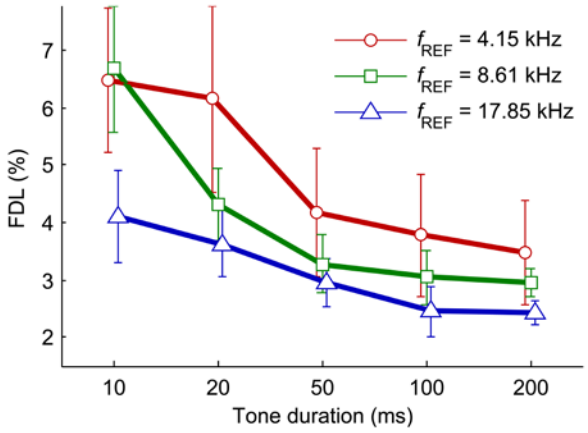

Figure 3.5. Population mean discrete tone task FDLs, expressed as Weber fractions, are plotted as a function of tone duration for the three tested reference frequencies. FDLs shown are determined irrespective of frequency change direction. Plots for each reference frequency are slightly offset to better show error bars, which represent 95% confidence intervals.

In the second phase of discrete tone testing, tone duration was varied between 10, 20, 50, 100, and 200 ms while reference frequency was varied over a more restricted set of 4.15, 8.61, and 17.85 kHz. Direction-irrespective FDLs are shown as a function of tone duration in Figure 3.5. A 4-way ANOVA showed a significant main effect of duration ($F_{4,353} = 48.28$, $p < 0.0001$) along with expected significant main effects of subject, reference frequency, and frequency change direction ($\alpha = 0.05$). There was significant interaction between tone duration and reference frequency ($F_{8,353} = 3.22$, $p = 0.0017$), but not between tone duration and subject ($F_{44,353} = 0.62$, $p = 0.97$) or tone duration and frequency change direction ($F_{4,353} = 1.29$, $p = 0.27$). FDLs decreased with increasing tone duration overall, but decreases were more pronounced for the two lower reference frequencies as tone duration increased. When FDLs are calculated for each tone duration without respect to reference frequency and plotted versus tone duration (not shown), the trend of increasing FDL with decreasing tone duration shows a 'knee' at 50 ms below which the slope of the

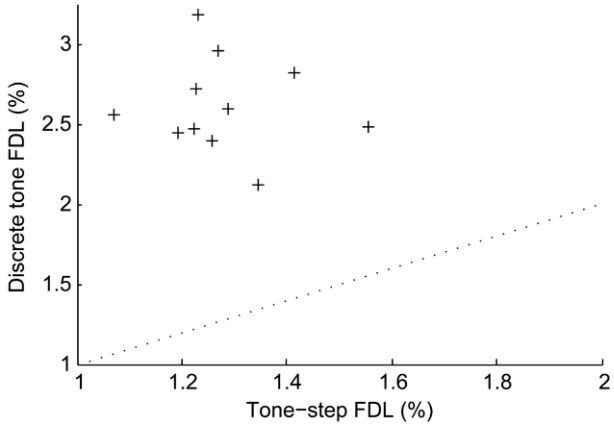

Figure 3.6. Overall FDLs, calculated as Weber fractions irrespective of reference frequency and frequency change direction, measured with the tone-step task are plotted against those measured with the discrete tone task for the 11 subjects that completed testing at both.

function increases. Individual subjects' FDLs for each combination of reference frequency and duration are presented in Appendix A, Table A.2.

3.3.3. Tone-Step vs. Discrete Tone Task

Overall tone-step FDLs and discrete tone FDLs, calculated irrespective of reference frequency and frequency change direction, from the 11 subjects that underwent testing in both tasks are plotted versus each other in Figure 3.6. Discrete tone FDLs are consistently higher than corresponding tone-step FDLs, but there is no significant correlation ($R = 0.12$, $p = 0.73$). Subjects' tone-step FDLs do not appear to be predictive of their subsequent discrete tone FDLs.

The absence of correlated FDLs from the tone-step task and discrete tone task might suggest that the two tasks depend on different detection abilities, a difference that might also be manifest in a comparison of response times for hit trials. Nosepoke withdrawal responses were monitored at a sampling rate of 100 kHz within the 600 ms response window. Plots of hit trial response time versus Δf for the tone-step and discrete tone tasks are shown in

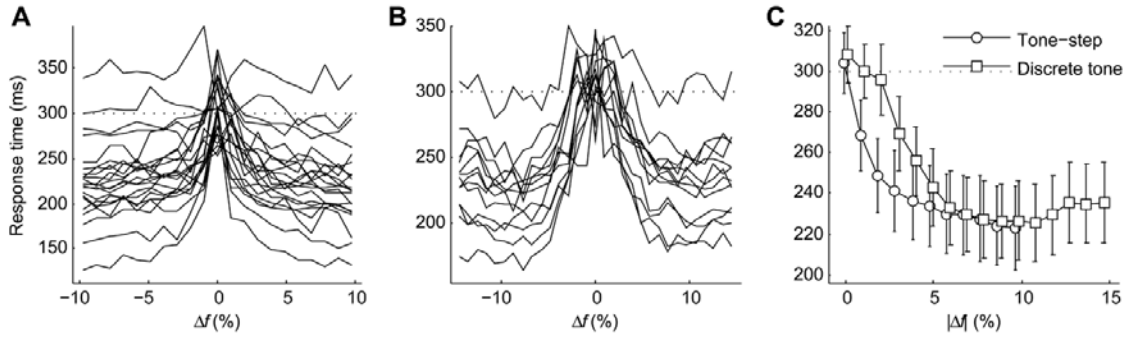

Figure 3.7. Mean hit trial/false alarm trial response times are plotted versus Δf for all subjects in the tone-step task **(A)** and discrete tone task **(B)**. Population mean response times, irrespective of frequency change direction, for the tone-step and discrete tone tasks are shown in **(C)**. The dashed line at 300 ms in each plot represents expected mean reaction time for purely guessing behavior within the 600 ms response window. Error bars represent 95% confidence intervals and are plotted slightly offset to show differences.

Figs. 3.7A and 3.7B, respectively. Mean population hit trial response times are shown as a function of absolute frequency change for both the tone-step and discrete tone task in Figure 3.7C. Plots of response time have the appearance of inverted hit rate performance curves, which is due to strong correlations between reaction time and false alarm-corrected hit rate in both tasks. In the tone-step task mean reaction time and hit rate were negatively correlated in all 22 subjects, 21 of which were significant correlations ($\alpha = 0.05$). In the discrete tone task all 13 subjects showed significant, negative correlations ($\alpha = 0.05$). If the processing of the stimuli are the same we would expect equivalent reaction times for equivalent hit rates regardless of the frequency change. However, for the 11 subjects that completed both tasks, linear regressions fit to reaction time versus hit rate were significantly different for all subjects (ANCOVA, $\alpha = 0.05$). Assuming equal slope in the regressions and controlling for false alarm-

corrected hit rate, discrete tone task response times were on average ~18 ms slower than those for the tone-step task.

To briefly examine the possibility that spectral splatter in the tone-step task served as cues, two subjects that had completed testing on the discrete tone task, RN23 and RN24, were further tested to compare FDLs for tone durations of 200 ms and 300 ms. The tone+ITI period was fixed at 300 ms for both tone durations, such that the ITI for 300 ms tone sequences was zero, which approximated a continuous tone except where the 5 ms offset and onset ramps from consecutive tones met. A single reference frequency of 17.85 kHz was used for these tests. FDLs calculated irrespective of reference frequency and frequency change direction for the 300 ms tones (separated by no ITI) were 2.33% on average for the two subjects, which is approximately in the range of tone-step FDLs at that reference frequency. The FDLs measured for 200 ms tones were 2.75% on average. Although the small sample size prevents any statistical analysis or conclusions, the measured FDLs suggest that increasing tone separation from 0 to 100 ms may have a large effect on frequency discrimination.

3.3.4. Effects of Reference Duration

It is possible in 'repeating standard' paradigms for subjects to gain an advantage by dynamically adjusting their detection strategies based on the relationship between time held in the nosepoke and target probability. During testing on either the tone-step or discrete tone task, the required hold time

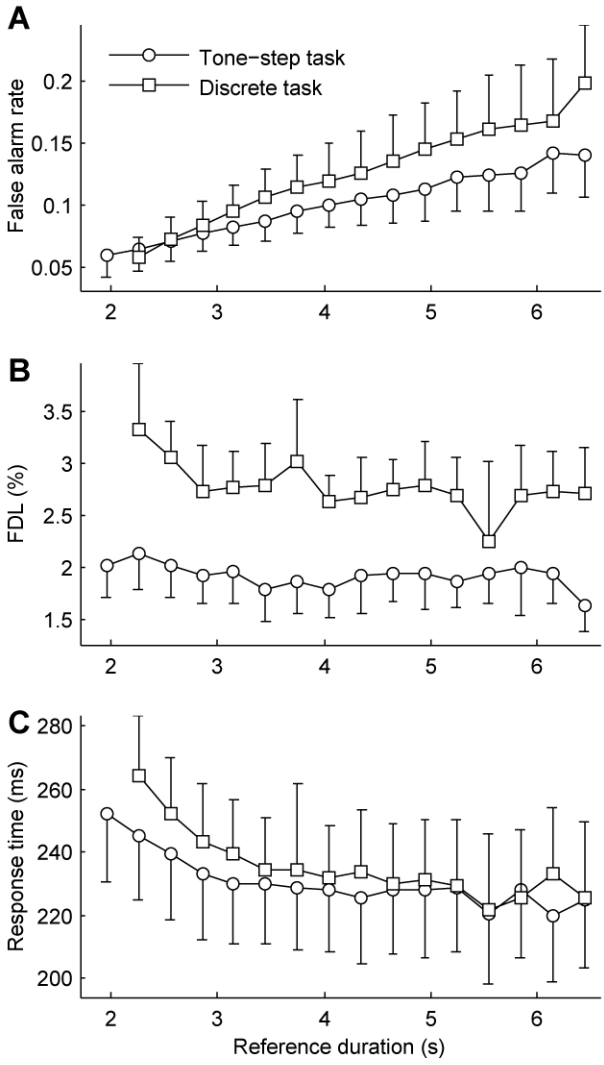

Figure 3.8. **A)** False alarm rate, **B)** frequency change direction- and reference frequency-irrespecive FDLs, and **C)** hit trial response time as a function of time held in the nosepoke, calculated for time bins of 300 ms from 1.8 to 6.9 seconds, for the tone-step task (circles) and the discrete tone task (squares).

before a target presentation is randomly set between bounds of 2 and 7 seconds. Therefore the longer a subject holds before being presented a target, the higher the probability that a target is about to occur. To determine if subjects' detection strategies varied with reference duration, false alarm rate was determined within 300 ms time bins from 1.8 to 6.9 seconds, corresponding to tone+ITI periods in the discrete tone task. Subjects appeared to recognize this increasing probability and showed increasing false alarm rates with increasing hold time (Fig. 3.8A). Increases in false alarm rate with reference

duration were significant for both the tone-step (ANOVA; $F_{15,351} = 5.62$, $p < 0.0001$) and the discrete tone task ($F_{14,194} = 5.57$, $p < 0.0001$). The trend was distinctly linear, with subjects showing false alarm rates of ~0.05 when hold time was 2 seconds up to false alarm rates of ~0.15 when hold time was 7 seconds.

To determine if increasing false alarm rates affected FDLs, FDLs were calculated irrespective of reference frequency or frequency change direction as a function of hold time within 300 ms bins from 1.8 to 6.9 seconds from the start of the trial. FDLs are shown as a function of hold time in Figure 3.8B. There was no significant effect of hold time on FDLs for either the tone-step (ANOVA; $F_{15,350} = 0.59$, $p = 0.884$) or discrete tone task ($F_{14,186} = 0.81$, $p = 0.662$). Subjects' increasing 'go' biases, evidenced in increasing false alarm rates, did not apparently confer any detection advantage. However, although FDLs did not decrease with increasing hold times, response times for hit trials (Fig. 3.8C) did significantly change as hold time increased (ANOVA; $F_{14,191} = 2.03$, $p = 0.018$), for the discrete tone task, although not for the tone-step task ($F_{15,351} = 0.73$, $p = 0.7578$). Decreases in response time were not due to the increasing 'go' bias with increasing hold time. Subjects' mean false alarm rates, irrespective of hold time, were not significantly correlated with hit trial response time for either the tone-step ($p = 0.33$) or discrete tone task ($p = 0.44$), indicating that a stronger bias did not result in faster responses. Therefore, decreasing response times with increasing hold times might indicate that processes underlying frequency discrimination become more efficient with increasing reference durations, albeit not more sensitive.

3.4. Discussion

3.4.1. Tone-Step Task FDLs

In this study we compared rat FDLs measured with a tone-step task that required detection of an abrupt frequency change in a continuous tone and a discrete tone task that required detection of a frequency change in a sequence of discrete pure tones. We found little evidence to suggest that the two tasks were measuring precisely the same behavioral ability. Overall FDLs measured with the tone-step task (mean 1.93 ±0.27%) were significantly lower than FDLs measured with the discrete tone task (mean 2.78 ±0.29%) and FDLs from the two tasks were not correlated in subjects that completed testing on both (Fig. 3.6). Further more, when hit rate was controlled for, response times in hit trials were ~18 ms (~10%) faster on average for the tone-step task, indicating that tone-step detection requires less processing time.

The tone-step task investigated in this study is somewhat unique in it's use of an abrupt, phase-matched frequency change, which to our knowledge was used in only one previous study on the lesser bulldog bat (Roverud, 1999). The bat's specialized auditory system makes any comparison with the present study in rats difficult. Apart from Roverud's study, the tone-step task might be most comparable to frequency discrimination studies that use detection of frequency modulation (FM). However, much like our results, studies directly comparing FM detection FDLs and two-tone discrimination FDLs in humans find that the two tasks seemingly measure different abilities (Jesteadt and Sims,

1975; Moore, 1976; Sek and Moore, 1995). The relationship between FM detection FDLs and frequency discrimination FDLs in humans (Sek and Moore, 1995) appears quite different from the relationship between tone-step detection FDLs and discrete tone FDLs seen in this study, suggesting that tone-step detection is also unrelated to FM detection.

One obvious distinction of the tone-step task from frequency discrimination or FM detection tasks is that frequency changes are instantaneous, which could create detectable spectral splatter for subjects to use as a cue. However, examination of the acoustic spectra at the frequency change (Fig. 3.1B) shows little spectral splatter relative to catch trial stimuli (Δf = 0%), especially near FDL thresholds (Δf = 2%). Roverud (1999) similarly saw little evidence of spectral splatter being used as a cue for tone-step detection. Aside from spectral splatter, the only cue available for subjects to detect in the tone-step stimulus is the frequency change, which would require frequency discrimination. Perhaps tone-step FDLs are not correlated with discrete tone FDLs because a common frequency discrimination process is confounded by processes of auditory short-term memory. In the discrete tone task subjects must retain frequency information throughout silent intervals separating tones. Talwar and Gerstein showed that FDLs in the rat did not increase when ITIs were varied between 0.1 to 2.1 seconds (1998), a range for which humans also show no considerable decrement (Harris, 1952; Clement *et al.*, 1999). However, Lyzenga *et al.* found that decreasing silent ITI from 200 ms to 0 ms significantly improved detection of frequency differences between

tones for humans (2007), with an effect size similar to the FDL improvements we saw in two rats when ITI was reduced from 100 ms to 0 ms in the discrete tone task. In a neurophysiological study in cat auditory cortex, Ulanovsky *et al.* found multiple concurrent timescales of adaptation for tracking auditory stimulus history, the two shortest having time constants of ~6.6 ms and ~150 ms (2004). Perhaps FDLs from the tone-step task and the discrete tone task are statistically unrelated because the auditory system must store frequency information in different-timescale memory stores which interact nonlinearly with processes of frequency discrimination, a possibility which could be investigated in future studies by testing short ITIs between 0 and 100 ms.

3.4.2. Comparison to Previous Studies

Regardless of the uncertain relationship between the tone-step and discrete-tone tasks, FDLs from the more traditional discrete tone task can be readily compared to existing literature on frequency discrimination in the rat. A graphical comparison of the discrete tone results of this study versus FDLs (in Hz) measured in other studies on the rat are presented in Figure 3.9. Overall the mean FDLs from our data appear to be considerably smaller than those measured by Kelly (1970), Syka *et al.* (1996), or Talwar and Gerstein (1999), but are comparable to Talwar and Gerstein's 1998 results. Talwar and Gerstein's 1998 study used a ‘repeating standard’ paradigm very similar to that used in this study, which they suggested results in lower measured FDLs due to a difference in task requirements. They argue that a ‘repeating standard’

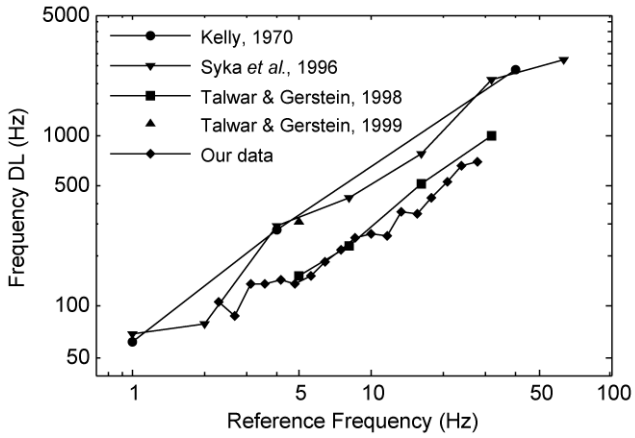

Figure 3.9. Comparison of measured FDLs for rats measured in other studies to the frequencies FDLs measured in this study.

paradigm presents a "detection" problem and two-tone stimulus, go/no-go trial paradigms, as used in other studies, present an "identification" problem, supported by evidence of different cortical processing pathways in frequency discrimination experiments in cats (Thompson, 1960; Cranford, 1978). Conceptually, however, the distinction between "detection" and "identification" seems marginal in go/no-go tasks that do not require directional classification of frequency differences. A more conspicuous difference between 'repeating standard' paradigms and two-tone go/no-go tasks is the long iso-frequency tone sequence prior to target presentation. In human subjects isofrequency tone sequences can induce a bias towards perceiving two auditory "streams" in subsequent alternating tone sequences, whereas without the inducing sequence subjects will perceive only a single stream (Rogers and Bregman, 1993; Beauvois and Meddis, 1997). Perhaps the FDLs measured with 'repeating standard' paradigms are smaller due to the priming of the auditory system with isofrequency sequences which have been shown to improve perception and segregation of frequency differences.

Effects of reference frequency and tone duration in our discrete tone results were consistent with previous studies in the rat. We used a larger set of reference frequencies, but tested within the same relative range, and our data show FDLs to be relatively flat for frequencies from 5 to 27.66 kHz. There was a small trend for FDLs to increase with decreasing frequency below 5 kHz, but this might be an artifact stemming from the use of a constant SPL intensity over a frequency range for which rat audibility thresholds increase by perhaps as much as 10 dB with decreasing frequency (Jamison, 1951; Gourevitch & Hack, 1966; Kelly & Masterton, 1977; Borg, 1982). In studies that explicitly adjusted intensity relative to rats' hearing thresholds there was no decreasing trend for FDLs with increasing reference frequency in the same range (Kelly, 1970; Syka *et al.*, 1996). However, increasing intensity from 30 to 50 dB above hearing threshold has also been shown to have little effect on rat FDLs (Syka *et al.*, 1996). Adjusting for the albino rat audiogram, the 60 dB tones used in this study were approximately between 40 and 60 dB above hearing threshold (Kelly & Masterton, 1977; Borg, 1982). In the second phase of discrete tone testing in which tone duration was varied, FDLs increased with decreasing tone duration, with an apparent inflection point at 50 ms equivalent to previous reports in the rat (Talwar and Gerstein, 1998) and relatively similar to FDL-duration functions in humans (Moore, 1973; Hall and Wood, 1984; Freyman and Nelson, 1986). The common effect of tone duration on frequency discrimination between rats and humans suggests that despite considerable differences in

sensitivity (see Fay, 1974), mammals may share a common process underlying frequency discrimination.

Response times in hit trials in this study had a more linear relationship to hit rate than that reported by Talwar and Gerstein in the rat (1998). Although they did see significant differences in response latency between threshold-level hit trials and suprathreshold-level hit trials, plots of response latency against frequency difference did not appear as the inverse of hit-rate curves (1998). Strong negative correlations between response time and hit rate should be expected, however, under B.F. Skinner's law of latency: response latency is inversely proportional to the salience of the stimulus (1938). The strong correlations seen in this study relative to previous studies is most likely due to the higher temporal precision of monitoring nosepoke withdrawal responses, i.e. much less movement by the animal is required to signal a decision. Nosepoke holding is also advantageous in auditory paradigms such as this because the stereotyped head position can standardize subjects' orientation to the sound source.

Despite general agreement with previous studies on the rat, our data present two unique results that bear future study. First, FDLs differed significantly with frequency change direction, showing lower thresholds for downward ($-\Delta f$) changes. Syka *et al.* found no significant effect of frequency change direction in pigmented rats, but for a relatively small sample size of three rats (1996). The one other study in the rat with sample sizes comparable to our data (Talwar and Gerstein, 1998) only tested with upward ($+\Delta f$)

frequency differences. Results from some studies in other mammals show similar magnitude directional differences, but without consensus as to the preferred direction. Cats (Brown *et al.*, 2004), *Cercopithecus* monkeys, and one rhesus monkey (Sinnott *et al.*, 1987) show lower FDLs for downward shifts similar to this study, but Japanese macaques and humans show lower FDLs for upward shifts (Sinnott *et al.*, 1987). Our analysis found significant interation between direction and reference frequency and between direction and subject, so procedural differences or within-species subject selection could perhaps account for conflicting results. Inclusion of both upward and downward frequency changes and multiple reference frequencies in the stimulus sets of future frequency discrimination studies could help clarify directional effects. Second, subjects appeared to dynamically adjust their decision strategies within trials to the increasing probability of a target presentation, evidence by significantly increasing mean false alarm rate. Rats have been shown to significantly adjust their decision strategies trial-to-trial in a two-tone go/no-go task (Talwar and Gerstein, 1999), but in the only previous 'repeating standard' frequency discrimination study in rats subjects did not appear to adjust decision strategies within trials based on time held (Talwar and Gerstein, 1998). However, Talwar and Gerstein's (1998) hold times were considerably longer (5-35 s) than those used in this study (2-7 s), so the probability of a target presentation increased at a faster rate and subjects may have been better able to track time held over the shorter reference durations. Future 'repeating

standard' studies could likely avoid dynamic adjustments in decision strategies by using longer reference durations than those used here.

3.5 Conclusions

Pilot studies with both the tone-step and discrete tone task showed that nearly all subjects could learn the tone-step, but only half could learn the discrete tone task. Subjects could also be trained to criterion on the tone-step task much more quickly. If the head-to-head comparison of tasks performed in this study had shown that the tone-step task measured frequency discrimination commensurate to the more difficult discrete tone task, then use of the tone-step task in subsequent behaving-recording experiments would presumably require less subjects overall and less training time. Since the analysis shows that the tasks are not equivalent, although perhaps related, we use the discrete tone task in the behaving-recording paradigm presented in the following chapter.

Results from discrete tone task suggest that the rat is an appropriate model for studying the neurophysiology of frequency discrimination with results that may be applicable to humans. Despite an evolutionary divergence that occurred ~70 million years ago, humans and rats appear to share a similar mammalian auditory system (Masterton *et al.*, 1969; Malmierca, 2003). Although overall human frequency discrimination ability is at least an order of magnitude more sensitive than the rat and most other mammals (Fay, 1974), the frequency-dependence and duration-dependence of FDLs measured in the rat with this discrete tone paradigm are analogous to trends seen in human

data. Furthermore, A1 has been shown to be necessary for fine-grained frequency discrimination in rats (Talwar *et al.*, 2001) as well as humans (Mendez & Geehan, 1988; Tramo et al., 2002). Therefore, the neural response properties in behaving rats we examine in the following chapter are likely similar to those we might expect to see in human A1.

To summarize, the subjects used in subsequent behaving-recording experiments have frequency discrimination abilities similar to other reports in the rat and are similarly analogous in stimulus-parameter-dependence, albeit not sensitivity, to human abilities. The discrete tone behavioral paradigm detailed in this chapter is well suited testing the psychophysics of threshold-level frequency change detection with associated A1 neural recordings in the following chapter.

Chapter 4

4. A1 Responses during Frequency Discrimination

4.1. Introduction

The frequency-selective receptive fields (RFs) of neurons in the primary auditory cortex (A1) and their ordered, tonotopic organization is perhaps one of the best biological examples of a continuous filter bank. The reliable representation of frequency in A1 neuron receptive fields largely along a single rostral-caudal axis has made the auditory cortex a popular locus for investigations into the relationship between demonstrated behavioral sensory sensitivity and sensory representation in the cortex. Studies linking behavioral frequency discrimination ability and A1 neural responses tend to fall into two categories: those that examine long-term plasticity in A1 response properties due to longer-term frequency discrimination training and those that examine dynamic changes in response properties primarily during performance of discrimination tasks.

Studies that examine long-term plasticity in A1 have found that receptive fields adapt to specific frequency comparisons when those comparisons are made relevant to awake subjects. When tones of specific frequencies predict aversive stimuli, A1 RFs rapidly adapt to increase responses to predictive frequencies and to decreases responses to non-predictive frequencies (Bakin &

Weinberger, 1990; Edeline & Weinberger, 1993; Bakin *et al.*, 1996; Ohl & Scheich, 1996; Ohl & Scheich, 1997). Similar long-term, frequency-specific enhancements in contrast are seen in appetitive frequency discrimination paradigms when subjects make fine-grained discriminations against a single reference frequency (Blake *et al.*, 2002; Witte & Kipke, 2005), which are associated with an enlarged cortical representation of the reference frequency in A1 compared to controls (Recanzone *et al.*, 1993; Polley *et al.*, 2006). While these studies do provide good evidence that changes in frequency discrimination acuity are paralleled by changes in A1 receptive fields, the importance of such changes can only be inferred when response properties are not tested during behavior itself. However, neural responses recorded from A1 of ferrets performing frequency discrimination show enhanced contrast adaptation similar to that seen in post-hoc testing in other studies, generated in the first few minutes of behavior, and the magnitude of the response change are correlated with associated behavioral performance (Fritz *et al.*, 2005, 2007).

Examination of shorter-term, during-behavior dynamic plasticity complements studies of longer-term plasticity by isolating effects of behavioral relevance and attentional state. Attention to stimuli has been shown to generally increase A1 responses to relevant stimuli in auditory tasks other than frequency discrimination (Hocherman *et al.*, 1981; Pfingst *et al.*, 1977; Benson and Hienz, 1978). Responses have also been shown to be modulated by attention and behavioral relevance during frequency discrimination (Gottlieb *et al.*, 1989; Durif *et al.*, 2003; Fritz *et al.*, 2007). However, it is difficult to

associate the attentional modulation of A1 responses seen in these frequency discrimination studies with attentional frequency acuity because discrimination was only tested with large frequency differences (> 0.25 octaves) well above discrimination thresholds. This study proposes to study A1 neural responses while subjects perform *fine-grained* frequency discrimination to A) examine how attention may modulate difference signals for psychophysical threshold-level frequency changes and B) to investigate possible neural correlates of behavioral frequency discrimination acuity.

4.2. Methods

4.2.1. Subjects and Surgery

The subjects in this study were 9 of the 13 female Sprague-Dawley rats that had successfully learned the discrete tone task described in the previous chapter. After the completion of all pre-implantation psychophysical testing, subjects were given free access to food for at least one week. Subjects were typically 6-9 months old and weighed 250-350 grams at the time of surgery. Subjects were implanted with microelectrode arrays into auditory cortex following the same surgical procedures detailed in Section 2.2.1. Following surgery, subjects were given a week to recover with free access to food before being returned to food deprivation, with free access to water, 24 hours prior to the first paired psychophysical testing and behavioral recording sessions. Subjects were then maintained at or above 85% of post-surgical weight. On

days when subjects were neither tested nor trained, they were given supplemental food. 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.

4.2.2. Apparatus

Paired psychophysical testing and neural recording sessions took place in the same psychophysical testing cages described in Section 3.2.2. Sessions were controlled by the same custom MatLab software on a PC computer interfacing with the external real-time digital analog processor (RP2.1; Tucker-Davis Technologies; Alachua, FL), and the program also controlled recording and storing of neural data. Pure tone stimuli were generated, calibrated, and played out by the same procedures described in Sections 3.2.2 and 3.2.3.

4.2.3. Behavioral Recordings

Neural signals were recorded from auditory cortex while subjects performed the discrete tone task described in Section 3.2.5. In the first sessions after surgery, subjects were re-tested with ±10-20% Δfs to determine if subjects still performed above criterion, a *d'* index of >1.96, following surgery. Successful resumption of performance better than criterion was presumed to be evidence that the small-scale cortical trauma inherent in electrode implantation surgery had not affected general task ability. After demonstrating criterion

performance, subjects were tested with threshold-level Δfs of ±1-15% with 0% change catch trials. Hold times were again randomly set between 2-7 seconds and were not reset by aborts.

Completed trials were again defined as those ending in a hit, miss, false alarm, or correct rejection, and subjects would typically complete ~300-500 trials per session. This relatively small number of trials, combined with the requirement to obtain adequate repetitions of a stimulus for statistical analysis of neural recordings, limited the size of the stimulus set that could be presented in a session. Therefore, two types of stimulus set were used to examine different effects. To examine the effects of attention and repetition during behavior on neural receptive fields, the first type of stimulus set contained all 18 standard reference frequencies with Δfs of ±1-15%. This yielded ~16 repetitions per reference frequency, but at most 1-2 repetitions of each of the 286 possible target frequencies. The second type of stimulus set contained only a single reference frequency with Δfs of ±1-15%, resulting in ~10-15 repetitions for each target frequency. This allowed for examination of neural encoding of specific frequency changes, but it should be noted that the use of a single reference frequency might induce within-session adaptation of responses, which must be investigated in later analysis.

4.2.4. Passive-Listening Recordings

Immediately prior to or following a behavioral session, subjects were tested with related pure tone stimulus sets during passive listening. The

passive-listening test cage was identical to behavioral cages, but with the nosepoke and feeder trays removed. Unlike the recording procedure described in Section 2.2.4, subjects were not restrained during recordings and were allowed to freely move around the cage, although subjects would typically sit quietly throughout testing after an initial exploration. A freely-moving paradigm was used in these passive-listening recordings to prevent possible influences of learned helplessness on emotional state or subsequent task motivation (for review see Seligman, 1972). Therefore it's important to note in comparisons between passive-listening and behavioral neural response properties that head orientation relative to the sound source is not controlled.

Two types of stimulus sets were used in passive-listening tests. Iso-intensity tuning curves (IsoTCs) were first used to examine neuronal frequency tuning. Frequency endpoints and spacings were varied dependent on the particular frequency range being examined, but in general endpoints were within the range of 2 to 32 kHz and frequency spacing was between 0.014 and 0.211 octaves. Tuning curves typically consisted of 200 ms duration tones, with 5 ms cosine rise/fall ramps, played at 60 dB to match behavioral stimulus intensity and repeated 50 times. Inter-tone interval (ITI) was uniformly randomized between 300 ms and 700 ms. The second type of stimulus used to examine passive-listening neural responses were comparison stimuli constructed to mimic behavioral stimuli. These were constructed similarly to behavioral stimuli, with 200 ms reference and target tones separated by 100 ms ITIs, but with a fixed reference duration of 1.8 seconds, such that each stimulus

contained 6 reference tones followed by two target tones. Passive-listening comparison stimuli (PLCs) tested either all 18 standard reference frequencies or just a single reference frequency, matching the corresponding behavioral stimulus set. When PLCs contained all 18 reference frequencies, only large Δfs of ±15% were tested. When PLC contained only a single reference frequency, all target frequency were tested for Δf steps from -15% to 15.

4.2.5. Electrophysiology

The microwire electrode arrays used in these experiments differed from those used in the experiments described in Chapter 1 only in the diameter of the tungsten microwires. The diameter was reduced from 50 to 35 µm, which substantially improved the signal-to-noise ratio of recorded action potentials by increasing average electrode impedance from ~60 kHz to ~1 MHz. During both behavioral and passive-listening recordings, a custom-built headstage amplifier (Tucker-Davis Technologies; Alachua, FL) was connected directly to the subject's implant. A cable ran from the headstage amplifier to a commutator in the center of the top of the cage, which was then connected to a preamplifier/digital-to-analog converter (Tucker-Davis Technologies). Neural signals were sampled continuously at 25 kHz and stored in buffers in the data acquisition hardware. When subjects completed a trial with a hit, miss, false alarm, or correct rejection, a snippet of the unfiltered signal was downloaded and saved to the local computer for offline processing and analysis. For each completed trial, the saved snippet length included 200 ms before and 2.2

seconds after the required nosepoke hold time. Recording snippets were not saved for aborted trials.

Raw neural signals were saved without filtering so that both local field potentials and action potentials could be extracted during offline processing. All offline processing was carried out using custom MatLab software. To extract local field potentials (LFPs), signals were digitally bandpass-filtered from 1 to 300 Hz and resaved at one-millisecond resolution. To extract action potentials, signals were first bandpass-filtered from 825 to 4000 Hz. Thresholds for spike crossings were automatically set for behavioral recordings at 3 times the root-mean-square (RMS) noise level of the signal. Thresholds from behavioral recordings were then applied to any passive-listening recordings taken during the same session, so that thresholding was consistent between any comparisons. Spikeshapes were saved for each threshold crossing as 64-sample snippets for spike-sorting. Spike-sorting was first performed with Quiroga *et al.*'s unsupervised superparamagnetic clustering program (2004). The results of the unsupervised clustering were manually verified and waveform templates were created for each identified cluster. A single set of waveform templates for each channel was applied to all behaving and passive-listening recordings within a session, such that spike-sorting was also consistent between any comparisons. Single units were resolvable in some recordings, evidenced by inter-spike intervals no less than a refractory period of 1-2 ms, but a majority of clusters were only resolvable to multi-unit clusters (MUCs).

4.2.6. Data Analysis

Behavioral recording sessions returned both psychophysical and neural data for analysis. Frequency discrimination limens (FDLs) were calculated for individual sessions according to false-alarm corrected hit rate as described in Section 3.2.6, but the false alarm rate used in calculations for this data was *estimated* false alarm rate, estimated from catch trial results, rather than *true* false alarm rate. Neural responses from trials ending in aborts were discarded to conserve memory, so psychophysical data from abort trials is also discarded to maintain accurate comparisons between psychophysical and neural results, preventing the calculation of *true* false alarm rate. Despite significant difference seen between upward ($+\Delta f$) and downward ($-\Delta f$) frequency changes in the psychophysical results in Chapter 3, the absolute value of frequency change direction is used in the majority of the following analysis to achieve adequate sample sizes for statistical analysis. The effect of frequency change direction was small, with upward FDLs 0.55% larger than downward FDLS on average, an effect size expected to have negligible impact on comparisons of psychophysical and neural data.

Neural data analysis began with an examination of LFPs to determine appropriate time windows for analysis of MUC data. The typical shape of evoked potentials recorded from A1 in response to simple stimuli is a small positivity (P1) followed by a large negativity (N1), which in turn is followed by smaller, alternating waves (P2, N2, etc.) that gradually diminish in amplitude (for review see Shaw, 1988). The P1 components in this data were generally

too small to analyze robustly, but for channels with LFP activity, the N1 component was consistently large and frequency-tuned. A nonparametric Wilcoxon's Matched-Pairs Signed-Rank (MPSR) test was used to determine if the N1 response for each electrode site was significantly driven LFP activity by comparing the magnitude of the N1 response, measured from 8 to 18 ms post-stimulus-onset, to a baseline measured over 50 ms prior to stimulus presentation. A Kruskal-Wallis one-way ANOVA, with groups of tone frequency, was used to identify significant frequency selectivity in the N1 response over the same time window.

The analysis of action potentials was carried out using methods similar to those described in Section 2.2.5, with two exceptions. First, spontaneous spikerate was calculated over the 50 ms prior to tone onset in this experiment, rather than 35 ms prior as in Chapter 1. Second, a less conservative α of 0.05 was used for statistical comparisons. Statistical results for individual units/MUCs were again obtained with nonparametric measures. Wilcoxon's Matched-Pairs Signed-Rank (MPSR) test was used for comparisons between particular frequency responses within set time windows, such as comparisons of driven rate with spontaneous rate. Kruskal-Wallis one-way ANOVAs, with groups of frequency, were used within fixed time windows to test for frequency-selectivity. Standard parametric statistics (1- and 2-way ANOVA, t-test; $\alpha = 0.05$) were used to analyze population effects. Normalization methods were similar to those described in Section 2.2.5, but the "normal" response definition was dependent on the particular comparison, which is detailed in the results.

4.3. Results

4.3.1. Post-Implantation Task Performance

Subjects typically demonstrated criterion-level performance on the frequency discrimination task, indicated by a *d'* index of >1.96 for Δfs of ±10-20%, within 1-2 sessions following implantation surgery. In subsequent behavior-recording testing sessions, subjects' overall psychophysical frequency difference limens (FDLs), calculated regardless of reference frequency or frequency change direction, were actually 0.085% less on average than their pre-surgical FDLs, a difference that was not significant (paired t-test, $p = 0.428$). Post-surgical performance curves for all 9 subjects, calculated without respect to reference frequency or frequency change direction, are shown in Figure 4.1. Implantation of microelectrode arrays into auditory cortex did not appear to result in any frequency discrimination deficits.

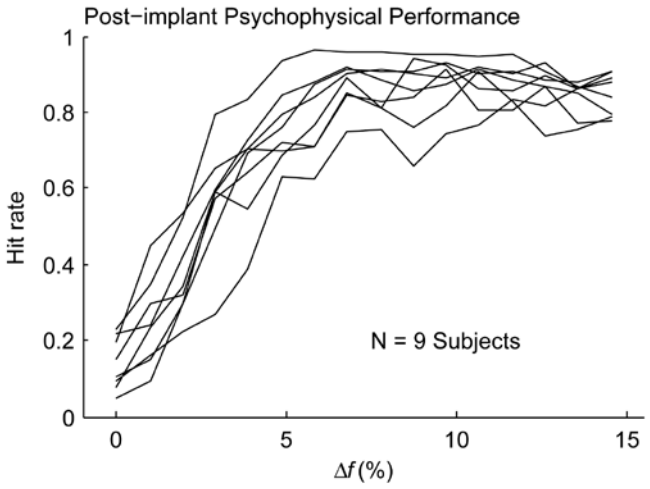

Figure 4.1. Post-implantation psychometric functions of hit rate as a function of Δf (expressed as Weber fractions) for all subjects (N = 9), with hit rate for each Δf combined across all reference frequencies without respect to frequency change direction.

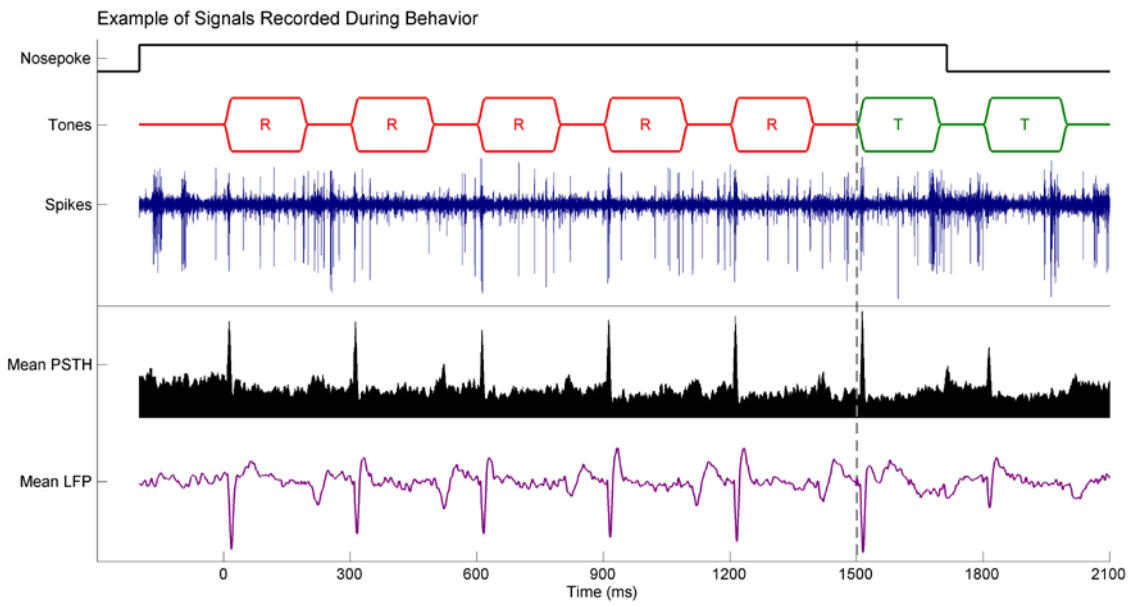

Figure 4.2. An example of signals recorded during frequency discrimination behavior from a single channel in subject RN19. Nosepoke engagement and tone timing are shown in the "Nosepoke" and "Tones" traces, respectively. Single unit activity recording during this trial is shown in the "Spikes" trace. The top-most of the two lower traces ("Mean PSTH") shows the mean peri-stimulus time histogram (PSTH) for action potential threshold crossings in all trials in this session for this channel. The bottom-most trace ("Mean LFP") shows the mean local field potentials from all trials in this session for the same channel.

4.3.2. Signal Quality

Neural recordings were recorded from electrodes implanted into primary auditory cortex (A1) during all behavioral sessions following implantation. An example of spike and LFP signals obtained from a single electrode site during behavior is shown in Figure 4.2. The stereotyped head position and stationary nosepoke-holding posture necessary for subjects to successfully perform the task resulted in relatively noiseless recordings during stimulus presentation. Action potentials frequently had high signal-to-noise ratios similar to that seen in the example, with short-latency tone-driven activity consistent with typical A1 response properties. Tone-driven LFPs were easily identifiable by a prominent

large negative trough followed a large positive peak, the N1 and P2 components, respectively. Smaller peaks and troughs that have been generally noted in previous reports (Shaw, 1988), such as P1 or N2, were less consistently seen.

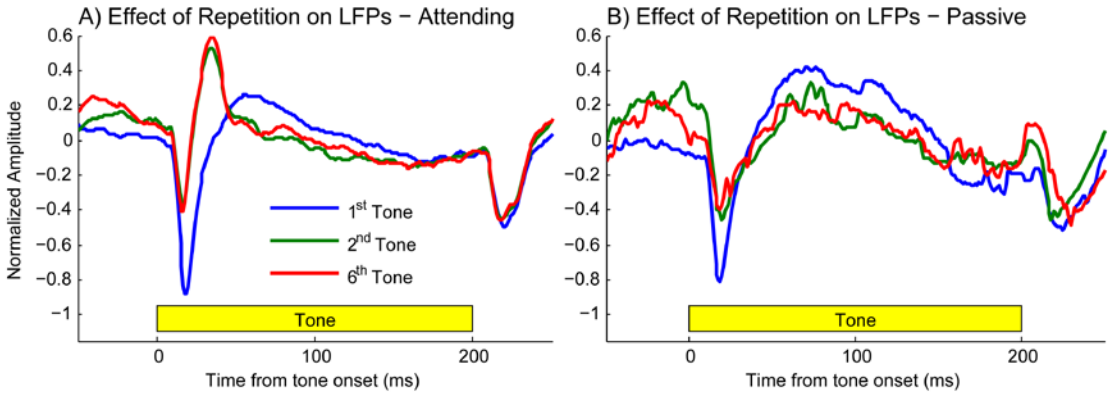

Figure 4.3. Mean LFP responses, normalized by N1 absolute amplitude, to the 1st (blue), 2nd (green), and 6th (red) repetition of a reference tone during **A)** attentional frequency discrimination behavior and **B)** passive-listening.

4.3.3. Local Field Potentials

Significantly tone-driven LFPs, as measured during the first reference tone presentation of all behavioral stimuli in a session, were seen at 94 different electrode sites in 9 subjects (MPSR, $\alpha = 0.05$). Generally, LFP responses to the first reference tone consisted of a large amplitude N1 component at the onset of the tone, lasting ~25 ms, which was followed by a long P2 component lasting from ~25 to ~125 ms after tone onset (blue line, Fig. 4.1A). However, the shape of the LFP response changed dramatically for repeated reference tones when subjects were performing the task. Beginning with the second reference tone, the N1 component decreased in amplitude by ~50%, and the following P2 component was both larger in amplitude and shortened in duration,

lasting from ~20 ms to ~50 ms after tone onset (green line, Fig. 4.1A). LFP responses to further reference tones during behavior, up to the presentation of the target, had the approximate shape of the responses to the second tone.

Passive-listening tests with comparison stimuli similar to behavioral reference-target tone sequences showed that changes in the amplitude and duration of the P2 component during the reference tones were not simply an effect of tone repetition. LFPs to the first reference tone of a stimulus during passive-listening had N1 and P2 components similar in amplitude and duration to those seen during the first reference tone during behavior (blue line, Fig. 4.3B). During the second reference tone during passive listening, however, the N1 component showed a ~50% decrease in amplitude again similar to that seen during behavior, but there was no corresponding modification of the P2 component. Rather, the P2 amplitude decreased while maintaining the same ~25 to ~125 ms post-tone-onset duration (green line, Fig. 4.2B). LFP responses to the following reference tones were similarly decreased in N1 and P2 amplitude. Decreases in both N1 and P2 amplitude during passive listening to the repeating isofrequency reference tones appear consistent with the decreasing contrast in neural responses during habituation seen in Chapter 2, whereas the increase in P2 amplitude during attentional behavior represents increased contrast.

During the behavioral task it was again the N1 and P2 components that showed the most conspicuous changes during the presentation of the target tone, difference in frequency from the reference tone by a frequency difference

Δf. For small, sub-threshold Δfs of ±0-2%, the N1 amplitude was generally decreased slightly during the first target tone relative to the response to the preceding reference (Fig. 4.4B), while P2 amplitude was relatively stable. For threshold-level (±3-4%) and suprathreshold Δfs (±5-10%), however, the N1 amplitude increased and the P2 amplitude decreased and lengthened in duration, such that for large Δfs LFP responses to the target tone most resembled responses to the first reference tone of the stimulus. The colormap in Figure 4.4A shows how LFP shape changed as a function of $|\Delta f|$ (absolute frequency change) from the final reference to the first target tone. Population mean amplitudes of the N1 and P2 components both show changes dependent on $|\Delta f|$ that parallel psychophysical performance, with the largest changes in responses over the threshold range of 0-5%. Response amplitude within the time window of ~50 to ~100 ms, perhaps containing the N2 and/or P3 components, also showed changes with $|\Delta f|$, but this may be more due to the lengthening of the P2 component into that time window.

Qualitative examination of LFPs served mainly to identify time windows of interest for analysis of spiking activity. Based on these results, onset activity over the first ~25 ms of the responses should be analyzed separately from post-onset activity from ~25 to ~50 ms post-tone-onset, since these two time windows are apparently differentially affected by attention and may show different dynamics during frequency changes.

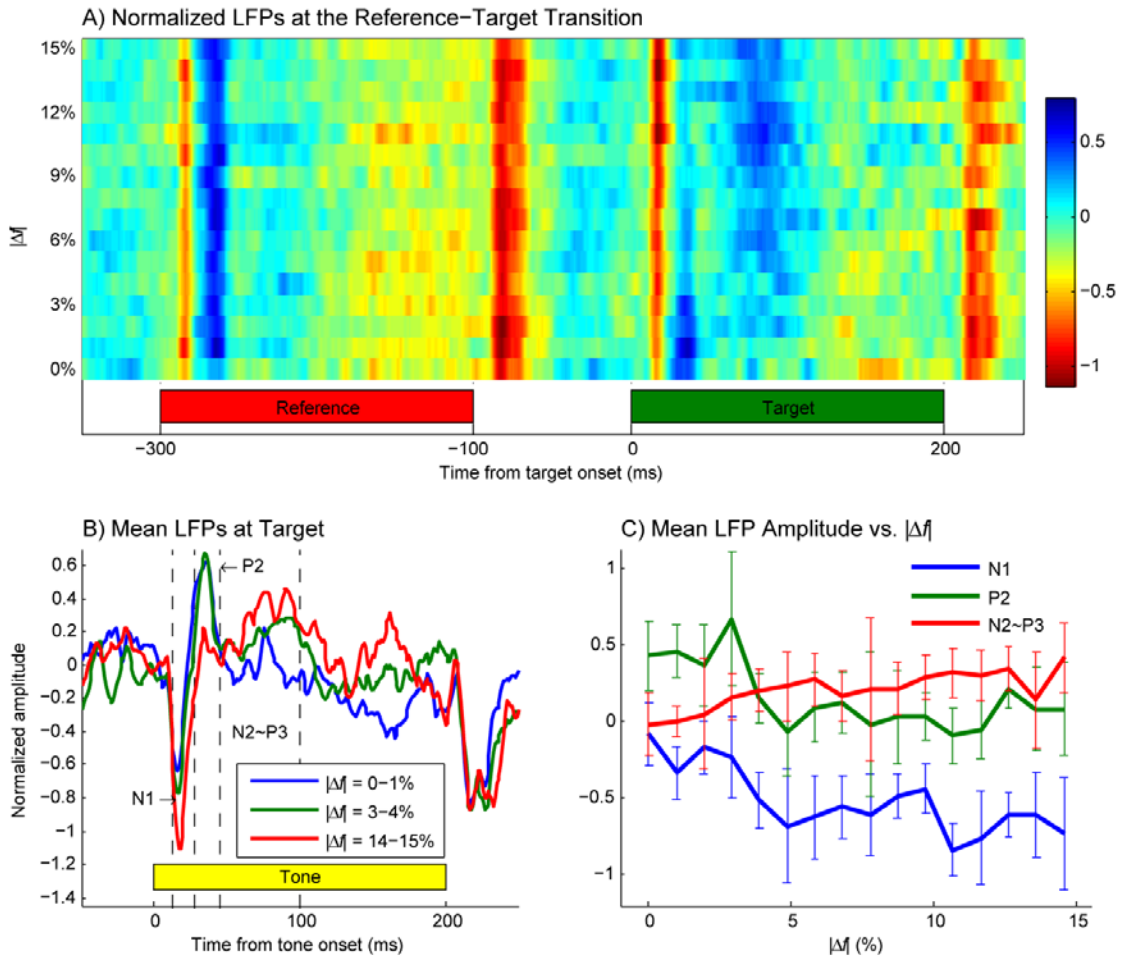

Figure 4.4. **A)** 10% trimmed mean normalized LFPs are shown as a colormap for the reference and target tones on each side of the reference-target transition for absolute frequency changes from 0 to 15%. Note the enhanced post-onset positivity that develops from ~30-100 ms after target tone onset for behaviorally suprathreshold (> 3-4%) frequency changes. **B)** Untrimmed mean normalized LFPs are shown for target tones with subthreshold ($|\Delta f|$ = 0-1%, blue), threshold-level ($|\Delta f|$ = 3-4%, green), and suprathreshold ($|\Delta f|$ = 14-15%, red) frequency changes. **C)** Mean LFP amplitude is averaged within the N1, P2, N2~P3 windows indicated in B) and shown as a function of $|\Delta f|$. Error bars show 95% confidence intervals.

4.3.4. Attentional Modulation of Spikerate Habituation

Significantly tone-driven, frequency-selective spiking activity, as measured during the first reference tone presentation of all behavioral stimuli in a session, was seen in 112 multi-unit clusters (MUCs) in A1 from 9 subjects (Puri-Sen 2-way ANOVA, $\alpha_{interaction} = 0.05$). The presence of significantly tone-

driven phasic or tonic responses was identified by comparing spikerate within windows from 8 to 22 ms and from 53 to 202 ms, respectively, against spontaneous spikerate measured in the 50 ms prior to tone onset (MPSR, α = 0.01). Of the 112 MUCs, 1 showed only a phasic response, 7 showed only tonic responses, and 104 had both phasic and tonic responses. Similar to neuronal firing patterns seen in Chapter 2, most MUCs displayed brief phasic excitation (~10-30 ms post-tone-onset), which was most often followed by tonic suppression throughout the duration of the tone, and further followed in some cases by offset excitation. The population mean peri-stimulus time histogram (PSTH) for the first reference tone of behavioral stimuli and passive listening comparison stimuli are shown as the blue lines in Figs. 4.5A and 4.5B, respectively.

The spikerate analysis windows suggested by LFP results were confirmed with examination of the effects of reference tone repetition on the population mean PSTH. Similar to changes seen in the LFPs, the mean PSTHs showed large changes between the first and second reference tone during behavior (green line, Fig. 4.5A). Phasic excitation decreased in a manner consistent with habituation results from Chapter 2, but both unlike habituation effect the spontaneous activity also decreased. There was also a notable change in the shape of the PSTHs in the post-phasic period separating the phasic and tonic responses. Post-suppression appeared to be enhanced in the response to the second reference tone relative to the response to the first. Further repetitions of the reference tone appeared to further decrease

spontaneous activity and to further enhance post-phasic suppression. The population mean PSTHs also showed decreases in tonic spikerate during the duration of the tone, but tonic were approximately equivalent to spontaneous decreases and did not appear to represent a change in the driven activity.

The population mean PSTHs for passive listening tests with reference-target comparison stimuli also showed decreases in phasic excitation during reference tone repetition consistent with habituation. However, during passive listening, spontaneous activity did not decrease during repetition and post-phasic suppression was not enhanced (Fig. 4.5B). Overall, the population mean PSTHs suggested that attention to the frequency discrimination task modulated both spontaneous and post-phasic activity.

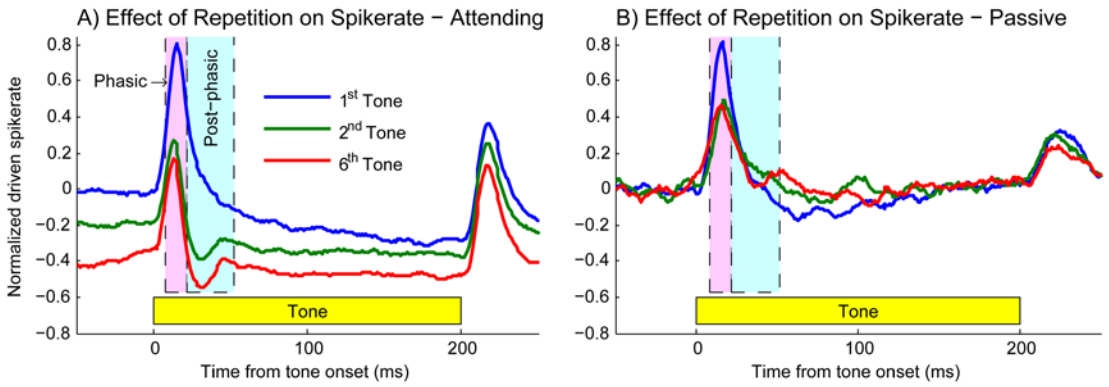

Figure 4.5. Mean PSTHs, collected in 10 ms moving-window bins and normalized by peak spikerate to the first reference tone, for the 1st (blue), 2nd (green), and 6th (red) repetition of a reference tone during **A)** attentional frequency discrimination behavior and **B)** passive-listening.

To quantify changes, phasic spikerate was averaged within an analysis window from 8 to 22 ms post-tone-onset and post-phasic spikerate within a window from 22 to 52 ms, shown as the purple and light blue boxes, respectively, in Figure 4.5. Note that the phasic analysis window used in this

analysis differs from the 8 to 22 ms window used in Chapter 2, to avoid overlap with post-phasic effects not seen in the habituation analysis. Spontaneous spike rate was average over the 50 ms preceding each tone.

Normalized mean changes in spontaneous, phasic, and post-phasic spikerates are shown as a function of reference tone number for the first six reference tones in Figure 4.6 for both the behaving and passive listening conditions. Significant population decreases are seen in the spontaneous (ANOVA; $F_{5,1745} = 48.26$, $p < 0.001$), phasic ($F_{5,1745} = 151.46$, $p < 0.001$), and post-phasic ($F_{5,1745} = 70.66$, $p < 0.001$) responses during behavior (Fig. 4.6A). Phasic and post-phasic responses decrease dramatically from the first to the second reference tone, by ~25%, after which the phasic response decreases only slightly while the post-phasic response continuous to gradual decrease with each successive tone. The rate of decrease in spontaneous activity is more consistent, decreasing by approximately equal steps with each successive repetition. By contrast, during passive listening only the phasic response shows a significant decrease ($F_{5,269} = 9.18$, $p < 0.001$), consistent with habitation. Mean spontaneous ($F_{5,269} = 0.19$, $p = 0.96$) and post-phasic spikerate ($F_{5,269} = 0.53$, $p = 0.75$) are unchanged during passively listening (Fig. 4.6B). Attention to the behavioral task appeared to decrease general, non-tone-driven activity, evidenced by the decreasing spontaneous rate, and to specifically decrease post-phasic spikerate, enhancing post-phasic suppression, but appeared to have little effect on phasic responses.

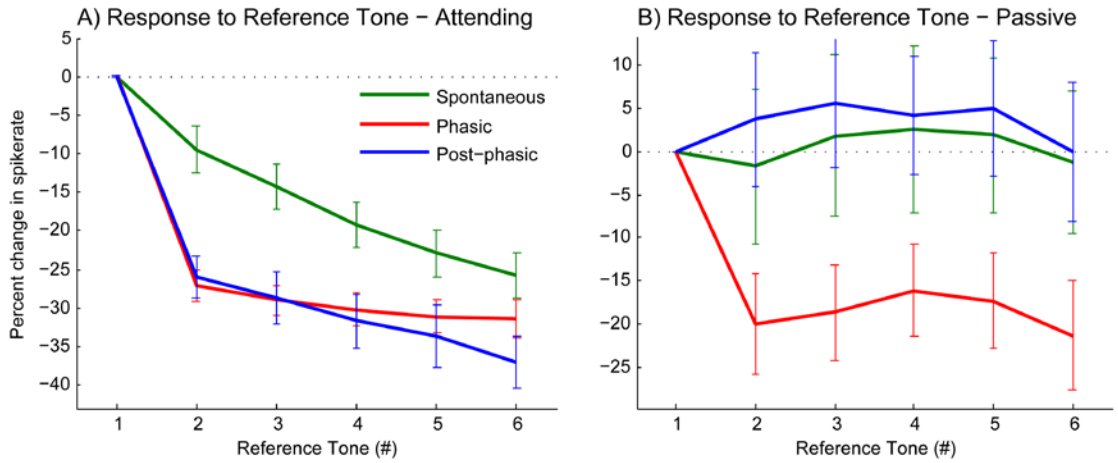

Figure 4.6. Mean population changes in absolute spikerate (not subtracting spontaneous activity) measured for spontaneous (green, -50-0 ms), phasic (red, 8-22 ms), and post-phasic (blue, 22-52 ms) analysis windows are plotted for the first six reference tones, normalized by the spikerate to the first tone, for responses recorded during **A)** attentional frequency discrimination behavior and **B)** passive-listening. Error bars show 95% confidence intervals.

4.3.5. Responses to Frequency Changes

Similar to the LFP results patterns of spikerate were most affected during target presentation within the same analysis windows that showed the most attentional effect during reference stimuli. A representative example of one isolated unit's response to catch trials (Δf = 0%) and to targets with large, psychophysically well-detected Δfs is shown in Figure 4.7. For this unit, responses to catch trials were similar to responses to preceding 3.59 kHz reference tones, as would be expected, with a small phasic response followed by post-phasic suppression. For target tones with large Δfs this unit showed strong excitatory responses during both the phasic and post-phasic periods, but did not show tonic firing through the entire duration of the tone. When spikerate is averaged over the entire phasic/post-phasic period for each Δf, the plot of

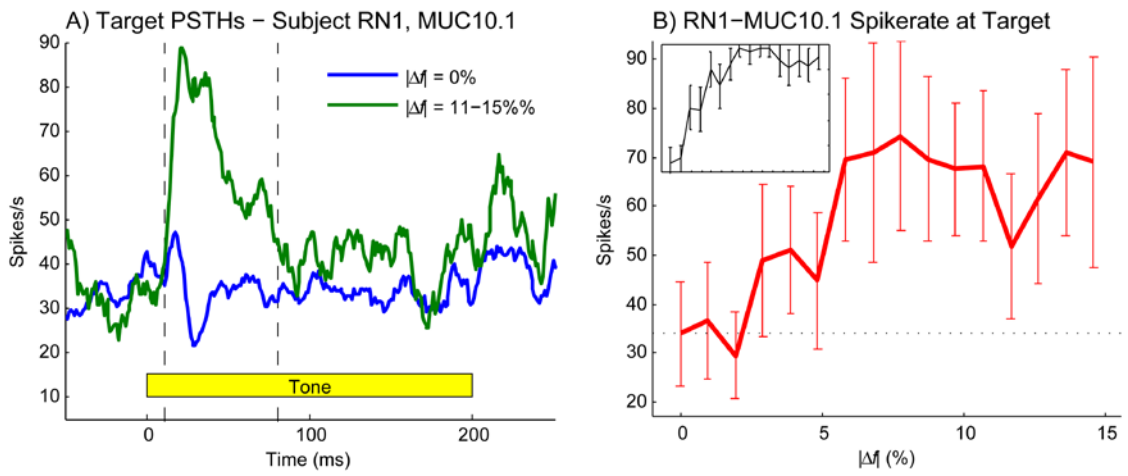

Figure 4.7. An example of spikerate dependent on the absolute frequency change ($|\Delta f|$) between the final reference and first target tone for one isolated unit. **A)** The mean PSTH, collected in 10 ms moving-window bins, is shown for catch trials (Δf = 0) and for large ($|\Delta f|$ = 11-15%) frequency changes. **B)** Spikerate is averaged within the time bracketed by the dashed lines in A), containing the phasic and post-phasic response periods, at each $|\Delta f|$ step. Note the correspondence between spikerate and the psychophysical hit-rate curve (inset). Error bars show 95% confidence intervals.

spikerate for this unit looks strikingly similar to the subject's psychophysical performance during the same session (Fig. 4.7B).

Population mean PSTHs showed changes at the target similar to the example in Figure 4.7. For catch trial target tones PSTHs appear similar to responses to preceding reference tones, with small phasic responses followed by post-phasic suppression (Fig. 4.8A). As $|\Delta f|$ increases from 0% to 15%, both phasic and post-phasic spikerate increase, but the difference between the PSTHs for the final reference and the first target tone is largest for the post-phasic response. The increase in the difference signal between the mean, normalized PSTHs for the first target and preceding reference tone is illustrated well in the difference colormap in Figure 4.8B.

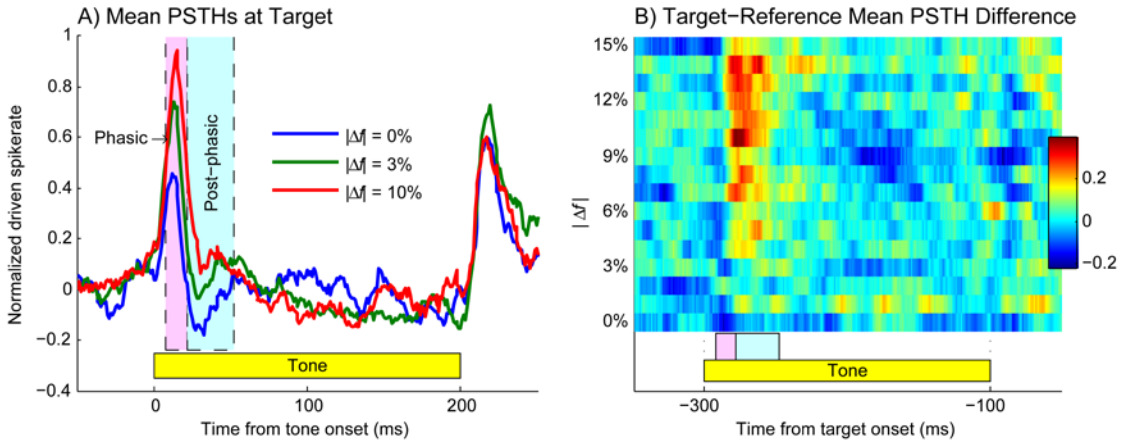

Figure 4.8. A) Mean PSTHs, collected in 10 ms moving-window bins and normalized by peak spikerate to the final reference tone, are shown for catch trial target tones ($|\Delta f|$ = 0%, blue) and for target tones with behaviorally threshold-level ($|\Delta f|$ = 3%, green) and suprathreshold ($|\Delta f|$ = 10%, red) frequency changes. Phasic (8-22 ms) and post-phasic (22-52 ms) analysis windows are indicated by purple and light blue rectangles, respectively. **B)** 10% trimmed-mean differences in PSTHs between the final reference and the first target tone are shown as a colormap for $|\Delta f|$ from 0 to 15%. Note the increase in post-phasic spikerate with increasing $|\Delta f|$.

Normalized mean changes in phasic and post-phasic spikerates during behavior are shown as a function of the $|\Delta f|$ between the reference and target tone in Figure 4.9A. As the $|\Delta f|$ of the target increases, both the phasic (ANOVA; $F_{15, 5186} = 2.62$, $p < 0.001$) and post-phasic ($F_{15,5185} = 2.29$, $p = 0.003$) response increase. The increasing mean change in the phasic responses from the final reference to the first target tone does not appear to represent an increase in excitation, but rather a lack of further habituation. Phasic responses to catch trials (Δf = 0%) are actually significantly decreased and for most larger $|\Delta f|$s phasic response change is not significantly different from zero (t-test, α = 0.05). Post-phasic responses, on the other hand, do show significantly more excitation during the target tone than during the reference tone with increasing $|\Delta f|$. Overall, mean changes in both phasic and post-phasic responses

mirrored overall psychophysical performance for $|\Delta f|$s, and subjects' hit rates, calculated without regard to reference frequency, were significantly correlated with change in the phasic ($\rho = 0.13$, $p < 0.001$) and post-phasic ($\rho = 0.11$, $p = 0.002$) response.

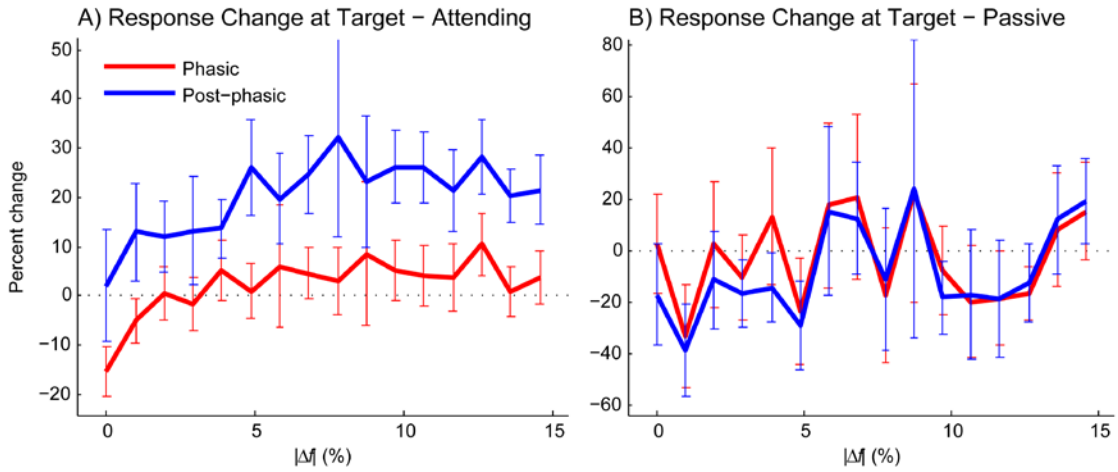

Figure 4.9. Mean population changes in absolute spikerate (not subtracting spontaneous activity), normalized by the spikerate to the final reference tone, measured for phasic (red, 8-22 ms) and post-phasic (blue, 22-52 ms) analysis windows during the target tone are shown as a function of $|\Delta f|$ for responses recorded during A) attentional frequency discrimination behavior and B) passive-listening. Error bars show 95% confidence intervals.

When subjects passively listened to similar reference-target stimuli in the same session, there was no such correspondence between psychophysical performance and responses to frequency changes in comparison stimuli (Fig. 4.9B). Note that phasic and post-phasic responses vary together with $|\Delta f|$ during passive listening because phasic excitation stretches into the post-phasic period due to the lack of substantial post-phasic suppression. Subjects' psychophysical hit rates for $|\Delta f|$s, calculated without regard to reference frequency, in associated sessions immediately preceding or following passive

listening testing were not correlated for either phasic (R = 0.15, p = 0.217) of post-phasic (R = 0.29, p = 0.097) responses. Therefore, the changes in A1 neurons' phasic and post-phasic responses correlated with fine-grained psychophysical performance likely arise from attentional modulation.

4.4. Discussion

This study examined neural responses in A1 to behavioral stimuli while subjects performed a repeating standard frequency discrimination paradigm. There were five main results. 1) When subjects attended to reference tones during frequency discrimination behavior prior to target presentation, spontaneous spiking activity decreased with reference repetition, likely reflecting global suppression of "noisy" activity in auditory cortex. 2) Attention to the behavioral task also enhanced post-phasic suppression in responses to reference tones. 3) Phasic responses decreased with reference repetition similarly in the attending and passive listening conditions. 4) Upon presentation of the target, phasic and post-phasic responses showed increases significantly correlated with psychophysical detection of the frequency change between the reference and the target. 5) Phasic and post-phasic responses to the same reference-target frequency changes during passive listening were not correlated with associated psychophysical performance in the same session.

The repeating standard reference sequence used in this frequency discrimination task might be expected to activate preattentive processes of deviant frequency detection. Even in anesthetized animals, A1 neurons will

show decreases to repetition of isofrequency tones and will show increased post-phasic responses to tones with differing frequency (Ulanovsky *et al.*, 2003, 2004), but only when frequency differences are sufficiently large (> 10%). This pre-attentive difference signal has been shown to arise from intracortical processing (Ulanovsky *et al.*, 2003). This mechanism of frequency change detection is strikingly similar to the results presented here in both the post-phasic time-course and the associated repetition suppression, except that significant difference signals are seen for psychophysical threshold-level frequency changes (~3%). Although care should be taken not to equate the anesthetized state in Ulanovsky *et al*.'s study with awake, passive listening, the comparison would seem to suggest that improvement in A1 neuronal acuity for frequency change seen here are attributable to effects of attention.

It might be somewhat surprising that the primary effect of attention shown in these results was an enhanced suppression of A1 responses when previous studies have shown attention-induced increases to behaviorally relevant sounds (Hocherman *et al.*, 1981; Pfingst *et al.*, 1977; Benson and Hienz, 1978). The tasks used in these studies, however, generally required simple detection of tones, such that enhancement of responses to those tones would be behaviorally beneficial. Furthermore, enhancement does not appear to be the only available method of attentional modulation. Tasks that involve some degree of frequency discrimination, but without repeating reference tones, have been shown to induce attentional modulation that enhances the responses of some A1 responses while suppressing others (Gottlieb *et al.*, 1989; Durif *et*

al., 2003). In the repeating standard paradigm used in this study, the most beneficial neuronal adaptation would be a decrease in A1 responses to the reference with elevated responses to the target, which indeed is shown in these results from the rat and results from a similar behavior-recording paradigm in ferrets (Fritz *et al.*, 2005, 2007).

4.5 Conclusions

The general hypothesis that the experiments comprising this thesis were based upon was that tonic responses in A1 encoded frequency resolution equivalent to psychophysical thresholds. The inherent definition of tonic responses presumes a response continuing through the duration of the stimulus. The difference signal we find in this experiment does not constitute a tonic response by that definition. However, the primary difference signal does appear to arise from intracortical processing as hypothesized, from connections that suppress the phasic response and mediate tonic frequency selectivity. Therefore, it might be said that frequency changes are encoded in the post-phasic initiation of tonic responses.

The encoding of frequency differences in a transient, post-phasic difference signal, rather than tonic encoding throughout the duration of a stimulus, may better explain psychophysical effects of tone duration. Based on these results, we might say that fine-grained frequency discrimination is encoded in the activation of A1 neurons from ~20 to ~50 ms post-tone-onset. In both human and animal studies, FDLs decrease (improve) with increasing

tone duration up to 50 ms, but improve little more for increasing tone duration beyond 50 ms, shown in Chapter 3 and in other studies (Moore, 1973; Hall and Wood, 1984; Hartmann et al., 1985; Freyman and Nelson, 1986; Talwar and Gerstein, 1998). If the high-resolution frequency change difference signal generally terminates at ~50 ms post-tone-onset, then downstream decision centers would receive no extra information for longer tones. The fact that 50 ms appears to be a critical duration for most tested mammals suggests that underlying processes of frequency discrimination seen here in the rat could be common to higher-order mammals such as humans.

Chapter 5

5. General Conclusions

5.1. Review of Main Findings

The unifying hypothesis throughout this thesis has been that tonic neural responses in primary auditory cortex, mediated by intracortical processing, are crucial for fine-grained frequency perception. In general, this hypothesis resulted in experimental designs well-suited to investigate neural correlates of frequency perception, but the overall results of these experiments show that while intracortical processing does likely mediate fine-grained frequency perception, frequency differences are encoded in transient, post-phasic responses that do not meet the definition of tonic activity sustained through the duration of stimuli.

The experiment detailed in Chapter 2 showed that neurons in rat primary auditory cortex show spectro-temporally complex responses to tones during non-attentive listening, with both phasic and tonic response components. When presented with a repeating, isofrequency series of tones, the phasic and tonic response components habituated differently, dependent on stimulus presentation rate, and lasting habituation was only somewhat frequency-specific. Psychophysical experiments in Chapter 3 showed that rats' can detect fine-grained frequency changes in similar repeating-tone sequences and that

rats' behavioral frequency discrimination acuity was comparable to that shown by other mammals. When neural responses were recorded during the frequency discrimination behavior in the experiments in Chapter 3, attention to the task enhanced post-phasic suppression of repeated reference tones in a manner not consistent with habituation during passive listening. Attention also reduced spontaneous, noisy activity. At the transition from reference to target frequency, the frequency change was found to be encoded in enhancement primarily of the post-phasic response, but this difference signal did not persist through the duration of the tone.

In retrospect, the hypothesis that frequency difference signals would be maintained in neuronal firing throughout the duration of target stimuli does not account for the decaying nature of neuronal memory traces. Neurons in auditory cortex can fire tonically throughout the duration of a stimulus because continuous thalamocortical input maintains the impetus driving intracortical processing. Detection of frequency changes between tones spaced in time, however, requires the comparison of incoming frequency information with a memory trace containing preceding frequency information. If we assume a single, general memory trace for frequency, then at the same time as input is being compared to the memory trace, the memory trace is being overwritten to contain current frequency information. The difference between the input and the memory trace can only persist as long as it takes for the preceding frequency information to decay or be overwritten. The duration of the transient difference signal seen in post-phasic responses suggests that memory for

previous frequencies either decays quickly upon presentation of new frequency information or perhaps it is quickly and actively discarded.

5.2. Directions for Future Research

The purpose of hypotheses in the scientific method is to present questions in a testable form, and experiments in which hypotheses are disproved often have more exciting implications than those in which they are upheld. Fine-grained frequency information was not found to be represented tonically in primary auditory cortex throughout the duration of the tone, but the discovered transient difference signal itself suggests interesting new questions for research. If frequency information is tested with a task other than the repeating standard paradigm used here, will the difference still be shown in transient intracortical activity? Will the primary effect of attention be enhanced intracortical suppression if tasks do not involve reference repetition? Can psychophysical performance be enhanced by methods or pharmacology that magnifies intracortical suppression?

In the introductory review, we showed that human frequency discrimination ability is correlated with learning disorders and with some cognitive abilities seemingly unrelated to the auditory modality. Our results suggest that frequency discrimination ability may be less determined by the steady-state response properties and receptive fields of cortical neurons than by how those properties are modified by attention. Deficits in frequency discrimination ability may reflect global deficits in attentional ability caused by

relatively weaker intracortical processes. Treatments that enhance intracortical inhibition, either by medication or perhaps through training, might then be expected to enhance both learning and frequency discrimination, such that frequency discrimination ability could serve as a litmus for the effectiveness of treatments.

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Appendix A: Corrected Hit Rate Frequency Difference Limens

Tables of individual albino rats' measured frequency difference limens (FDLs) for the discrete tone task detailed in Chapter 3 are presented here, calculated from false alarm-corrected hit rate. Table A1 shows corrected hit rate FDLs as a function of reference frequency for 200 ms tones. Table A2 shows corrected hit rate FDLs as a function of reference frequency for tone durations of 10, 20, 50, 100, and 200 ms.

Reference frequency (kHz)	RN1	RN3	RN8	RN10	RN11	RN15	RN16	RN17	RN18	RN19	RN20	RN23	RN24
2.31	+4.26	+5.54	+3.61	+5.11	+2.04	+2.37	+3.68	+6.15	+1.94	+1.66	+2.37	+1.88	+4.37
	−9.16	OR	OR	−6.86	−3.20	−2.84	−7.43	OR	−1.93	−1.61	−2.01	−2.76	−7.40
	6.32	**6.59**	**5.73**	**5.63**	**2.49**	**2.51**	**6.10**	**10.33**	**1.94**	**1.63**	**2.28**	**2.21**	**6.13**
2.68	+1.47	+2.63	+3.45	+3.45	+1.72	+2.18	+2.81	+3.23	+2.24	+3.46	+1.71	+1.88	+4.81
	−13.15	−10.54	OR	OR	−2.64	−2.76	−2.65	−1.99	−2.39	−2.40	−2.14	−1.61	−6.34
	2.47	**7.17**	**3.83**	**5.06**	**2.20**	**2.44**	**2.70**	**2.66**	**2.32**	**2.75**	**1.84**	**1.77**	**5.48**
3.10	+2.55	+11.62	+7.75	+12.95	+2.31	+2.56	+3.83	OR	+2.29	+5.25	+3.64	+1.88	+2.84
	−2.72	−7.61	−4.84	−2.77	−1.66	−1.55	−1.71	−0.98	−1.54	−2.34	−1.61	−2.60	−3.71
	2.66	**11.48**	**5.28**	**13.39**	**1.85**	**2.15**	**3.24**	**2.09**	**1.73**	**4.63**	**2.92**	**2.29**	**3.64**
3.59	+4.29	+7.36	+3.04	+7.78	+2.19	+2.60	+2.78	+4.70	+1.90	+3.42	+1.61	+2.20	+4.63
	−4.72	−9.74	−6.97	−7.57	−2.39	−2.36	−3.71	−4.48	−2.06	−2.65	−1.81	−1.60	−4.45
	4.42	**7.51**	**3.54**	**7.67**	**2.26**	**2.55**	**3.09**	**4.65**	**1.96**	**2.81**	**1.70**	**1.96**	**4.59**
4.15	+4.40	+11.79	+5.23	+7.54	+2.08	+2.56	+3.86	+4.90	+2.24	+2.41	+2.36	+1.82	+3.10
	−4.49	−5.41	−3.21	−3.22	−1.80	−2.73	−3.11	−4.38	−1.97	−4.05	−2.49	−2.31	−2.56
	4.43	**6.94**	**3.81**	**5.42**	**1.89**	**2.64**	**3.52**	**4.57**	**2.13**	**2.60**	**2.43**	**2.05**	**2.85**
4.80	+5.56	+4.09	+5.45	+4.86	+2.16	+3.01	+5.30	+3.82	+3.10	+2.82	+1.84	+2.30	+1.22
	−4.51	−4.15	−1.98	−2.98	−1.50	−3.25	−3.39	−1.75	−2.15	−2.34	−1.58	−1.55	−3.61
	5.17	**4.12**	**2.61**	**3.55**	**1.83**	**3.16**	**3.56**	**2.90**	**2.43**	**2.58**	**1.67**	**1.67**	**1.79**
5.55	+4.35	+2.56	+1.44	+3.28	+1.69	+2.05	+3.52	+2.49	+2.34	+2.32	+1.67	+1.96	+3.08
	−3.48	−3.62	−3.74	−4.90	−2.00	−2.65	−2.55	−2.59	−1.84	−2.41	−2.20	−2.48	−3.72
	3.76	**3.05**	**2.81**	**3.71**	**1.82**	**2.41**	**2.81**	**2.55**	**2.12**	**2.36**	**1.95**	**2.18**	**3.43**
6.43	+4.09	+4.28	+2.17	+3.85	+1.69	+1.81	+1.72	+1.70	+1.48	+2.13	+1.63	+1.62	+2.36
	−4.74	−2.89	−3.75	−3.63	−2.21	−3.15	−3.56	−4.17	−1.35	−2.61	−1.49	−1.75	−2.84
	4.36	**4.23**	**3.53**	**3.80**	**1.90**	**2.55**	**3.15**	**3.60**	**1.44**	**2.56**	**1.55**	**1.70**	**2.62**
7.44	+3.27	+2.44	+3.46	+4.42	+2.32	+3.15	+3.40	+3.92	+2.43	+3.28	+2.45	+2.44	+2.36
	−3.74	−4.10	−2.19	−3.37	−1.93	−3.09	−3.46	−1.69	−1.49	−2.34	−1.61	−4.08	−4.01
	3.42	**2.73**	**2.86**	**3.85**	**2.42**	**3.12**	**3.43**	**3.45**	**1.98**	**2.75**	**2.06**	**2.66**	**2.58**
8.61	+2.15	+3.13	+1.99	+2.42	+2.36	+2.82	+1.87	+2.77	+3.29	+3.08	+2.71	+2.91	+0.82
	−4.38	−3.68	−5.27	−6.62	−2.14	−4.19	−2.84	−3.61	−3.66	−2.83	−2.47	−2.71	−2.63
	2.73	**3.37**	**2.92**	**3.61**	**2.27**	**3.36**	**2.76**	**3.18**	**3.39**	**3.01**	**2.61**	**2.78**	**2.41**
9.96	+1.98	+2.83	+1.77	+2.27	+2.18	+2.52	+2.76	+2.48	+3.23	+2.30	+2.47	+2.54	+2.62
	−4.17	−3.42	−2.22	−3.39	−1.70	−1.74	−1.78	−2.48	−2.29	−3.03	−2.25	−1.35	−4.92
	3.18	**3.14**	**1.86**	**3.12**	**1.88**	**2.36**	**2.57**	**2.50**	**2.52**	**2.47**	**2.39**	**2.10**	**4.12**
11.52	+1.44	+1.84	+1.82	+2.82	+1.81	+2.84	+1.72	+2.05	+1.62	+1.87	+2.27	+3.06	+3.14
	−6.51	−2.94	−4.46	−4.20	−2.32	−2.74	−1.96	−1.84	−2.23	−1.90	−2.31	−1.61	−2.44
	2.16	**2.18**	**2.60**	**3.23**	**2.03**	**2.75**	**1.84**	**1.93**	**1.81**	**1.88**	**2.29**	**1.80**	**2.63**
13.33	+2.33	+2.80	+2.55	+2.83	+1.66	+2.35	+2.45	+1.66	+1.79	+1.92	+1.86	+2.32	+1.91
	−4.96	−3.70	−3.34	−3.65	−3.32	−3.42	−3.52	−4.52	−2.70	−3.09	−3.09	−3.73	−0.90
	3.26	**3.43**	**2.82**	**3.31**	**2.17**	**2.82**	**2.91**	**2.39**	**2.21**	**2.84**	**2.22**	**2.93**	**1.35**
15.43	+2.08	+2.41	+2.91	+2.70	+2.28	+3.18	+2.63	+2.55	+2.36	+3.13	+4.57	+1.67	+1.50
	−3.62	−2.58	−3.03	−3.21	−1.81	−2.03	−1.28	−1.36	−1.23	−1.29	−1.35	−0.63	−2.58
	2.85	**2.44**	**2.96**	**2.90**	**2.11**	**2.73**	**1.74**	**1.55**	**1.67**	**1.71**	**3.20**	**1.14**	**2.18**
17.85	+2.00	+2.21	+2.27	+3.19	+2.19	+2.05	+1.92	+2.16	+2.18	+2.48	+2.44	+0.60	+1.57
	−3.26	−3.98	−3.91	−4.00	−1.89	−2.46	−3.00	−4.12	−2.52	−2.19	−1.85	−2.54	−3.81
	2.31	**2.48**	**2.65**	**3.50**	**2.11**	**2.26**	**2.18**	**2.40**	**2.36**	**2.42**	**2.20**	**2.41**	**2.23**
20.66	+1.66	+2.24	+2.13	+1.82	+2.07	+2.40	+2.52	+2.46	+2.67	+2.47	+2.92	+0.73	+2.20
	−3.08	−4.88	−4.63	−3.55	−2.37	−4.67	−1.65	−2.46	−2.27	−2.30	−1.71	−3.29	−2.32
	2.00	**3.24**	**2.60**	**3.05**	**2.23**	**3.47**	**1.93**	**2.46**	**2.54**	**2.40**	**2.34**	**2.94**	**2.27**
23.90	+1.75	+2.34	+1.35	+2.44	+2.47	+3.40	+1.98	+1.41	+2.99	+3.59	+4.04	+2.58	+2.02
	−2.32	−5.37	−2.89	−4.20	−1.75	−3.73	−3.31	−2.48	−2.70	−3.78	−3.74	−2.69	−3.11
	1.99	**2.69**	**1.92**	**3.42**	**1.95**	**3.52**	**2.62**	**2.10**	**2.78**	**3.65**	**3.88**	**2.62**	**2.34**
27.66	+1.47	+2.22	+1.67	+2.72	+1.76	+3.00	+2.06	+2.06	+2.11	+2.73	+1.75	+1.66	+1.79
	−2.87	−3.37	−2.93	−2.51	−2.70	−3.85	−3.95	−3.54	−3.75	−4.69	−4.07	−2.71	−1.81
	2.14	**2.70**	**2.31**	**2.63**	**2.06**	**3.49**	**2.53**	**2.71**	**2.53**	**3.23**	**2.44**	**1.92**	**1.80**

Table A1. Discrete tone task FDLs calculated with false alarm-corrected hit rates as a function of reference frequency for 200 ms tones for 13 subjects. FDLs for upward shifts are indicated with a '+', FDLs for downward shifts with a '-', and direction-irrespective FDLs are shown in bold. Stimuli for which FDLs could not be determined because subjects did not exceed threshold with tested Δfs of -15% to 15% are indicated as out of range ('OR').

Reference frequency	Tone duration (ms)	RN1	RN3	RN8	RN10	RN11	RN15	RN16	RN17	RN18	RN19	RN20	RN23	RN24
4.15 kHz	10	+10.60	OR	+14.33	OR	+4.80	+4.78	+6.77	+6.42	+4.55	+4.60	+5.61	+9.57	+9.12
		−6.03	−11.34	−5.29	−10.34	−3.85	−4.63	−8.26	−8.13	−4.43	−3.46	−6.82	−6.43	−1.62
		10.14	**OR**	**7.17**	**OR**	**4.77**	**4.71**	**8.13**	**6.62**	**4.49**	**4.19**	**5.76**	**7.14**	**8.06**
	20	+8.08	OR	+12.78	+14.43	+3.46	+4.60	+5.03	+6.33	+3.60	+3.98	+4.90	+3.30	+8.08
		−5.68	−7.46	−4.21	−5.45	−3.21	−5.52	−6.50	−5.50	−4.34	−3.68	−6.21	−4.83	−2.24
		7.21	**12.32**	**5.23**	**11.22**	**3.36**	**5.36**	**5.89**	**5.75**	**3.93**	**3.79**	**5.42**	**4.92**	**5.64**
	50	+4.95	+12.37	+11.08	+10.53	+2.38	+4.34	+5.14	+6.62	+2.59	+2.62	+2.54	+2.18	+4.90
		−3.46	−5.58	−4.22	−3.65	−1.86	−2.82	−3.44	−6.00	−2.33	−2.90	−3.39	−3.20	−2.49
		4.51	**8.36**	**4.67**	**6.55**	**2.19**	**3.99**	**3.87**	**6.06**	**2.41**	**2.72**	**2.94**	**2.63**	**3.51**
	100	+4.28	+8.14	+5.47	+9.33	+1.91	+2.85	+5.69	+4.30	+2.42	+2.08	+1.94	+1.63	+3.45
		−5.27	−5.54	−3.89	−3.52	−1.79	−4.13	−4.26	−4.95	−2.27	−2.23	−2.30	−2.09	−2.20
		5.80	**7.28**	**4.34**	**5.40**	**1.85**	**3.49**	**5.23**	**4.50**	**2.37**	**2.15**	**2.22**	**1.77**	**2.75**
	200	+4.40	+11.79	+5.23	+7.54	+2.08	+2.56	+3.86	+4.90	+2.24	+2.41	+2.36	+1.82	+3.10
		−4.49	−5.41	−3.21	−3.22	−1.80	−2.73	−3.11	−4.38	−1.97	−4.05	−2.49	−2.31	−2.56
		4.43	**6.94**	**3.81**	**5.42**	**1.89**	**2.64**	**3.52**	**4.57**	**2.13**	**2.60**	**2.43**	**2.05**	**2.85**
8.61 kHz	10	+2.81	+3.79	+1.74	+7.26	+6.59	+3.80	+5.27	+4.08	+4.53	+3.81	+5.84	+7.58	+5.50
		−9.72	−9.90	OR	OR	−3.51	−8.81	−9.19	−11.02	−10.08	−6.49	−8.99	−7.48	−2.72
		7.18	**10.12**	**OR**	**9.69**	**6.40**	**5.46**	**6.55**	**5.41**	**5.80**	**4.68**	**6.55**	**7.56**	**4.64**
	20	+3.48	+6.72	+2.26	+6.67	+4.48	+3.26	+3.95	+3.21	+3.62	+3.91	+3.43	+3.92	+0.71
		−6.63	−2.80	−7.48	−7.35	−2.67	−6.25	−5.09	−5.58	−5.99	−4.15	−3.45	−5.64	−2.42
		4.36	**4.18**	**3.74**	**7.21**	**2.87**	**5.06**	**4.68**	**4.17**	**4.41**	**4.04**	**3.44**	**4.47**	**3.46**
	50	+2.53	+4.88	+1.51	+1.86	+2.60	+3.14	+2.29	+3.12	+3.52	+2.46	+3.15	+2.85	+0.55
		−4.45	−4.71	−5.62	−5.58	−2.39	−4.89	−3.95	−3.85	−3.66	−3.92	−2.19	−3.35	−2.54
		3.11	**4.82**	**1.85**	**4.65**	**2.46**	**3.77**	**2.72**	**3.40**	**3.60**	**3.41**	**2.69**	**3.20**	**2.96**
	100	+1.83	+3.44	+1.49	+2.62	+2.06	+3.45	+2.58	+2.68	+3.93	+3.00	+2.87	+2.74	+0.72
		−2.88	−5.41	−4.54	−5.31	−1.85	−4.35	−3.33	−4.41	−2.82	−3.11	−2.11	−3.11	−2.06
		2.53	**3.61**	**2.25**	**4.72**	**1.92**	**4.01**	**3.05**	**3.49**	**3.30**	**3.06**	**2.49**	**2.94**	**2.16**
	200	+2.15	+3.13	+1.99	+2.42	+2.36	+2.82	+1.87	+2.77	+3.29	+3.08	+2.71	+2.91	+0.82
		−4.38	−3.68	−5.27	−6.62	−2.14	−4.19	−2.84	−3.61	−3.66	−2.83	−2.47	−2.71	−2.63
		2.73	**3.37**	**2.92**	**3.61**	**2.27**	**3.36**	**2.76**	**3.18**	**3.39**	**3.01**	**2.61**	**2.78**	**2.41**
17.85 kHz	10	+3.19	+2.85	+4.38	+6.77	+3.11	+3.03	+3.28	+3.23	+3.61	+2.71	+3.37	+3.37	+1.55
		−5.78	−10.42	OR	−7.29	−3.38	−3.35	−3.76	−4.62	−3.47	−2.67	−1.99	−5.23	−5.11
		4.43	**5.47**	**5.61**	**7.39**	**3.29**	**3.13**	**3.45**	**3.60**	**3.55**	**2.69**	**3.18**	**4.05**	**3.35**
	20	+2.89	+3.56	+3.38	+4.51	+2.35	+3.11	+3.16	+2.79	+2.71	+2.83	+2.85	+0.83	+1.80
		−5.24	−8.70	−5.33	−6.18	−2.88	−2.77	−2.67	−5.43	−3.09	−2.58	−2.48	−4.67	−4.61
		4.28	**4.44**	**4.29**	**5.46**	**2.53**	**3.05**	**3.00**	**3.23**	**2.87**	**2.71**	**2.64**	**4.69**	**4.00**
	50	+2.33	+3.16	+2.08	+3.64	+2.19	+2.55	+2.62	+2.42	+2.29	+2.86	+2.32	+0.65	+1.77
		−4.18	−5.78	−5.21	−4.60	−3.15	−2.62	−3.17	−4.08	−2.47	−2.33	−1.53	−3.34	−4.22
		3.44	**4.32**	**3.09**	**4.25**	**2.33**	**2.59**	**2.80**	**2.65**	**2.39**	**2.55**	**1.90**	**3.12**	**2.91**
	100	+1.25	+2.12	+1.46	+3.03	+2.29	+2.49	+2.05	+2.29	+2.16	+3.02	+2.60	+0.59	+1.83
		−3.91	−4.38	−4.21	−4.44	−1.75	−2.66	−2.27	−2.83	−2.20	−2.59	−1.55	−2.59	−4.39
		2.25	**3.49**	**2.06**	**3.71**	**2.14**	**2.55**	**2.17**	**2.48**	**2.18**	**2.84**	**2.23**	**0.87**	**2.82**
	200	+2.00	+2.21	+2.27	+3.19	+2.19	+2.05	+1.92	+2.16	+2.18	+2.48	+2.44	+0.60	+1.57
		−3.26	−3.98	−3.91	−4.00	−1.89	−2.46	−3.00	−4.12	−2.52	−2.19	−1.85	−2.54	−3.81
		2.31	**2.48**	**2.65**	**3.50**	**2.11**	**2.26**	**2.18**	**2.40**	**2.36**	**2.42**	**2.20**	**2.41**	**2.23**

Table A2. Discrete tone task FDLs calculated with false alarm-corrected hit rates as a function of reference frequency and tone duration for 13 subjects. FDLs for upward shifts are indicated with a '+', FDLs for downward shifts with a '-', and direction-irrespective FDLs are shown in bold. Stimuli for which FDLs could not be determined because subjects did not exceed threshold with tested Δfs of -15% to 15% are indicated as out of range ('OR').

Appendix B: Signal Detection Frequency Difference Limens

S.1. Signal Detection Analysis

The main analysis of psychophysical results in Chapter 3 uses Heffner and Heffner's (1988) formula to correct hit rate for false alarm rate. A better approach to account for bias may be to use signal detection indices. In their signal detection analysis of frequency discrimination in the rat, Talwar and Gerstein (1999) found that the nonparametric index *A'* was the most suitable measure of acuity. Therefore, we will also use the *A'* index to determine signal detection frequency difference limens (FDLs) to compare to false alarm-corrected hit rate FDLs. The A' index is calculated from hit rate (H) and false alarm rate (F) as follows:

$$A' = \tfrac{1}{2} + [(H - F)\cdot(1 + H - F)]/[4\cdot H\cdot(1 - F)], \qquad \text{(Grier, 1971).}$$

An *A'* index of 0.85 roughly corresponds to 50% performance with a false alarm rate of 0.05 (Talwar & Gerstein, 1999), and that value was used to determine FDLs from performance curves of *A'* versus Δf.

Table B1 shows signal detection FDLs as a function of reference frequency for 200 ms tones. Table B2 shows signal detection FDLs as a function of reference frequency for tone durations of 10, 20, 50, 100, and 200 ms. False alarm-corrected hit rate FDLs and signal detection FDLs were approximately equivalent.

Reference frequency (kHz)	RN1	RN3	RN8	RN10	RN11	RN15	RN16	RN17	RN18	RN19	RN20	RN23	RN24
2.31	+4.50	+5.87	+3.81	+5.29	+2.29	+2.41	+5.94	+7.02	+2.34	+1.93	+2.61	+2.23	+4.65
	−9.36	OR	OR	−7.10	−3.50	−2.84	OR	OR	−2.65	−1.89	−2.58	−3.33	−12.45
	8.04	**8.41**	**6.00**	**5.94**	**2.83**	**2.54**	**6.79**	**OR**	**2.45**	**1.91**	**2.59**	**2.67**	**6.70**
2.68	+1.54	+2.86	+3.50	+3.61	+1.78	+0.94	+3.36	+3.34	+2.56	+3.74	+1.91	+2.19	+5.08
	−13.21	OR	OR	OR	−2.68	−0.94	−2.84	−2.06	−2.66	−2.64	−2.51	−1.88	−6.61
	2.56	**13.58**	**5.57**	**5.79**	**2.22**	**0.94**	**2.89**	**2.72**	**2.61**	**6.07**	**2.29**	**2.10**	**5.93**
3.10	+2.60	+12.32	+8.14	OR	+2.33	+2.60	OR	OR	+4.21	+5.65	+3.88	+4.58	+4.11
	−2.75	−13.23	−4.93	−3.06	−1.73	−1.54	−3.05	−1.12	−1.80	−2.64	−1.94	−3.93	−3.87
	2.69	**12.55**	**5.51**	**OR**	**1.89**	**2.12**	**3.80**	**2.48**	**2.19**	**5.62**	**3.33**	**4.40**	**4.38**
3.59	+4.33	+7.66	+3.17	+8.25	+2.27	+2.76	+3.56	+6.14	+2.20	+6.69	+1.83	+2.45	+4.82
	−4.73	−10.19	−7.12	OR	−2.52	−2.42	−5.84	−5.61	−2.41	−2.83	−2.57	−1.87	−5.03
	4.45	**10.08**	**3.71**	**9.64**	**2.36**	**2.63**	**5.48**	**6.11**	**2.27**	**7.54**	**1.92**	**2.23**	**4.88**
4.15	+4.58	+12.38	+5.29	+12.32	+2.14	+2.58	+6.29	+4.98	+2.52	+2.59	+2.57	+2.24	+3.20
	−4.87	−5.70	−3.27	−3.39	−1.88	−2.74	−4.05	−4.43	−2.32	−4.23	−2.68	−2.75	−2.67
	4.66	**7.68**	**3.87**	**6.12**	**1.93**	**2.66**	**4.75**	**4.62**	**2.44**	**2.85**	**2.63**	**2.50**	**2.93**
4.80	+5.59	+4.54	+5.62	+5.04	+2.26	+3.01	+8.63	+3.86	+3.43	+2.90	+2.13	+2.87	+1.32
	−4.54	−4.71	−2.12	−3.30	−1.61	−3.27	−3.69	−1.81	−2.44	−2.45	−1.76	−1.78	−3.82
	5.20	**4.61**	**2.80**	**3.80**	**1.91**	**3.17**	**5.33**	**2.98**	**3.04**	**2.69**	**1.83**	**1.93**	**1.92**
5.55	+4.52	+2.86	+1.53	+3.45	+1.83	+2.09	+4.06	+2.53	+2.61	+2.43	+1.81	+2.26	+3.19
	−3.57	−3.97	−3.80	−5.12	−2.18	−2.71	−2.87	−2.66	−2.21	−2.55	−2.34	−2.86	−3.84
	3.83	**3.49**	**3.01**	**3.86**	**1.93**	**2.47**	**4.06**	**2.60**	**2.47**	**2.47**	**2.09**	**2.50**	**3.57**
6.43	+4.15	+4.61	+2.31	+4.00	+1.85	+1.87	+2.49	+1.79	+1.70	+3.24	+1.75	+1.90	+2.55
	−4.80	−4.71	−3.83	−3.95	−2.34	−3.17	−5.13	−4.25	−1.86	−4.21	−1.61	−1.93	−3.00
	4.43	**4.64**	**3.72**	**3.99**	**2.03**	**2.57**	**3.89**	**3.68**	**1.71**	**3.93**	**1.68**	**1.91**	**2.79**
7.44	+3.37	+2.76	+3.53	+4.67	+2.37	+0.96	+3.70	+3.97	+2.66	+3.47	+2.58	+2.66	+2.49
	−3.97	−4.50	−2.26	−3.63	−3.31	−3.04	−3.70	−1.76	−1.74	−2.51	−1.81	−4.47	−4.31
	3.54	**3.62**	**3.00**	**4.09**	**2.51**	**3.07**	**3.70**	**3.53**	**2.41**	**2.92**	**2.22**	**2.87**	**2.72**
8.61	+2.30	+3.32	+2.05	+2.64	+2.36	+2.82	+3.36	+2.85	+3.56	+3.21	+2.88	+3.29	+0.94
	−4.90	−3.98	−5.35	−7.55	−2.11	−4.20	−4.29	−3.70	−4.16	−3.44	−2.72	−2.94	−2.77
	2.98	**3.58**	**3.06**	**4.54**	**2.25**	**3.36**	**3.65**	**3.26**	**3.72**	**3.21**	**2.82**	**3.20**	**2.59**
9.96	+2.03	+3.05	+1.82	+2.58	+2.17	+2.62	+4.25	+2.60	+3.58	+2.40	+2.55	+2.76	+2.77
	−4.22	−3.62	−2.08	−3.55	−1.78	−1.80	−3.04	−2.52	−2.55	−3.33	−2.31	−1.64	−5.07
	3.25	**3.36**	**1.87**	**3.33**	**1.91**	**2.33**	**3.36**	**2.55**	**2.92**	**2.58**	**2.46**	**2.43**	**4.45**
11.52	+1.54	+2.01	+1.92	+3.10	+1.89	+2.75	+2.11	+2.07	+1.82	+1.94	+2.36	+3.36	+3.39
	−6.62	−3.12	−4.61	−4.37	−2.43	−2.78	−2.56	−1.87	−2.54	−2.04	−2.41	−1.89	−2.61
	2.32	**2.54**	**2.86**	**3.82**	**2.14**	**2.72**	**2.28**	**1.94**	**2.08**	**1.98**	**2.38**	**2.43**	**2.80**
13.33	+2.37	+3.24	+2.59	+2.99	+1.70	+2.43	+2.71	+1.75	+1.93	+2.62	+1.91	+2.60	+2.07
	−4.98	−3.92	−3.35	−3.79	−3.35	−3.50	−3.77	−4.66	−2.89	−3.43	−3.18	−4.47	−1.03
	3.29	**3.72**	**2.83**	**3.47**	**2.10**	**2.87**	**3.30**	**2.53**	**2.45**	**3.08**	**2.32**	**3.46**	**1.55**
15.43	+2.22	+2.70	+2.98	+2.86	+2.40	+3.18	+3.23	+2.98	+2.58	+3.25	+4.61	+1.91	+1.66
	−3.75	−3.26	−3.11	−3.38	−1.89	−2.03	−1.56	−1.48	−1.39	−1.41	−1.39	−0.87	−2.76
	3.07	**2.83**	**3.04**	**3.10**	**2.18**	**2.75**	**2.36**	**1.68**	**1.87**	**1.83**	**3.24**	**1.65**	**2.35**
17.85	+2.12	+2.54	+2.39	+3.50	+2.27	+2.09	+2.30	+2.28	+2.43	+2.63	+2.57	+0.85	+1.77
	−3.56	−4.44	−4.03	−4.26	−2.00	−2.54	−3.49	−4.33	−2.76	−2.79	−1.96	−2.84	−4.14
	2.50	**2.93**	**2.78**	**3.87**	**2.20**	**2.33**	**2.76**	**2.56**	**2.61**	**2.64**	**2.33**	**2.67**	**2.55**
20.66	+1.67	+2.50	+2.21	+2.08	+2.15	+2.48	+2.99	+2.56	+2.89	+2.58	+2.97	+0.93	+2.37
	−3.06	−5.39	−4.76	−3.80	−2.46	−4.71	−1.88	−2.59	−2.62	−2.42	−1.85	−3.57	−2.48
	1.95	**3.58**	**2.69**	**3.30**	**2.32**	**3.50**	**2.79**	**2.56**	**2.80**	**2.51**	**2.43**	**3.46**	**2.43**
23.90	+1.31	+2.57	+1.41	+2.67	+2.67	+3.44	+2.45	+1.50	+3.44	+3.70	+4.09	+2.78	+2.20
	−2.40	−5.88	−2.93	−4.37	−1.85	−3.74	−4.04	−2.57	−2.90	−3.92	−3.81	−4.27	−3.46
	2.09	**2.98**	**1.97**	**3.71**	**2.17**	**3.55**	**3.15**	**2.20**	**3.45**	**3.76**	**3.93**	**2.84**	**2.58**
27.66	+1.64	+2.48	+1.79	+2.86	+1.65	+3.08	+2.36	+2.10	+2.31	+3.03	+1.86	+1.49	+2.07
	−3.01	−3.65	−3.02	−2.69	−2.99	−3.93	−4.84	−3.60	−4.09	−4.90	−4.17	−4.18	−3.11
	2.29	**3.10**	**2.48**	**2.78**	**2.36**	**3.59**	**2.82**	**2.76**	**2.80**	**4.29**	**2.55**	**2.71**	**2.18**

Table B1. Discrete tone task FDLs calculated with the nonparametric index *A'* as a function of reference frequency for 200 ms tones for 13 subjects. FDLs for upward shifts are indicated with a '+', FDLs for downward shifts with a '-', and direction-irrespective FDLs are shown in bold. Stimuli for which FDLs could not be determined because subjects did not exceed threshold with tested Δfs of -15% to 15% are indicated as out of range ('OR').

Reference frequency	Tone duration (ms)	RN1	RN3	RN8	RN10	RN11	RN15	RN16	RN17	RN18	RN19	RN20	RN23	RN24
4.15 kHz	10	+13.17	OR	+14.42	OR	+4.78	+4.81	+8.43	+6.43	+4.89	+4.69	+5.73	OR	+11.09
		−7.33	OR	−5.40	−10.72	−3.83	−4.67	−8.68	−8.14	−4.79	−3.56	−6.93	−7.02	−2.97
		10.46	**OR**	**7.33**	**OR**	**4.72**	**4.74**	**8.56**	**6.63**	**4.83**	**4.30**	**7.05**	**OR**	**8.51**
	20	+8.61	OR	+13.19	+14.54	+3.51	+4.65	+5.47	+6.21	+3.84	+4.03	+5.07	+5.73	+8.40
		−7.12	−10.51	−4.44	−5.65	−3.22	−5.57	−7.76	−5.47	−4.66	−3.74	−6.35	−5.24	−2.44
		7.49	**OR**	**5.36**	**14.23**	**3.39**	**5.41**	**7.41**	**5.71**	**4.39**	**3.84**	**5.74**	**5.42**	**6.98**
	50	+5.30	+14.34	+11.23	+11.06	+2.43	+4.42	+5.54	+7.79	+2.84	+2.71	+2.71	+2.59	+5.47
		−3.67	−7.79	−4.32	−3.99	−1.91	−2.86	−3.84	−6.04	−2.54	−4.00	−3.57	−3.57	−2.70
		5.09	**10.61**	**4.78**	**9.25**	**2.23**	**4.05**	**5.57**	**6.11**	**2.62**	**2.80**	**3.15**	**3.06**	**3.78**
	100	+6.44	+11.41	+5.63	+13.23	+1.23	+2.89	+6.39	+4.37	+2.64	+2.22	+2.38	+1.85	+3.62
		−5.59	−6.66	−3.96	−3.68	−1.06	−4.19	−6.17	−5.02	−2.71	−2.40	−2.50	−2.57	−2.35
		6.17	**8.31**	**4.44**	**6.70**	**1.10**	**3.60**	**6.26**	**4.60**	**2.64**	**2.31**	**2.47**	**2.17**	**2.89**
	200	+4.58	+12.38	+5.29	+12.32	+2.14	+2.58	+6.29	+4.98	+2.52	+2.59	+2.57	+2.24	+3.20
		−4.87	−5.70	−3.27	−3.39	−1.88	−2.74	−4.05	−4.43	−2.32	−4.23	−2.68	−2.75	−2.67
		4.66	**7.68**	**3.87**	**6.12**	**1.93**	**2.66**	**4.75**	**4.62**	**2.44**	**2.85**	**2.63**	**2.50**	**2.93**
8.61 kHz	10	+4.98	+11.40	+1.80	+7.44	+6.50	+3.86	+5.70	+4.10	+4.90	+3.97	+5.92	OR	+7.25
		−10.23	−10.51	OR	OR	−3.49	−8.94	−11.57	−11.05	−10.63	−6.65	−9.10	−14.15	−2.90
		7.67	**12.06**	**OR**	**13.52**	**6.21**	**5.53**	**7.48**	**5.45**	**6.31**	**5.93**	**6.68**	**OR**	**5.33**
	20	+3.71	+7.64	+2.32	+7.13	+4.17	+3.32	+4.53	+3.21	+3.86	+4.07	+3.56	+4.31	+0.89
		−8.36	−4.41	−7.55	−7.51	−2.70	−6.30	−5.36	−5.58	−6.61	−4.29	−3.58	−8.53	−2.68
		4.63	**10.81**	**3.82**	**7.44**	**2.86**	**5.12**	**5.25**	**4.13**	**4.70**	**4.19**	**3.57**	**5.50**	**3.83**
	50	+2.86	+5.15	+1.66	+2.38	+2.64	+3.21	+2.77	+3.13	+3.82	+2.62	+3.34	+3.25	+0.78
		−5.17	−7.01	−5.76	−5.98	−2.44	−4.93	−4.90	−3.85	−4.04	−4.09	−2.38	−3.60	−2.73
		3.53	**5.36**	**3.54**	**5.23**	**2.51**	**3.81**	**4.44**	**3.41**	**3.87**	**3.86**	**2.87**	**3.49**	**3.53**
	100	+2.04	+3.76	+1.65	+3.10	+2.04	+3.47	+3.11	+2.70	+4.21	+3.08	+3.09	+3.06	+0.89
		−4.35	−6.06	−4.68	−5.60	−1.89	−4.38	−3.72	−4.45	−3.33	−3.20	−2.31	−3.41	−2.24
		2.72	**4.72**	**2.49**	**5.15**	**1.92**	**4.01**	**3.50**	**3.49**	**3.92**	**3.14**	**2.70**	**3.30**	**2.43**
	200	+2.30	+3.32	+2.05	+2.64	+2.36	+2.82	+3.36	+2.85	+3.56	+3.21	+2.88	+3.29	+0.94
		−4.90	−3.98	−5.35	−7.55	−2.11	−4.20	−4.29	−3.70	−4.16	−3.44	−2.72	−2.94	−2.77
		2.98	**3.58**	**3.06**	**4.54**	**2.25**	**3.36**	**3.65**	**3.26**	**3.72**	**3.21**	**2.82**	**3.20**	**2.59**
17.85 kHz	10	+3.50	+4.77	+4.48	+9.14	+3.24	+3.07	+3.52	+3.30	+3.85	+2.86	+3.48	+3.61	+1.75
		−8.54	−13.35	OR	−7.52	−3.47	−3.43	−5.61	−4.76	−3.83	−2.84	−2.18	−5.50	−5.30
		5.29	**10.01**	**5.76**	**10.17**	**3.39**	**3.19**	**3.75**	**3.67**	**3.84**	**2.85**	**3.27**	**5.12**	**4.94**
	20	+3.23	+3.82	+3.47	+4.71	+2.40	+3.16	+3.39	+2.86	+2.88	+2.97	+3.01	+5.22	+1.94
		−5.50	OR	−5.42	−6.38	−2.88	−2.82	−3.35	−5.64	−3.36	−2.78	−2.64	−4.91	−4.80
		4.69	**8.07**	**4.46**	**5.71**	**2.57**	**3.11**	**3.37**	**3.35**	**3.16**	**2.88**	**2.81**	**5.08**	**4.29**
	50	+2.73	+3.53	+2.17	+4.04	+2.29	+2.67	+2.84	+2.53	+2.53	+2.99	+2.46	+0.90	+1.94
		−4.35	−6.23	−5.32	−4.79	−3.24	−2.74	−3.66	−4.19	−2.69	−2.48	−1.69	−4.19	−4.50
		3.74	**4.96**	**3.30**	**4.50**	**2.46**	**2.70**	**3.21**	**2.78**	**2.62**	**2.71**	**2.05**	**3.56**	**3.97**
	100	+1.51	+2.50	+1.60	+3.26	+2.34	+2.62	+2.51	+2.43	+2.39	+3.16	+2.80	+0.84	+2.06
		−4.09	−7.29	−4.35	−4.66	−1.84	−2.80	−2.69	−3.38	−2.58	−2.76	−1.80	−2.82	−4.70
		2.43	**4.21**	**3.05**	**4.13**	**2.17**	**2.68**	**2.61**	**2.66**	**2.45**	**3.00**	**2.52**	**2.80**	**3.48**
	200	+2.12	+2.54	+2.39	+3.50	+2.27	+2.09	+2.30	+2.28	+2.43	+2.63	+2.57	+0.85	+1.77
		−3.56	−4.44	−4.03	−4.26	−2.00	−2.54	−3.49	−4.33	−2.76	−2.79	−1.96	−2.84	−4.14
		2.50	**2.93**	**2.78**	**3.87**	**2.20**	**2.33**	**2.76**	**2.56**	**2.61**	**2.64**	**2.33**	**2.67**	**2.55**

Table B2. Discrete tone task FDLs calculated with the nonparametric index *A'* as a function of reference frequency and tone duration for 13 subjects. FDLs for upward shifts are indicated with a '+', FDLs for downward shifts with a '-', and direction-irrespective FDLs are shown in bold. Stimuli for which FDLs could not be determined because subjects did not exceed threshold with tested Δfs of -15% to 15% are indicated as out of range ('OR').