EXPERIENCE DEPENDENT CHANGES

IN THE AUDITORY CORTICAL REPRESENTATION

OF NATURAL SOUNDS

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LIST OF SYMBOLS AND ABBREVIATIONS

dBSPL	Decibel Sound Pressure Level
g	Grams
Hz	Hertz
kHz	Kilohertz
ks	Kilosample
um	Micrometer
mm	Millimeter
MΩ	Megaohm
ms	Millisecond
s	Second

A1	Primary Auditory Field
A2	Secondary Auditory Field
AAF	Anterior Auditory Field
ABR	Auditory Brainstem Recording
AC	Auditory Cortex
ANF	Auditory Nerve Fiber
BF	Best Frequency
DP	Dorsal Posterior Field
FIR	Finite Impulse Response
FSL	First Spike Latency
IC	Inferior Colliculus

LFP	Local Field Potential
LIEFTS	Leaky Integration Event Formation Temporal Summation
PDF	Probability Density Function
PSTH	Peri-Stimulus Time Histogram
RMS	Root Mean Square
RMSE	Root Mean Square Error
SE	Standard Error
SU	Single Unit
UF	Ultrasound Field

SUMMARY

Vocal communication sounds are an important class of signals due to their role in social interaction, reproduction, and survival. However, it is still unclear how our auditory system detects and discriminates these sounds. The auditory cortex is thought to play a role in this process, because loss of this area can cause deficits in the vocalization discrimination in primates and speech comprehension in humans. In addition, the auditory cortex can undergo both rapid and long-term changes under classical and operant conditioning. But unlike these conditioning paradigms, the behavioral relevance of communication sounds are acquired through social interaction. Thus, the question remains as to how the auditory cortex changes its neural representation of sounds that are socially acquired.

To address this question, we used a neuroethological model system, which allowed us to study the neural mechanisms underlying natural behavior. This model system consists of ultrasonic whistles emitted by pups and are thought to be communicative in nature. The calls can elicit a search and retrieval behavior in mothers, and are recognized as behaviorally relevant by mothers, but not pup-naïve virgins. Therefore, this mouse ultrasonic communication system provides the opportunity to understand how the brain encodes natural sounds, and how the neural representation changes for vocalizations learned through social interaction.

In this dissertation, we recorded single neurons from the auditory cortex of fully awake, head-restrained mice, and began by assessing the changes in the cortical

neurons and local field potentials of animals that either do (mothers), or do not (naïve virgins) recognize pup ultrasounds as behaviorally relevant. We then evaluated the role that pup experience and the maternal physiological state played in this cortical plasticity. Following these results, we explored the behavioral relevance of these neural changes using a two-alternative choice task. Finally, we developed a model to predict the response latency to natural sounds with the intent to define cortical neurons and their roles in processing acoustic features.

Our results show that the auditory cortex in animals that have had pup experience differ in their pup call-evoked inhibition, that the physiological changes associated with motherhood act to affect the long-term retention of this plasticity, and that these changes are correlated with call recognition behavior. In addition, we find that by using a model to predict the response to these vocalizations, there is a distinct subset of cortical neurons that preserve the peripheral mechanism for onset encoding, and a subset that represents a sound's behavioral meaning. Taken together, this research emphasizes the importance of the primary auditory cortex in processing natural vocalizations, demonstrates how it changes to represent behavioral relevance, and creates a framework for studying functionally how these changes contribute to behavior.

CHAPTER 1

INTRODUCTION

Sitting at my desk, pondering this dissertation, I turn to my dog and call out his name, "*Patches*", I say. He is sleeping, but his ears flicker, and he slowly moves his head to look at me. Just a few months prior when we first adopted him from the shelter, we had given him a new name, and at this time, he showed no recognition or behavioral response to the sound, "*Patches*". Clearly then, the meaning of this sound has changed, but the question is, how does our brain account for this change?

Our sensory systems govern our perception of the world, and within our acoustic environment, sounds are continuous, resulting from physical vibrations. Yet, these vibrations can possess meaning, and what might be familiar to some can be unfamiliar to others. How our auditory system transforms these acoustic features into behaviorally relevant sounds and how we utilize these signals for localization, detection, and discrimination is not completely understood. It is amazing to think that our auditory system performs one, if not all of these operations in parallel and does so with both a speed and accuracy unmatched by any current computational approach.

In an attempt to understand how acoustic information gives rise to perception and behavior, there has been a strong push towards investigating the auditory cortex as a central component to this complex operation. It has numerous connections to higher cognitive areas, such as the prefrontal cortex (Fritz et al., 2010), changes its representation to facilitate learning and memory (Bieszczad and Weinberger, 2010;

Reed et al., 2011), and can alter its encoding to improve behavioral performance based on the expectation of sound (Jaramillo and Zador, 2011). While these studies have been instrumental in our understanding of auditory cortical function, they do not entirely explain how the auditory cortex encodes communication sounds, which are learned through social interaction. This difference in how we learn sounds is important because it can affect the resultant neural representation. In fact, a recent study demonstrated that the direction of plasticity depended on whether the training task used positive or negative reinforcement (David et al., 2012). Thus, the goal of this thesis is to understand the encoding of socially acquired vocalizations, and how this might subserve perception and behavior.

The subject matter below is intended to build a basic understanding of the methods and approaches used in this dissertation. Section 1.1 will briefly describe how a sound signal goes from the periphery to the auditory cortex. Then 1.2 will introduce communication sound processing, motivate neuroethology as a way to study the goal of this thesis, and in 1.3, detail the approach we have chosen. In 1.4, we will discuss what we currently know about experience dependent plasticity, and in 1.5, explain the importance of studying this in the awake animal. Finally, 1.6 will review the aims and goals of this dissertation.

1.1: The auditory system

Within our environment, sounds are dynamic in nature, constantly changing as a function of time. As these mechanical waves arrive at our ears, our brain transforms

these inputs into electrical signals, and performs a variety of operations that allow us to perceive and react to these cues. Yet, the understanding of how our auditory system represents a sound's behavioral relevance and facilitates perception remains unsolved. Below, we will briefly discuss how a sound is transformed into neural signals, how the physical characteristics of sounds are transmitted, and motivate the auditory cortex as a starting point to understand how the brain changes to encode a sound's behavioral relevance.

1.1.1: Peripheral processing and the transduction of sounds

From a physical perspective, sound is a mechanical wave created by a vibrating object and moves outward as particles in the medium (typically air) are compressed and spread apart. The characteristics of these vibrations in air and the way in which it physically affects our eardrum underlie how we describe a sound's acoustic features. This consists of attributes such as its frequency (number of vibrations of a particle in a fixed time), amplitude (energy in which the vibrating object imparts to the medium), and place in time (the start/duration of time in which this occurs).

As this sound wave travels through our auditory canal, it causes the eardrum to vibrate. The eardrum is connected to three bones in the middle ear called ossicles, and act to transfer this signal to the oval window. At this stage, these signals enter the cochlea, a fundamental first step in the transfer of mechanical to electrical energy (Moore, 1997). Here, as the oval window vibrates, the fluid within the cochlea moves resulting in the movement of the basilar membrane. A key feature in this process is that

the basilar membrane separates sounds into different frequencies because of its intrinsic mechanical properties. At one end it is relatively narrow and stiff (high frequency responding), and at the other it is wider and less rigid (low frequency responding). This frequency place representation of the neural activity represents our auditory system's transformation of spectral into spatial information and is called tonotopy. Downstream auditory nerve fibers reflect this tonotopic representation, and additionally encode the sound's intensity, timing, and amplitude envelope using action potentials. It does this through both the rate of spiking discharges (Ruggero, 1992), and their timing (Frisina et al., 1985; Heil and Irvine, 1997). However, while the auditory periphery encodes acoustic features, it is unknown how these signals give rise to perceptual information (Rauschecker, 1998).

As this neural representation ascends the auditory system, these signals project upstream in a number of parallel pathways through the cochlear nuclei, superior olivary nuclei, inferior colliculus, and medial geniculate body of the thalamus (Kandel, 2000). One might expect that with the many levels of processing between the periphery and cortex, neurons at higher levels perform increasingly complex operations to transform simple features into how we perceive sounds. Indeed, we know that the former is true when comparing the computations of the nerve fiber to the inferior colliculus. In addition to the preservation of tonotopy (Merzenich and Reid, 1974), the inferior colliculus also plays roles in sound localization (Benevento and Coleman, 1970; Wenstrup et al., 1986), and the encoding of sound duration, direction of frequency sweep, and amplitude modulation (Covey and Casseday, 1999; Eggermont, 2001).

However, while increasingly complex in its representation, it is thought that these subcortical structures primarily perform feature extraction rather than auditory object recognition (Ulanovsky et al., 2004). In fact, a recent study by Chechik et al. (2006) provided support for this idea by recording neural responses to vocalizations in the inferior colliculus, thalamus, and auditory cortex. Measuring responses in these three structures, they found that auditory cortical neurons, compared to those in the inferior colliculus, carry much less information about the spectral and temporal structure and more about the "*abstract notion of stimulus identity*" (Chechik et al., 2006). Therefore, based on these ideas and the role of cortical structures in higher cognitive functioning (Rauschecker, 1998; Naatanen et al., 2001; Scheich et al., 2007; Bieszczad and Weinberger, 2012), we chose the auditory cortex as a starting point for us to understand how changing a sound's behavioral relevance alters its neural representation, and how this subserves higher order areas in the detection, discrimination, and recognition of sounds.

1.1.2: The auditory cortex

In this section, we first discuss how studies have physiologically defined the different auditory fields. Doing so will help provide an understanding of how we characterize and distinguish the core areas of auditory cortex from higher order non-core auditory areas.

Similar to other sensory cortical areas, the auditory cortex shows a distinct laminar structure (Linden and Schreiner, 2003). In total, there are six layers that can be

anatomically defined, and different layers have distinct cell types, input and output connections (for detailed review see: Linden and Schreiner, 2003; Winer et al., 2005). The layers are numbered I-VI, with the former being the most superficial. For the core areas of auditory cortex, the predominant thalamic input comes from the ventral medial geniculate body and arrives at cortical layers III and IV (Cruikshank et al., 2002). In these layers, neurons are organized tonotopically and show robust responses to tones, have narrower frequency tuning curves, and respond to specific best frequencies (Merzenich et al., 1975; Reale and Imig, 1980; Hackett et al., 2011). In contrast, non-core or higher auditory areas receive the majority of its thalamic inputs from the medial or dorsal divisions, and typically respond more poorly to tones, but more robustly to noise and show more complex frequency tuning (Clarey, 1992; Stiebler et al., 1997; Kaas and Hackett, 1998). These distinct differences are fundamental to how studies characterize the auditory cortex and divide it into its core and non-core areas.

As we mentioned above, the different auditory fields and their locations are characterized physiologically by their tonotopic structure. This is performed using a frequency mapping procedure, which consists of measuring the single and/or multi-unit responses to tones in layers III/IV, and obtaining a tonotopic map by defining a best/characteristic frequency at each location. The best frequency is the frequency that produces the strongest response across different intensities (Hackett et al., 2011), and the characteristic frequency is the frequency that can produce a response at the lowest intensity (Merzenich et al., 1975). For the most part, both methods yield similar results, where core areas show tonotopic organization, and non-core areas do not.

Using this mapping procedure, specific auditory fields within core and non-core areas have been distinguished in different animal species based on their responses to sounds. Across all mammals, the primary auditory field (A1) is considered a core area as it is tonotopically organized and responds robustly to tones. Outside of A1, the number of fields, anatomical location, and roles in sound processing becomes more complex depending on animal species. For example, in the mouse, macaque, cat, and gerbil, the anterior auditory field (AAF) is similar to A1 but typically lies rostral and shows an opposing frequency gradient (Stiebler et al., 1997). However, in the cat, mapping studies show the presence of two additional tonotopic fields, the posterior auditory field, and ventral posterior auditory field, both of which are nonexistent in the mouse and macaque monkey (Reale and Imig, 1980). The diversity of physiological or anatomical differences in the designation of auditory fields suggests that species differences may reflect the varying environmental or behavioral influences. Because this dissertation uses the mouse model to investigate auditory cortical processing in core areas, we will focus primarily on how the mouse auditory cortex has been defined.

One of the first complete studies on this came from Stiebler et al. (1997) who used characteristic frequency and defined the parcellations based on earlier work in the cat auditory cortex (Andersen et al., 1980; Reale and Imig, 1980). By recording multi-units from anesthetized mice, they found five different fields based on the frequency arrangement and response characteristics (Fig. 1.1). They were defined as A1, AAF, ultrasound field (UF), dorsal posterior field (DP), and the secondary auditory field (A2). The locations of A1 and AAF were characterized by their robust responses to tones and

distinct rostral to caudal frequency gradients. This was confirmed by a more recent study that used a high density mapping procedure showing a clear increase in a multi-unit's best frequency from caudal to rostral in AAF, and then a clear reversal in the gradient going rostral to caudal in A1 (Hackett et al., 2011). In the dorsal-rostral area of auditory cortex, Stiebler and colleagues defined UF as a region that did not show a clear tonotopic gradient, but had much higher frequency responses (~46-70 kHz) compared to A1 and AAF. Although not tonotopic, UF was considered a core area because of its similarities to other core areas in its connections with the ventral medial geniculate body and inferior colliculus (Hofstetter and Ehret, 1992). Finally, both DP and A2 fields were considered non-core areas because they had more complex frequency tuning, lacked tonotopic arrangement, and their characteristic frequencies were difficult to determine. Thus, based on the physiological characteristics of multi-unit responses, the mouse auditory cortex has a number of distinct fields, several of which are analogous to other animal models (i.e. cat, gerbil, macaque).

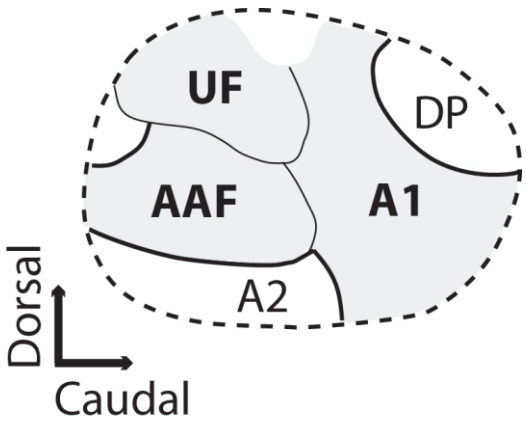


Figure 1.1: Schematic of the auditory cortical fields in the mouse. The primary areas consist of AAF, A1, and UF. Light grey areas represent the core areas of auditory cortex.

1.2: The auditory cortex and sound processing

Although we have a basic knowledge across animal species about the physiological organization of auditory cortex according to best frequency, we still do not completely understand its role in sound processing. Early lesion studies made great progress in developing our knowledge of the function of auditory cortex. David Ferrier was one of the early pioneers who would lesion brain areas found by generating an auditory startle response through stimulating the cortex (Heffner, 1987). His initial experiments suggested that bilateral lesions in monkeys would render them unresponsive to sounds, while unilateral lesions would render only the contralateral ear unresponsive to sounds. However, a number of later studies demonstrated that these lesions produced more specific changes that were species dependent (Heffner and Heffner, 1986, 1990; Peretz et al., 1994; Ohl et al., 1999). For example, in a localization task, bilateral auditory cortical lesions affected the behavioral performance in cats (Strominger, 1969), macaques (Heffner and Masterton, 1975), and ferrets (Kavanagh and Kelly, 1987), but not in rats (Kelly and Glazier, 1978; Kelly and Kavanagh, 1986) or hedgehogs (Ravizza and Diamond, 1974). This difference in behavioral performance suggests that the importance of sound localization may be species specific, and emphasizes the need to study auditory cortical function utilizing model systems that exploit natural behavior. In the following sections, we will briefly discuss a general hypothesis about the role of auditory cortex in encoding sounds, motivate the need to study species-specific vocalizations, and introduce the importance of using a neuroethological approach.

1.2.1: Communication sound processing

The mammalian auditory system has been studied extensively, and much of this comes from recording cortical responses to specific acoustic features such as changing tone frequencies (Merzenich et al., 1975; Merzenich et al., 1976; Kelly et al., 1986), frequency sweeps (Whitfield and Evans, 1965; Glaser, 1971; Mendelson and Cynader, 1985; Mendelson et al., 1993), amplitude modulations (Creutzfeldt et al., 1980; Phillips and Hall, 1987), intensities (Schreiner et al., 1992; Taniguchi and Nasu, 1993; Heil et al., 1994), and durations (Galazyuk and Feng, 1997; He et al., 1997). Through these studies and numerous others, current data supports the idea that auditory cortical neurons are diverse in their responses and can be temporally precise (Heil and Irvine, 1997) or "*sluggish*" (Ulanovsky et al., 2004), and can respond linearly or non-linearly to different sound features and their combinations (Bar-Yosef et al., 2002; Machens et al., 2004). Because of this response variability, it has been suggested that the auditory cortex may play a role in the representation of auditory objects or the behavioral relevance of sounds (Nelken et al., 2003; Nelken, 2004; King and Nelken, 2009).

This diversity in responses may reflect the role of auditory cortex in tasks such as communication. Communication sounds are a class of signals that are acoustically complex in their spectral and temporal features, and carry biological importance (Wang, 2000; Kanwal, 2006; Suta et al., 2008). These distinct characteristics of vocalizations could mean that the neural representation for these sounds differ from other non-behaviorally relevant acoustic signals. Indeed, in the primary auditory cortex of the awake marmoset, neurons respond more strongly to vocalizations compared to

equivalently complex time-reversed versions of these sounds (Wang et al., 1995). This suggests that specific neurons in the marmoset show combination sensitivity between the spectral and temporal features of the species-specific calls. In addition, Wang and colleagues also demonstrated that in contrast to the marmoset, auditory cortical neurons in the cat showed no differences between the call and its time-reversed pair (Wang and Kadia, 2001). This selectivity demonstrates the importance of studying species-specific vocalizations, and motivates the use of a neuroethological approach to studying communication.

1.2.2: Neuroethology and studying sound processing

Previously, we discussed a number of species-specific differences in auditory processing. This included evidence of species-specific neural processing of vocalizations, lesions that produce species-specific deficits in sound localization, and distinct differences in the physiological organization of the auditory cortex. This species variability provides strong evidence that to study communication processing, a neuroethological approach is necessary. For review, neuroethology is the study of the neural mechanisms underlying natural behavior, and takes into consideration the hypothesis that neural responses to the same sounds can differ because of evolutionary pressures or experience-dependent factors (Gentner and Margoliash, 2002). These factors could explain the neural response differences between the cat and marmoset in response to marmoset vocalizations mentioned previously (Wang et al., 1995; Wang and Kadia, 2001). Therefore, because the goal of this thesis is to understand the auditory

cortical representation of socially acquired vocalizations, it is necessary to use a model system where the vocalizations are communicative and naturally acquired during an individual lifetime.

It is argued that a disadvantage with this approach is its inability to probe the entire acoustic space of neural responses. We contend that studying neural processing using complex vocalizations and fixed acoustic features are equally important. Together, they provide greater insight into how the auditory cortex integrates the representation of both acoustic structures and auditory objects, and how this integration plays a role in sound perception. However, in studying the neural coding of communication sounds, a neuroethological approach may be the most appropriate. This is based on the idea that, "*one cannot study how the cortex codes Chinese in a native English speaker who never learned Chinese.*" (Wang, 2000).

1.3: Mouse ultrasonic communication system

The mouse ultrasonic communication system provides a neuroethological approach to address how the auditory cortex changes to encode behaviorally relevant sounds. In the mouse, studies have shown that the animals communicate through sound and use these signals to convey either their current physiological state or the environmental conditions (Brudzynski, 2005; Ehret, 2005; Portfors, 2007). Both pups and adults emit ultrasonic vocalizations (USVs), and these sounds are thought to be communicative in nature because they have been described as signals that transfer the sender's affective state to the receiver, subsequently altering the receiver's behavior

(Ehret and Haack, 1981; Hammerschmidt et al., 2009; Shepard and Liu, 2011). For example, a mouse pup displaced from its nest will emit ultrasonic isolation calls. These calls are thought to initiate searching behavior in the mother to retrieve the pup back to the nest (Noirot, 1972; Smotherman et al., 1974). Unlike virgins with no pup experience, these calls have been shown to be behaviorally important to the mother (Ehret, 1982, 1987). Thus, the mouse ultrasonic communication system presents an opportunity to understand not only how natural sounds are encoded in the brain, but how this representation changes when a sound acquires behavioral relevance through social interaction.

1.3.1: Infant and adult mouse ultrasounds

Pups and adults both emit USVs, but are distinct in their perceptual and acoustic representation. The adult USV is thought of as a courtship call that is produced by the male when an adult female is nearby (Holy and Guo, 2005), and plays a role in attracting the female (Pomerantz et al., 1983). In support of this, a recent study demonstrated that virgins were attracted to the playback of male vocalizations, but not to artificial pup calls (Hammerschmidt et al., 2009). This is likely because the behavioral response to adult calls is innate (Shepard and Liu, 2011), and virgin females do not recognize pup calls until they have received sufficient pup experience (Ehret et al., 1987).

Unlike adult calls, pup USVs are sounds emitted from newborn mice and are evoked by changes in their body temperature, isolation from the nest, contact with adults and littermates, and rough handling. In particular, the isolation sounds are

communicative and facilitates a mother's ability to localize a pup to perform retrieval back to the nest (Ehret, 2005). In addition, while adult calls are innately recognized, pup calls are acquired through pup experience during post-partum days 3-13. Pups call very little in the first three days after birth, but then increase their call rate on day four (Noirot, 1972). From day 4-13, the calling rate exhibits an inverse U-shaped function with the peak between days 7 and 9 (Hahn et al., 1998). Through this experience, mothers and cocarers (virgins with pup experience) recognize and preferentially approach a speaker playing back pup calls over a neutral sound (Ehret, 1982, 1987; Ehret et al., 1987). In both the pup and adult call communication paradigms, the female mouse acts as the receiver. Therefore, because the two calls differ in how they are acquired (innate versus experience), we can begin to understand how a change in the pup calls' behavioral relevance selectively affects auditory coding.

Studies now clearly show that pup isolation calls can elicit a search and retrieval by the mother, and will respond to a large variety of ultrasounds. In particular, the lactating mother has been shown to prefer USVs and synthetic calls with specific acoustic parameters (Ehret and Haack, 1981; Ehret, 1987). This suggests that the acoustic features are important and may facilitate the discrimination of pup and adult ultrasounds. In support of this, a study by Liu et al. (2003), demonstrated that the two USVs were acoustically separable in its spectral and temporal features (Figure 1.2; Liu et al., 2003). Figure 1.2 shows the contour plots of the joint distributions for all the recorded pup isolation calls between post-partum days 5-12 and adult encounter vocalizations. In addition, Liu and colleagues found that the calls were different in their

call repetition rates (100 ms for adults, 180 ms for pups). Clearly then, both pup and adult vocalizations could be distinguished by auditory cortical neurons based on these differences in their spectral and temporal features. However, it is not yet understood whether the auditory cortex is solely representing these acoustic features or if its encoding reflects the differences in perceptual relevance of the USVs.

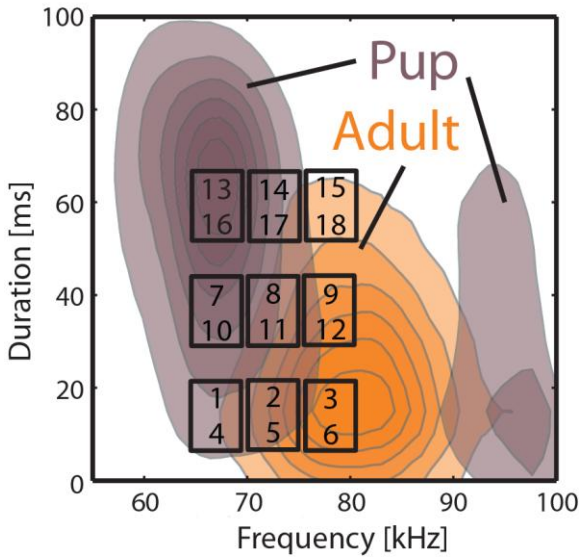


Figure 1.2: Contour plots of the joint distributions of call frequencies and durations of adult (orange) and pup (brown) USVs. The contours represent 0.19, 0.37, 0.56, 0.75, and 0.93 probabilities. The two classes of calls are distinct in their acoustic features, but overlap in the frequency-duration space. The numbers in the boxes correspond to the 18 pup calls used in this thesis for playback to the animal (see Appendix B). Adult calls used in this thesis were taken from the same regions as the pup calls in this frequency-duration space. Figure was adapted with permission from Robert C. Liu from the paper: Liu RC, Schreiner CE (2007) Auditory cortical detection and discrimination correlates with communicative significance. PLoS Biol 5:e173.

1.3.2: The role of experience and hormones on pup call preference

In the auditory system, reproductive hormones can play a significant role in regulating response properties in humans (de Boer and Thornton, 2008; Song et al.,

2008), rodents (Coleman et al., 1994; Willott et al., 2006), birds (Maney et al., 2006; Tremere et al., 2009; Remage-Healey et al., 2010; Tremere and Pinaud, 2011), frogs (Goense and Feng, 2005; Miranda and Wilczynski, 2009), and fish (Sisneros and Bass, 2003). For example, reproductive hormones contribute to the maintenance of cognitive function in verbal memory (Sherwin, 2003) and facilitate auditory processing for species-specific vocalizations (Maney and Pinaud, 2011). However, much less is known about how these intrinsic physiological factors work with extrinsic factors like pup experience, to affect the sensory representation of behaviorally relevant stimuli.

Unlike past studies which have focused independently on the effects of either learning paradigms or reproductive hormones on cortical processing, this model system provides the opportunity to explore how both these factors work together to alter the representation for stimuli learned through social interaction. While both mothers and cocarers can behaviorally recognize pup ultrasounds, a natural question is how the physiological changes associated with motherhood affect this learning. In rats and mice, it is now understood that while mothers can retrieve and perform maternal behavior on post-partum day one, virgins that are co-housed with mothers during pregnancy and parturition (cocarers) need several days of pup experience before exhibiting equivalent maternal behavior (Ehret, 1989; Numan and Stolzenberg, 2009; Stolzenberg and Rissman, 2011). In addition, Ehret and Koch (1989) demonstrated in a sound recognition task that both mothers and virgins preferentially approach a speaker playing back ultrasounds after pup weaning (21 days of pup experience); however, 1 month later, only mothers could still recognize the calls. Overall, these results show that both the

onset and maintenance of maternal behavior or pup call recognition are affected by the physiological differences between mothers and cocarers. Whether these physiological differences affect auditory processing itself remains an open question.

1.4: Plasticity and the auditory cortex

Thus far, we have discussed the basics of how a sound is transformed into neural signals, the importance of using species-specific vocalizations in studying the representation of sounds, and a unique model system that will help us explore these areas. However, one of the primary goals of this thesis is not just to understand how natural sounds are encoded, but also how this neural code changes when a sound acquires behavioral relevance. In the following sections, we will briefly introduce our current understanding of different forms of experience dependent plasticity. Then we will discuss evidence of plasticity related to species-specific vocalizations and focus in more detail on the maternal model.

1.4.1: Experience dependent plasticity

There is evidence now that the adult auditory cortex is not static, but that different forms of experience can alter its response. Early on, much of this understanding came from studying learning-induced plasticity using classical conditioning. Classical conditioning is a form of learning that consists of presenting a conditional stimulus (CS, e.g. sound) with an unconditional stimulus (US, e.g. foot shock) in succession until the CS evokes the same response as the US. Using this paradigm,

evidence of auditory cortical plasticity was found in the cat by Galambos et al. (1955), where pairing an auditory CS with a shock led to increases in the amplitude of CS-evoked field potentials across auditory cortex. These findings were further strengthened by a number of fear conditioning studies from Weinberger and colleagues, showing frequency response plasticity in single neurons, multi-units, and local field potentials (Diamond and Weinberger, 1989; Bakin and Weinberger, 1990; Galvan and Weinberger, 2002). In particular, these studies demonstrated that there was both an increase in the response to the frequency of the CS, but also a decrease in the pre-training best frequency response. Additionally, they demonstrated that these changes were not a result of random sounds and shocks that were unpaired. While this work helped to establish the idea that learning could result in adult auditory cortical plasticity, a number of questions remain. Most importantly, whether this form of plasticity is conserved across all types of learning, and how these changes might facilitate perception and behavior.

In addition to classical conditioning, a number of studies have explored the effects of operant conditioning and task dependence on the auditory cortical plasticity. Operant conditioning refers to the animal reinforcing its behavior by either the removal or introduction of a positive or negative consequence. Using this paradigm, Fritz and colleagues trained ferrets to perform either tone detection or a two-tone discrimination in an active avoidance conditioning task (Fritz et al., 2003; Fritz et al., 2005). In this particular task, the animals were allowed to lick a waterspout, and trained to stop when the target sound was played to avoid a shock. Recording auditory cortical neurons in the

awake ferret auditory cortex, they found that in the tone detection task there was an enhancement of the response at the target frequency (Fritz et al., 2003; Fritz et al., 2005). In contrast, neurons in the two-tone discrimination task changed to enhance its response at the target frequency and suppress its response at the reference frequency (Fritz et al., 2003; Fritz et al., 2005). Therefore, these studies presented evidence of short-term adult auditory cortical plasticity using operant conditioning in the awake animal, and that the nature of the neural changes depended on the specific behavioral task.

Behaviorally, it is clear that the way in which an animal learns a sound's meaning under an operant or classical conditioning task differ. In spite of this, both approaches showed the same general changes in demonstrating an enhancement of the neural response at the target frequency. This similarity suggests that the neural changes may reflect the fact that both involve associative learning. That is, the animal learns to associate a behavior with a particular stimulus. Therefore, auditory cortical plasticity may depend on the specific stimulus feature (tone frequency in both paradigms) that yields the most favorable behavioral outcome. Indeed, there is evidence that frequency-specific neural plasticity is not the only outcome of learning, but that auditory cortical plasticity correlates with the most behaviorally salient features. For example, studies have found auditory cortical changes in the neural responses to sound intensity (Polley et al., 2006), frequency modulation (Wetzel et al., 1998; Ohl et al., 1999; Ohl et al., 2001), and temporal modulation (Beitel et al., 2003; van Wassenhove and Nagarajan, 2007). While these changes were more indicative of the target stimulus, recent work by

David et al. (2012) also demonstrated that the auditory cortex changed in ways that were specific to how the sound was learned. Using an aversive and an appetitive instrumental learning task, they found that responses to the target sound changed but in opposite directions. Specifically, there was an enhancement in the neural response after aversive conditioning and suppression after appetitive conditioning. Clearly then, these studies demonstrate that the adult auditory cortex can be "retuned" to account for changes in behavioral relevance, and that the type of plasticity reflects the most behaviorally salient acoustic feature.

Even with the vast evidence of auditory cortical plasticity, there is still a limited understanding of how these changes relate to perception and behavior. A number of more recent studies have begun to clarify this by recording neurons from awake behaving animals. One study in particular linked changes in the auditory cortex with behavioral performance. Using a positive reinforcement task, Jaramillo and Zador (2010), explored how expectation of a sound could alter behavioral performance in a discrimination task, and whether the auditory cortex would reflect these changes. Altogether, they found that temporal expectation of the target stimulus improved behavioral performance, silencing auditory cortex by applying a $GABA_A$ receptor agonist decreased this performance, and that neuronal activity in the auditory cortex correlated with each animal's behavioral ability. These findings further support the idea that auditory cortex is important in sound recognition, and that changes in the sound task can result in auditory cortical plasticity, which may be important for improving behavioral performance.

1.4.2: Auditory cortical plasticity and communication processing

Based on the studies mentioned previously, it is evident that the adult auditory cortex can undergo changes in its representation of specific acoustic features, and that these changes can depend on the behavioral task. How this translates to our learning of vocalizations is still not understood. Species-specific vocalizations are generally complex in their spectral and temporal characteristics (Liu et al., 2003). In addition, while training paradigms typically involve a single behavior, such as detection or discrimination, communication in real contexts involves both tasks and is learned through social interaction. For these reasons, a primary goal of this thesis is to begin to understand how the auditory cortex changes in response to socially acquired vocalizations.

A number of studies have demonstrated that mothers, but not pup-naïve virgin females, recognize the communicative significance of pup USVs (Ehret, 1982, 1987; Ehret et al., 1987), but our understanding is still limited as to whether this difference is correlated with neural changes as early as sensory cortex. Much of the work thus far has consisted of recordings from groups of neurons (multi-unit, MU) in the anesthetized mouse auditory cortex of both mothers and virgins. In two separate studies, Liu and colleagues demonstrated that there were distinct differences in the MU activity between mothers and virgins in their cortical entrainment, and their detection and discrimination information of pup calls (Liu et al., 2006; Liu and Schreiner, 2007). While these results were the first to show that changes in the auditory cortex correlate with the pup vocalizations' behavioral relevance, there remains a number of open questions.

Importantly, future studies should address whether these changes between mothers and virgins persist when looking specifically at single neurons in the awake animal.

1.5: The auditory cortex of awake animals

Much of our understanding about how neurons in the auditory cortex process sounds comes from studying animals under anesthesia. In electrophysiology, anesthesia is generally used to facilitate our ability to record single neurons from animals *in vivo*. However, a number of studies have begun to uncover the distinct differences in how auditory areas encode stimuli when comparing neural activity in the anesthetized to the awake state (Gaese and Ostwald, 2001; Syka et al., 2005; Ter-Mikaelian et al., 2007). This issue of sensory processing under different anesthetic conditions is not isolated to the auditory system, as studies in other areas such as the visual and somatosensory systems have also demonstrated unique stimulus responses when comparing the anesthetized to the awake preparation (Chen et al., 2005; Greenberg et al., 2008). In fact, the study by Greenberg et al. (2008) in the visual cortex stated explicitly that, "*brain activity during wakefulness cannot be inferred using anesthesia*", further emphasizing the importance of studying how the brain works in the awake state. While it is important not to ignore these effects in the interpretation of cortical activity and its functional importance, a number of studies have also shown similarities between the two states depending on the brain area and type of stimulus encoding being studied (Ter-Mikaelian et al., 2007; Schumacher et al., 2011). This suggests that both preparations can provide valuable information about how sensory stimuli are encoded;

but it is imperative to understand the type of question asked, under what experimental conditions brain area the study is conducted, and how this affects the interpretation.

Compared to subcortical structures, work investigating the effects of anesthesia on primary auditory cortical processing of both tones and more complex sounds, such as vocalizations, suggest the need for using an awake preparation. In response to tones, both Gaese and Ostwald (2001) and Zurita et al. (1994) demonstrated that there is an overall suppression of intrinsic excitability as well as a sharpening of frequency responses in neurons, which may depend on anesthesia. To more complex sounds, such as sinusoidally amplitude-modulated tones, Ter-Mikaelian et al. (2007) demonstrated that when considering the spike timing and variability, anesthesia increased the auditory cortical temporal precision. Finally, in response to species-specific vocalizations, both Syka et al. (2005) and Huetz et al. (2009) demonstrated differences in the temporal coding and strength of responses in the auditory cortical coding of communication sounds. Taken together, these studies suggest that recording the neural activity in the awake animal is critical in our exploration of auditory cortical processing of natural vocalizations and how this encoding transforms to reflect a sound's change in behavioral relevance. We thus have employed a technique that allows us to record from the auditory cortex of awake-restrained mice in our study of communication sound processing.

1.6: Summary and Objectives

The aim of this dissertation is to utilize the mouse ultrasonic communication system to investigate how the auditory cortical representation of natural sounds is transformed when they become behaviorally relevant. In order to study this question, we record both neurons and local field potential responses to sounds in the awake mouse auditory cortex.

We begin chapter 2 by first exploring the differences in cortical responses to pup calls for mice that do (mothers) and do not (virgins) recognize pup calls as behaviorally relevant. We demonstrate that in the awake mouse, the auditory cortical call-inhibited responses undergo changes that correlate with the behavioral relevance and hypothesize a possible function for this plasticity. In chapter 3, we build off this result by uncovering how pup experience during motherhood (mothers) or experience alone (cocarers), influence the inhibitory plasticity. Based on these differences, we design a closed loop feedback behavioral experiment to test whether the cortical changes are correlated with behavioral performance. Then, in chapter 4, we begin to explore how call-excited neurons encode vocalizations. We proceed by creating a model that predicts the first spike latency of natural calls. We find that this model segregates out a specific subset of excited neurons that are “acoustically faithful” and another that reflect the difference in a sound’s behavioral meaning. In chapter 5, we summarize the results of the dissertation, discuss the significant contributions, and demonstrate the design for an auditory cortical implant that can record single neurons to address future studies.

CHAPTER 2

INHIBITORY PLASTICITY IMPROVES THE CORTICAL DETECTION OF VOCALIZATIONS

All results in this chapter were published previously in Neuron: Galindo-Leon, E.E., Lin, F.G.*, Liu, R.C. (2009) Inhibitory plasticity in a lateral band improves cortical detection of natural vocalizations. Neuron 62: 705-716 (*co-first authors). Any citation of work in this chapter should properly cite the above article.*

An important task of the auditory cortex is to process behaviorally relevant vocalizations. Much progress has been made in advancing our understanding of how the auditory system encodes the acoustic features of such sounds (Wang et al., 1995; Nelken et al., 1999; Nagarajan et al., 2002; Wallace et al., 2005; Huetz et al., 2009). However, it is still unclear how encoding changes as vocalizations become behaviorally relevant. To study this, we used a previously described ultrasonic communication system between mouse pups and adult females (Ehret et al., 1987; Ehret, 2005). In this chapter, we explored the response differences in single units (SU) and local field potentials (LFP) in the awake mouse auditory cortex, and presented pup isolation calls to animals that either do (mothers) or do not (virgins) recognize the sounds as behaviorally relevant. By identifying the neural plasticity that correlates with the change in behavioral relevance of pup calls, we hope to expand our understanding of how the

brain changes its representation of communication sounds learned through social interaction, and how this might functionally facilitate detection or discrimination.

2.1: Introduction

The perception of species-specific vocalizations is a critical neural process due to its roles in social interaction, reproduction, and survival (Seyfarth et al., 1980; Barfield and Thomas, 1986; Brudzynski and Chiu, 1995; Wang, 2000; Ehret, 2005). The auditory cortex is thought to be essential in the processing of these sounds (see 1.2). Recent studies have begun to improve our understanding of how auditory cortical neurons at both the population and single neuronal level encode sounds and contribute to these processes. At the population level, studies suggest that distributed cortical excitation can help improve signal-to-noise for downstream processing (Medvedev and Kanwal, 2004; Wallace et al., 2005; Wang et al., 2005; Liu and Schreiner, 2007). At the cortical neuronal level, precisely-timed inhibitory input (Razak and Fuzessery, 2006, 2010) and facilitatory combination-sensitivity both help shape excitatory selectivity for calls (Fitzpatrick et al., 1993; Rauschecker et al., 1995; Razak et al., 2008; Washington and Kanwal, 2008). However, this picture ignores a possible role for call-evoked inhibition at the population level – an issue that has been overlooked in most vocalization studies.

Inhibition plays an important role in the encoding of sound stimuli and can shape the spectral and temporal response properties of auditory cortical neurons (Wehr and Zador, 2003; Zhang et al., 2003; Tan et al., 2004; Sadagopan and Wang, 2010; O'Connell et al., 2011). Although most experience-dependent plasticity studies have targeted how

excitatory responses change their sound encoding, there is a growing interest in how this also affects inhibition. For example, a recent study in rats trained on a sound detection task found that engagement in the auditory task suppressed overall responses in the auditory cortex (Otazu et al., 2009). These results led them to suggest that task-engaged suppression may allow the primary auditory cortex to inhibit neurons irrelevant to the task. In addition, a study in ferrets trained on a conditioned avoidance tone detection task, found that while cells tuned near the target tone were enhanced, those tuned far from it became suppressed, suggesting that this differential plasticity enhances the representation of the target tone (Atiani et al., 2009). Together, these studies demonstrate that response suppression is an outcome of experience dependent plasticity and plays an important role in the distributed sound processing in primary auditory cortex. However, the question remains whether these forms of plasticity reflect what occurs when communication sounds are learned within a natural context.

Auditory cortical plasticity can depend on how sounds are learned and responses can be enhanced or suppressed based on the reinforcement conditioning (David et al., 2012). In addition, species-specific vocalizations can invoke a differential cortical response when comparing two different animal species (Wang and Kadia, 2001). As most communication sounds are learned through social interaction, these studies emphasize the importance of studying how the auditory cortex encodes behaviorally relevant vocalizations through a neuroethologically motivated approach. By utilizing a mouse model communication system (see 1.3), we begin to address this question by

recording both excitatory and inhibitory SU responses in the auditory cortex of awake mice.

Here, we report on electrophysiological recordings in the auditory cortices of fully awake, head-restrained mice. We focus on how neural responses could contribute to the collective detection of a class of natural pup calls, by contrasting pooled responses to all calls between virgins and mothers. We found that communication sounds could generally excite as well as purely inhibit cortical spiking. Comparing animal groups, pooling the various forms of evoked excitation did not reveal a significant response difference during the calls. However, the timing and strength of call-evoked inhibition was systematically altered in mothers – particularly for the frequency band *lateral* to the ~60-80 kHz frequency of the pup whistles. We suggest this lateral band inhibitory plasticity as a mechanism to enhance the signal-to-noise in the neural population representation of a pup call, and hypothesize this would improve the downstream detection of calls.

2.2: Methods

The electrophysiological recording, surgical methods, acoustic stimuli, and statistical tests used are described in appendices A, B, and C. In this chapter, the data from both SUs and LFPs comes from eight virgin females and seven mother CBA/CaJ mice, all between 14 and 24 weeks old at the time of surgery. For mothers, recordings were taken 0 to 2 weeks following pup weaning. An animal name list of the associated animals used for this chapter in appendix A.

2.2.1: Single unit analyses

In response to pure tones at 60 dBSPL, a BF and tuning bandwidth was characterized for each excited SU. To define the BF, the PSTH response (5 ms bin width) to all 40 logarithmically spaced frequencies (6.5-95 kHz) was used to manually identify a response window. An excitatory response window was determined to be the times at which the spike rate begins to increase and when it returns to the spontaneous rate. The frequency yielding the maximum spike rate response in this window was then classified as the SU's BF. Using the BF, a half-max value was then determined by finding the halfway point between the spontaneous rate and the maximum spike rate.

In response to natural vocalizations, latency and duration of the PSTH, and the strength of response were characterized for each SU. SU response latency was determined by finding the half-max or half-min of the smoothed spike rate (convolution of individual spikes with a Gaussian smoothing function, 5 ms standard deviation). The half-max (half-min) was determined based on the spike rates at stimulus onset and at the maximum (minimum). The response latency was the time relative to stimulus onset for the smoothed, pooled spiking response to reach the halfway point. The duration of SU inhibition was the time over which the smoothed spike rate stayed below the half-min value. Similarly, the duration of SU excitation was the time over which the smoothed spike rate stayed above the half-max value.

To determine if there were differences between animal groups in the pooled spike rate for call-excited or -inhibited SUs, each smoothed, time-dependent spike rate function was normalized by the average spontaneous rate during the blank trial and

then averaged the SUs together. The strength of SU excitation or inhibition was quantified by integrating the actual spike count over a period from 205 to 265 ms. This accounts for the shortest neural delay to the auditory cortex, and the longest duration pup call, 60 ms plus any offset responses.

2.2.2: Local field potential analyses

The LFP is usually analyzed in spectral bands – such as theta (~4-10 Hz), beta (~10-35 Hz) and gamma (~35-90 Hz) – consistent with an oscillatory view of neural activity. We took a complementary approach by instead studying the wide-band (2-100 Hz) signal. Although the LFP was spectrally peaked around 4-10 Hz, this nevertheless better preserves the shape of transients, such as those induced by the acoustic stimulation (Shah et al., 2004). Our analysis applied the Hilbert transform to each LFP trace to generate its *unique* analytic signal in the complex domain (Boashash, 1992; Pikovsky et al., 2001). We focused on the Hilbert phase trajectory, where specific phases approximately corresponded to specific shape features in the signal.

LFP phase precision is defined at each time point by the mean resultant length of the trial-by-trial wide-band Hilbert phases over the *N* trials:

$$\overline{R(t)} = \left| \frac{1}{N} \sum_{k=1}^{N} e^{i\phi_k(t)} \right| \qquad (2.1)$$

This quantity has also been called phase concentration (Lakatos et al., 2005), and is algebraically related to the circular variance (Mardia and Jupp, 2000) or phase reliability (Montemurro et al., 2008).

For tone responses, the percentage increase in tone-evoked phase precision over the virgin was defined as:

$$\%\ \text{Increase} = \frac{\int_{205}^{275} \overline{R_{mothers}(t)}dt - \int_{205}^{275} \overline{R_{virgins}(t)}dt}{\int_{205}^{275} \overline{R_{virgins}(t)}dt} \qquad (2.2)$$

The integration period was from 205 to 275 ms to account for the duration, including onset/offset ramps, of the tone. To determine whether there was a significant increase in the integrated phase precision, we performed a bootstrap. We sampled each distribution of sites for mothers and virgins separately with replacement 1000 times and found the 95% confidence interval. Differences were taken as significant when the confidence bound did not include 0.

2.3: Results

In order to investigate cortical responses to communication sounds in awake animals, we developed a head-restrained, electrophysiology preparation for mice (see Appendix A). We targeted recording locations using a stereotaxically-laid grid of holes

over auditory cortex. SUs and LFPs were first characterized by their responses to tones (Fig. 2.1, middle row). We classified SUs as tone-excited or -inhibited depending on whether spiking increased or *only* decreased following tone presentation, respectively; some SUs were tone-nonresponsive or not isolated during tone stimulation (Table 2.1). A best frequency (BF) was selected for each tone-excited SU by finding the frequency eliciting the greatest spike rate in a window around its peri-stimulus time histogram (PSTH) peak. A BF for each LFP site was determined similarly based on the largest average negative deflection within the first 100 ms after tone onset. In our data set, mothers and virgins were mostly similar in their distributions of both SU and LFP BFs. Recording sites were then similarly characterized by their responsiveness to a pool of 18 different pup ultrasounds (Appendix B) as pup call-excited (Fig. 2.1A and B), -inhibited only (Fig. 2.1C), or -nonresponsive.

2.3.1: Plasticity in SU responses

We found a larger proportion of SUs in mothers (35/47) compared to virgins (21/39) that were either excited or inhibited by pup calls (z-test, $z=1.81$, $p<0.05$, 1-tailed). In *both* animal groups though, about equal proportions of these responsive SUs showed either pure inhibition or some form of excitation (18 excited vs. 17 inhibited in mothers, z-test, $z=0$, $p>0.05$, 2-tailed; 10 vs. 11 in virgins, z-test, $z=0$, $p>0.05$, 2-tailed), indicating a previously under-reported prevalence of communication call-evoked cortical inhibition (Table 2.1). Focusing first on excited responses, we found a significantly higher proportion of tone-excited SUs in mothers (12/18) compared to

virgins (8/26) which were also pup call-excited (z-test, z=2.04, p<0.05, 2-tailed). Previous anesthetized MU studies found that nearly all excitation occurred near sound onset, and that mothers showed a better temporal alignment across BF ranges than virgins (Liu and Schreiner, 2007). By contrast, we now found that latencies to the excitation onset (half-maximum) varied over a wide range of times (t-test, t=0.43, df=25, p>0.05). This

Table 2.1: Classification of Single Unit Responses.

	Mothers	Virgins
Total Recorded SU's	47	39
Pup Call-Responsive	35/47	21/39
No Tone Data	9	3
& Pup Call-Excited	4	0
& Pup Call-Inhibited	3	0
& Pup Call-Nonresponsive	2	3
Tone-Responsive	32/38	35/36
Tone-Excited	18/32	26/35
& Pup Call-Excited	12/18	8/26
& Pup Call-Inhibited	1/18	6/26
& Pup Call-Nonresponsive	5/18	12/26
Tone-Inhibited	14/32	9/35
& Pup Call-Excited	2/14	2/9
& Pup Call-Inhibited	10/14	5/9
& Pup Call-Nonresponsive	2/14	2/9
Tone-Nonresponsive	6/38	1/36
& Pup Call-Excited	0	0
& Pup Call-Inhibited	3	0
& Pup Call-Nonresponsive	3	1

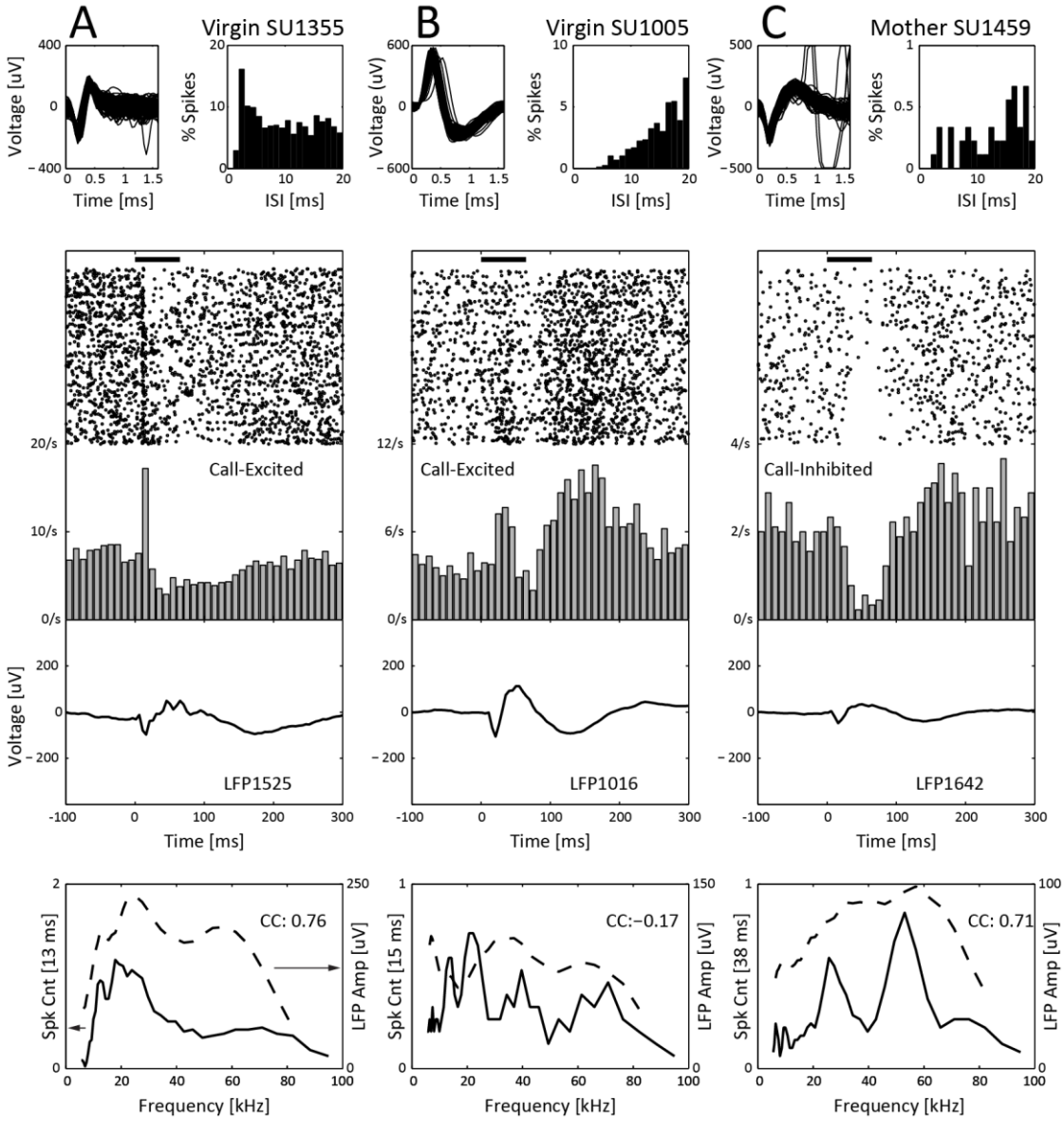


Figure 2.1: Three examples of different SU and LFP pairs co-recorded off the same electrode in response to pup calls and tones demonstrate the quality of our recordings. (A-C top row) Responses to pup calls. Trial-by-trial SU spike raster (upper) and 10 ms-binned PSTH (middle) for call-excited (A and B) and -inhibited SUs. The mean call-evoked LFP responses (bottom) at the same site are also shown. Stimuli presented during the interval denoted by the horizontal black bar. (A-C bottom row) Tonal tuning curves for SU spike count (solid black line, left axis) and the amplitude of the negative LFP deflection (dotted black line, right axis. Correlation coefficients (CC) between the full frequency tuning curves for SU and LFP varied from -0.58 to 0.95 over our tone-excited population of SUs, suggesting these neural signals do not reflect the same processes.

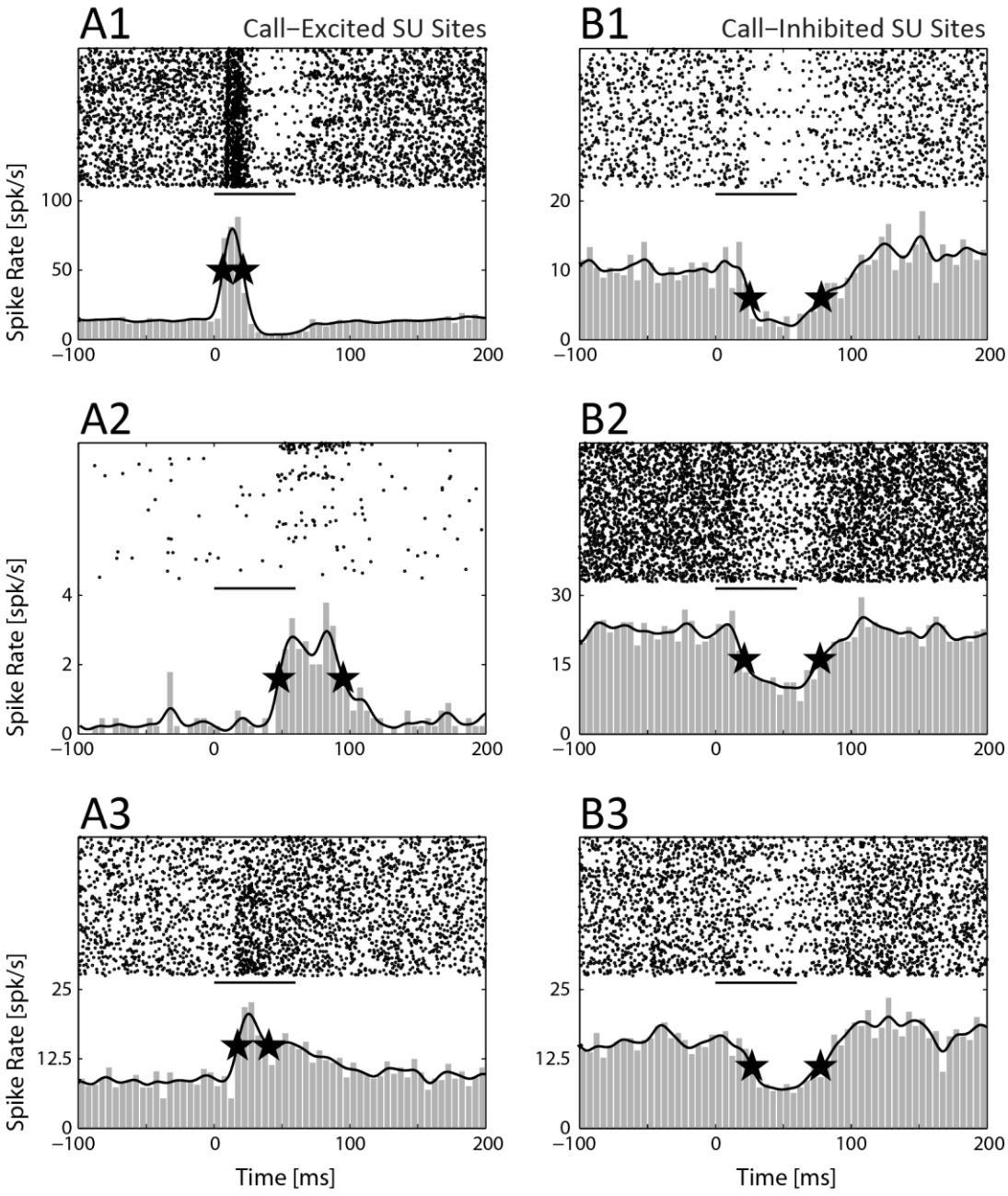


Figure 2.2: Examples of the diversity of call-excited SU responses (A1-A3) and the more stereotypical nature of call-inhibited responses (B1-B3) to pup calls. Black bar represents the stimulus period and stars show the half-maximum latency for call-excited responses, and the half-minimum latency for call-inhibited responses. Time between the two stars represents the duration of response. Examples show the raw (gray bars, 5 ms bins) and Gaussian-smoothed (black line) PSTH's.

reflected the many different ways by which excitation occurred in the awake animal, such as transient onsets and delayed offsets (for examples see Fig. 2.2). Furthermore, this variability in call-excited responses was also reflected in the strength of SU excitation, which was computed by integrating the normalized spike rate over the stimulus period (black bar in Fig. 2.3A1). We found no significant differences between mothers and virgins in the strength or duration of call-excited responses. Therefore, the distributions overlapped between the two animal groups (Fig. 2.3A2). In addition, by pooling all the call-excited responses in both mothers and virgins, we found that mothers had a greater magnitude of the time-dependent, population-averaged spike rate, but it was not significant (ANOVA $F = 0.74$, $p>0.05$). Although we did not find significant changes in the duration and strength of the pooled cortical excitation in awake animals, we cannot exclude the possibility that our methods may have failed to uncover more subtle changes in excitation due to the variability in spiking responses. Turning to inhibited cells, the time course of responses was more stereotyped (Fig. 2.2B1-B3), in contrast to excited cells (Fig. 2.2A1-A3).

The proportion of call-inhibited SUs in mothers compared to virgins was higher but not significantly so, whether all SUs (20/47 for mothers, 11/39 for virgins, z-test, $z=1.2$, $p>0.05$, 2-tailed) or only tone-inhibited SUs (10/14 for mothers, 5/9 for virgins, z-test, $z=0.33$, $p>0.05$, 2-tailed) were considered. More importantly, unlike excitation, the strength of each call-inhibited SU response (Fig. 2.3B1) was significantly stronger, and the duration of inhibition was significantly longer, so that mothers and virgins occupied distinct regions in the latency-duration plane (Fig. 2.3B2). Comparing the call-inhibited

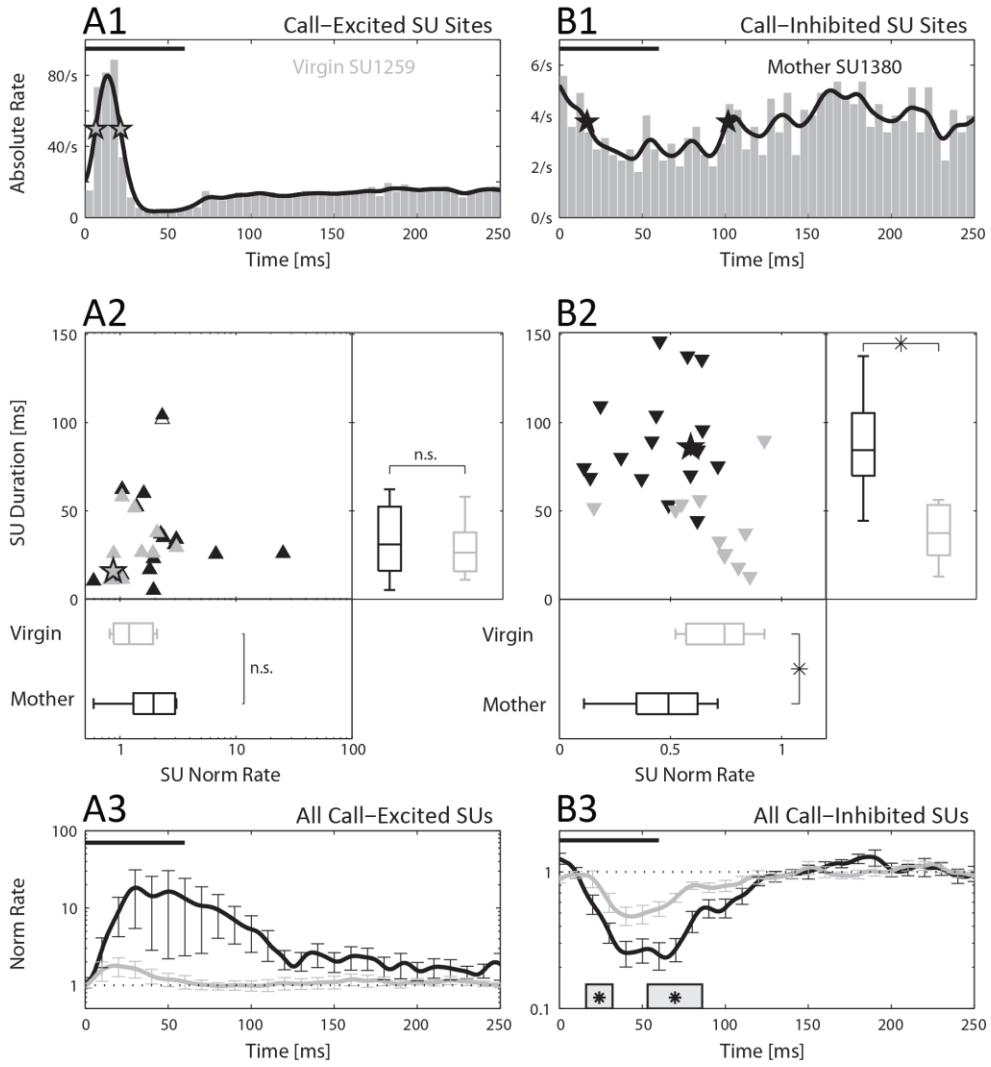


Figure 2.3: Mothers show stronger and longer call-inhibited SU responses compared to virgins. (A1 and B1) Examples of raw (gray bars, 5 ms bins) and Gaussian-smoothed (black line) PSTH's for a call-excited and -inhibited SU. The stars indicate half-max or -min values for calculating the latency and duration. (A2 and B2 scatter plot) Call-excited (upward triangles) and -inhibited (downward triangles) latencies and durations for mothers (black) and virgins (gray). Stars refer to SUs depicted in A1 and B1. Excited $N_{mothers}$=16, $N_{virgins}$=10. Inhibited $N_{mothers}$=17, $N_{virgins}$=11. (A2 and B2 bottom box plots) Group comparison of call-evoked excitatory and inhibitory normalized rate (SU strength). For this and later figures, box plots show lines at the lower quartile, median and upper quartile, and whiskers extending out to extreme data points that are not outliers. The difference between mothers and virgins for call-excited SUs was not significant (Mann-Whitney, U=80, $N_{mothers}$=16, $N_{virgins}$=10, $p>0.05$, 2-tailed), but was significant for call-inhibited SUs (t-test, t=2.9, df=26, $p<0.01$). For this and later figures, n.s./asterisk indicates a non-significant/significant comparison. (A2 and B2 right box plots) Group comparison of call-evoked excitatory and inhibitory durations. Mothers and virgins were not significantly different for call-excited SUs (t-test, t=.54, df=24, $p>0.05$), but were for call-inhibited SUs (t-test, t=4.7, df=26, $p<0.0001$). (A3 and B3) Population-averaged time course of spike rate normalized by the spontaneous rate, for call-excited and -inhibited SUs. In this and later figures, the gray rectangles marked by asterisks denote regions where significant differences were found between traces, and the error bars represent standard errors. Significant differences occurred only for call-inhibited responses. Dotted lines represent the baseline spontaneous rate.

PSTH, we also found that the inhibition was significantly deeper in mothers on a time point by time point basis (Fig. 2.3B3). Thus, in the awake mouse, average inhibition of cortical spiking by the class of pup calls was systematically longer and stronger in mothers compared to virgins.

What function might these changes have, and how specific are they for processing pup calls? Because our SU data alone did not permit us to fully address these questions, as explained below, we turned next to analyzing the LFP. This allowed us to both corroborate and extend our evidence for functionally-relevant inhibitory plasticity.

2.3.2: Plasticity in LFP responses

What causes a neuron to be inhibited or excited depends on the nature of its inputs from other neurons. To monitor this, we used the LFP, which is sensitive to the slow currents generated at excitatory and inhibitory synapses (Lopes da Silva and Kamp, 1987), and to spiking afterpotentials from neurons across this network (Logothetis, 2003). While distant, synchronous currents contribute to this signal, a recent study suggests that local contributions within ~250 um are dominant (Katzner et al., 2009). In principle, such local currents could be different around SUs that are being inhibited versus excited, not least of all because of the absence of the SUs own spiking in the former case. Indeed, simultaneous *in vivo* intracellular and extracellular recordings have shown that a SUs membrane potential often mirrors the LFP, so that depolarizations (hyperpolarizations) co-occur with relative negativities (positivities) in the extracellular potential (Kaur et al., 2005; Poulet and Petersen, 2008).

Given this possibility, we separately examined LFPs depending on whether a co-recorded SU was excited or inhibited by calls, in case the local network supporting each response type changed in a systematic way that would be reflected in the LFP. For this limited purpose, we focused only on the wide-band LFP (up to 100 Hz), rather than consider specific spectral bands (e.g. theta-band) individually (Galindo-Leon and Liu, 2010). We used standard time domain methods to generate the Hilbert phase time-series for each trial's LFP signal (Fig. 2.4A). This describes *when* various shape features in a signal – such as local minima (~π), maxima (~0 and 2π) and zero-crossings (~0.5π and 1.5π) – occur.

Using this analysis, we discovered plasticity in the call-evoked variability of the local network activity around call-inhibited SUs. To characterize variability, we constructed at each recording site a time-dependent histogram of the Hilbert phase trajectories across the different trials of all the calls (Fig. 2.4B). Before stimulus onset, the instantaneous Hilbert phase was essentially random. However, shortly after the onset of the calls, the Hilbert phase began concentrating near 0.5π to π, corresponding to the descent of the LFP toward its valley. The Hilbert phase distribution then became very sharp, and eventually widened back to a uniform distribution. We quantified the trial-by-trial variability of this local network response by a phase precision measure indicating how well aligned the instantaneous Hilbert phases from different trials were: a value of 1 at a particular time implies that all trials had exactly the same phase, while randomly distributed phases would yield a value of 0 (Fig. 2.4C). Examples of the LFP phase precision at call-excited and -inhibited sites are shown in the upper panels of Fig.

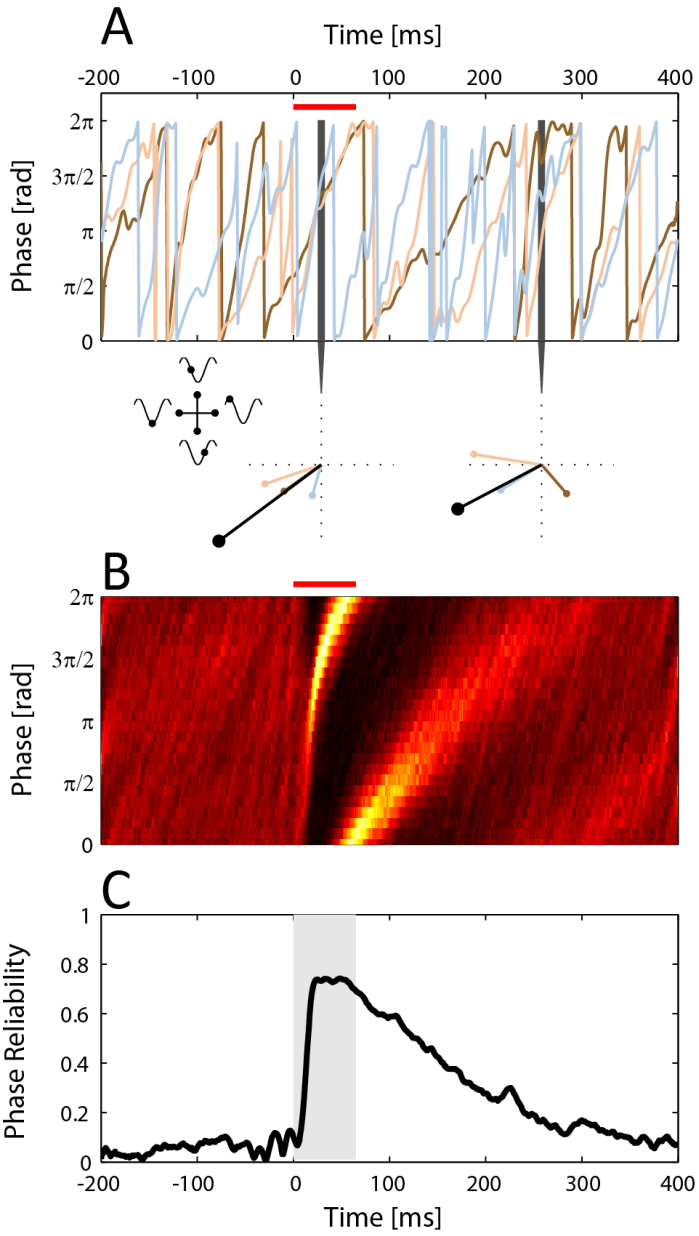


Figure 2.4: Example demonstrating the computation of the Hilbert phase time series for each LFP signal and the resultant phase reliability measure. (A) Examples of three LFP trials in response to pup call stimuli (blue, tan, brown), and at a given time (black vertical line), the three trials show distinct phase values at either 30 ms after the call stimuli (left coordinate axes) or at 260 ms (right coordinate axes). (B) The variability in the LFP shape depicted as a time-dependent probability histogram of trial-by-trial Hilbert phases. Brighter (darker) colors indicate higher (lower) probabilities for a specific phase at a specific time. (C) Based on the time-dependent probability histogram a phase precision value at each time point is computed, with one being the maximum value and indicating that all trials have the same Hilbert phase at a specific time point.

2.5A1 and B1, respectively. In general, call onset reliably reset the wide-band LFPs Hilbert phase and drove a rapid increase in the precision of the local network response at each site. Over time, this phase drifted as intrinsic, non-stimulus-locked fluctuations began dominating the signal again.

In order to detect systematic differences in local network variability, we compared the durations, max precision values, and the population-averaged phase precision time courses between animal groups. Mirroring the results found when comparing SU firing between mothers and virgins, the LFPs at call-excited SU sites (Fig. 2.5A2 and A3) were not different in any of these measures. In contrast, the LFP phase precision at call-inhibited sites in mothers reached a higher phase precision peak value, and stayed higher for longer, even beyond the duration of the pup call stimulus (Fig. 2.5B3). Thus, the local network near call-inhibited SUs in mothers responded trial-by-trial with more stereotyped activity than in virgins.

Furthermore, the LFP phase precision at call-inhibited SU sites in mothers increased even beyond the level of precision at call-excited sites. For call-inhibited sites in mothers, the precision became greater after about 18 ms, and stayed higher until 190 ms after stimulus onset (comparison not illustrated). This result indicates that local network level changes between virgins and mothers consistently increased the precision of presumed synaptic and membrane currents associated with inhibiting cortical neurons. In fact, on a site-by-site basis, we found that the stronger the SU inhibition, as measured by a lower normalized spike rate averaged across all calls (integrated over the maximum call duration), the greater the peak LFP phase precision (corrcoef, cc=-0.55,

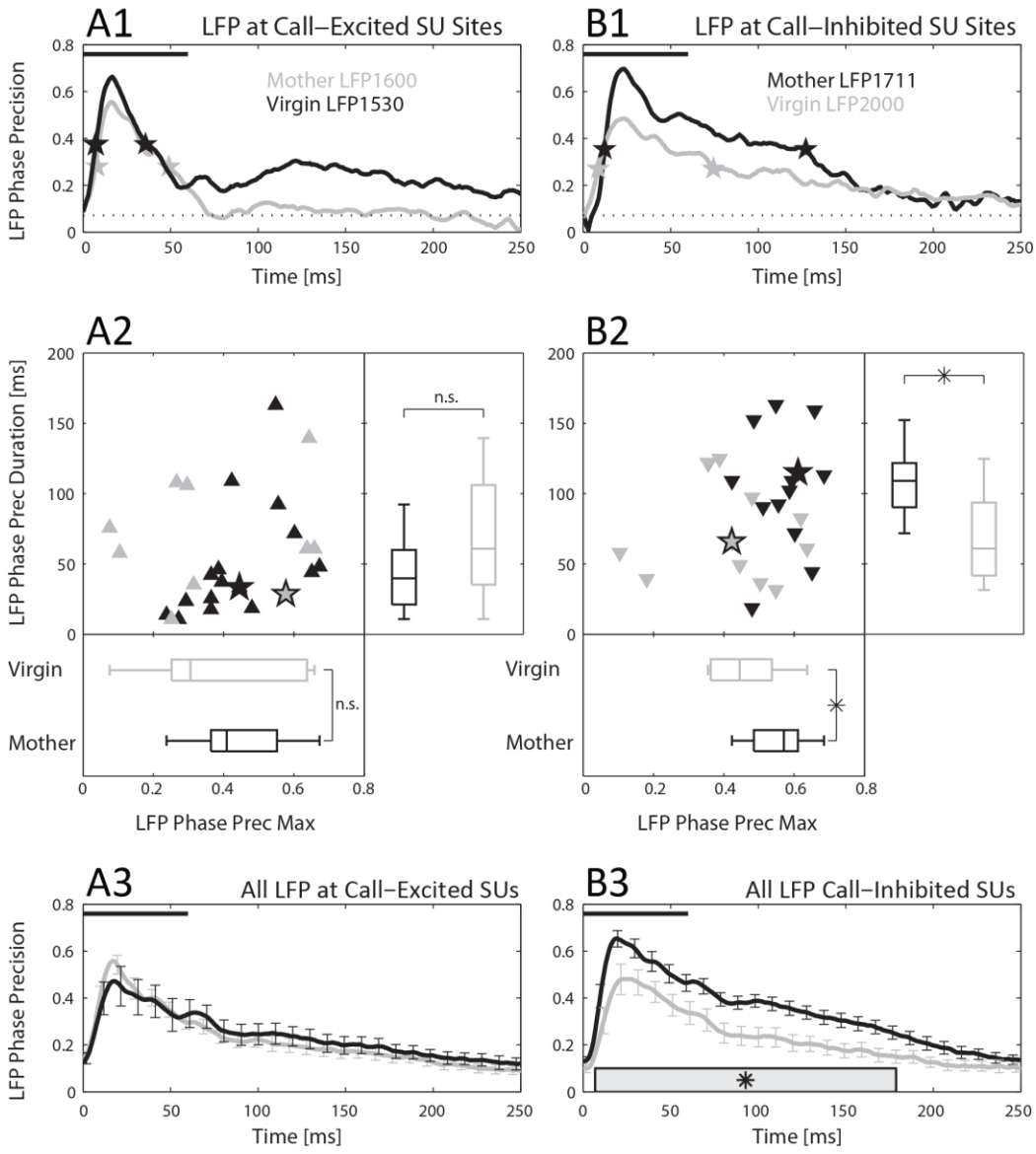


Figure 2.5: Mothers have a longer and greater LFP phase precision at call-inhibited but not -excited SU sites. (A1 and B1) Example LFP phase precision trajectories and their durations (stars) for both a call-excited and -inhibited site in a mother and virgin. Phase precision values lying above the dashed line are significant. (A2 and B2) Peak values and durations of the LFP phase precision at call-excited (upward triangles) and -inhibited SU sites (downward triangles) for mothers and virgins. Excited $N_{mothers}$=16, $N_{virgins}$=10. Inhibited $N_{mothers}$=14, $N_{virgins}$=11. (A2 and B2 bottom box plots) Group comparison of call-evoked LFP phase precision max values. Differences between mothers and virgins were not significant at call-excited SU sites (t-test, t=0.83, df=24, p>0.05, 2-tailed), but were significant at call-inhibited SU sites (t-test, t=2.4, df=23, p<0.05, 2-tailed). (A2 and B2 right box plots) Comparison of call-evoked LFP phase precision durations. Differences between groups were not significant at call-excited SU sites (Mann-Whitney, U=54, $N_{mothers}$=16, $N_{virgins}$=10, p>0.05, 2-tailed), but were significant at call-inhibited SU sites (t-test, t=2.3, df=23, p<0.05, 2-tailed). (A3 and B3) Population-averaged phase precision trajectories. LFPs from call-excited SU sites did not show a significant difference between mothers and virgins. LFPs from call-inhibited sites in mothers had a significantly higher phase precision trajectory than virgins beginning near sound onset until more than 100 ms after sound offset.

df=26, p<0.005, 2-tailed). This did not occur for excitation (cc=0.33, df=24, p>0.05, 2-tailed). Thus, longer and deeper inhibition of SU spiking in mothers correlates with more sustained and higher phase precision in the LFP.

2.3.3: Plasticity dominated by laterally tuned sites

The SU and LFP data both suggest significant changes in the nature of call-evoked inhibition in the mother's auditory cortex. Is this plasticity globally distributed, or might changes in inhibition between virgins and mothers depend on the specificity of a recording site's tuning to the frequencies in pup calls? We addressed this by separating our SUs and LFPs at call-excited and -inhibited sites depending on the LFP BF. Sites with LFP BF<50 kHz (lateral band) nevertheless responded to high-ultrasonic calls presented at moderate sound levels. These sites showed a significant difference in the degree of call-evoked SU inhibition between mothers and virgins (Fig. 2.6B1 Inset). In parallel, there were large differences in the strength of the phase precision for LFPs recorded around call-inhibited SUs (Fig. 2.6B1). Differences were not apparent for call-excited SUs (Fig. 2.6A1 and Inset).

In fact, the lateral BF range was mainly responsible for the population differences in normalized SU firing (Fig. 2.3A2 and B2, lower box plots) and LFP phase precision (Fig. 2.5A3 and B3). When we compared LFP sites tuned to high-ultrasonic frequencies (BF>50 kHz), the differences in SUs and LFPs at both call-excited and -inhibited SU sites (Fig. 2.6A2 and B2) were not significant. For the call-excited SU responses, the median SU normalized firing rate was higher in mothers, but not

significantly so. Mirroring the SU results, the LFP phase precision at call-excited and -inhibited sites also did not show significant differences between mothers and virgins. Thus, using both SUs and LFPs, we conclude that there is a robust plasticity in call-evoked inhibition within auditory cortical regions tuned to frequencies *lower* than the high-ultrasonic frequencies where pup calls are found.

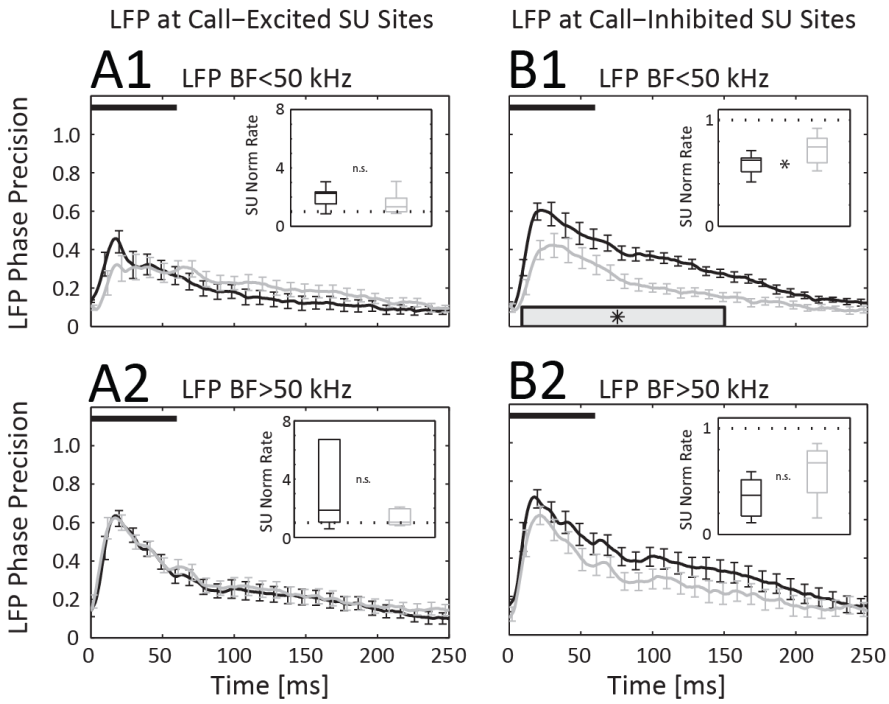


Figure 2.6: Plasticity from call-inhibited sites tuned to lateral frequencies. Significant differences in LFP phase precision mainly arose from call-inhibited sites with LFP BF<50 kHz. SUs from these same lateral band sites had greater call-evoked inhibition in mothers than in virgins. (A1 and A2) Population-averaged phase precision trajectories for LFPs at call-excited SU sites, grouped by LFP BF. No significant differences in trajectories were found between mothers and virgins for either lateral (A1) or high-ultrasonic (A2) band sites. Similarly, differences in the normalized, integrated SU firing (see Fig. 2.3A2 and B2) did not reach significance for either the lateral (t-test, t=.98, df=10, p>0.05, 2-tailed) or the high-ultrasonic band (Mann-Whitney, U=16, $N_{mothers}$=10, $N_{virgins}$=5, p>0.05, 2-tailed). (B1 and B2) Population-averaged phase precision trajectories for LFPs at call-inhibited SU sites, grouped by LFP BF. The trajectory for mothers was significantly higher than virgins at lateral (B1) but not high-ultrasonic (B2) band sites. Similarly, differences in the normalized, integrated SU firing were significant for the lateral (t-test, t=2.3, df=13, p<0.05, 2-tailed) but not the high-ultrasonic band (t-test, t=1.7, df=11, p>0.05, 2-tailed; $N_{mothers}$=9, $N_{virgins}$=4).

The LFP plasticity may partially reflect a change in the reliability of feed-forward inputs into the lateral band. This conclusion is based on the peak phase precision at the onset of vocalizations. The high-ultrasonic regions in both virgins and mothers presumably receive strong inputs from pup calls, and the peak phase precision values for both animal groups at call-excited and –inhibited sites were correspondingly higher and not significantly different (ANOVA $F(3,21) = 0.58$, $p>0.05$). On the other hand, pup calls do not normally drive very reliable inputs to the lateral band, since the phase precision here was significantly lower compared to the high-ultrasonic band for all sites (t-test, $t=3.3$, $df=49$, $p<0.01$). This changed in mothers, particularly for call-inhibited sites in the lateral band, so that mothers had more reliable evoked responses whose precision rose higher than in virgins. This more uniform peak phase precision across both the high-ultrasound and lateral bands at call-inhibited SU sites in mothers could thus result in a more robust neural response to pup calls across the auditory cortex.

2.3.4: Lateral band plasticity enhanced for pup call frequencies

Both the SU and the LFP data suggest that the main changes in call-evoked inhibition occurred for neural sites tuned to lateral frequencies. We next asked whether these changes were in any way specific for pup calls, or whether they reflected a more generic difference in auditory processing in mothers. To address this, we turned to pure tonal stimuli, since tone frequency is one of the main parameters that defines these whistle-like pup calls (Liu et al., 2003). We looked to see whether the lateral band's inhibitory plasticity was specific for stimulus frequencies in the high-ultrasonic range,

and analyzed tone responses from lateral sites the same way we did for natural pup calls. However, because we had far fewer trials for our tone stimuli (~15/tone) compared to our pup call stimuli (~50/call x 18 calls), we had to pool responses for 5 adjacent, logarithmically-spaced tones. Even still, the reduced trials made our normalized SU firing rate estimates noisy. Moreover, because there was a floor in SU spiking, relative changes in the strength of SU inhibition were harder to quantify. Thus, since we found that SU inhibition was correlated with LFP phase precision, we relied on the latter for this analysis.

Comparing LFP phase precisions for high-ultrasonic tones between 60-80 kHz, we found a significant increase in mothers compared to virgins at lateral band call-inhibited SU sites (Fig. 2.7B1, top panel). This was a more than 50% improvement, computed by taking the (bootstrap) mean difference in the group-averaged phase precisions during the tone. This enhancement was relatively specific for the natural pup call frequency range: varying the center tone frequency outside of this range caused the improvement to drop from its peak (Fig. 2.7C). Some weak frequency generalization was nevertheless apparent. When tone frequencies between 30-40 kHz were examined, a group difference was still found (Fig. 2.7B1, lower panel), but it was smaller, and the time interval for significance was shorter. Thus, call-inhibited SU sites in mothers had a significant increase in tone-evoked phase precision for frequencies starting above ~30 kHz, with greatest enhancement spanning the natural pup call range. In contrast, at call-excited SU sites, the phase precision time course for 60-80 kHz tones was not different

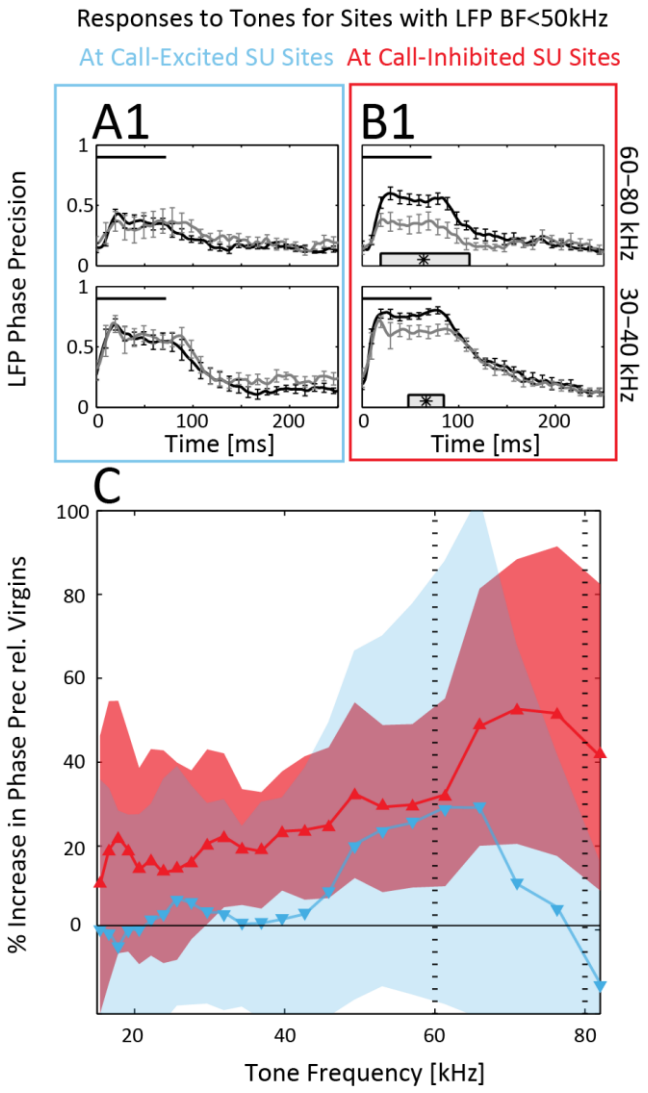


Figure 2.7: Differences between mothers and virgins were particularly enhanced for tone frequencies falling in the natural pup call range. (A1 and B1) Comparison of 60-80 kHz (upper panels) and 30-40 kHz (lower panels) tone-evoked LFP phase trajectories, averaged across lateral band LFPs. The magnitude of phase precisions was higher for the latter frequencies since lateral band sites should be more responsive to them. No significant differences between mothers and virgins were found for call-excited sites for either tonal stimulus. However, mothers and virgins did differ significantly at call-inhibited sites for both tonal stimuli. Excited $N_{mothers}$=7, $N_{virgins}$=5. Inhibited $N_{mothers}$=7, $N_{virgins}$=6. (B) Frequency-dependence of tone-evoked LFP phase precision enhancement in mothers compared to virgins for call-excited (blue) and -inhibited (red) sites. Each point pooled trials from 5 logarithmically-spaced tone frequencies centered on that point. Population-averaged phase precision trajectories were computed separately for call-excited and -inhibited SU sites from each animal group. The average difference (solid lines) between trajectories (mother – virgin) over the duration of the tone quantified the precision enhancement relative to virgins. Shaded bands represent 95% confidence intervals computed by bootstrapping across sites. We found a significant increase only for LFPs at call-inhibited SU sites, with the greatest differences for frequencies falling in the natural pup call range (dashed vertical lines). A smaller but still significant difference was also apparent for tone frequencies above ~30 kHz.

between virgins and mothers (Fig. 2.7A1, top panel). The percentage increase relative to virgins was also non-significant across all tone frequencies (0% line lies within the 95% confidence interval, blue band in Fig. 2.7C). Finally, neither call-excited nor -inhibited sites with LFP BF>50 kHz exhibited significant differences (data not shown).

2.4: Discussion

Earlier studies of auditory cortical communication sound encoding have almost entirely focused on excitatory neural responses (Wang et al., 1995; Medvedev and Kanwal, 2004; Syka et al., 2005; Wallace et al., 2005; Liu et al., 2006; Liu and Schreiner, 2007; Recanzone, 2008; Huetz et al., 2009). Inhibition has previously been considered only in so far as it shapes an individual cortical neuron's receptive field and excitatory responsiveness to calls (Narayan et al., 2005; Razak and Fuzessery, 2006). This work on ultrasonic call processing in the awake mouse demonstrates an alternative role for inhibition in the distributed cortical representation of species-specific vocalizations. Its importance was revealed through a plasticity that yielded more robust inhibition to ultrasonic pup call frequencies by neural sites tuned to lateral frequencies. The data suggests lateral band inhibition can enhance the cortical contrast in the population representation of a communication call. Here we relate this work to prior studies, interpret our plasticity data, and propose its function for improving call detection in background noise.

2.4.1: Relation to prior studies

This study took advantage of a known behavioral change in the recognition of a natural communication call to search for auditory cortical correlates of its behavioral relevance, a strategy previously applied in anesthetized mice (Liu et al., 2006; Liu and Schreiner, 2007). Here we investigated cortical coding in awake mice for the first time, and focused only on neural changes relevant for detection by pooling responses across calls. Some conclusions remained consistent. Both the current and previous work suggest plasticity in feedforward activity because mothers showed a more "synchronized" response onset across the auditory cortex. Nevertheless, the data in this study are fundamentally different and could not have been predicted from the anesthetized work. Whereas earlier conclusions were based on changes in excitatory neural responses, here we found that half of the SUs that responded to calls did so in a purely inhibitory manner (Table 2.1). In fact, call-evoked pure inhibition has rarely been reported, perhaps for methodological reasons. MU recordings may obscure the inhibition of individual neurons by sounds. Ketamine anesthesia may disinhibit (Bergman, 1999; Behrens et al., 2007) or otherwise modulate (Syka et al., 2005) or synchronize (Greenberg et al., 2008) cortical excitation. Neuron search strategies may also differ. Most studies first characterize units by their excitatory tone response area and BF, yet many of our call-inhibited SUs did not have excitatory tonal responses (18 of 25, Table 2.1); a "best" frequency for all SUs was instead based on the surrounding populations' response to tones (i.e. LFP BF). Even when inhibited SU responses to natural calls have been reported in an awake animal though (Recanzone, 2008), the

fraction of neurons has been very small, and has varied according to the call (0-10%). Hence, a final possibility is that single frequency ultrasonic calls may be more likely to evoke pure inhibition than the broad-band calls used in other studies.

2.4.2: Robust plasticity in inhibition rather than excitation

Our data suggests this inhibition may be functionally relevant for detecting pup calls since it systematically changed in its strength, particularly in the lateral band, in a manner that correlated with the call's behavioral significance. These results were observed both directly in the SU data (Fig. 2.3B, 2.6B inset), and indirectly through associated changes in the surrounding local network (Fig. 2.5B, 2.6B). Whether the changes here arise from pup experience, hormonal changes associated with pregnancy or lactation, or attention remains to be investigated.

Despite the robust plasticity in evoked SU inhibition, our data did not demonstrate a significant change between virgins and mothers in pooled SU excitation (Fig. 2.3A) for either the high-ultrasonic or lateral frequency bands (Fig. 2.6A Insets), although there may be a trend towards greater strengths for high-ultrasonic SUs in mothers. In other words, enhancing the "average" excitation per "typical" SU may not be necessary to improve the detection of pup calls. Distributed excitation may serve a different role in communication processing, such as discriminating calls.

There are two qualifications to this. First, cortical neurons are not all identical, and we likely do not record equally from all types. In particular, our high-impedance tungsten electrodes may be less sensitive to spikes from smaller interneurons than to

larger pyramidal cells (Towe and Harding, 1970; Gold et al., 2006). The latter is likely the "typical" SU that we detect, as they make up 70-80% of cortical neurons (DeFelipe and Farinas, 1992). Such a recording bias may explain why we did not see a systematic change in SU excitation arising from inhibitory interneurons that presumably generate the stronger inhibition we reported here. In support of this, a recent study by Cohen et al. (2011) demonstrated that in the anesthetized auditory cortex of mothers, fast spiking neurons (putative inhibitory interneurons) show a more robust response to pup vocalizations in mothers but not virgins. This finding agrees with the idea that the stronger call-inhibited SUs observed in this study consist mostly of pyramidal cells.

Second, lack of change in "average" excitation does not preclude differential plasticity that depends on systematic variations within this study's pool of "typical" SUs. This may be especially relevant for high-ultrasound-tuned excitatory neurons. For example, three SUs in mothers had noticeably higher normalized spike rates (affecting the PSTH in Fig. 2.3A3 and the box plot in the inset of Fig. 2.6A2). These may be part of a specialized neuronal subcategory that emerged in mothers. The high variability in types of excitation makes it difficult to distinguish these. Thus, we did not further sub-classify SUs based on their excitatory response time courses, since sample sizes would have been too small to make reliable conclusions. In support of possible subtle changes in excitation that elude our current methods, we did find more offset (latency >40 ms) and sustained (duration >50 ms) SUs in mothers. Furthermore, individual excitatory receptive fields may also be changing, which could improve call discrimination. This could occur without affecting overall evoked excitation and call detection.

In parallel with these SU results, our LFP data did not show changes in the network activity associated with SU excitation, although it did for inhibition (Fig. 2.5, 2.6). Although this is a serendipitous finding for our plasticity analysis, it is not entirely clear why a coarse measure of neural activity such as the LFP would show different changes depending on the response of a co-recorded SU. Instead, we mention here two possible, non-exclusive scenarios. First, there may be spatial clustering in SU response types, as has been reported for vocalization responses in the anesthetized guinea pig (Wallace et al., 2005). Second, the dominant sources contributing to the LFP may arise from a spatially restricted region (Katzner et al., 2009), and these sources differ depending on whether the co-recorded SU is inhibited or excited. For example, inhibition of a pyramidal cell can come from fast perisomatic inhibition by nearby basket cells (Freund and Katona, 2007). Each basket cell initiates synchronous inhibitory postsynaptic potentials in many pyramidal cells within its localized region of innervation (Miles et al., 1996). Such currents may be less consistent or weaker around pyramidal cells that are excited by calls. If plasticity occurs primarily in the inhibitory network, the LFP around excited SUs might not then easily reveal this.

A difference between excitation and inhibition was also apparent in how the LFP phase precision correlates to the strength of SU spiking, irrespective of the plasticity between animal groups. Phase precision measures response variability across trials, which can arise here from either random neural noise for each call, or systematic variation for acoustically different calls. If different calls elicit similar neural responses, then the latter component is minimized. This is the case for call-inhibited but less so for

-excited SU sites. Most of our call-inhibited SUs were uniformly inhibited by most, if not all, the calls, whereas the response to different calls by call-excited SUs was typically more varied. Hence, our pooled phase precision at call-inhibited SU sites reflects neural noise more directly, and reveals an intriguing correlation with the strength of SU inhibition: LFP trajectories, which include both synaptic and spiking contributions, become less variable as the co-recorded SU's spiking drops to zero. This relation justified interpreting increases in tone-evoked phase precision as indicative of enhanced inhibitory strength (Fig. 2.7). In further support of this, differences between virgins and mothers were similar even when we compared average call-specific (instead of call-pooled) phase precision trajectories. This suggests that mean response differences across calls were not a major contribution to the LFP variability at call-inhibited sites. For call-excited SUs, systematic variations in the pattern of mean firing for individual calls could degrade a site's pooled LFP phase precision, independent of the SU's excitatory strength. Thus, these measures were uncorrelated at call-excited sites.

2.4.3: Hypothesized role of enhanced inhibition in the lateral band

How might a more robust inhibitory response at the population level functionally improve communication sound detection? Accumulating evidence suggests the auditory cortex changes to more powerfully represent sounds that acquire behavioral meaning (Fritz et al., 2003; Weinberger, 2004). Inhibitory plasticity may help achieve this by enhancing the neural contrast in a sound's distributed cortical code. A model based on our results illustrates how this might work (Fig. 2.8). An emitted pup call (upper right)

excites the ultrasound region of the basilar membrane, and is transduced into a neural signal that feeds forward through subcortical stations to the auditory cortex, evoking a distributed response spanning both the high-ultrasonic (solid bars) as well as lateral (hatched bars) frequency bands. In each region, (presumed) pyramidal cell activity is divided into call-excited or -inhibited classes. Normalized evoked spike rates (relative to spontaneous activity) for a virgin (gray) or mother (black) are represented by bar heights. If pooled spike rates are not significantly different between groups, rates are depicted as equal for simplicity. Hence, only the call-inhibited sites in the mother's lateral band are shown as significantly lower. Assuming simple one-to-one integration of call-excited and -inhibited activity in a frequency band-specific fashion, this would produce a downstream representation with a greater contrast in mothers between the frequency region that should represent the pup call (high-ultrasound) and lateral frequency areas.

Why would enhancement of population-level contrast be advantageous for call processing? If calls were emitted in the presence of broadband background noise, call-evoked lateral band inhibition could help suppress this noise, helping the neural activity in the high-ultrasonic band to stand out more clearly. In fact, we actually found some generalization in mothers of the enhanced inhibition at laterally-tuned sites for lower frequencies as well (Fig. 2.7). Whether this inhibition can add to the call-evoked inhibition must be tested in future two-tone or masking experiments. Finally, such a coding scheme is reminiscent of attention-related gain changes of auditory cortical neurons during a tone-in-broadband noise detection task in ferrets (Atiani et al., 2009).

That study observed stronger suppression of excitatory activity for neurons tuned to sites “far” from the target frequency. On the other hand, our main effect was stronger inhibition at these sites to the target sound (pup call) alone. In both scenarios, the hypothesized outcome would be enhanced neural contrast for the target.

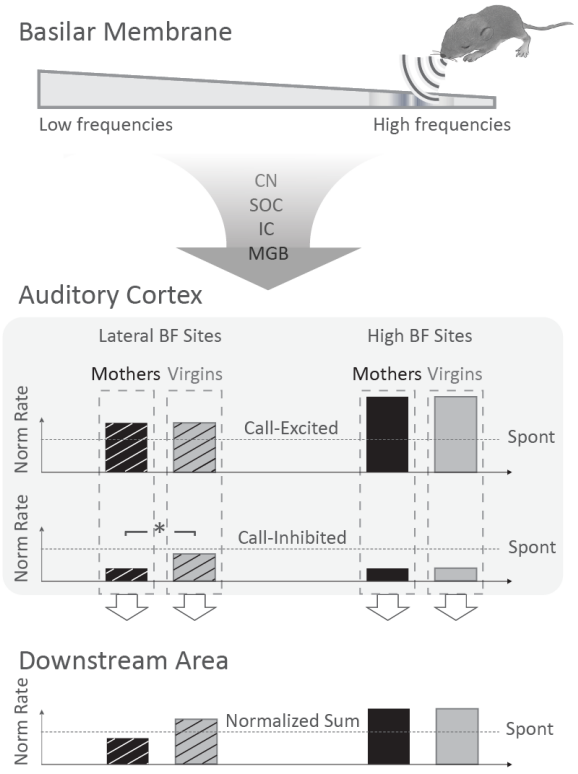


Figure 2.8: Hypothesized model to enhance a pup call’s neural contrast. CN: cochlear nucleus; SOC: superior olivary complex nuclei; IC: inferior colliculus; MGB: medial geniculate body. See text for details.

CHAPTER 3

DIFFERENTIAL ROLES OF EXPERIENCE AND PHYSIOLOGICAL STATE ON PLASTICITY

In the previous chapter, we demonstrated that socially acquired natural vocalizations resulted in call-evoked inhibitory plasticity in the auditory cortex of mothers. While these changes could have resulted from pup experience alone, intrinsic factors such as physiological state (hormones, arousal, and attention) may have also played a role. In this chapter, we begin to study how these factors contribute to plasticity by recording neural responses in co-caring females, and comparing them to mothers and virgins. Cocarers are adult virgins that gain experience with pups while helping a mother care for her litter and show pup call recognition behavior (Ehret et al., 1987). However, they physiologically differ from mothers during this experience because they do not undergo pregnancy or parturition. Using the same techniques in chapter 2, we recorded single neurons and their responses to pup vocalizations in the primary auditory cortex of awake mice. We used the SU call-evoked inhibition found previously as a proxy for detecting plasticity, and explored the role of maternal physiological state and experience on sensory cortical plasticity. In addition, we assessed the relevance of these cortical changes by testing the behavioral performance of each animal group in a two-choice alternative test that compared their approach responses to pup calls versus neutral tones.

3.1: Introduction

The adult auditory cortical representation of sounds can be dynamic, undergoing plasticity to reflect changes in behavioral relevance. It is clear now that plasticity can depend on the most salient acoustic feature learned (see 1.4.1). However, while experience dependent plasticity is a well-studied phenomenon in the auditory cortex, we still do not fully understand how this plasticity is affected by how sound experience is acquired, or how our intrinsic state affects this process. A recent study by David et al. (2012) has begun to uncover the former; by changing the valence of the stimulus (appetitive versus aversive), both training procedures resulted in plasticity, but with opposite sign. Thus, differences in plasticity can occur from how an animal learns the significance of a sound, but how does an animal's intrinsic physiological state affect these changes?

The physiological state of the animal such as the hormonal state and its effects on the auditory system have been well studied, and can regulate processing in a number of animals (see 1.3.2). For example, estrogen is a hormone associated with pregnancy and maternal behavior that also affects neural excitability in the auditory system of birds (Tremere et al., 2009). In fact, a number of studies have demonstrated that the application of estrogen to the caudomedial nidopallium in the bird, a cortical-like auditory region, can facilitate auditory processing for species-specific vocalizations (Tremere et al., 2009; Remage-Healey et al., 2010; Maney and Pinaud, 2011; Tremere and Pinaud, 2011). However, much less is known about its influence in the mammalian auditory cortex, and its effects on the learning of sounds.

The maternal model of acoustic communication between offspring and adult females offers the opportunity to explore this question and dissociate how hormones and experience contribute to auditory cortical plasticity. After birth, mouse pups emit bouts of USVs that are behaviorally relevant to the mother but not the pup-naïve virgin (Ehret et al., 1987; Ehret, 1989, 2005). Neural correlates for this behavioral difference have been demonstrated in the auditory cortex of both anesthetized (Liu and Schreiner, 2007; Cohen et al., 2011) and awake animals (see Chapter 2). In particular, we demonstrated in chapter 2 that awake-restrained mothers, measured after weaning, have a greater pup call-evoked SU inhibition compared to virgins. The question then is whether these changes are a result of pup experience alone, or whether the maternal physiological state also plays a role. Cocarers, which are virgins with pup experience, do not undergo the hormonal changes associated to motherhood, but still show behavioral differences from pup-naïve mice in an ultrasound recognition task (Ehret et al., 1987). Thus, to begin to dissociate the effects of hormones and experience on cortical plasticity, we first recorded from cocarers at the same time point as mothers, after pup weaning. These results then motivated further experiments discussed in this chapter on cocarers that were recorded early during their pup experience (early cocarers).

Here, we confirm using SU recordings only that mothers have greater call-evoked inhibition when compared to both virgins and cocarers in the “lateral band” (A1 and AAF, but not UF). Next, recording from early cocarers during pup experience, we find that their call-evoked inhibition was more similar to mothers. This led us to suggest that inhibitory plasticity is not maintained in cocarers, and that the maternal physiological

state contributes to the retention of auditory cortical changes. Based on this, we find that this retention may occur through a decrease in the spontaneous activity and hypothesize that this change acts as a mechanism to preserve the experience-dependent cortical plasticity. Finally, we provide support for this hypothesized mechanism for maintaining call-recognition by demonstrating that cortical changes correlate with differences in behavioral performance across animal groups.

3.2: Methods

The electrophysiological recording, surgical methods, acoustic stimuli, and statistical tests used in this chapter are located in appendix A, B and C. In addition, details of the SU analyses used in this chapter can be found in section 2.2.1. In this chapter, the electrophysiology data comes from 47 female CBA/CaJ mice all between 14 and 24 weeks old at the time of surgery. From these animals, we recorded 393 SUs, 352 that were in core auditory areas. For the behavioral experiments, 34 female mice were used for 197 number of test trials. A list of all the animals used in this chapter can be found in appendix A.

3.2.1: Auditory brainstem recordings

ABRs were performed only on those animals used in our behavioral tests. ABRs were conducted inside a soundproof anechoic chamber to test each animal's threshold of hearing to different tone frequencies. Animals were first anesthetized using a mixture of Ketamine and Xylazine (4 parts 100 mg/kg Ketamine: 1 part 5 mg/kg Xylazine), and a

maintenance dosage was prepared (6 parts Ketamine: 1 part Xylazine). Once the animal showed no reflexive response to a light toe pinch, it was placed onto a Styrofoam bed with its right ear 11 cm from a speaker on a vibration-isolation table. Subdermal needles connected to a RA4LI headstage (Tucker Davis Technologies) were used to record the ABRs with the ground placed ventral lateral to the left external pinna, the reference electrode ventral lateral to the right external pinna, and the recording electrode at the vertex of the skull. The brainstem signals were recorded at 25 kS/s and band pass filtered from 100 Hz to 3 kHz. Stimuli were played 500 times presented at a rate of 19 Hz and in 5 dB steps to obtain an averaged ABR for a specific tone frequency at different amplitude levels. The threshold was then defined as the lowest amplitude level that could evoke an auditory response.

3.2.2: Estrus cycle monitoring

The animal's estrus state was monitored by vaginal cytology using methods described previously (Shepard and Liu, 2011). Following each behavioral experiment, a pipette with 0.9% sterile saline was used to flush the animal's vagina. Several drops of this solution was then ejected onto a pre-cleaned glass microscope slide, and visualized under a light microscope at 4X magnification. The animals typically cycled through four different phases: proestrus, estrus, metestrus, and diestrus, with each phase marked by a distinct mixture of cornified epithelial cells, leukocytes, and cuboidal cells. During proestrus, the uterine line is building up and generally has a low epithelial cell density (Figure 3.1A). Estrus occurs when the uterine lining has been built up, and therefore the

cornified epithelial cell density will be high, but the amount of leukocytes will be low (Figure 3.1B). Following this, metestrus occurs as the female is shedding her uterine lining, and the samples will show a high density of epithelial cells, leukocytes, and cuboidal cells (Figure 3.1C). Finally, during diestrus, the uterine lining has been shed and the slide will show a greater abundance of leukocytes and cuboidal cells, but a very low density of epithelial cells (Figure 3.1D).

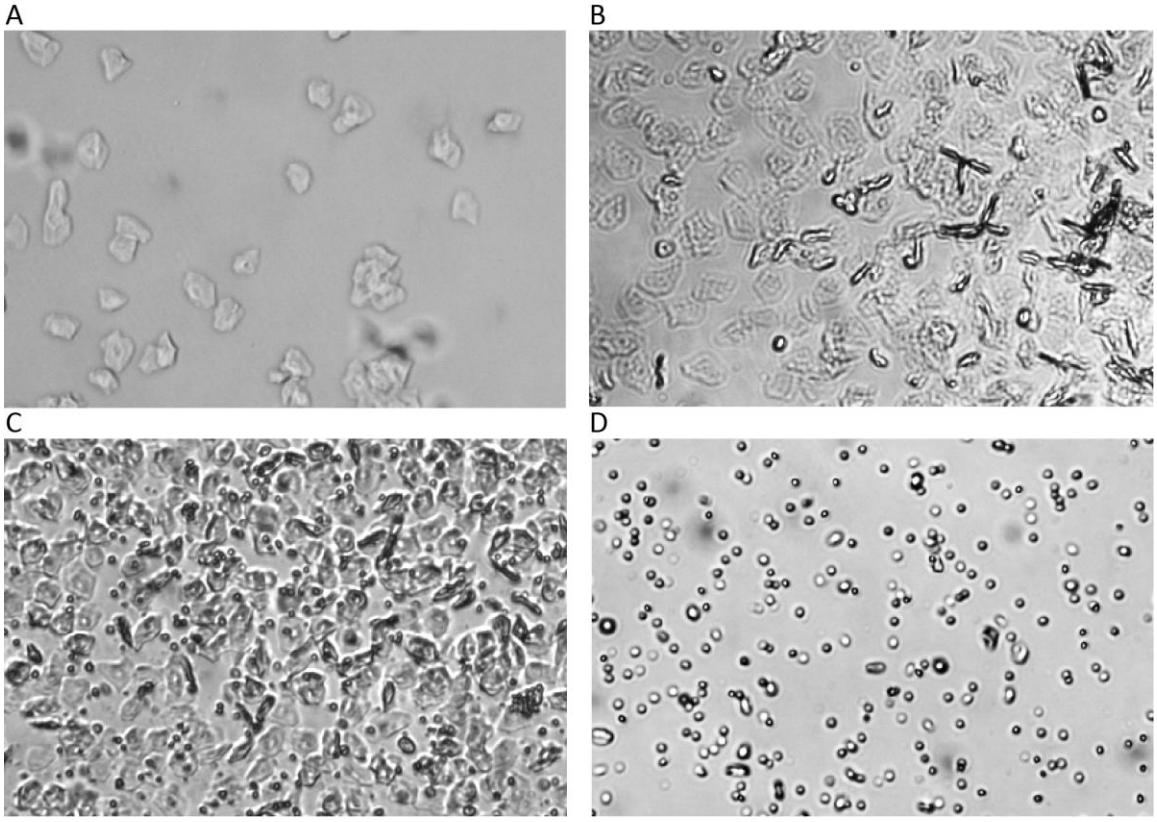


Figure 3.1: Vaginal cytology identification. This figure shows the four phases, (A) proestrus with the lower cell density, (B) estrus with the high density of cornified epithelial cells and low density of leukocytes, (C) metestrus which follows estrus and typically shows a greater number of leukocytes, and (D) diestrus where there is typically a higher density of leukocytes and a lower density of either the epithelial or cuboidal cells.

3.2.3: Two alternative choice task

All behavior tests were performed inside a soundproof anechoic chamber under dim red light. In the text below, the x-direction refers to the horizontal axis in figure 3.3, and the y-direction refers to the vertical axis in figure 3.3. Animals were tested on an elevated W-maze (35.5 cm from ground, 80 cm length, 3.5 cm high walls, 33 cm from long edge to nest in the y-direction, 30 cm from long edge to open end in the y-direction) with speakers located 60 cm away from the nest depression (Fig. 3.2). In addition, there were two servo-controlled doors (Hitec HS-7950TH, Poway, CA) that were 24.5 cm long and 13.5 cm high. The stands holding up each door were located 18 cm from the maze in the x-direction, and 5 cm from the maze in the y-direction.

The doors were controlled remotely using a digital pulse width signal from an RX5-2 (Tucker Davis Technologies). Servos were first reprogrammed using an HPP-21 (Hitec HS-7950TH, Poway, CA) where a 1ms pulse width command was set to 0 degrees, and the 2.1ms pulse width command was set to 180 degrees. Code written in RPvdsEx allowed the user to control the position of the doors in real time. During the experiment, a 1ms pulse width sent to the servos would close the doors around the nest, 2.05ms was used to open the doors, and 1.9ms was used to open the doors at approximately 160 degrees. Only when the doors were in the 160-degree position, could the animal explore the entire maze. To prevent the animal from being able to burrow underneath the doors in the fully open or closed configuration, three plastic barriers were attached to the maze using Neodymium magnets (K&J Magnetics Inc., Jamison,

PA). The barriers were 3 cm tall and located underneath where the doors were fully open or closed (Fig. 3.3, green line).

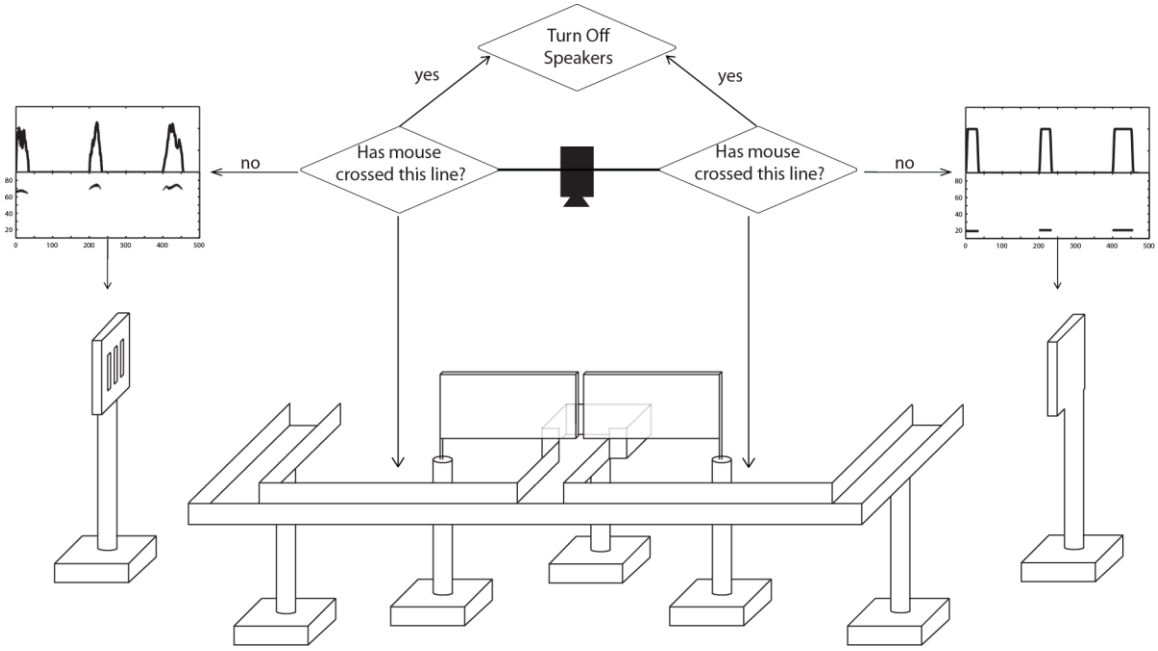


Figure 3.2: The two-alternative choice behavioral setup. The figure shows the design of the elevated W-maze and the placement of the doors and speakers. The black block in the center represents the camera mounted above the maze recording the animal's movement and feeding back this information to the Cleversys computer program. When the test starts, the animal's position in the nest (region located behind the 2 doors) cues the sound playback to begin, and a sequence of three pup calls and three tones are played back from opposing speakers. The sequence of three calls (amplitude envelopes on top, frequencies on bottom) is shown above the left speaker. The corresponding bout of three tones is shown above the speaker on the right. During the playback, the sequence of sounds were alternated, with a silent gap between each bout. The period of time from the start of one bout to the start of the second was 1.6 seconds. In addition, for each behavior experiment, the playback of calls and tones were alternated between the left and right speakers to eliminate possible left/right room biases. In this image, the doors are at the 0-degree position.

Mothers, late cocarers, and early cocarers were tested for three consecutive days with four pups aged P6-P8. For post-weaned mothers and cocarers, the four pups came from a donor litter, while for early cocarers, the four pups came from the litter in

which they were co-caring. Prior to each test day, 70% ethanol was used to wipe down the elevated W-maze, and clean Alpha-Dri bedding was added. The mouse was then placed onto the W-maze with four pups in the nest area for a one hour habituation (Nest Area, Fig. 3.3). During this time, the experimenter remained outside the anechoic sound chamber while the mouse was habituated to the sound of the servo-controlled doors. Periodically, the experimenter would enter the chamber and displace pups from the nest to motivate retrieval behavior. The number of times this occurred per animal varied, but each animal would perform at least two retrievals prior to the test. The goal was to motivate the mouse to retrieve pups from various areas of the maze until they could consistently retrieve multiple pups at the ends of the W-maze back to the nest.

Following habituation, two sound preference tests were performed, spaced approximately 1 hour apart. Prior to each sound test, we first motivated the mouse by scattering pups and eliciting a retrieval response. Two pups were placed on the left and right sides of the nest depression at equivalent distances, and the mouse was monitored using video tracking software (TopScan by Cleversys, Reston, VA). If during this test, a female mouse did not retrieve within 10 minutes, we did not continue with the sound preference test. For mice that did retrieve, we allowed them to explore the W-maze post-retrieval. We closed the servo-controlled doors following their return to the nest. Once the doors closed, two pups were removed from the nest, and then the two-alternative choice test began (for sound stimuli see Appendix B). The doors opened after approximately 30 seconds of sound playback to allow the mouse time to perceive each sound. Once the mouse made a decision and moved 18 cm from the center of the maze

towards one speaker, both speakers were switched off (dotted line, Figure 3.3). In total, we collected data for at most six pup retrieval and sound preference tests for each animal.

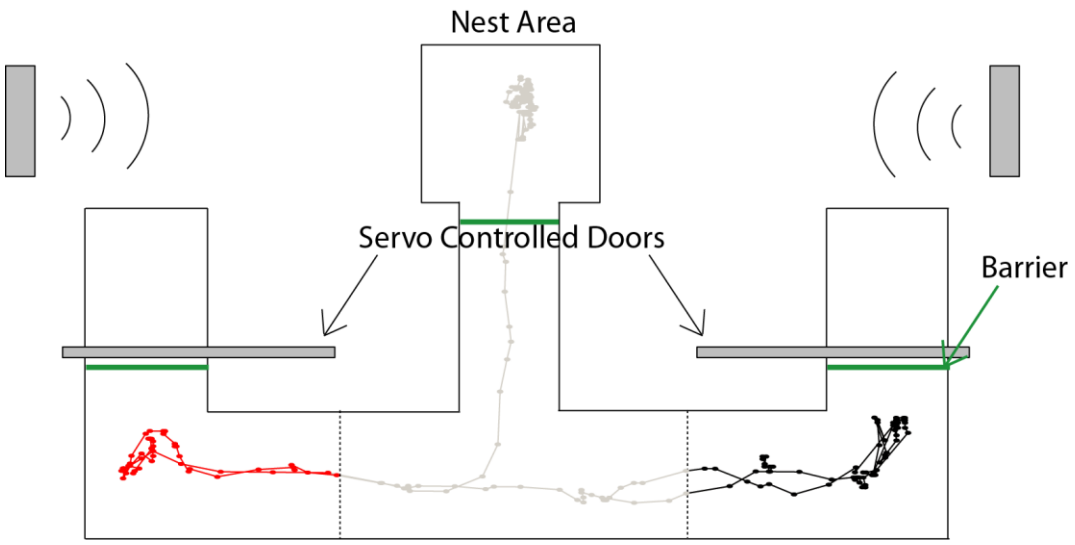


Figure 3.3: Visual tracking of the animal in the behavioral setup. The figure shows a top down schematic of the behavioral setup. The gray boxes in the upper left and right are the speakers playing back either tones or pup vocalizations. The gray line shows the animal's travel in the nest area prior to making a decision to go towards the left side first (red trace). After moving left first, the animal proceeds to go to the right side (black trace). Each dot in the trace represents 0.33 seconds of time, and the entire trace represents the first 150 seconds of an experiment. Dotted vertical lines in the maze show the point at which left and right sides are defined.

Visual tracking the animal's center of mass during the behavioral tests provided quantitative information about the animal's behavior. During each two-alternative choice test, the animal's first decision to move towards one speaker or the other was scored. Once the servo-controlled doors opened, the animal had to cross a line 18 cm away from the center of the maze to count as a decision. Figure 3.3 illustrates this event by the points where the gray trace changes to the colored trace.

During each experiment, Cleversys would output in real time the location of the animal. We used this to quantify the amount of time the animal spent in each arm, and the time spent exploring the maze before returning to the nest. In this setup, the nest area was considered the square in the middle. A measure of behavior that we computed was the time spent exploring the maze. This consisted of the time between when an animal left the nest following a test until their first return back to the nest. For example, following the retrieval of all four pups, if the animal did not leave the nest to explore the maze then the exploration time was 0ms. However, if the animal left the nest following retrieval to explore the maze and returned 1 minute later, then the exploration time was 60ms. This exploration time was computed for animals searching the maze post-retrieval of all pups, and during the pup call test. We also quantified pup retrieval times for each test (up to 6 total pup retrievals per animal). This was computed by marking the time at which all pups were scattered, and noting when the animal retrieved all pups back to the nest area.

Estrus cycle was monitored to investigate its potential effect on behavioral performance. To compute whether the two-alternative choice task was dependent on the estrus state, the proportion of responses to the pup call speaker was measured on each day and labeled as either proestrus, metestrus, estrus, or diestrus (see Figure 3.1). For example, if an animal in metestrus went towards the pup call speaker twice out of two experiments, the proportion would be 1. Alternatively, if the animal went to the tone speaker twice out of two experiments, the proportion would be 0. This allowed us to create a distribution of proportions for the different estrus states (diestrus, estrus,

metestrus, proestrus) to analyze the effects of the reproductive cycle on behavioral performance.

3.2.4: LFP and SU classification and analysis

Pup call and tone responsive SU PSTH's were classified using methods previously described (Galindo-Leon et al., 2009). Briefly, SU response duration was determined by finding the half-min of the smoothed spike rate (convolution of individual spikes with a Gaussian smoothing function, 5 ms standard deviation), and determining the time over which the smoothed spike rate stayed below the half-min value. To determine if there were differences between animal groups in the pooled spike rate for call-inhibited SUs, each smoothed, time-dependent spike rate function was normalized by the average spontaneous rate during the blank trial and then pooled over SUs. The strength of SU inhibition was quantified by integrating the actual spike count over a period from 5 to 65 ms (accounting for the longest duration pup call plus an initial neural delay and any offset responses) and dividing by the spontaneous rate.

Using each SUs call-inhibited strength and duration of responses, we created a contour plot of bootstrapped means. For each animal group, we sampled from the SU data set with replacement, and then used this to get a mean of the strength and duration of the samples. We performed this operation 10,000 times and then used the mean values to create a 2D histogram. The bin widths of the 2D histogram were computed in a way so that each bin would on average cover approximately 5% of the

data. We then performed a 2D smoothing procedure that is shown below in equation 3.1. In this case, N is the resultant smoothed matrix, and V is the original input matrix.

$$N(x_i, y_j) = \frac{V(x_i, y_j)}{\sum_{n=i-1}^{i+1} \sum_{m=j-1}^{j+1} V(x_n, y_m)} \quad (3.1)$$

To compute the spontaneous rate for both calls and tones for responsive and non-responsive SUs, we took the spiking activity in the 200 ms prior to stimulus onset for all trials (900 trials of pup calls, 600 trials for tones).

To correct for LFP phase delays introduced by data acquisition filters, and action potential contamination, techniques were used similar to those introduced previously (Galindo-Leon et al., 2009; Galindo-Leon and Liu, 2010). The LFP tuning curves were computed based on its response to the tone frequencies (6.5 to 95 kHz) at 60 dBSPL. First, the n^{th} mean LFP response was computed by averaging all the trials from the *n-1:n+1* frequencies (Fig. 3.6B). Then, the value of the largest deflection of the mean LFP between stimulus onset to onset+70ms was taken. The LFP tuning curve was then defined as this deflection magnitude as a function of stimulus frequency (Fig. 3.6D). The LFP BF was defined as the frequency with the largest negative deflection. In addition, the LFP tuning curve was used to create a frequency color map (Fig. 3.7A) to approximate anatomical regions such as UF, AAF, A1, A2, and DP. These regions were defined based on three factors: the recording site's LFP BF, the bandwidth of the tuning curve, and its frequency relative to the surrounding recording sites. Each auditory field

was defined based on the characteristics of each region previously found in Stiebler et al. (1997). A UF recording hole generally had an LFP BF greater than ~45 kHz and a more localized excitatory response to the ultrasonic frequencies. AAF was typically defined as the holes ventral to UF holes, and showed LFP BFs below ~45 kHz with excitatory responses across a broad frequency range. The approximation of UF and AAF helped to define A1, which was caudal relative to AAF and following the tonotopic reversal. Finally, recording sites that were located in the middle of the rostral to caudal tonotopic reversal were labeled as joint A1 and AAF. Any recording holes that demonstrated no reliable LFP responses to the frequency tones but showed auditory responsiveness to pup calls were labeled as DP if caudal to A1, and A2 if ventral to A1 and AAF holes.

3.3: Results

3.3.1: Long-term inhibitory plasticity is not a result of pup experience alone

To investigate whether an animal's intrinsic state influences cortical inhibitory plasticity, we recorded SUs from core AC (Kaas and Hackett, 2000) of 14 cocarers to compare to 13 mothers and 14 naïve virgins. In our experiments, naïve virgins were female mice that had no breeding experience or contact with pups during adulthood. In contrast, cocarers were virgin mice that had the same amount of exposure to pups as a mother, by being co-housed with a mother while caring for its pups. In this chapter, both mothers and cocarers were mice that had 21 days of pup experience (from

parturition to weaning), but cocarers differed from mothers because they did not undergo the physiological changes due to pregnancy, parturition and lactation. Over all the electrophysiology experiments, we recorded a total of 323 core SUs between 300-700um depth. Considering all units, we found no significant differences in the spontaneous activity across animal groups (Fig. 3.4A and B).

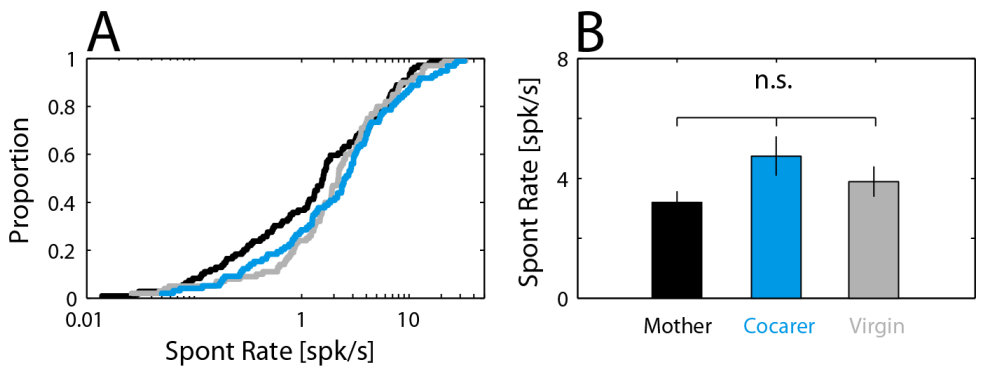


Figure 3.4: Animal groups show no differences in spontaneous rates of firing for all recorded SUs. (A) Shows the proportion of spontaneous rates among all SUs that were recorded from mothers (black, N = 109), virgins (gray, N = 100), and cocarers (blue, N = 98). (B) Animal groups show no differences in their spontaneous rates (ANOVA, $F(2,304) = 2.4$, $p > 0.05$). Note: Data was square-root transformed prior to performing the ANOVA test (see Appendix C).

Consistent with our previous study Galindo-Leon et al., 2009), we found no significant differences in the proportion of call-inhibited SUs across recordings: 36/114 (32%) SUs in mothers, 22/100 (22%) in cocarers, and 27/108 (25%) in virgins (Pearson χ^2 (2, N=322) = 2.7, $p > 0.05$). Moreover, when we pooled the call-inhibited SU responses to the 18 different pup ultrasounds, we found that the PSTH strength and duration were similar between cocarers and virgins (Fig. 3.5A). In contrast, mothers had significantly greater duration and strength of inhibition when compared to virgins (Fig. 3.5B and

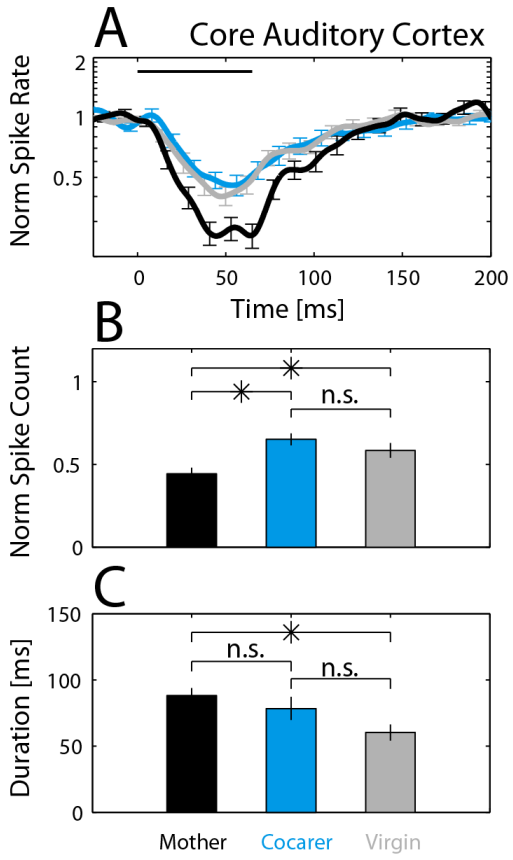


Figure 3.5: Comparing SU call-evoked inhibition in core areas UF, A1, and AAF. (A) Mothers (black) show a stronger call-evoked inhibition when compared to virgins (gray) and cocarers (blue) in their population-averaged spike rate normalized by the spontaneous rate. (B) Mothers show a significantly lower normalized rate derived by integrating the spike count over the stimulus period and dividing by the spontaneous rate (Kruskal-Wallis, χ^2 (2,82) = 13.0, $p < 0.01$, Fisher's LSD post hoc: M-V, $p<0.05$; M-C, $p<0.05$; C-V, n.s.). (C) Mothers show significantly longer durations of inhibition compared to virgins, but cocarers were not different from mothers or virgins (Kruskal-Wallis, χ^2 (2,82) = 11.6, $p < 0.01$, Fisher's LSD post hoc: M-V, $p<0.05$; M-C, n.s.; C-V, n.s.). The number of SUs used in each figure is the following: $N_{mothers}$=37, $N_{virgins}$=23, $N_{cocarers}$=27

C). Interestingly, although SUs from mothers were more deeply inhibited compared to cocarers, they were not significantly different in their durations. Hence, these results demonstrate that although the overall call-inhibited SU responses of cocarers were more similar to virgins, they were not entirely like one group or the other. Therefore,

pup experience alone cannot account for all of the differences in call-evoked inhibition between mothers and virgins.

3.3.2: Inhibitory plasticity dominated by sites in A1 and AAF

Given that the pup vocalizations are in the ultrasonic frequency range (60-80 kHz), we wondered whether the inhibitory plasticity occurred uniformly in all core auditory cortical areas. In the mouse auditory cortex, Stiebler et al. (1997) demonstrated that multi-units in UF generally respond to frequencies above 45 kHz, whereas those in A1 and AAF have lower BFs and opposing caudal to rostral frequency gradients. Our recordings in the awake mouse consisted of mainly SUs (for neural coding studies) and their co-recorded LFPs. Since tonotopy in the auditory cortex is mainly visible at a coarse population and much more variable at the SU level (Bandyopadhyay et al., 2010), we assumed the LFP would be a better guide for visualizing the BF map. We evaluated this assumption by comparing the BFs and tuning curves for pairs of SUs and their co-localized LFPs recorded along the same cortical column at different depths (between 300-700 um) in the same electrode penetration.

In total, we found 37 such pairs of recordings, and their average depth difference was 121.1 um with a standard deviation of 94.2 um. For each SU, we first defined the interval to count spikes to create a tuning curve (see 2.2.1). The BF was then determined by the frequency that evoked the greatest number of spikes during this period (Fig. 3.6A and C). For each co-recorded LFP, a tuning curve was created by taking the value of the greatest negative deflection that occurred during the sound stimulus (0-70ms, black

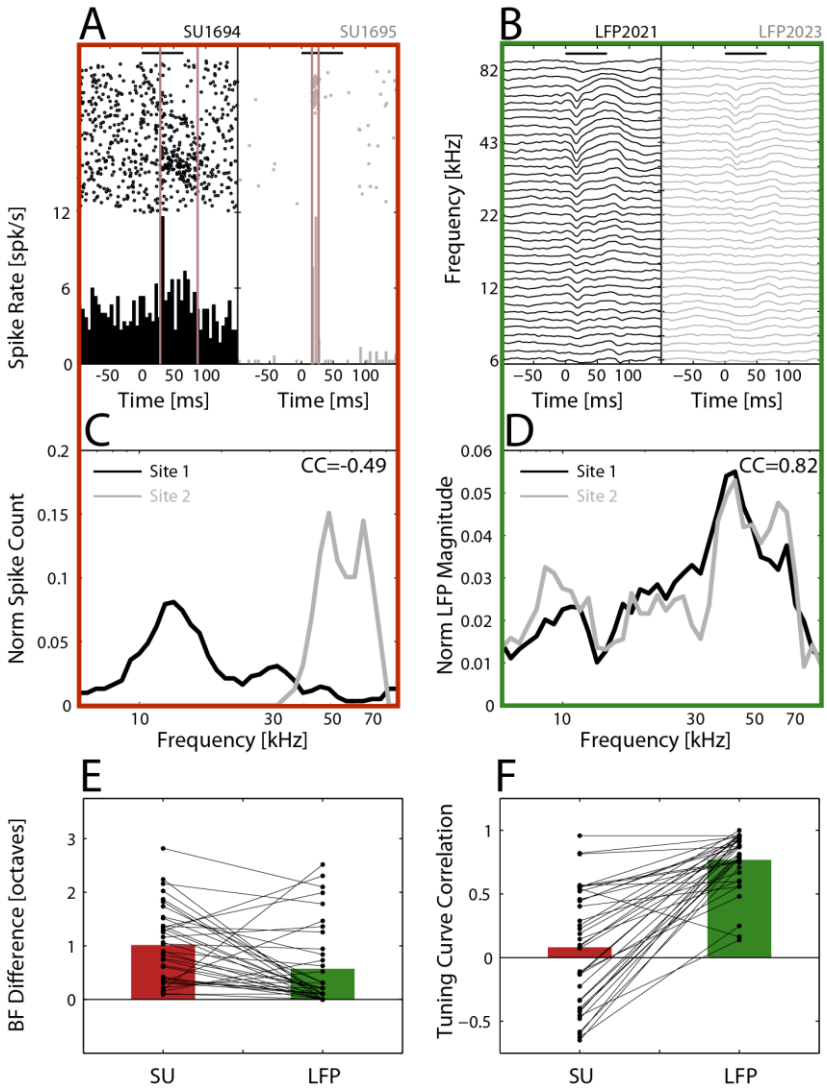


Figure 3.6: SU frequency response within each recording location is more variable compared to the co-recorded LFP. Red color represents the recorded SUs, and the green color represents the co-recorded LFPs. (A) Example SUs from one recording location. SU1694 was recorded at a depth of 483 um, and SU1695 was recorded at a depth of 584 um. The black bar at the top shows the start and duration of the tone stimulus, and the vertical brown bars represent the period in which spikes are counted to produce the SU tuning curve. (B) Figure shows the co-recorded LFPs (green) for SU1694 (LFP2021) and SU1695 (LFP2023). Each trace shows the average LFP response at that frequency with 0 ms being the onset of the stimulus. (C) Based on the window of spiking activity in the panels of A, tuning curves are generated for both SUs with the black line representing SU1694 and the gray line representing SU1695. The tuning curves are then normalized so that the area under the curves sum to one. (D) The corresponding LFP tuning curves, where the black line represents LFP2021 and the gray line represents LFP2023. The tuning curves are also normalized so that the area under the curves sum to one. (E) The BF difference in octaves $\log_2(BF1/BF2)$, is computed between each pair of recordings. Co-recorded LFPs show significantly lower BF differences compared to SUs (Wilcoxon Sign Rank, $z=3.1$, $N_{SU}= N_{LFP} =37$, $p < 0.01$). (F) For each pair of recordings, a tuning curve correlation coefficient (CC) is computed. Examples of these values are in C and D, where the SUs have a CC of -0.49, and the LFPs have a CC of 0.82. The LFPs show a much higher tuning curve correlation compared to the two recorded SUs (Tuning Curve Correlation: Wilcoxon Sign Rank, $z=5.1$, $N_{SU}=37$, $N_{LFP}=37$, $p < 0.001$). $N_{SU}=37$, $N_{LFP}=37$.

bar at top of Fig. 3.6B) for each frequency. The LFP BF was then defined as the frequency that evoked the largest negative deflection (Fig. 3.6D).

To compare the variability between SU and LFP BFs within each recording column, we measured the octave difference in BF between the two SUs or LFPs as $\log_2(BF_1/BF_2)$, where BF1 (BF2) was the larger (smaller) of the two BFs. For example, two BFs that were identical would produce a difference of zero. We found that SUs were significantly more variable in their BF values within a column compared to LFPs (Fig. 3.6E). To assess differences in the tuning curves themselves, we computed correlation coefficients between the 37 pairs of SU or LFP recordings, and compared these between SUs and LFPs. LFP tuning curves were significantly more similar across sites compared to SUs (Fig. 3.6F). Interestingly, the BF difference and tuning curve correlation measures for both SUs and LFPs were not correlated with the depth difference between recordings (corrcoef, Tuning Curve Correlation: $cc_{SU} = 0.07$, $cc_{LFP} = -0.05$; BF change: $cc_{SU} = -0.11$, $cc_{LFP} = 0.07$, all $p>0.05$). These results confirm that in the awake mouse, SU tone responses in a given auditory cortical column are more variable compared to their co-recorded LFPs, and making the LFP more useful in identifying the likely cortical field (UF, AAF, A1, and AAF/A1) for recording sites in the awake animal.

Using the LFP, we found a similar tonotopic progression to Stiebler et al. (1997). This occurred mainly in the second row of holes in our stereotaxically prepared recording grid, with the BF gradient reversal (see 1.1.2) occurring in the second or third column corresponding to 60% or 70% respectively of the distance between Bregma and Lambda (Fig. 3.7A). This row generally covered the rostral to caudal transition from AAF

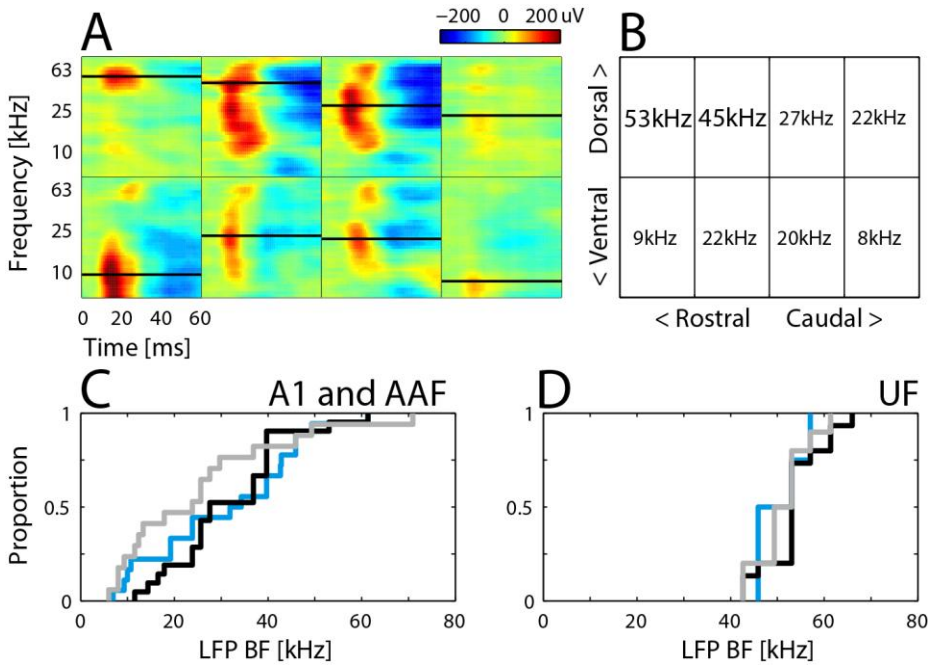


Figure 3.7: (A, B) Each box represents a recording location in the mouse auditory cortex, and shows the left portion of figure A as a color plot, with negative LFP deflections as red and positive deflections as blue. The color limits are set to the maximum positive and negative deflections found in the entire map. The black line shows the LFP BF for that recording hole. The high frequency value in the upper left represents UF. Note the tonotopic reversal in the second row, which shows the rostral to caudal transition from AAF to A1. (C, D) Cumulative density functions show that the distributions of each SU recording site's LFP BF is not different between mothers (black), cocarers (blue), or virgins (gray) in regions defined as A1 and AAF or UF (UF: Kruskal-Wallis, χ^2 (2,26) = 1.3, $p > 0.05$; A1 and AAF: ANOVA, F (2,53) = 1.1, $p > 0.05$). For A1 and AAF: $N_{mothers} = 21$, $N_{cocarers} = 17$, $N_{virgins} = 18$, for UF: $N_{mothers} = 15$, $N_{cocarers} = 10$, $N_{virgins} = 4$.

to A1. Due to the similarities between AAF and A1 in their tone responses, and our lower density recording grid (~400 um), we were not able to identify the exact end of AAF and start of A1. Thus, these areas were labeled as AAF/A1. In the top row, we found that the more caudal holes were typically associated with either A1 or AAF/A1 depending on the broadness of its frequency response and LFP BF, whereas the more rostral 2 holes usually contained sites with high frequency BF's above 45 kHz, corresponding to UF. Occasionally, a more caudally located hole in rows one and two

would show a break from the tonotopic gradient with a much higher frequency BF, and/or a much weaker relative LFP response, suggestive of the DP field. Thus, we were able to use the LFP response to tone frequencies as a way to determine whether recorded SUs came from more primary core areas (A1, AAF, UF) or more secondary non-core areas (A2, DP). Those SUs that were defined as being in non-core areas were subsequently excluded from all the data analysis. To analyze the responses in the core areas, we defined the areas with BFs lateral to the pup call range as A1 and AAF, which consisted of any recordings from AAF, A1, or AAF/A1; and we defined contiguous more rostral-dorsal areas with BFs in the pup call range as UF. It should be noted that during the designation of auditory fields for each animal, we had no prior knowledge of the locations of recorded SUs.

Over UF, A1 and AAF, we found no differences in each animal group's distribution of LFP BFs in either auditory field for all SUs (UF: Kruskal-Wallis, χ^2 (2,91) = 2.6, $p > 0.05$; A1 and AAF: Kruskal-Wallis, χ^2 (2,197) = 2.2, $p > 0.05$) or call-inhibited SUs only (Fig. 3.7C and 3.7D). In addition, we found no changes in the LFP tuning curves between the animal groups for UF or A1 and AAF (data not shown). For all groups the mean and standard deviation of call-inhibited UF LFP frequencies was 52.2 ± 6.1 kHz, and for LFP frequencies in A1 and AAF it was 29.3 ± 15.4 kHz. Looking at all recorded SUs, the LFP BF mean and standard deviation in UF was 52.5 ± 9.7 kHz, and for A1 and AAF 26.2 ± 16.5 kHz. To compare our LFP BF results to the multi-units CF results found previously, we looked at the proportion sites tuned greater or less than 50 kHz. In the UF region, we found 69% of sites with LFP BFs greater than 50 kHz, whereas Stiebler

found 76% of recording sites above 50 kHz. In the A1 and AAF regions, we found 91% of sites with LFP BFs less than 50 kHz, whereas Stiebler found 100% of their recording sites below 50 kHz. Hence, our data shows similarities to those found previously, where the differences could result from the state of the animal during experiments (awake versus anesthetized), animal model (CBA/CaJ versus NMRI), and recording technique (MU versus LFP).

Using the above auditory field segregation, we compared the call-inhibited SU responses in mothers, cocarers, and virgins separately for UF and for A1 and AAF together. In contrast to UF, A1 and AAF were included together because these regions had similar LFP BF ranges, and consisted of BFs more lateral to the pup call frequency range. In UF, we found no differences between mothers, cocarers, and virgins (Duration: Kruskal-Wallis, χ^2 (2,26) = 3.9, $p > 0.05$; Strength: ANOVA, $F(2,26) = 1.6$, $p > 0.05$). However, the call-inhibited differences we found previously in figure 3.5 were consistent with those SUs located in A1 and AAF. Again, the mothers had significantly stronger (Fig. 3.8B) and longer duration (Fig. 3.8C) of call-evoked inhibition compared to virgins, whereas cocarers did not. These results demonstrated that there was a greater plasticity in the call-inhibited SU responses in A1 and AAF, auditory fields with BFs lateral to the pup call frequencies. This suggests that pup experience alone (cocarers) does not account for the differences between mothers and virgins, and that the maternal physiological state must play a role in the observed cortical inhibitory plasticity.

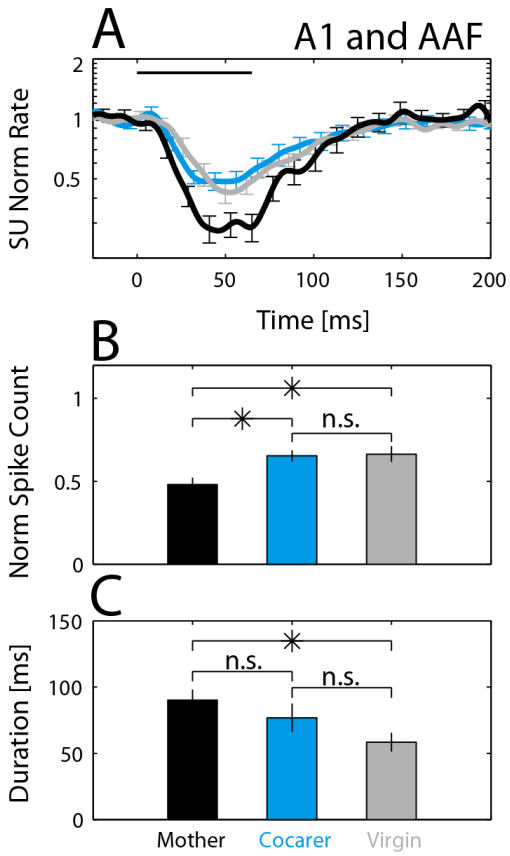


Figure 3.8: Greatest differences in call-evoked SU inhibition occurs in A1 and AAF. (A) Mothers (black) show a stronger call-evoked inhibition when compared to virgins (gray) and cocarers (blue) in their population-averaged spike rate in fields A1 and AAF. (B) Mothers show a significantly lower normalized rate (Kruskal-Wallis, χ^2 (2,53) = 10.2, $p < 0.01$, Fisher's LSD post hoc: M-V, $p<0.05$; M-C, $p<0.05$; C-V, n.s.). This rate is derived by integrating the spike count over the stimulus period and dividing by the spontaneous rate. (C) Mothers show significantly longer durations of inhibition compared to virgins, whereas cocarers are not different from mothers or virgins (Kruskal-Wallis, χ^2 (2,53) = 8.1, $p < 0.05$, Fisher's LSD post hoc: M-V, $p<0.05$; M-C, n.s.; C-V, n.s.). For A1 and AAF: $N_{mothers} = 21$, $N_{cocarers} = 17$, $N_{virgins} = 18$.

3.3.3: Physiological state influences maintenance of cortical plasticity

The similarity of cocarers to virgins was a surprise. This was because previous work showed that both a mother and a cocarer with at least 5 days of pup experience could behaviorally recognize pup vocalizations over a neutral sound (Ehret, 1989).

However, they also found that call recognition deteriorated in an ovariectomized cocarer one month after pup weaning, but not in the mother. Given this, we wondered whether the lack of difference between late cocarers and naïve virgins was because experience had no effect on cortical plasticity, or if changes might have occurred early during pup experience, but had since decayed. This therefore motivated new experiments on early cocarers during the initial stages of their experience with pups. Here, we will refer to cocarers recorded post-weaning as late cocarers, and those during pup experience as early cocarers.

For this study, early cocarers had pup experience prior to surgery through post-parturition day 6, near the peak in pup vocal activity, before being isolated for surgery and recording. Recordings were taken from post-parturition days 9 through 11 (Fig. 3.9A). In total, we collected data from six animals, isolating 45 SUs, 13/45 of which were call-inhibited (29%) in the core fields at depths between 300-700um, and 8/13 of which were designated as A1 and AAF sites. Because of the lower N size (N=8) in our early cocarer group, we employed a bootstrap method to estimate the population mean and confidence intervals for our two measures of call-evoked inhibition (strength or SNR, and duration). Briefly, we performed a bootstrap where we sampled each group's SU data set with replacement, preserved the starting N size, and computed a mean duration and strength for this sampled data set. This was iterated 10,000 times, and we created a 2D contour of the 95% confidence interval around the bootstrap means for these measures. We found that for these two features, the confidence regions for the early cocarers and mothers lie on top of each other, whereas late cocarers are shifted

towards weaker SNRs (Fig. 3.9B). Interestingly, we also found a subset of call-inhibited SUs in the early cocarers with durations of inhibition much longer than the other animal groups, lasting well beyond the stimulus period (bottom right inset, Fig. 3.9B).

While these results clearly illustrate how early cocarers are more similar to mothers, for completeness we also quantified the measures of inhibition used in the previous figures in 3.9C, D, and E. We found that early cocarers were not significantly different from mothers, but significantly different from late cocarers in their call-inhibited SU PSTH responses and strength of inhibition (Fig. 3.9C and D). While both mothers and early cocarers showed higher average durations of inhibition compared to late cocarers none of the animal groups were statistically different from one another (Fig. 3.9E). Hence, recent experience with pups can result in auditory cortical inhibitory plasticity, but the maternal physiological state during this experience must be important in maintaining this plasticity over longer periods. The fact that the strength of inhibition, but not the duration, between early and late cocarers are different suggests that pup experience and the maternal physiological state may affect specific features of inhibition to varying degrees.

3.3.4: Physiological state selectively reduces spontaneous activity

Although our recordings in mothers are performed after the hormones related to pregnancy and parturition have returned to levels more similar to virgins, we wondered whether the changes caused by the maternal physiological state during pup experience could have also affected other spiking characteristics of auditory cortical

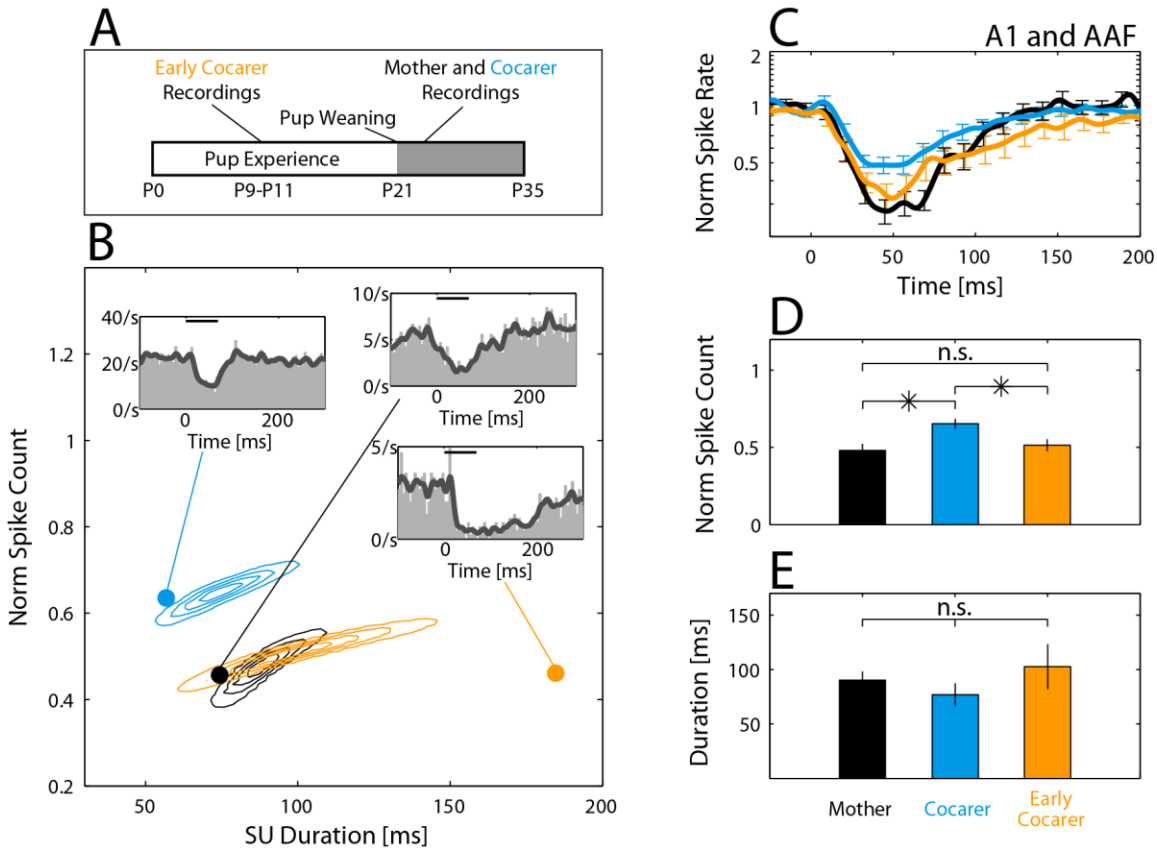


Figure 3.9: Early cocarers are more similar to mothers in their call-inhibited SU responses in fields A1 and AAF. (A) Time line shows the period where early cocarers (orange) are recorded versus the mothers (black) and late cocarers (blue). Early cocarers are recorded from post-parturition day 9 through 11 whereas both mothers and late cocarers are recorded post-weaning. (B) Plots (see Methods) show contours for 25, 50, 75, and 95% of the bootstrapped means for duration and strength. Early cocarers, but not late cocarers, overlap with mothers in this SU strength/duration space. Insets show example PSTH responses to all 18 calls and dots show their measured duration and strength. (C) Both mothers and early cocarers show stronger averaged call-inhibited SU responses when compared to late cocarers. (D) Mothers and early cocarers show a significantly lower normalized rate when compared to late cocarers (Kruskal-Wallis, χ^2 (2,45) = 10.3, $p < 0.01$, Fisher's LSD post hoc: M-C, $p<0.05$; M-EC, n.s.; C-EC, $p<0.05$). (E) Although both mothers and early cocarers have mean durations higher than late cocarers, the groups are not different (Kruskal-Wallis, χ^2 (2,45) = 2.6, $p > 0.05$). For A1 and AAF: $N_{mothers}$ = 21, $N_{cocarers}$ = 17, $N_{early\ cocarers}$ = 9.

neurons. A number of studies have demonstrated that reproductive hormones can affect the spontaneous activity in a number of different areas including the amygdala (Schiess et al., 1988) cerebellum (Smith et al., 1988), and barrel cortex (Kis et al., 2001). Based on this, we measured the spontaneous firing rates (see Methods 3.2.4) in our data for neurons that were call-responsive, and those that were not. Interestingly, we found that there was a significant interaction between the animal group and call-responsiveness $F(3,344) = 4.7$, $p<0.01$, and an overall main effect of responsiveness $F(1,344) = 35.7$, $p<0.001$ (Fig. 3.10A). The main effect of responsiveness indicated that considering all animals together, a SU was more likely to respond to vocalizations if its spontaneous firing rate was higher. However, the significant interaction tells us that this effect of responsiveness was not consistent across all animal groups. For call-responsive neurons in mothers, there was a significantly lower spontaneous firing rate compared to virgins, late cocarers, and early cocarers, while the latter three animal groups were not different from each other (Fig. 3.10C, left bars). In contrast, spontaneous rates for non call-responsive SUs were not significantly different across groups (Fig. 3.10C, right bars).

To test whether these changes were specific to call-responsive SUs, we used the same set of SUs to identify neurons that were responsive or non-responsive to pure tones from an equivalent octave range of frequencies as our pup calls, namely 20-28 kHz. Specifically the five tones presented in this range were 20.6, 22.2, 23.9, 25.7, 27.6 kHz. Figures 3.10B and D show that while we still found a significant main effect of responsiveness, $F(1,323) = 49.2$, $p<0.001$, there was now no significant interaction between group and tone-responsiveness, $F(3,323) = 0.3$, $p>0.05$. Importantly, unlike

calls, the effect of tone-responsiveness in mothers was similar to the other animal groups (Fig. 3.10D). Taken together, this data suggests that the maternal physiological state plays a role in selectively suppressing the spontaneous firing rates of neurons that are call-responsive, possibly as a way to facilitate the maintenance of the long-term cortical inhibitory plasticity in mothers.

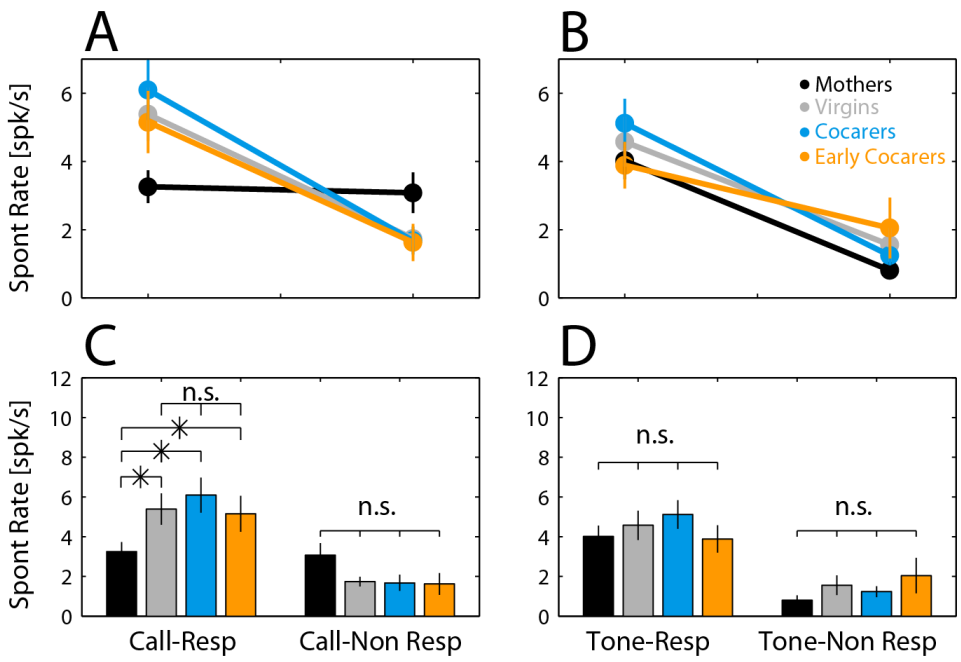


Figure 3.10: Mothers (black) differ from all other animal groups in its intrinsic firing rates for neurons that are call-responsive, but not for tone-responsive SUs. (A, C) For virgins (gray), late cocarers (blue), and early cocarers (orange), a neuron that is call responsive generally has a higher intrinsic firing rate. However, for mothers, the call-responsive SUs have a significantly lower firing rate compared to the other 3 groups. For call-non-responsive SUs, there were no differences in the intrinsic firing rate. For call-responsive: $N_{mothers}$ = 72, $N_{virgins}$ = 59, $N_{cocarers}$ = 68, $N_{early\ cocarers}$ = 30 (Fisher's LSD post hoc: M-V/M-C/M-EC, $p<0.05$; V-C/V-EC/C-EC, n.s.). For call non-responsive: $N_{mothers}$ = 37, $N_{virgins}$ = 41, $N_{cocarers}$ = 30, $N_{early\ cocarers}$ = 15 (Fisher's LSD post hoc: M-V/M-C/M-EC/V-C/V-EC/C-EC, n.s.). (B,D) When comparing to tone-responsiveness to 20-28kHz tones (4 tones total), all groups showed a higher intrinsic firing rate for those that are tone-responsive, compared to those that are tone non-responsive. We found no group differences in these two categories. Note: To perform statistical tests, we used a square root transformation of the data (see Appendix C for explanation). For tone-responsive: $N_{mothers}$ = 69, $N_{virgins}$ = 77, $N_{cocarers}$ = 67, $N_{early\ cocarers}$ = 27. For tone non-responsive: $N_{mothers}$ = 29, $N_{virgins}$ = 19, $N_{cocarers}$ = 27, $N_{early\ cocarers}$ = 16.

3.3.5: Physiological state influences long-term salience of pup calls

The call-inhibited SU data suggests that both mothers and cocarers undergo significant auditory cortical changes that correlate with prior experience with pups. In addition, the data demonstrates that the physiological changes that a mother experiences from pregnancy and parturition may play a role in retaining this inhibitory plasticity. Thus, we were motivated to test whether these differences between mothers and cocarers correlate with call recognition behavior.

To test the call-evoked behavioral response in mothers, late cocarers, and early cocarers, we implemented a two-alternative pup call detection task (see Methods, 3.2.3) designed to provide more automation compared to the classic paradigm used by Ehret (Ehret et al., 1987; Ehret, 1989). Briefly, animals were first habituated to the testing apparatus for approximately one hour prior to each experiment and then we performed a pup retrieval test. This consisted of scattering pups symmetrically throughout the maze so as not to introduce a left right bias. Following retrieval of all pups back to the nest, we allowed the animal to explore the maze until she returned to the nest for at least thirty seconds. At this point, we remotely activated the servo-controlled doors to keep the animal in the nest area and then began the two-alternative choice test. Once the test started we kept the animal in the nest for thirty seconds while we played back bouts of pup calls from a speaker positioned on one side of the maze, which alternated with a speaker placed on the opposite side playing back tones centered around 20 kHz. We did this to ensure that the animals were likely to listen to the sounds before making a choice. After thirty seconds, we remotely sent a signal to

open the servo-controlled doors allowing the animal to exit the nest and then scored whether it moved towards the pup call or tone speaker. In total, we recorded 75 experiments from 13 mothers, 74 experiments from 13 late cocarers, and 48 experiments from 8 early cocarers.

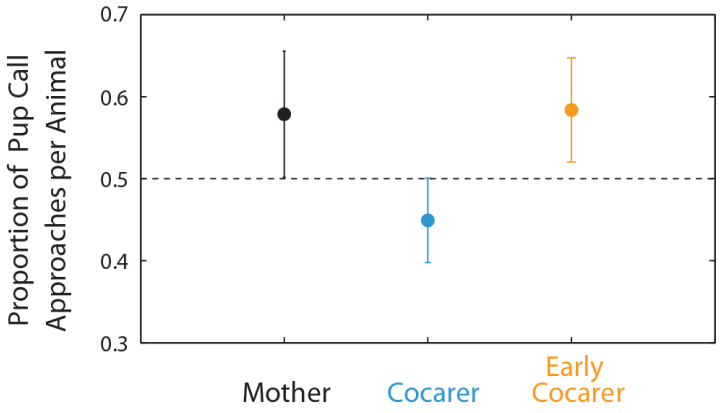


Figure 3.11: The proportion of responses towards the pup call speaker per animal in mothers (black), early cocarers (orange), and late cocarers (blue). Figure shows the mean and standard error for each animal group. Animal groups show no differences in their proportion of approaches (Kruskal-Wallis, $\chi^2(2,31) = 3.8$, $p > 0.05$). Notice though that both mothers and early cocarers show a higher proportion of call approaches compared to the late cocarers.

We first evaluated the total proportion of approaches towards the pup call speaker in each animal group. Although we found no statistically significant differences between the animal groups, our data showed that both mothers and early cocarers approached the pup call speaker a greater number of times compared to late cocarers (Fig. 3.11). In addition, 7/13 (54%) mothers and 5/8 (63%) early cocarers performed the call recognition task above the 0.5 chance level, while only 2/13 (15%) late cocarers performed greater than chance. From this, it is clear that mothers and early cocarers

may be more responsive to the pup calls compared to late cocarers. In support of this, we found that late cocarers had approximately twice the number of experiments where they made no response to the sound playback. This consisted of 5 experiments in mothers (6.7%), 11 experiments in late cocarers (14.9%), and 4 experiments in early cocarers (8.3%).

How then does this relate to the call recognition studies by Ehret? Comparing our analysis above to that of Ehret, we found distinct differences in their method of quantifying pup call approaches. Importantly, they included only those trials where an animal approached a speaker, and compared the total number of approaches towards the pup call or tone playback speaker across all animals studied (Ehret, 1982). Using Ehret's method of quantifying pup call approaches, we first removed the experiments where the animal made no decision. We then evaluated the total number of times the animals within each group approached the pup call speaker compared to the tone speaker. We found that both mothers and early cocarers approached the pup call speaker a significantly greater number of times when compared to chance. In contrast, the late cocarers showed no differences from chance level performance (Fig. 3.12). These results would suggest that the differences in SU inhibitory plasticity found earlier between mothers, early cocarers, and late cocarers may be correlated with the animal group's pup call detection and approach behavior. Interestingly, although our mother and early cocarer results match those found by previous studies (Ehret et al., 1987; Ehret, 1989), our late cocarer results differ. Ehret previously demonstrated in a number of tests that mothers, early cocarers, and late cocarers approached a speaker playing

back 50 kHz tone bursts over a speaker playing back 20 kHz tones significantly greater than chance. This difference in the performance of late cocarers could be attributed to the use of different mouse strains (inbred CBA/CaJ versus outbred NMRI), testing methods (W-maze with servo controlled doors versus straight platform), sound playback type (synthesized pup vocalizations versus 50 kHz tone bursts), and habituation time or tests per day (1 hour habituation and 2 tests per day versus 6 hours habituation and 6 tests per day). Regardless of these differences in testing procedures, it is important to note that Ehret also demonstrated that cocarers tested one month after pup weaning no longer significantly responded to the speaker playing back ultrasounds, whereas mothers did. Hence, consistent with our results, there is still a difference in NMRI mice in the maintenance of pup call salience between mothers and cocarers.

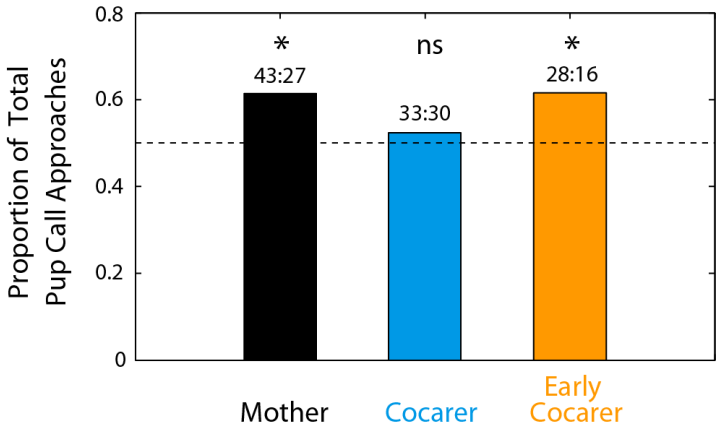


Figure 3.12: The number of approaches towards the pup call versus tone speaker in mothers (black), early cocarers (orange), and late cocarers (blue). Mothers approach the pup call speaker significantly more than the tone speaker (43 call: 27 tone, 61.4% call side, Binomial Test $z=1.9$, $p<0.05$, 1-tailed). Cocarers do not show a preferential approach response to either side (33 call: 30 tone, 52.3% call side, Binomial Test, $z=0.4$, $p>0.05$, 1-tailed). Early cocarers show preference for the pup call speaker (28 call: 16 tone, 63.4% call side, Binomial Test, $z=1.8$, $p<0.05$, 1-tailed). The black line represents chance level performance.

While Ehret's studies looked mostly at the proportion of approaches towards ultrasounds, we wondered whether the animal groups differed in other measures of behavior, such as exploration times or pup call retrieval. To quantify whether the sound playback influenced an animal's exploration we measured the amount of time each animal spent searching the W-maze immediately following retrieval of pups back to the nest (3.13A, post-retrieval) and the two-alternative choice sound test (Fig. 3.13A, post-call test). In figure 3.13A, the y-axis represents the amount of time spent searching between when the animal first leaves the nest (following retrieval or call playback test) to its first return back to the nest area. We compared these two exploration times using a mixed between-within subjects repeated measures ANOVA and found a significant interaction between the animal group and test (ANOVA, $F(2, 165) = 5.3$, $p<0.01$). Given this significant interaction, we proceeded to do a multivariate ANOVA on the exploration times and found no significant differences in the post-retrieval times, but differences between mothers, early cocarers, and late cocarers in their post-call test exploration times (Fig. 3.13A). In addition, figure 3.13B shows the difference in exploration times within each experiment, and again demonstrates that both early cocarers and mothers spend a significantly longer time searching following the call playback test. These results suggest that mothers and early cocarers, but not late cocarers, have a greater motivation to search for pups. However, is this greater motivation simply due to each animal's intrinsic level of motivation to retrieve pups? We addressed this question by quantifying the average amount of time the animal took to retrieve each pup back to the nest. Overall, we found no differences in the pup

retrieval times (Kruskal-Wallis, χ^2 (2,187) = 2.0, p > 0.05, Fig. 3.14B). This further supports the idea that the cortical inhibitory plasticity in mothers and early cocarers may be correlated with the behavioral relevance of pup vocalizations and that reproductive experience influences the long-term saliency of these sounds.

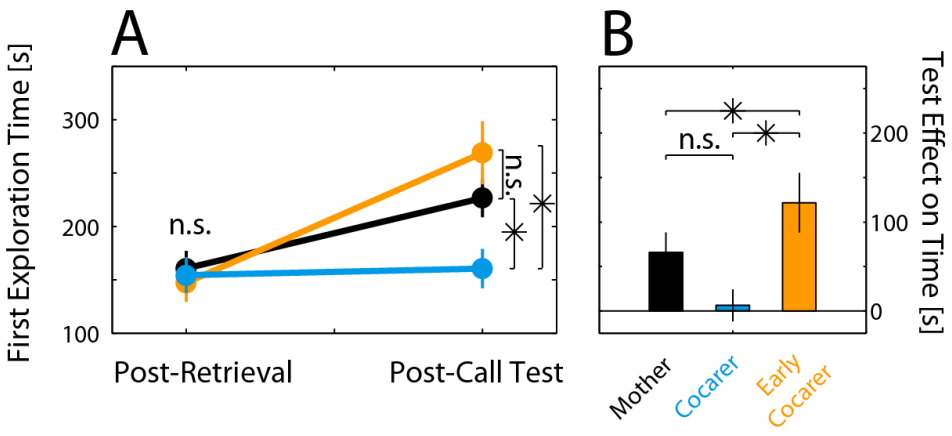


Figure 3.13: During the call test, mothers (black) and early cocarers (orange) show greater exploration times during their first search of the maze compared to late cocarers (blue). (A) The figure demonstrates the amount of time the animal explores the maze prior to their first return to the nest in the W-maze. Following the retrieval of pups back to the nest, the three groups show no differences in the amount of time exploring the maze. However, during the call playback test, both mothers and early cocarers explore the maze for a longer period of time when compared to late cocarers (Post-retrieval test: ANOVA, F(2, 165) = 0.08, p>0.05; Post call-test: ANOVA, F(2, 165) = 8.4, p<0.001; M-C:p<0.05, EC-C:p<0.05, EC-M: n.s.). (B) Bars demonstrate the within subjects difference in post-call test exploration time versus post-retrieval exploration time. A mean of zero indicates that the animal spends an equivalent amount of time exploring the maze following both tests, and a positive mean indicates that the animal spends more time exploring the maze following the call-test compared to the retrieval test. Both mothers and early cocarers explore for a greater amount of time following the call test compared to late cocarers (ANOVA, F(2, 165) = 4.5 p<0.05; M-C: p<0.05, EC-C:p<0.05, M-EC: n.s.).

Although we found clear differences in mothers and early cocarers' proportion of approaches and exploration times, there is the possibility that alternate intrinsic factors may play a role in driving these changes. This includes the estrus cycle of the animal, habituation to the apparatus, the animal's threshold of hearing, and their

motivation to perform pup retrieval. In mice, the estrus state has been shown to affect an animal's perception of acoustic meaning (Ehret and Schmid, 2009), and can even alter the brainstem auditory neural responses in humans (Elkind-Hirsch et al., 1992). Therefore, to study whether the estrus state of the animal influenced its behavioral performance, we recorded the estrus cycle in a subset of animals (10 mothers, 8 early cocarers, 10 late cocarers). If the cycle state could not be defined as one of the four stages (see Methods, Fig. 3.1), data for that day was not included in this analysis. In our results, we found no consistent estrus dependent effects on both the two-alternative choice task (Table 3.1) and no influence on the animal's exploration time. Specifically, there were no interaction effects between the estrus state and the exploration times ($F(3,126)=.109$, $p>0.05$), or between the animal group and exploration times ($F(6,126)=.361$, $p>0.05$). Although our measures of behavior showed no dependent effects, it is possible that the estrus state influenced behavior in a more specific way. Nonetheless, we can conclude that for the differences found previously, the estrus state did not play a role in these behavioral measures.

In our experimental design, the mouse remains in the nest area for thirty seconds while two speakers are playing back both ultrasounds and tones centered around 20 kHz. Given that the speakers were placed equidistant from the nest, it is possible then that an animal's threshold of hearing ultrasounds versus 20kHz could affect both the proportion of approaches and exploration times. To test this hypothesis, we recorded the ABR thresholds to both 64kHz and 20kHz in every animal (see Methods 3.2.1). Overall, we found no interaction effect between group and the ABR response

thresholds using a repeated measures ANOVA (ANOVA, $F(2,31) = 0.82$, $p>0.05$, Fig. 3.14A). These results further support those found previously in our lab (Miranda et al., *in review*) that the ABR thresholds to a series of tones (8, 16, 32, 64, and 80 kHz) were not different between mothers, cocarers, and virgins. Here we showed that early cocarers also did not differ in their response thresholds. In addition, we found no correlation between the behavioral performance or the exploration times and the threshold response to 20 or 64 kHz (data not shown). In conclusion, we demonstrate here that pup experience alone can result in the acquisition of the behavioral relevance of pup calls. However, the distinct physiological changes that occur associated with pregnancy and parturition affect the long-term saliency of pup calls both cortically and behaviorally.

Table 3.1: Table shows the proportion of approaches towards the pup call speakers dependent on the test days and the estrus cycle.

	Mother	Cocarer	Early Cocarer
Test Day 1	15/26 (58%)	10/15 (67%)	10/22 (45%)
Test Day 2	14/24 (58%)	8/15 (53%)	13/24 (54%)
Test Day 3	14/20 (70%)	10/14 (71%)	10/17 (59%)
Proestrus	8/10	5/11	6/11
Estrus	7/10	3/11	3/5
Metestrus	7/13	7/14	4/6
Diestrus	11/22	11/14	13/17

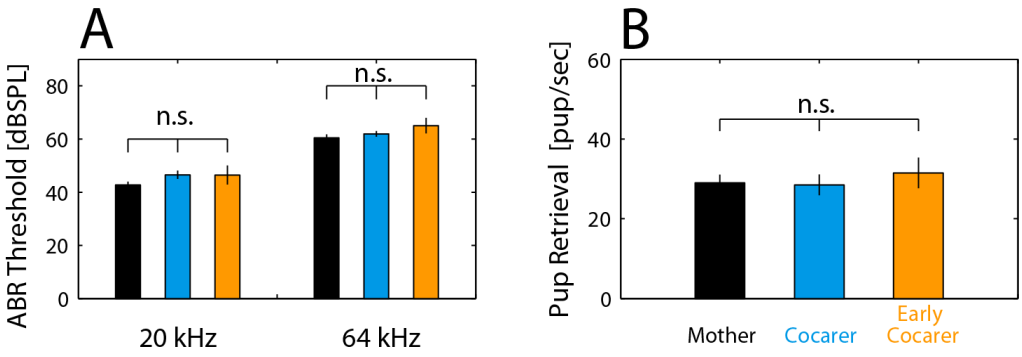


Figure 3.14: Figures demonstrate that there are no differences between the animal groups in their threshold of hearing 20 and 64 kHz, and in their average pup retrieval times. Mothers are black, late cocarers are blue, and early cocarers are orange. (A) Animal groups show no significant differences in their ABR thresholds to 20 kHz or 64 kHz tones. These tones represent the approximate frequencies of the sounds played back during the experiment. (B) Testing the animal's ability to retrieve pups displaced from the nest, groups show no significant differences in the pup retrieval times.

3.4: Discussion

Our results above have begun to dissociate the effects of experience and physiological state on inhibitory plasticity in the sensory cortex of awake (head-restrained) mice. Similar to the work in chapter 2 comparing pup call-evoked cortical responses, we found that mothers showed significantly stronger and longer durations of SU call-evoked inhibition compared to virgins, primarily in "lateral band" auditory fields A1 and AAF. Mothers undergo distinct physiological changes during pup experience, and to understand how pup experience alone influences auditory plasticity, we recorded from cocarers. We found that cocarers were more similar to virgins in their average PSTH and strength of pup call-evoked inhibition, but were not different from mothers or virgins in their duration of inhibition.

Although cocarers showed auditory cortical responses more similar to virgins, previous behavioral studies demonstrated that cocarers early in their pup experience do show pup call recognition (Ehret et al., 1987). Those results combined with the fact that our neural recordings in cocarers and mothers occurred 12-23 days after pups stopped emitting ultrasounds (Noirot, 1972; Scattoni et al., 2009), led us to study cocarers at an earlier time point. By recording from early cocarers (6 days pup experience), we found that their average PSTH and strength of call-evoked inhibition were more similar to mothers, but not late cocarers. These results suggest that experience can produce plasticity in call-evoked cortical responses; however, the maternal physiological state likely helps maintain these changes for the long term.

How might the cortex mediate the retention of these changes? Investigating further, we found suppression in the spontaneous activity of call-responsive neurons in mothers. We hypothesize that while the strength of call-evoked inhibition decays in the cocarer, maternal physiological state acts to maintain these changes in the mother by decreasing the spontaneous activity, specifically for those neurons that are pup call-responsive. Indeed, studies have shown that increasing spontaneous activity can result in faster decay of plasticity in the amygdala (Li et al., 2009), and in retinotectal neurons after the induction of LTP/LTD (Zhou et al., 2003).

While the call-inhibited SU data supports this hypothesis, the question remains whether these differences are correlated with each animal group's behavioral recognition of pup calls. To address this, we used a two-alternative choice test and found that mothers and early cocarers, but not late cocarers, approached the pup call

speaker significantly more times than the tone speaker, and explored the maze for a significantly longer period of time. Therefore, we find that while very recent pup experience can result in cortical plasticity for pup calls, the maternal physiological state facilitates long-term maintenance of these changes. We further suggest that this maintenance could be mediated by selectively suppressing the spontaneous activity of call-responsive neurons, as observed in mothers to "protect" against plasticity decay. Finally, our data supports the idea that the observed lateral band inhibitory plasticity is a functionally relevant correlate of call recognition. Specifically, mothers and early cocarers, but not late cocarers showed preferred approach to pup calls, which maps onto the pattern of cortical inhibitory plasticity.

3.4.1: Relation to prior studies

Our previous study in chapter 2 demonstrated that mothers had stronger and longer call-evoked SU inhibition compared to virgins, and by analyzing the LFP, these differences were primarily attributed to auditory cortical regions tuned to frequencies lower than the ultrasonic pup calls. These findings led us to suggest that the changes in call-evoked inhibition enhance the contrast between the activity of ultrasonic and lateral frequency neural populations to improve the detection of pup calls. Our current study confirms this result using only SUs. Whereas the SU call-inhibited strength and duration were not significantly different in UF (auditory field with BFs closer to the pup call range), mothers showed greater call-evoked SU inhibition in A1 and AAF (auditory fields with BFs much lower than the pup ultrasounds).

In addition to the auditory cortical work, the effects of motherhood and pup experience have also been studied electrophysiologically in the somatosensory cortex. Mapping the somatosensory cortex (areas serving the ventrum skin) in the anesthetized rat, Rosselet et al. (2006) found plasticity in receptive field size and cortical map organization of the ventral abdomen in lactating and nursing mothers compared to virgins. In addition, Xerri et al. (1994) demonstrated that this plasticity was not derived solely from the physiological changes associated with pregnancy and parturition, but were due to the mother's interaction with pups during the lactating period. Although the previous studies did not find long-term retention of cortical plasticity in mothers, our findings may not be directly comparable due to differences in the specific measures studied (excitation versus inhibition), sensory systems, species, or because our cortical recordings are taken from awake animals. In our model system, the effects of anesthesia itself have been demonstrated in the auditory cortex, as mothers differed from virgins in their excitatory response to pup calls in the anesthetized animal (Liu and Schreiner, 2007), and in their inhibitory responses in the awake preparation (Galindo-Leon et al., 2009). Regardless, studies in both somatosensory and auditory cortices demonstrate that pup experience dynamically changes the mother's sensory representations of pup stimuli.

How might call-evoked SU inhibition be mediated in the auditory cortex? Given our use of high-impedance tungsten electrodes, which is thought to be more sensitive to recording pyramidal neurons (Towe and Harding, 1970; Gold et al., 2006), and the fact that they make up 70-80% of cortical neurons (DeFelipe and Farinas, 1992), our call-

evoked inhibited SU data likely reflects the responses of these cells. One source of inhibition onto pyramidal cells comes from inhibitory interneurons (fast spiking cells) (Gonzalez-Burgos et al., 2005; Freund and Katona, 2007), and thus the prolonged and deepened inhibition in the mother and early cocarer could be a result of stronger feed-forward inhibition from fast-spiking inhibitory interneurons. Under this assumption, our results agree with a recent study from Cohen et al. (2011), which found that in the anesthetized mother, auditory cortical fast spiking neurons respond more robustly to pup calls.

Aside from the natural communication context, other recent studies have also demonstrated plasticity in stimulus-evoked suppression as a consequence of instrumental conditioning. Two studies in particular, were performed in the awake auditory cortex of ferrets, which were trained on either a negative reinforcement task (Atiani et al., 2009), or both positive and negative reinforcement tasks (David et al., 2012). In Atiani et al. (2009), ferrets were conditioned to stop licking a waterspout upon detection of a target tone within a background noise to avoid a shock. Interestingly, if the target tone was at a frequency away from the BF of a recorded neuron, then that neuron's activity was generally suppressed. To some degree, their suppression results parallel our data, since SUs tuned to frequencies away from the "target" pup call range, in our case A1 and AAF, showed greater call-evoked inhibition. In addition, David et al. (2012) recently demonstrated in the same animal model (ferrets), that changing the reinforcement task (positive versus negative) directly altered the neural response at the target tone (enhanced inhibition versus excitation). It is clear then that the auditory

cortex can undergo stimulus-evoked plasticity in SU inhibition, but that the way in which this occurs can depend specifically on how an animal learns a sound's behavioral relevance.

3.4.2: Role of maternal physiological state in plasticity maintenance

There is now evidence that plasticity can differ in the adult auditory cortex dependent on the sound experience, but the question remains as to how the physiological state affects this process. In the rodent model of motherhood, pup experience alone can induce maternal behavior. However, the physiological changes that occur during pregnancy and parturition facilitate its onset and maintenance (Moltz et al., 1970; Numan et al., 1977; Bridges, 1984; Orpen and Fleming, 1987; Fleming and Sarker, 1990; Scanlan et al., 2006; Stolzenberg et al., 2007; Stolzenberg and Rissman, 2011). Indeed, pup experience and maternal hormones have been shown to play critical roles in the behavioral recognition of pup ultrasounds (Ehret, 1982; Ehret et al., 1987; Ehret, 1989; Koch and Ehret, 1989). In fact, a study by Ehret and Koch (1989) demonstrated in a two-alternative sound preference test that both mothers and ovariectomized cocarers, but not pup-naïve virgins, preferentially approached a speaker playing back ultrasounds. Interestingly, one month after weaning, this ultrasound preference was retained in mothers, but not in ovariectomized cocarers. Consistent with this, our study found that both call-evoked inhibitory plasticity and behavioral call recognition decayed in the cocarer. Incidentally though, the time period for recognition decay in Ehret's work differed from both our cortical and behavioral findings. The fact

that they observed decay only after one month, and we observed it as early as 1-2 weeks could be due to mouse strain differences (NMRI versus CBA/CaJ), since strains can differ in their maternal behavior (Priebe et al., 2005). Nevertheless, both results indicate that pup call recognition can be acquired through experience, but that the maternal physiological state during acquisition helps to retain the changes in the behavioral and cortical representation of pup ultrasounds.

Certainly, maternal experience plays an important role in lifelong plasticity and can facilitate future rearing behavior (Bridges, 1975; Scanlan et al., 2006). Additionally, motherhood can improve memory task performance when compared to virgin females, even when tested long after maternal experience has ended (Kinsley et al., 1999; Gatewood et al., 2005; Love et al., 2005; Lemaire et al., 2006; Pawluski et al., 2006; Macbeth et al., 2008). Our current study further supports these ideas that motherhood imparts long lasting changes and extends this to the auditory cortex of awake mice. During pregnancy, parturition, and lactation, the mother undergoes fluctuations in hormones and neuromodulators such as estrogen, progesterone, prolactin, oxytocin, and dopamine, all of which may contribute to this plasticity (Miranda and Liu, 2009).

The mesolimbic dopaminergic system and its interaction with maternal hormones may play an important role in our findings. Dopamine has been shown to be important for long-term maternal memory (Byrnes et al., 2002; Afonso et al., 2008; Parada et al., 2008; Afonso et al., 2009; Numan and Stolzenberg, 2009), and has direct effects on auditory cortical plasticity (Stark and Scheich, 1997; Bao et al., 2001; Kudoh and Shibuki, 2006; Schicknick et al., 2008; Hui et al., 2009). In fact, Schicknick et al.

(2012) recently demonstrated in the gerbil that auditory cortical dopamine activity after learning to discriminate sounds improved memory consolidation. These studies lead us to speculate that the dopaminergic system may be modulated by maternal hormones (estrogen, progesterone, prolactin) to help prime the neural circuits that receive pup stimuli and contribute to the retention of this auditory cortical plasticity.

3.4.3: Decreased spontaneous activity involved in plasticity retention

A surprising finding from our work was the observation that spontaneous activity in call-responsive neurons was suppressed in mothers compared to the different virgin groups. The fact that this difference was not apparent for mothers' tone-responsive neurons suggests that this suppression is selective for pup calls, and may be functionally important for call processing or memory in the mother. In line with our findings, there is evidence suggesting that a suppression of spontaneous rate could play a role in the retention of cortical plasticity. In studying synaptic plasticity in developing circuits, Zhou and colleagues demonstrated that spontaneous activity of tectal neurons rapidly reversed the long-term potentiated and depressed synaptic modifications of retinotectal neurons (Zhou et al., 2003). This attests to the detrimental effects of spontaneous firing for maintaining synaptic plasticity. Presumably, higher spontaneous rates would produce faster decays. This notion has been validated through modeling. First, Li and colleagues created a biophysical network simulation of the lateral amygdala to model conditioned fear memory (Li et al., 2009). They found that increased spontaneous firing resulted in a faster decay of plasticity and hypothesized that low

spontaneous firing, typically observed in the lateral amygdala, acts to preserve fear memory through decreased Hebbian weakening.

Why does the decrease in spontaneous rate only affect a mother's call-responsive neurons? A recent study by Masquelier and colleagues provided evidence for this by modeling the response properties of a single neuron that underwent spike timing dependent plasticity (STDP). Using a simple leaky integrate-and-fire neuron and a population of stochastically and independently firing afferents, they found that the neuron converged to a state where a specific pattern of inputs was required for the post-synaptic neuron to fire. Importantly, the selectivity for a specific pattern of inputs left the neurons with very low spontaneous firing rates, whereas neurons with higher spontaneous rates were less selectively activated (Masquelier et al., 2008). Together these studies suggest that lowering a neuron's spontaneous rate might be an effective mechanism to preserve synaptic plasticity for specific stimuli. To our knowledge, we are the first to demonstrate this experimentally through the result that suppressed spontaneous activity, specifically in call-responsive neurons in mothers, correlates with longer maintenance of lateral band inhibitory plasticity.

Finally, since dopamine facilitates the long-term consolidation of maternal memory and auditory learning, as discussed above, is there evidence that it alters neural spontaneous activity? Indeed, studies in the basolateral amygdala have demonstrated that dopamine can play a role in the increase and decrease of spontaneous activity (Rosenkranz and Grace, 1999, 2002). In addition, it was found that the application of dopamine suppressed the spontaneous firing rates of pyramidal tract neurons in the

rodent motor cortex (Awenowicz and Porter, 2002). Therefore, based on these studies and dopamine's known roles in auditory cortical plasticity, we hypothesize that dopamine plays a role in suppressing spontaneous activity of call-responsive neurons in the mother; and this consequently helps to preserve the SU response to pup vocalizations.

In conclusion, we have shown that very recent pup experience alone can result in auditory cortical inhibitory plasticity, though the maternal physiological state helps to maintain this plasticity. We found that these cortical changes correlate with differences in behavioral recognition of pup calls, and we hypothesize that the maintenance of this long-term plasticity is mediated by selectively suppressing the spontaneous firing of call-responsive SUs. Our results from a natural behavioral context emphasize the need to understand how the intrinsic state of the animal can influence experience-dependent plasticity and the need to study this in the awake animal. Moreover, we speculate that these changes in cortical inhibition lead to behavioral improvements in pup call detection, and that the maternal state helps in the consolidation of cortical plasticity to provide more rapid induction of future maternal behavior.

CHAPTER 4

DISTINCT SUBSETS OF CORTICAL NEURONS ENCODE THE ACOUSTIC FEATURES AND PERCEPTUAL RELEVANCE OF NATURAL SOUNDS

Much of the work in this chapter was published in Journal of Neurophysiology: Lin, F.G., Liu, R.C. (2010) Subset of thin spike cortical neurons preserve the peripheral encoding of stimulus onsets. Journal of Neurophysiology 104 (6): 3588-3599. Portions of this chapter were not included in this manuscript and will be published elsewhere.

Pup calls are an acoustically variable class of sounds and can differ in their frequency, duration, and amplitude envelope. In addition, for some animals, these vocalizations also carry behavioral significance. An important question in auditory neuroscience is how neurons in our cortex encode these features. Previously in chapter 2, we demonstrated that call-evoked excitation was variable and showed onsets, offsets, and sustained responses to calls. This is not entirely surprising, as auditory cortical neurons are complex in their response properties and can be temporally precise and sluggish (Nelken, 2004), possibly as a way to represent the variety of acoustic features. In this chapter, we utilize this idea to segregate the diversity in our call-excited cortical responses. We use a modeling approach to explore how the neural representation of sound features is transformed from the periphery to the auditory

cortex. In theory, doing this identifies whether there are different populations of neurons: those that faithfully encode the acoustic features, and those that process more complex properties of the sound (e.g. salience, context)

4.1: Introduction

The neocortex is marked not only by a stereotypy in its microcircuitry (Kozloski et al., 2001; Silberberg et al., 2002), but also by a diversity in the cell types making up these circuits (DeFelipe and Farinas, 1992; DeFelipe, 1993; Kawaguchi and Kubota, 1997). It may not be too surprising then that a variety of spiking patterns, which may contain transient (Hromadka et al., 2008) and/or sustained (Wang et al., 2005) components, is often observed across a population of neurons in response to a single class of stimuli (Evans and Whitfield, 1964; Chechik et al., 2006; Bartho et al., 2009; Galindo-Leon et al., 2009). In auditory cortex, such different responses presumably reflect diversity in how neurons are sensitive to acoustic features within sounds (Sadagopan and Wang, 2009; Atencio and Schreiner, 2010), and different complex nonlinearities (Ahrens et al., 2008; Atencio et al., 2008; Sadagopan and Wang, 2009) probably underlie how these neurons encode subtle variations in stimuli (Bar-Yosef et al., 2002; Bar-Yosef and Nelken, 2007). Nevertheless, the degree to which diversity in acoustic sensitivity actually maps onto cortical cell type diversity, or instead reflects an encoding strategy amongst presumed pyramidal cells (Chechik et al., 2006; Wang, 2007), is poorly understood.

Addressing this problem requires identifying cell types from *in vivo* recordings in (ideally awake) animals listening to systematically varying sounds, while sampling

enough neurons to quantify diversity. The latter favors extracellular methods, where a common strategy is to sort SUs based on their extracellular action potential duration (Bartho et al., 2004; Atencio and Schreiner, 2008; Hromadka et al., 2008; Wu et al., 2008). Intracellular studies with morphological assessments have demonstrated that this duration correlates with various types of cortical neurons (McCormick et al., 1985; Gray and McCormick, 1996; Nowak et al., 2003). For example, many thin spike SUs (those with short peak-peak durations) are thought to correspond to suspected inhibitory interneurons (Swadlow, 2003), thereby providing one means to approach the cataloging of cellular diversity.

To dissect the variety in acoustic sensitivity, we started with the concept that successive stages of processing likely create more nonlinear neurons (Ahmed et al., 2006; Atencio et al., 2009). The corollary of this is to expect that cortical neurons would be highly nonlinear compared to auditory nerve (AN) fibers, potentially encoding acoustic features very differently. Indeed, temporal modulation is one example where the encoding is systematically transformed from the periphery to the cortex (Eggermont, 2001). In contrast, sound onset encoding can actually be quite comparable between AN fibers and at least some auditory cortical neurons (Phillips and Hall, 1990; Heil and Irvine, 1997; Heil and Neubauer, 2003). Specifically, the first spike latency (FSL) in both areas depend similarly on the acceleration of the amplitude envelope (Heil and Irvine, 1997), likely reflecting a common mechanism for nonlinear amplitude envelope integration (Heil, 2004). However, these conclusions were based on studies in anesthetized animals, and a recent report comparing cortical FSLs in anesthetized versus

awake conditions has demonstrated coding differences between the two (Ter-Mikaelian et al., 2007). How closely then might cortical FSLs in *awake* animals follow the mechanism for onset sensitivity observed at the AN, and can the degree of similarity be meaningfully used to classify encoding diversity?

To answer these questions, we directly modeled the nonlinear envelope integration mechanism that can be found at the auditory periphery (Neubauer and Heil, 2008), and asked how well the same mechanism, when extended to the auditory cortex, predicted its FSLs. We applied this to cortical neurons from awake mice listening to species-specific communication calls. Such natural vocalizations are thought to be discriminated in auditory cortical activity based on the temporal pattern of spiking (Gehr et al., 2000; Schnupp et al., 2006; Liu and Schreiner, 2007; Huetz et al., 2009), thereby motivating the study of FSLs for this class of sounds. We found that the error our model produced in predicting call-evoked FSLs spanned a large range, but was not randomly distributed across SUs. There were systematic differences in sound encoding properties of neurons whose call-evoked FSLs were best versus poorly predicted by the model, presumably due to higher nonlinearities in the latter group. Most notably, the neurons whose call-evoked FSLs were best predicted tended to have thin spikes, potentially indicative of their being putative fast spiking interneurons. In addition, the subset of poorly predicted neurons demonstrated dissimilar responses to pup calls versus adult calls, two sounds that share similar acoustic features but are perceptually distinct. Hence, our forward model and data leads us to hypothesize that 1) neurons that best preserve the peripheral mechanism for onset encoding in the auditory cortex may

actually be inhibitory rather than excitatory, and 2) those neurons that do not play a role in encoding acoustic features may be recruited through behavioral experience to represent a sound's perceptual relevance.

4.2: Methods

In this chapter, the model used SU data from (55 SUs that satisfied criterion) 9 virgin females, 7 mothers, 9 cocarers. In section 4.3.4 and 4.3.5, we used principles learned from the model to segregate neurons in our entire population of recorded SUs from 14 virgin females, 13 mothers, 14 cocarers CBA/CaJ mice. All mice were between 14 and 24 weeks old at the time of surgery. An animal name list for this chapter is listed in appendix A. Methods on the electrophysiology recordings, surgery, and acoustic stimuli are located in appendices A and B. The methods listed below are specific to this chapter.

4.2.1: SU analyses

The bandwidth of the frequency tuning curve was identified as the difference between the highest and lowest frequencies with spike rates exceeding this half-max value. The tuning quality was then defined as the SU's BF divided by the bandwidth. To estimate the temporal precision of first spikes across trials for each call, a measure analogous to the vector strength (Goldberg and Brown, 1969) was used. First, a period was mapped from 0-100 ms relative to t_{onset} to phases from 0 to 2π. Across all the trials for a particular call, each first spike was converted into a unit vector with a phase

corresponding to its latency, and then these were vector averaged together. For example, if for all trials, the first spike occurred at 10 ms post-stimulus, the length of the resultant vector for that call would be 1; if the first spikes were uniformly dispersed throughout the period, the vector would be equal to 0. Additional standard SU analyses can be found in chapter 2.2.1.

The SU action potential waveform was also used to separate neurons into thin (putative inhibitory interneurons) and thick spiking groups (putative pyramidal neurons). The peak-to-peak time of the average spike waveform was used (Wu et al., 2008), and the entire population exhibited a clear bimodal distribution (Fig. 4.1E). This resulted in thin and thick spiking SUs being classified as having peak-to-peak times less than or greater than 0.35 ms, respectively. Throughout the recordings, thick spiking cells were encountered more frequently (65%), as might be expected if they correspond to cortical pyramidal neurons (Markram et al., 2004).

4.2.2: Van Rossum metric

To estimate the dissimilarity between the spiking responses to two different calls, the van Rossum metric was used (van Rossum, 2001). To compute the van Rossum distances, each spike train was first convolved with a decaying exponential function (equation 4.1).

$$V(t) = \sum_{i}^{M} H(t - t_i) e^{[\frac{-(t-t_i)}{\tau}]} \qquad (4.1)$$

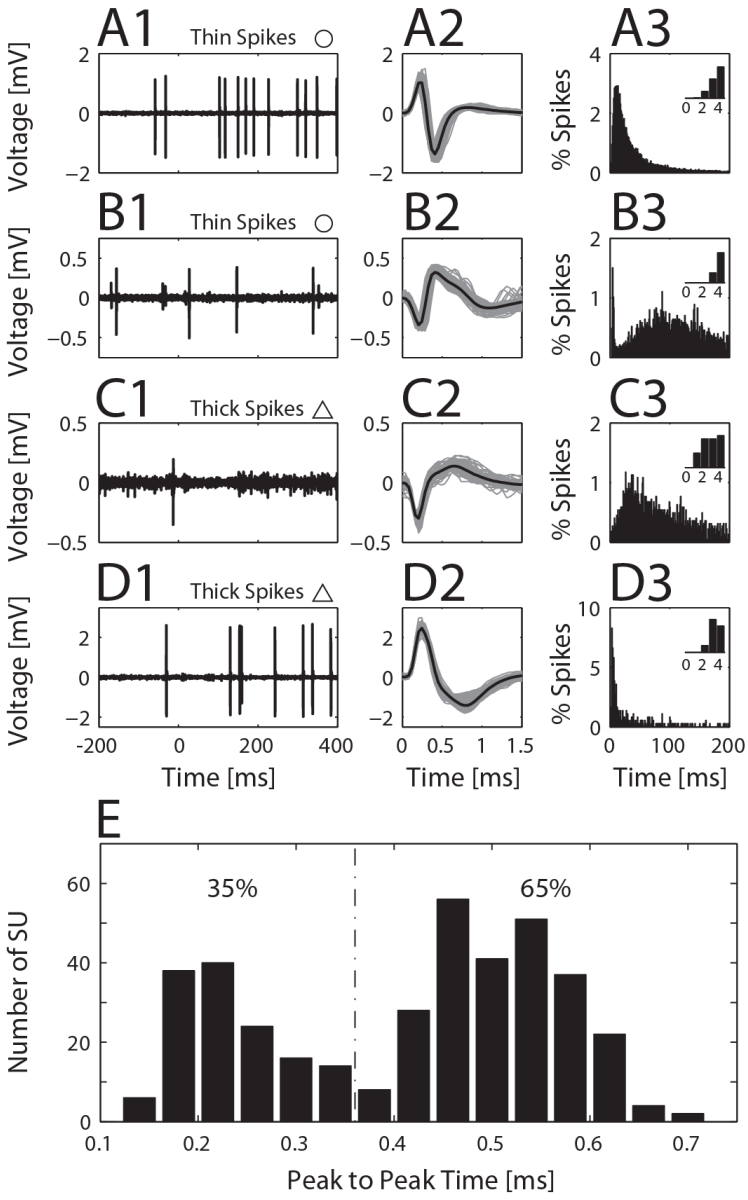


Figure 4.1: SU Recordings. Example (A1) single trial, (A2) spike waveform, (A3) ISI distribution with (inset) expansion of the region between 0-5 ms, for (A) SUnitID 1823; (B) SUnitID 1719; (C) SUnitID 1498; and (D) SUnitID 1421. Mean spike waveform shown in black, with gray lines displaying all spikes. (E) The histogram inset demonstrates the percentage of thin and thick spikes in our entire population of cells. The figure shows the distribution of thin and thick spikes for the cells used to predict the first spike response to pup calls. Based on the overall population, we called spikes with a peak to peak time less than 0.35ms thin spikes, and those with greater than 0.35ms, thick spikes.

In equation 4.1, H(t) is the Heaviside step function, M is the total number of spikes in the spike train, and τ is the exponential decay constant. From here, a distance was computed between two spike trains by equation 4.2, where D_{ij} is the van Rossum distance (VR distance) between spike trains *i* and *j*, and T is the length of the window.

$$D_{ij} = \frac{1}{\tau}\int_0^T [V_i(t) - V_j(t)]^2 dt \qquad (4.2)$$

D_{ij}, which we will call the VR distance, was used to compare how well call-responsive SUs can distinguish between two different call vocalizations versus how well it can distinguish the two calls from its spontaneous activity. The reference spontaneous activity consisted of a trial in our recordings in which no sound was presented.

To do this, we measured both the inter-call distance (discrimination) and the call-to-blank distances (detection). For example, in figure 4.4 we computed a VR distance between calls 13 and 18, and divided this by the average of the distances for call 13-blank and call 18-blank. Thus, if this fraction exceeds 1, the spike patterns between calls are greater in their difference than the spike patterns between the calls and spontaneous. Given that the longest pup vocalizations are ~60ms in length, we used only spikes from onset to onset+100ms to account for any offset type neural activity. However, the short time window combined with the low spiking rates of some neurons resulted in a number of spike trains with only zero or one spike. Recall that for our call playback, up to 50 trials of a SU's response was recorded to each pup call. Thus, to

account for the low spike numbers, but retain the stimulus information present in the 100ms time window, a distance was computed for a "collapsed" spike train. This is a spike train that includes all spikes in all trials. Specifically, this involved collapsing the spike times in all trials of the same stimulus into a single spike train, and then computing a VR distance. If in the spike train there were two spikes with identical times, one of the spike times was jittered by the smallest amount possible bounded by our sampling rate of the spiking data (0.0001 ms). To give an example of this effect, we used a time constant of 10ms, and took a spike train with 10 spikes from our data. We compared this spike train to a jittered version of itself and produced a distance of 7.7×10^{-5}. This is several orders of magnitude less than the smallest distance in figure 4.4A, and thus does not bias the actual computed distances.

To evaluate the inter-call, call-to-blank, or the detect/discrimination measures, we used a time constant of 10 ms similar to the values used in two previous studies (Narayan et al., 2006; Wang et al., 2007), which derived this value as the optimal time constant for neural discrimination of conspecific songs. The 10 ms time constant was implemented because the resultant V(t) function (Eq. 4.1) is well matched to the temporal nature of excitatory post-synaptic potentials in the auditory cortex (Wehr and Zador, 2005).

4.2.3: Estimating first spike latencies

To estimate the FSL in response to a stimulus, different techniques have been applied in the literature (Chase and Young, 2007; Ter-Mikaelian et al., 2007; Pawlas et

al., 2010). While each has advantages and disadvantages, the choice of algorithm appeared not to be critical for our purposes since different methods we implemented produced similar conclusions. Here we present results based on a binless method previously described by (Chase and Young, 2007), since it implements a statistical criterion that in principle accounts for the possibility that spontaneously arising first spikes can bias the latency estimate. Briefly, it collapses spike trains across trials into a list of all spikes, and essentially asks whether the number of spikes occurring within a particular post-onset interval exceeds what would be expected based on a Poisson distribution with a mean rate defined by spontaneous activity and accounting for the number of trials (measured here between -150 to 0 ms re. stimulus onset, t_{onset}). The intervals progressively increment in time steps defined by the difference between the nth post-onset spike (t_n) and our reference time (fixed here at $t_{ref} = t_{onset} + 5$ ms, safely accounting for the shortest possible synaptic delays to the cortex, e.g. (Heil and Irvine, 1997)). We chose n to start at 6, comparable to that used by (Chase and Young, 2007). The FSL was defined as the time t_n at which the probability that at least n spikes in $t_n - t_{ref}$ could have been due to spontaneous Poisson firing first dropped below a threshold $p \leq 0.01$. Spike times after $t_{onset} + 100$ ms were not included, since this was beyond when even offset spiking to the longest stimuli would occur. We found our choice of parameters provided fairly consistent yet more rigorous estimates of what would be identified as the FSL "by eye" for both pure tone and call data sets. If the spiking response to a particular pup call never satisfied the $p \leq 0.01$ criterion, no statistically valid FSL was defined.

4.2.4: Cortical LIEFTS model

While different models could be implemented to predict cortical FSLs, we chose to use a "functional" peripheral model (Neubauer and Heil, 2008), which has been physiologically validated for AN fiber FSLs (Heil et al., 2008), and which can be straightforwardly applied to the cortex. We preferred this over more complex subcortical models (Fishbach et al., 2001; Fishbach et al., 2003; Dugue et al., 2010), which can involve more parameters to predict cortical activity, since our purpose was to focus on how functionally similar the nonlinear envelope integration mechanism was between the periphery and cortex for encoding sound onsets.

The LIEFTS model (Neubauer and Heil, 2008) we applied accounts for a physical delay (L_{min}) between the external stimulus and the inner hair cell response, integrates the amplitude envelope with an exponentially decaying weighting function ($P_{li}(t^{\bullet})$), and applies a biophysically plausible cubic nonlinearity (Heil and Neubauer, 2003; Heil et al., 2008) to derive a stimulus-driven rate of neurotransmitter release:

$$R(t^{\bullet}) = k * \left[\frac{1}{\tau} \int_{-\infty}^{t^{\bullet}} P_{li}(u) e^{\frac{-(t^{\bullet}-u)}{\tau}} du \right]^3 \qquad (4.3)$$

The sensitivity *k* captures all linear proportionality factors up to this stage, $t^{\bullet}$ is the time shifted by L_{min}, and τ represents the integration time constant. $R(t^{\bullet})$ constitutes

the hazard function of the distribution function for first spikes (Neubauer and Heil, 2008), and can be used to generate the FSL probability density function (PDF),

$$\frac{dF(t^{\bullet})}{dt} = [R(t^{\bullet}) + R_{spont}]e^{-\int_{-\infty}^{t^{\bullet}}[R(u)+R_{spont}]du} \quad (4.4)$$

R_{spont} represents the spontaneous discharge rate, which can be estimated from the spontaneous spiking in the absence of stimuli.

To test whether this model can predict *cortical* first spike responses to natural calls, we increased the physical delay L_{min}, and generalized the sensitivity across frequencies *k(f)*. Intuitively, these changes presumed that the timing of first cortical spikes elicited by a call can be explained by a similar nonlinearity as observed in ANFs, after accounting for a longer transmission distance, and a change in gain for different frequency channels. This frequency-dependent gain could arise either from the intrinsic frequency-dependent sensitivity of individual ANFs, or in the convergence of different frequency channels in the feed-forward circuit to the cortex. Only responses to pure tone stimuli were needed to build the model, which was then tested for pup call stimuli. We have implemented the cortical LIEFTS model by optimizing different combinations of essential parameters (L_{min}, k, and τ) (Heil et al., 2008). For simplicity and because our conclusions are not essentially altered, we describe here just the results for when τ was

set to 1.4 ms (based on an estimate of the time constant of the membrane of the inner hair cell (Raybould et al., 2001)), and L_{min} and k were determined as described below.

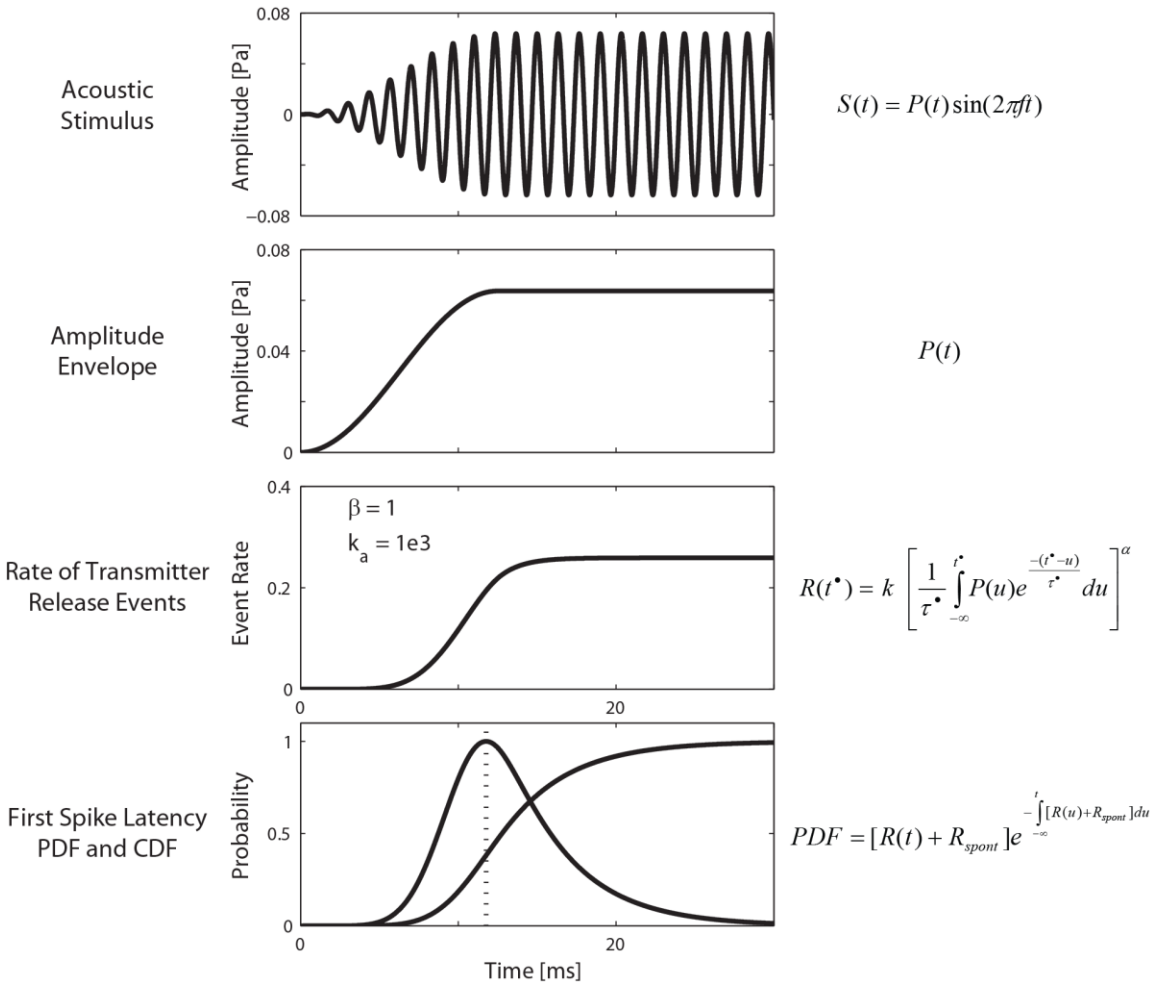


Figure 4.2: Cortical LIEFTS model. Schematic diagram of the cortical LIEFTS model.

We began by using the (Chase and Young, 2007) statistical method to find the FSLs for BF tones of varying amplitudes (Fig. 4.3A and B), allowing us to construct a latency-level function (open squares, Fig. 4.3B). To minimize the number of parameters to fit, we set L_{min} to 0.75x the estimated FSL for the loudest tone at 70 dBSPL (dashed

line, Fig. 4.3B). In a limited number of cases (e.g. SUs that were clearly non-monotonic), we set L_{min} to 0.75x the shortest FSL. R_{spont} was set to the average spontaneous rate prior to the stimulus for all trials. We then solved for the value of *k* at BF through Eqs. 4.3 and 4.4 by using an unconstrained nonlinear optimization based on the Nelder-Mead simplex method (MATLAB Fminsearch). We took the time at the mode of the PDF as the predicted FSL. We compared this (filled black circles, Fig. 4.3B) to the experimental FSL (open squares, Fig. 4.3B) to find the optimal value for *k* that minimized the RMS of the logarithm (base 10) of their ratio (root mean-square error, RMSE) averaged across dBSPL.

At frequencies away from a neuron's BF, we determined *k(f)* by scaling the sensitivity to match FSL predictions to the derived FSLs for pure tones at different frequencies (Fig. 4.3C). Experimental latencies were generally longer at the highest ultrasonic frequencies, indicating that cochlear delays were not responsible for the observed spread in latencies at these frequencies. If for a specific tone frequency f_o, no first spike was statistically found, we set $k(f_o)$ to a SU-specific lower bound value. This was determined by when the peak probability of a predicted, acoustically driven first spike just exceeded the probability for a spontaneously generated first spike. FSLs for communication calls were then predicted (Fig. 4.3D) by interpolating a *k(f)* that matched the starting frequency of a call. Starting frequencies were used because the single frequency calls themselves exhibited little frequency modulation near the onsets.

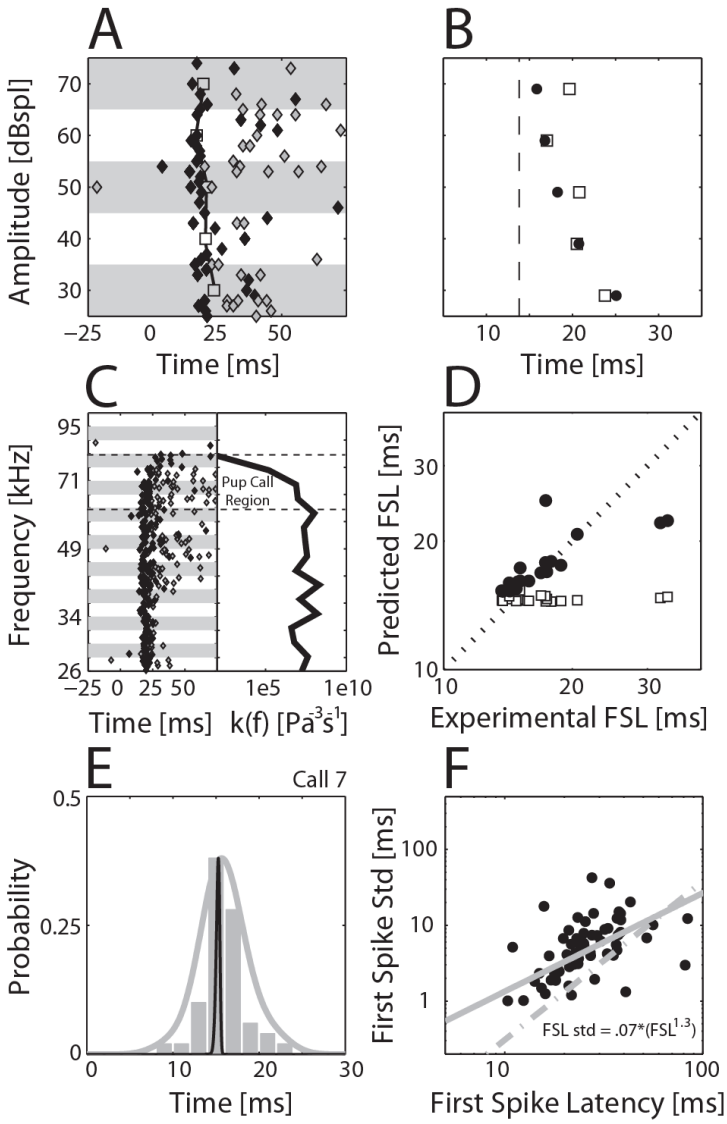


Figure 4.3: Cortical LIEFTS model (A) The figure shows the spiking response to different amplitudes (30-70 dBSPL, alternating gray and white bars) at its BF. The raster plot illustrates the first spike after stimulus onset (black diamonds) and the spikes during the spontaneous period and after the first spike (gray diamonds). The open rectangles represent the FSL as defined by (Chase and Young, 2007) method. (B) The figure shows the first spike latencies at each dBSPL (open squares) and the fitted response (black circles) with the defined L_{min} (dotted line). (C) The frequency response is shown on the left with the first spikes following stimulus onset (black) and the following spikes (gray). We played pure tones and each stimulus is shown in alternating gray and white bars. We also show the corresponding linearly interpolated *k(f)* fitted to correct for changes in FSL dependent on frequency plotted logarithmically. (D) The figure shows the cortical LIEFTS model-predicted versus experimental FSL with (closed circles) and without (open squares) *k(f)* dependence. (E) For call #7, we show the Gaussian smoothed experimental first spikes (gray), and the cortical model prediction. (F) The figure demonstrates the relationship between the FSL and its latency variability for all SUs (black dots,). We found a similar power law fit (solid gray line) to a previous cortical FSL study in anesthetized animals (Heil and Irvine), shown as the dotted line.

Although implementing the model as described above could be effective for predicting FSLs based on the PDF mode, the predicted PDF width systematically underestimated the observed trial-by-trial variability in FSLs (e.g. Fig. 4.3E). This underestimation of the PDF width may be a result of the fact that the cortical LIEFTS model does not account for the firing rate saturation that may occur at higher stimulus amplitudes (Heil et al. 2008), and/or because temporal precision likely deteriorates after the multiple synapses leading to higher auditory stations. Indeed, consistent with previous reports (Heil and Irvine, 1997; Ter-Mikaelian et al., 2007), we found a systematic increase in the FSL standard deviation with increasing FSL. For this, we extracted a distribution of experimental first spike times for each stimulus (pure tones eliciting evoked first spikes for 50, 60, and 70 dBSPL) for SUs whose spontaneous spike rates <5 Hz, and whose post-stimulus first spike time distributions significantly differed from spontaneous first spike times (re. arbitrary reference time during silence, Kolmogorov-Smirnov test, $p<0.05$). This led to a power law relationship (Fig. 4.3F, solid line): $\sigma(FSL) = 0.07*(FSL^{1.3})$. Combining all of the above steps together, we arrived at a call-by-call prediction for each SU (Fig. 4.3D). Overall prediction quality for each SU was then measured by the relative RMSE (defined above) taken between predicted and experimental pup call FSLs, but averaged only across calls with significant experimental FSLs.

4.3: Results

We recorded SUs from awake, head-restrained mice, which included call-excited, -inhibited, and non-responsive neurons. Previous results demonstrated that while call-inhibited SUs showed a consistent suppressed response to the pup calls, call-excited SUs were much more variable (Fig. 2.2 and 2.3). In light of this, we wondered whether the greater diversity in call-excited SUs could play a more important role in encoding the pup calls' acoustic features. We investigated this by using the van Rossum metric, a method that evaluates the dissimilarity between two spike trains.

We used our population of call-excited and –inhibited SUs, and computed an inter-call (discrimination) distance, and a call-to-blank (detection) distance. To determine how well the two groups can differentiate between acoustic features, we chose to compare two pup calls that were different in both their frequencies and amplitude envelopes (calls 13 and 18, Fig. B.1). In this comparison, we found that call-inhibited SUs showed a greater detection as most of their points were below the unity line (Wilcoxon Sign Rank, $z=5.4$, $N=84$, $p<0.00001$, Fig. 4.4A). In contrast, most of the call-excited SUs were above the unity line (Wilcoxon Sign Rank, $z=2.6$, $N=84$, $p<0.05$, Fig. 4.4A). We further evaluated these differences by defining a value that showed how well SU spike trains could differentiate between two calls relative to their ability to differentiate between a call and its spontaneous activity (Fig. 4.4B). These results suggest that in comparison to the call-inhibited SUs, the call-excited SUs may play a greater role in encoding the acoustic features of different vocalizations.

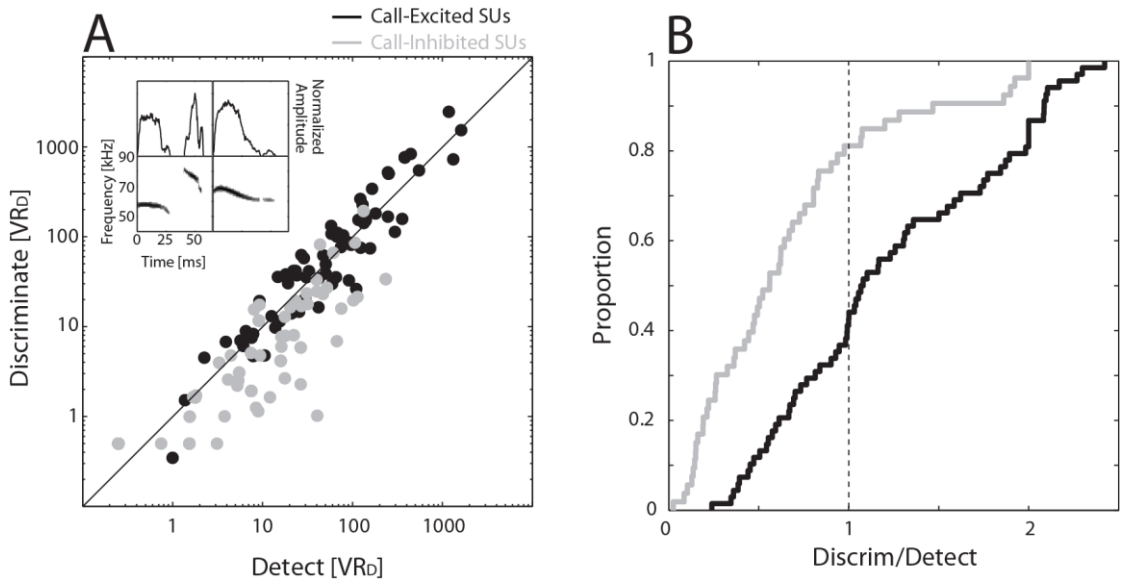


Figure 4.4: The VR inter-call and call-to-blank distances for call-excited (black) and call-inhibited (gray) SUs. (A) The figure shows the inter-call distances on the y-axis and the corresponding call-to-blank distances on the x-axis. Notice that for inhibited SUs (gray), most of the points lie below the unity line suggesting that inhibited SUs generally have a greater call-to-blank distance compared to their inter-call spike pattern distance. The insets in the figure show the frequency and amplitude envelope of the two calls compared here. These correspond to calls 13 and 18 (see Appendix B). (B) The cumulative distribution function shows the values of the van Rossum inter-call distance divided by the call-to-blank distance for each SU.

While this example clearly demonstrates a difference in call-excited and –inhibited SUs in their ability to differentiate between vocalizations, these two calls differ dramatically. The question remains as to how the two types of SUs encode the sounds when only a single parameter is changed, i.e. duration, frequency, or amplitude envelope. For example, by comparing calls 15 and 16, the frequency and durations are relatively similar, but the amplitude envelopes differ. Doing this, we found that in fact, the populations of both call-excited and –inhibited SUs have similar discrimination/detect values (Kolmogorov-Smirnov, $D = 0.21$, $p>0.05$). Although in this comparison the groups did not differ, call-excited neurons in general showed greater

inter-call distances relative to their call-to-blank distances. In support of this, looking at how the two groups encode durations, we could make six comparisons in the pup calls (1-13, 2-14, 3-15, 4-16, 5-17, 6-18, see Appendix B). Using the Kolmogorov-Smirnov test, we found that in 4/6 of these comparisons, call-excited SUs had higher discrimination/detect values, and in 2/6 they were not different from call-inhibited SUs (data not shown). Thus, in general, the call-excited neurons may play a greater role in encoding the diversity of acoustic features in pup calls.

How then do auditory cortical SUs encode acoustic features using excitation? It has been suggested that sensory stimuli can be represented as either a firing rate based on the average number of spikes per unit time, or a temporal code, which depends on the precise timing of spikes. While studies have demonstrated that both methods can represent different stimulus features, the latency of the first spike following stimulus onset is a particularly rapid and reliable way of encoding information in the auditory, somatosensory, and visual systems (Heil, 2004; Johansson and Birznieks, 2004; Gollisch and Meister, 2008). Therefore, in an attempt to understand how neurons in the awake mouse auditory cortex encode natural vocalizations, we characterized the first spike latency of pup-call excited SUs and modeled their dependence on specific acoustic features. To do so, we required a SU to have an overall excitatory response to three stimulus sets, including pure tones of different frequencies at 60 dBSPL, pure tones of the same frequency (BF) at different dBSPLs, and a collection of 18 ultrasound pup calls at 65 dBSPL. Only 55 of the SUs in our database satisfied our restriction criterion, since many SUs could be overall inhibited (Galindo-Leon et al., 2009) or nonresponsive to one

or more stimulus set. For each SU, we fitted the cortical LIEFTS model to predict its FSLs for pup calls that elicited statistically significant experimental FSLs by the (Chase and Young, 2007) method. Of the 55 SUs, four were further excluded from population summaries because for them, no individual pup call evoked a statistically significant experimental FSL, even though they had an overall excitatory response.

4.3.1: Prediction errors segregated distinct groups of SUs

The prediction error, characterized by the RMSE, spanned a large range equivalent to a 7.2% to 167% difference between predicted and experimental FSLs. Figure 4.5A-C shows these on a per-call (x) as well as an averaged per-SU (solid symbols) basis for SUs segregated into RMSE quartiles: Q_{Best} contained the 25% of SUs with the lowest prediction errors for these pup calls; Q_{Poor}, 25% with the largest prediction errors; Q_{Mid}, 50% around the median. Points for both calls and SUs were close to the diagonal for the Q_{Best} group, but began deviating from it for the Q_{Mid} and Q_{Poor} groups. If predicted and experimental FSLs were uncorrelated for all SUs regardless of the size of the error, then the model would simply be inappropriate for capturing the mechanisms that generate cortical first spikes (Bar-Yosef and Nelken, 2007). However, we found that Q_{Best} SUs had a strong correlation between prediction and experiment (Fig. 4.5A, cc = 0.83, $p<0.001$), and even Q_{Poor} had a weak correlation (Fig. 4.5C, cc = 0.21, $p<0.05$). This indicates that the cortical LIEFTS model can at least partially explain the mechanism for FSLs, albeit to varying degrees for different cortical SUs.

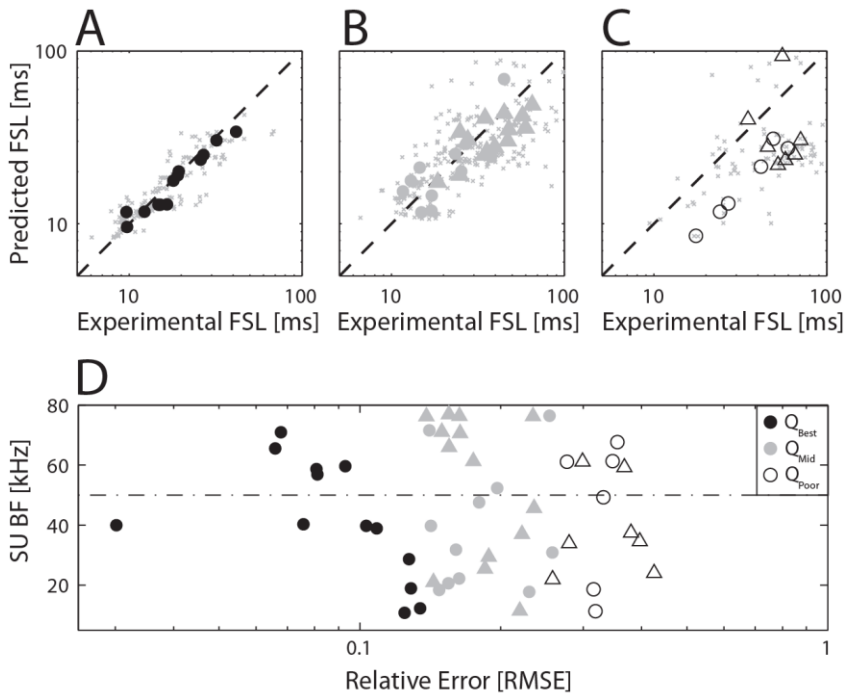


Figure 4.5: Prediction errors segregate SUs. (A, B, C) For each group, the figure illustrates the individual predicted versus experimental FSL (crosses), and the mean predicted FSL versus the mean experimental FSL for each SU (circles – thin spikes, triangles – thick spikes). (D) The figure demonstrates the relationship between the relative RMS prediction error and the SU's BF. The dotted line represents 50 kHz and shows that the proportions of cells above and below 50 kHz are no different between the three groups. There were a total of 13 SUs in Q_{Best} (black symbol), 25 cells in Q_{Mid} (gray symbol), and 13 cells in Q_{Poor}(open symbol). The group designation refers to whether a SU's pup call-excited response is well predicted (Q_{Best}, top quartile), poorly predicted (Q_{Poor}, bottom quartile) or within the middle 50% (Q_{Mid}) of prediction errors across the population.

This result was not trivially due to better ultrasound responsiveness on the part of Q_{Best} SUs. The range of BFs was not different between groups (ANOVA, $F(2,48) = 0.47$, $p>0.05$), and 38-44% of SUs in each group actually had BFs above 50 kHz (Fig. 4.5D), where SUs typically showed clear responses to at least a few of the calls in the 60-80 kHz pup call range. On the other hand, whether the magnitude of a SU's prediction error was small or large also did not appear to be random. In particular, a spike waveform analysis revealed that all Q_{Best} SUs had thin spikes (circles in Fig. 4.5D), even though both

Q_{Poor} and Q_{Mid} contained an approximately equal mix of SUs with thin and thick (triangles) spikes (binomial, Q_{Mid}: 11/25 thin; Q_{Poor}: 6/13 thin, $p>0.05$, two-tailed). This suggests that SUs with the smallest prediction errors may be biophysically distinct from those with large errors (Hasenstaub et al., 2010).

This led us to ask whether prediction error correlated with differences in other characteristics. Indeed, Q_{Best} SUs were distinct in the nature of their nonlinear encoding of sound onsets. Figure 4.6 shows the rasters and pooled PSTHs to 3 calls (upper half of each panel) and 3 frequency- and duration-matched pure tones (lower half) for 6 SUs tuned to frequencies above (A-C) or below (D-F) 50 kHz, falling into either the Q_{Best} (A and D), Q_{Mid} (B and E) or Q_{Poor} (C and F) groups. The two Q_{Best} examples (Fig. 4.6A and D) exhibited robust onset responses for both tones and calls, with the response strength dropping as frequency increased. Because of the slight differences in mean amplitude (60 dBSPL for pure tones versus 65 dBSPL for calls) and amplitude onsets (10 ms $\cos^2$ ramp for pure tones versus rapid onsets for calls), call and tone responses were not always identical (compare call 17 to the 76 kHz tone response for SUnitID 1773). Importantly though, the nonlinearities in the cortical LIEFTS model correctly accounted for these acoustic differences.

In the case of Q_{Mid} and Q_{Poor} though, some calls elicited responses that were completely unexpected based on the tone response, even though the calls were single frequency whistles. For example, SUnitID 1363 (Fig. 4.6C) responded transiently to tone onsets, but fired robustly near call offsets. Similarly, the response of Q_{Mid} SUnitID 1355

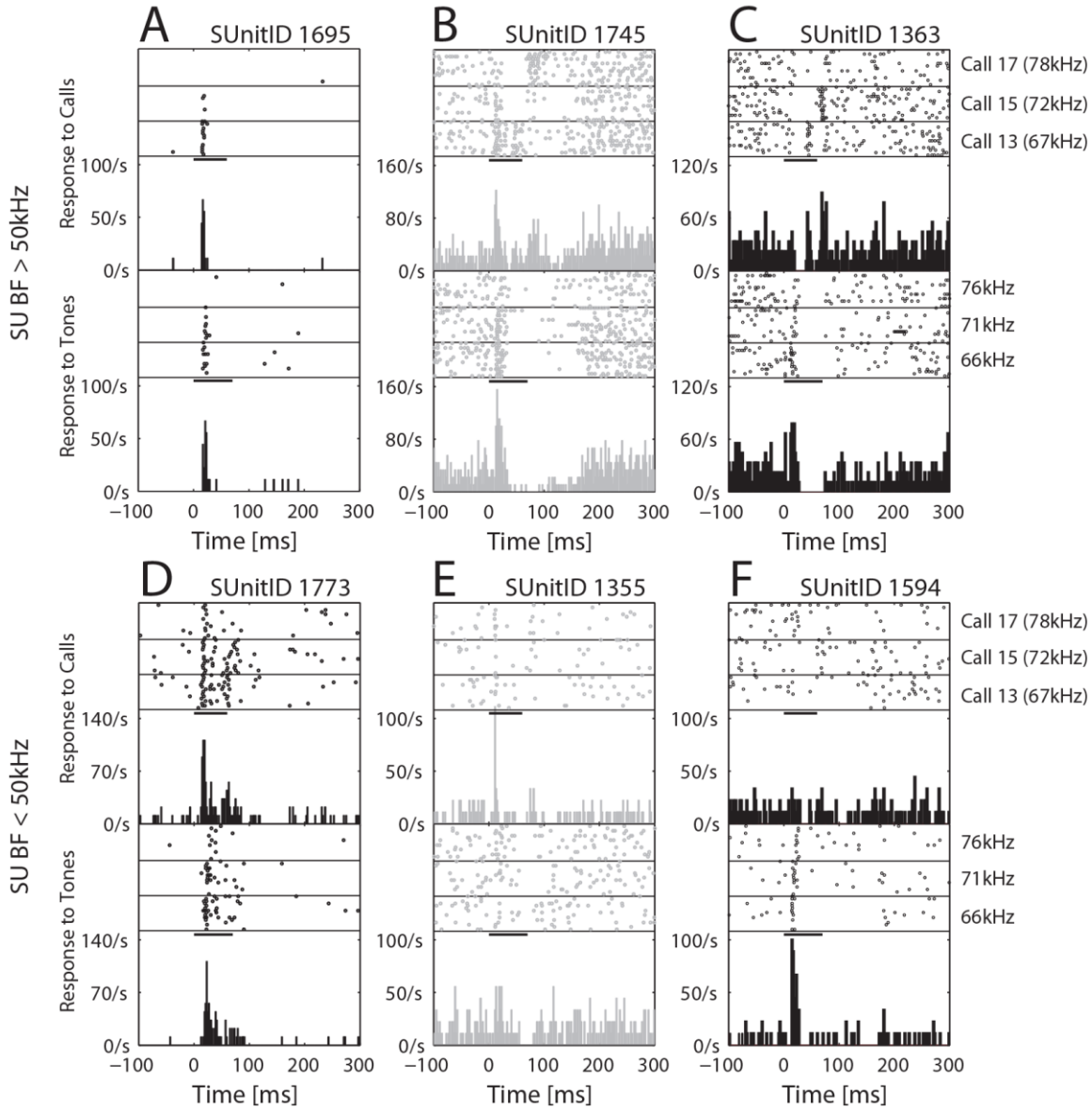


Figure 4.6: Correspondence between tone and call responses varied systematically across groups. (A-F) This figure illustrates two examples from each group (Q_{Best} – left column, Q_{Mid} – middle column, Q_{Poor} – right column), SUs with BF's greater than 50kHz (top row), and less than 50kHz (bottom row). For each example, the figure shows the response to three different frequency calls with the longest durations (upper panel), and the corresponding response to pure tone frequencies (lower panel). Within each panel, the raster plot and a binned representation of the spikes (2 ms bins) is shown with stimuli separated by lines. The bar above the binned histogram represents both the start and duration of the stimuli (pure tones ~ 70ms and longest duration calls ~60ms).

(Fig. 4.6E) to tones was imprecise, but was well locked to sound onset for calls. On the other hand, Q_{Poor} SUnitID 1594 (Fig. 4.6F) fired transiently and reliably for tones, but was unresponsive to calls 13, 15, and 17. We also observed call-evoked onset inhibition in some SUs, like Q_{Mid} SUnitID 1745 in response to call 17 (Fig. 4.6B). Such inhibition was not accounted for by our purely excitatory model. Hence, the failure of the cortical LIEFTS model for Q_{Mid} and Q_{Poor} SUs coincided with cases where the response to pure tones could not be extrapolated to explain actual call-evoked responses, suggesting more complex sensitivities to the acoustics of a sound's onset.

4.3.2: SU groups differed in their response to natural call onsets

Indeed, sensitivity to onset frequency was noticeably more consistent for Q_{Best} compared to Q_{Poor} SUs, based on the statistically estimated FSL for individual natural calls. Q_{Best} and Q_{Mid} SUs had clearly delayed FSLs for higher versus lower frequency calls (Fig. 4.7A, black and gray dots, signed-test, $z_{QB} = 5.1$, $N_{QB} = 39$, $p < 0.001$, $z_{QM} = 3.9$, $N_{QM} = 48$, $p < 0.001$), whereas FSLs for Q_{Poor} SUs were not systematically different (open dots, signed-test, $z_{QP} = 0.6$, $N_{QP} = 11$, $p > 0.05$). Similarly, Q_{Best} and Q_{Mid} SUs showed significantly later FSLs for slower amplitude onsets compared to faster onsets (Fig. 4.7B, black and gray dots, respectively, mostly above the diagonal, signed-test, $z_{QP} = 2.8$, $N_{QP} = 19$, $p < 0.01$, $z_{QM} = 3.5$, $N_{QM} = 2$, $p < 0.001$). However, FSLs for Q_{Poor} SUs across their populations were not significantly different for the two types of sounds (open dots, signed-test, $z_{QP} = 0$, $N_{QP} = 5$, $p > 0.05$), suggesting that the shape of the ultrasounds' envelope onset was not a reliable driver of their first spikes.

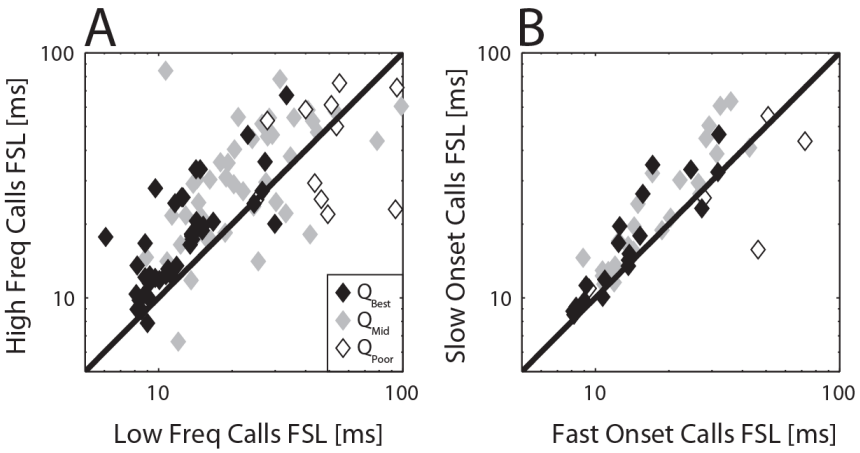


Figure 4.7: Systematic acoustic differences reflected across the population of cortical FSLs. (A) For all the predicted cells, we plotted the FSL to low (calls 1, 2, 7, 8, 13, 14) versus high (calls 5, 6, 11, 12, 17, 18) frequency pup calls. For example, the FSL to call 1 would be plotted against call 5, and the FSL to call 2 would be plotted against call 6 and so on. Those calls that did not evoke a FSL were not included. The overall population shows a latency dependence on frequency as the majority of points lie above the unity line. (B) For the same cells and significant first spike distributions, we show the first spike response to both a fast (calls 7, 13) and a slow (calls 8, 14) amplitude envelope onset calls with similar starting frequencies. Again, responses to call 7 were plotted against 8, and 13 against 14. The population shows a significant later latency for the slower amplitude envelope onset ramp.

Figure 4.7 also suggests that the absolute latencies of pup-excited responses may be systematically different between groups. Q_{Best} FSLs were generally earlier than Q_{Poor} FSLs on a per-call basis (Fig. 4.8A), and averaged per-SU (ANOVA, $F(2,48) = 10.7$, $p < 0.001$, Fisher's LSD post hoc: Q_{Best}-Q_{Mid}, $p<0.05$; Q_{Best}-Q_{Poor}, $p<0.05$; Q_{Mid}-Q_{Poor}, $p<0.05$). Moreover, first spikes were also more precisely timed trial-by-trial (quantified by vector strength, see Methods) for Q_{Best} compared to Q_{Poor}, on a per-call basis (Fig. 4.8B), as well as averaged per-SU (ANOVA, $F(2,48) = 3.2$, $p < 0.05$, Fisher's LSD post hoc: Q_{Best}-Q_{Mid}, n.s.; Q_{Best}-Q_{Poor}, $p<0.05$; Q_{Mid}-Q_{Poor}, n.s.). Another difference between the best and worst predicted SUs arose in the selectivity for individual calls. Q_{Best} SUs generally exhibited statistically significant FSLs to far more of the calls than Q_{Poor} SUs (Fig. 4.8C). This result

could not be explained simply by a difference in spontaneous firing between groups (Fig. 4.8D).

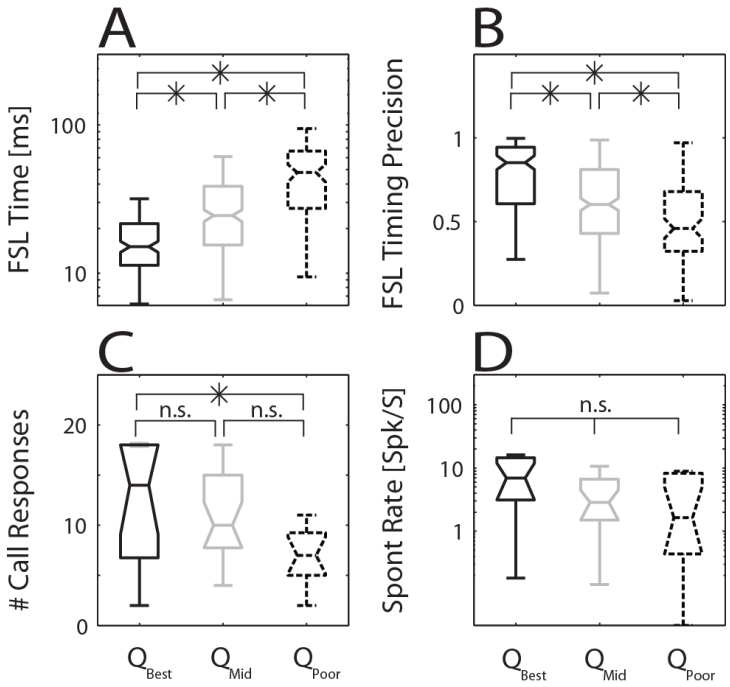


Figure 4.8: Group differences are nontrivial. (A) We took the mean of the FSLs per-call and found a significant difference between each group. Q_{Best} is the solid black line, Q_{Mid} is the solid gray line, and Q_{Poor} is the dotted black line (Kruskal-Wallis, χ^2 (2,509) = 135.3, $p < 0.001$, Fisher's LSD post hoc: Q_{Best}-Q_{Mid}, $p<0.05$; Q_{Best}-Q_{Poor}, $p<0.05$; Q_{Mid}-Q_{Poor}, $p<0.05$). (B) We computed the timing precision for each SU based on a measure analogous to the vector strength (see Experimental Procedures), and found that there was a significant difference between each group of SUs (Kruskal-Wallis, χ^2 (2,509) = 79.3, $p < 0.001$, Fisher's LSD post hoc: Q_{Best}-Q_{Mid}, $p<0.05$; Q_{Best}-Q_{Poor}, $p<0.05$; Q_{Mid}-Q_{Poor}, $p<0.05$). (C) For each SU, we computed the number of pup calls in which there was a statistically significant FSL response (Kruskal-Wallis, χ^2 (2,48) = 7.3, $p < 0.05$, Fisher's LSD post hoc: Q_{Best}-Q_{Mid}, n.s.; Q_{Best}-Q_{Poor}, $p<0.05$; Q_{Mid}-Q_{Poor}, n.s.). We found a significant difference between Q_{Best} and Q_{Poor}.(D) The figure shows that the spontaneous rate was not significantly different between each group of SU's (, Kruskal-Wallis, χ^2 (2,48) = 4.1, $p > 0.05$).

4.3.3: SUs differed in their discharge patterns and spike waveforms

Strikingly, FSL prediction error also correlated with systematic differences in spiking after the first spike. This is evident from comparing the call-evoked PSTHs for the

3 groups (Fig. 4.9A, population-averaged and pooled over all 18 calls, normalized by spontaneous rate and smoothed). Q_{Best} SUs (thick black line) consistently showed strong transient firing at sound onset. Some SUs, such as SUnitID 1773 (Fig. 4.6D), also produced sustained firing, but this was not that common. On the other hand, the averaged discharge pattern for Q_{Poor} SUs (thin dashed line) was somewhat delayed, lacked a strong onset, and instead held a more stable level of firing during and beyond the stimulus. Finally, Q_{Mid} SUs exhibited a more heterogeneous group of responses that seemed to exhibit response characteristics between Q_{Best} and Q_{Poor}. These data indicate that SU groups differed not only in terms of how they fired first spikes, which was the basis for classifying them, but also in terms of how they fired subsequent spikes.

The population differences were apparent even at the individual SU level. Evaluating call-pooled, normalized PSTHs on a SU-by-SU basis, Q_{Best} SUs (Fig. 4.9B, black symbols) had significantly shorter half-max PSTH durations (right panel) and earlier half-max PSTH latencies (bottom panel) compared to Q_{Poor}. The majority of Q_{Best} SUs therefore clustered tightly towards the lower left corner of Fig. 4.9B, where transient onset SUs should lie, while Q_{Poor} SUs were generally found more to the right and upper portions of Fig. 4.9B, where offset and more sustained SUs should lie. Q_{Mid} SUs were scattered throughout this plane, and were not different from Q_{Poor} SUs in PSTH duration, but were for PSTH latencies. As a result, having a transient onset response to calls was *not sufficient* for a SU to belong to Q_{Best} (gray and black symbols in lower left quadrant of Fig. 4.9B, <20 ms latency and <20 ms duration), although it was an important factor (11/13 black symbols are in that quadrant). A summary of direct comparisons between

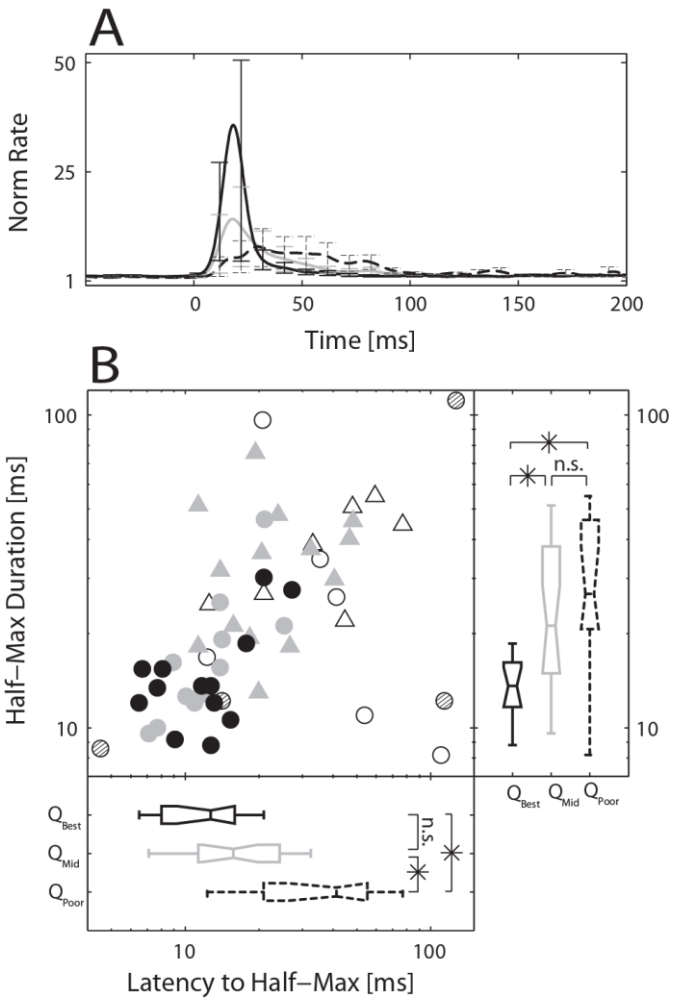


Figure 4.9: SU groups differ in their discharge pattern and spike waveforms. (A) For each group, we pooled their SU PSTH responses to all 18 pup calls normalized by the spontaneous rate. Q_{Best} is the solid black line, Q_{Mid} is the solid gray line, and Q_{Poor} is the dotted black line. (B) The scatter plot shows the latency from stimulus onset to the half-max of the PSTH to all 18 calls, and the duration is the amount of time the PSTH remains above this value. The circles and triangles correspond to thin or thick spikes (see Methods), and the hatched symbols mark those cells that were pup call-excited but did not have first spike distributions significantly different from spontaneous. The lower boxplot represents the median and range of the latency to the half-max for all three groups (Kruskal-Wallis, χ^2 (2,48) = 15.9, $p < 0.01$, Fisher's LSD post hoc: Q_{Best}-Q_{Mid}, n.s.; Q_{Best}-Q_{Poor}, $p<0.05$; Q_{Mid}-Q_{Poor}, $p<0.05$). The Q_{Best} cells had significantly earlier pup call responses compared to Q_{Poor}, but not Q_{Mid}. In addition, there was also a significant difference between Q_{Mid} and Q_{Poor}. The right boxplot represents the median and range of the duration of the response for all three groups. We found that Q_{Best} had significantly shorter durations of responses compared to Q_{Mid} and Q_{Poor}, but Q_{Mid} and Q_{Poor} did not show significant differences (Kruskal-Wallis, χ^2 (2,48) = 9.3, $p < 0.01$, Fisher's LSD post hoc: Q_{Best}-Q_{Mid}, $p<0.05$; Q_{Best}-Q_{Poor}, $p<0.05$; Q_{Mid}-Q_{Poor}, n.s.).

Q_{Best} and Q_{Poor} properties is shown in Table 4.1, Box 1, reiterating the systematic call encoding differences that emerged simply by grouping SUs according to their relative FSL prediction error under the cortical LIEFTS model. By using *relative* error, we did not artificially bias our results towards SUs with earlier, less variable FSLs (Fig. 4.3F). Indeed, two Q_{Best} SUs in Fig. 4.9B fell outside of the onset-transient group because they had noticeably later and longer responses. Thus, it is interesting to find that the majority of Q_{Best} SUs nevertheless were among the earliest firing in response to calls.

Table 4.1: Using relative or absolute error segregates a similar population of Q_{Best} and Q_{Poor} SUs. The table shows the mean and standard error values for each of the analyses that were done previously for groups segregated using RMS relative error (box 1), and RMS absolute error (box 2). Box 3 represents those SUs that were segregated by both methods. For significance: *-p<0.05,**-p<0.01,***-p<0.001, n.s. - not significant.

	Segregated by Relative Error			Segregated by Absolute Error			Intersected SUs		
	Q_{Best}		Q_{Poor}	Q_{Best}		Q_{Poor}	Q_{Best}		Q_{Poor}
# of SUs	13		13	13		13	9		10
# of Call Responses	12.2 ± 1.6	*	6.8 ± 0.8	13.6 ± 1.4	**	7.9 ± 0.9	13.7 ± 1.9	*	7.3 ± 0.9
FSL per-call [ms]	18.0 ± 0.8	***	48.0 ± 2.4	14.4 ± 0.4	***	50.7 ± 2.1	14.2 ± 0.5	***	53.2 ± 2.4
FSL per-SU [ms]	20.1 ± 2.6	***	46.2 ± 4.6	14.7 ± 0.9	***	51.7 ± 3.1	15.0 ± 1.2	***	53.2 ± 3.4
FSL Precision per-call	077 ± .02	***	0.53 ± .01	0.79 ± .05	***	0.49 ± .07	0.85 ± .01	***	0.47 ± .03
FSL Precision per-SU	0.71 ± .05	*	0.53 ± .06	0.75 ± .04	*	0.55 ± .07	0.81 ± .04	**	0.53 ± .08
Call PSTH Half-Max [ms]	13.0 ± 1.7	***	43.7 ± 7.6	9.6 ± 0.7	***	39.2 ± 5.0	9.8 ± 0.9	***	39.3 ± 6.1
Call PSTH Duration [ms]	15.4 ± 1.8	*	35.0 ± 6.5	12.2 ± 0.6	***	42.5 ± 5.4	12.6 ± 0.8	***	42.0 ± 7.0
% Thin Spk SUs	100	**	46	100	***	31	100	**	30
Spont Rate [spk/s]	11.8 ± 3.7	n.s.	5.5 ± 2.0	14.3 ± 3.7	***	2.0 ± 0.8	13.9 ± 5.1	**	2.3± 1.0
Tuning Width [BF/BW]	1.7 ± 0.2	*	2.3 ± 0.2	1.4 ± 0.2	***	2.5 ± 0.2	1.7 ± 0.2	*	2.5 ± 0.2

This raises the question of whether absolute timing error might be more pertinent for segregating SUs. To address this, we re-segregated SUs into quartiles based on the RMS absolute FSL difference between prediction and experiment (Table

4.1, Box 2), keeping all fits and predictions the same. Importantly, we reached the same overall conclusions concerning differences between properties of Q_{Best} and Q_{Poor} SUs, in some cases at even greater levels of significance. Moreover, 9 (10) SUs remained in the lowest (highest) error quartile regardless of whether relative or absolute measures were employed (Table 4.1, Box 3). All differences between these intersected groups of Q_{Best} and Q_{Poor} SUs remained significant. These results provide confidence that one subgroup of short latency, precisely firing, mostly transient cortical SUs likely encode the acoustic onsets of these calls in a similar way as at the auditory periphery.

Finally, since the Q_{Best} SUs all had thin spikes, we evaluated criteria that might indicate whether thin spike SUs correspond to suspected fast-spiking interneurons (Table 4.2). Ignoring error grouping, we found that our thin spike SUs had significantly higher spontaneous firing rates and were recorded at a shallower depth than thick spike SUs. Moreover, the bandwidth of the tonal frequency response curves was significantly larger for the former (smaller quality factor, defined by BF/BW, see Experimental Procedures). In response to pup calls, FSLs were also significantly earlier for thin spike SUs. These results suggest that as a population, thin spike SUs have characteristics consistent with being possible fast-spiking interneurons, although we cannot definitively know this for any one SU without intracellular recordings. Importantly though, the differences between Q_{Best} and Q_{Poor} SUs was not just due to a change in the balance between thin and thick spike SUs. Restricting only to thin spike SUs in both groups, we found that the FSL and FSL precision per-SU and per-call, as well as the PSTH half-max were still significantly different, even though spontaneous activity and tuning bandwidth

were not (presumably since all SUs had thin spikes). Although we did not find a significant difference in the number of call responses, a trend similar to the one found in Table 4.1 was present (T-test, t = 2.0, df = 17, p = 0.06; Q_{Best}: 12.2 ± 1.6; Q_{Poor}: 7.2 ± 1.2). Hence, the degree to which a SU's onset response to pup calls follows the cortical LIEFTS model appears to segregate out a distinct subpopulation of thin spiking neurons, some of which may be putative fast spiking inhibitory interneurons.

4.3.4: Call responses in a subset of Q_{Poor} neurons are selectively modulated by a sound's behavioral relevance

Q_{Best} and Q_{Poor} neurons clearly show distinct differences in how they encode pup vocalizations. Indeed, we demonstrated previously that Q_{Best} neurons are a distinct subset and represent the simple acoustic features of pup calls, such as its amplitude envelope and onset frequency. There is evidence that in addition to these acoustically faithful representations of sound in the auditory cortex, there are also neurons that carry more information about higher order acoustic features of the stimulus (Chechik et al., 2006). Thus, given the model's poor ability to predict how Q_{Poor} SUs encode simple acoustic features, we wondered whether these SUs instead play a role in encoding other higher order features.

The mouse ultrasonic communication system provides the opportunity to study this question because both pup and adult calls are sounds that are semantically distinct (see 1.3.1), but overlap in their simple acoustic features of onset frequency and duration (see Appendix B). To understand whether Q_{Poor} SUs encode higher order acoustic

features, we compared their responses to a set of pup and adult calls, stimuli that were acoustically matched in onset frequency and duration, but differed in both the frequency modulation and behavioral relevance. We looked first at the responses of the 10 intersected Q_{Poor} SUs (Table 4.1, box 3), which consisted of 6 mother, 4 cocarer, and 0 virgin neurons.

Given the differences in call recognition and cortical inhibitory plasticity found previously between mothers and cocarers, we wondered whether they would also show differences their encoding of pup and adult calls. In principle, if the neurons were faithful in their basic encoding of sound onset frequency and duration, the overall responses to pup and adult calls were expected to be the same (Figure B.1, pup call *i* is acoustically matched to adult call *i+18*). In support of this, we found that our Q_{Best} SUs indeed showed no differences in their overall responses to pup or adult calls (Wilcoxon Sign Rank, W=22, $p>0.05$), and no animal group dependencies. Interestingly though, when comparing the intersected Q_{Poor} SUs between animal groups, we found that mothers but not cocarers showed a greater response to pup calls compared to adult calls (Fig. 4.10A3 and B3, Mothers N=6: Wilcoxon Sign Rank, W=0, $p<0.05$; Cocarers N=4: Wilcoxon Sign Rank, W=4, $p>0.05$).

Figure 4.10 suggests that a subset of neurons in the awake auditory cortex of mothers may be differentially encoding the higher order acoustic differences between pup and adult calls. To further study this, we wondered whether this difference in call responsiveness occurred among other neurons within our data set with Q_{Poor}-like response characteristics (i.e. spike shape, response latency, and spontaneous rate). We

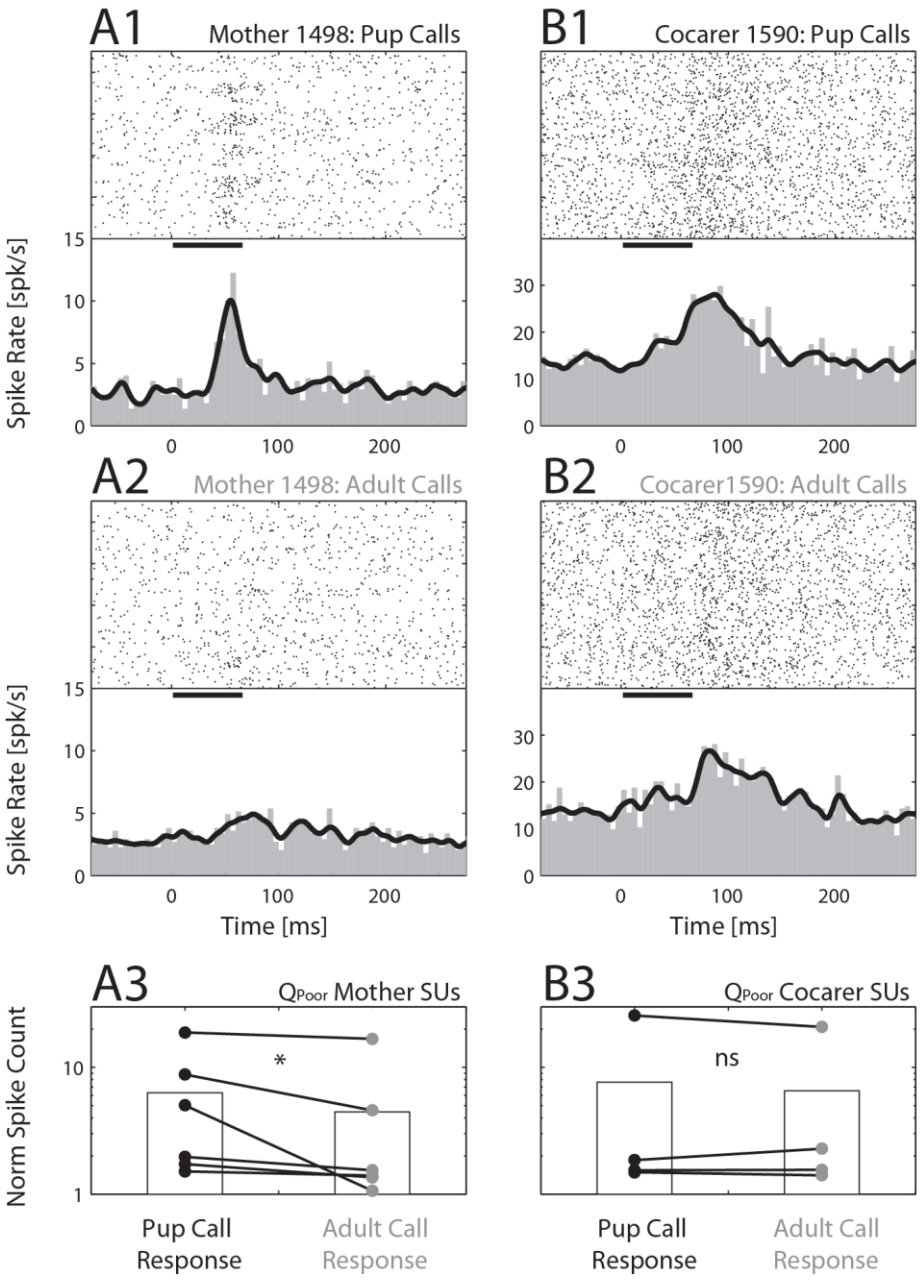


Figure 4.10: Mothers Q_{Poor} SUs show selective enhancement for the class of more salient pup calls versus less salient adult calls, which is not observed in cocarers. (A1, B1) Figures show examples of the responses to the 18 different pup calls (A2 and B2) and the responses to the 18 different adult calls. Notice that in the mother there is a large difference between its response to pup and adult calls whereas the cocarer show more similar responses. Both SUs presented here were classified as thick spiking. The black bar starting at 0 time shows the length of the longest call stimulus. (A3) Dividing the number of spikes from 0-100ms by the number of spikes during an equivalent spontaneous period, we found that the 6 Q_{Poor} SUs in mothers showed stronger responses to the pup calls (black dots) compared to the adult calls (gray dots). (B1) In contrast, the 4 Q_{Poor} SUs in cocarers did not show differences between the pup and adult calls.

selected for units by restricting our larger data set, finding those neurons within 1 standard deviation of the Q_{Poor} mean for three characteristics (Table 4.1, box 3, thick spike only, mean ± 1*standard deviation: 19.9<PSTH_onset<58.7ms; 0<spont rate<5.3spk/s).

From this, we were able to segregate out 11 mother, 3 early cocarer, 8 cocarer, and 7 virgin SUs. Based on the more similar nature of evoked responses in mothers and early cocarers discussed in chapter 3, we combined their responses (N = 13), and contrasted them against cocarers and virgins (N = 10), which were also more similar to each other in evoked responses. We found that mothers and early cocarers together had a much larger response to pup calls and in fact showed a significant enhancement in their call-excited response to pup over adult calls (Fig. 4.11A1 and A2, Wilcoxon Sign Rank, W=2, p<0.001). Importantly, 13 out of the 14 SUs (93%) in this group showed a greater response to pup calls compared to adult calls (Fig. 4.11A2). In contrast, we found no significant differences in the call-excited responses for cocarers and virgins (Fig. 4.11B1 and B2, Wilcoxon Sign Rank, W=30, p>0.05). In addition, we found that these changes were not a result of SU BF differences when comparing the neurons in mothers and early cocarers to those in virgins and cocarers (t-test, t=1.01, df=20, p>0.05). This suggests that a subset of call-excited neurons in mothers and early cocarers selectively enhances its response to a sound dependent on its behavioral saliency.

We next wondered whether these changes were specific to those SUs with Q_{Poor} characteristics. To do this, we restricted our larger data set in a similar way to find those

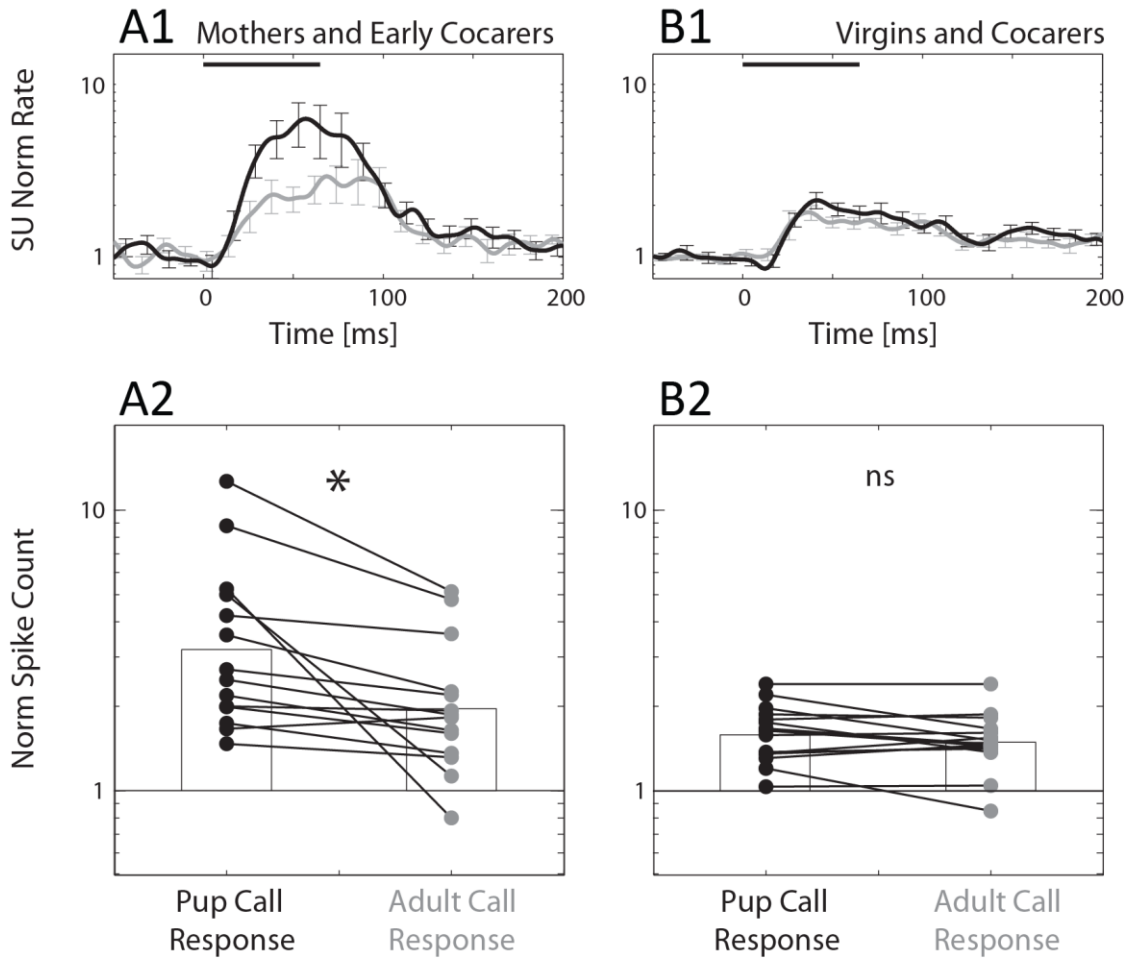


Figure 4.11: Mothers and early cocarers differentially respond to pup and adult calls when compared to virgins and cocarers. (A1) Shows the mean PSTH response ($N_{mothers}$ = 11, $N_{early\ cocarers}$ = 3) to the 18 pup calls (black line) and 18 adult calls (gray line). There is a clear difference in the mean PSTH responses, normalization was performed by dividing the intrinsic firing rate. (A2) Demonstrates that for these SUs, they respond preferentially to pup calls over adult calls. (B1) The mean PSTH response ($N_{virgins}$ = 7, $N_{cocarers}$ = 8) to the 18 pup calls and 18 adult calls. For virgins and cocarers, we found no difference in their responses to pup and adult calls. The normalized spike count was computed by integrating the spikes from 200-300ms based on the mean length of responsiveness in A1 and B1.

neurons within 1 standard deviation of the Q_{Best} mean for the same three characteristics (Table 4.1, box 3, thin spike only, mean ± 1*standard deviation: 7.1< PSTH_onset <12.5ms; 0<spont rate<29.2spk/s). Doing this, we were able to segregate out 6 mother, 2 early cocarer, 10 cocarer, and 1 virgin SU. Similar to figure 4.11, we combined the responses of mothers and early cocarers (N=8), and contrasted them against cocarers and virgins (N=11). Unlike for Q_{Poor} SUs, we found no differences in how the animal groups respond to pup and adult calls in their overall PSTH (Fig. 4.12A1 and B1). In addition, the animal groups also showed no differences in their normalized spike counts in response to either pup or adult calls (Fig. 4.12 A2 and B2, M/EC: Wilcoxon Sign Rank, W=7,p>0.05; V/C: Wilcoxon Sign Rank, W=27, p>0.05). Similar to the Q_{Poor}-like SUs, the Q_{Best}-like SUs also showed no differences in their SU BFs when comparing mothers and early cocarers to virgins and cocarers (t-test, t=1.64, df=13, p>0.05). This suggests then that for the Q_{Best} SUs, our model could accurately predict their first spike responses to the simple acoustic features, and that changing the behavioral relevance of the stimulus did not affect this process. In contrast, we found that how Q_{Poor} SUs encoded the calls were affected by their behavioral relevance, and may be more sensitive to specific higher order features possibly to facilitate downstream areas in the discrimination of pup calls.

To further test this, we explored whether the spike patterns of the Q_{Poor} SUs might also be more sensitive to the acoustic differences between pup and adult calls. Although the pup and adult calls we used in playback were matched in their onset frequency and durations, figure B.1 clearly shows distinct differences in other acoustic

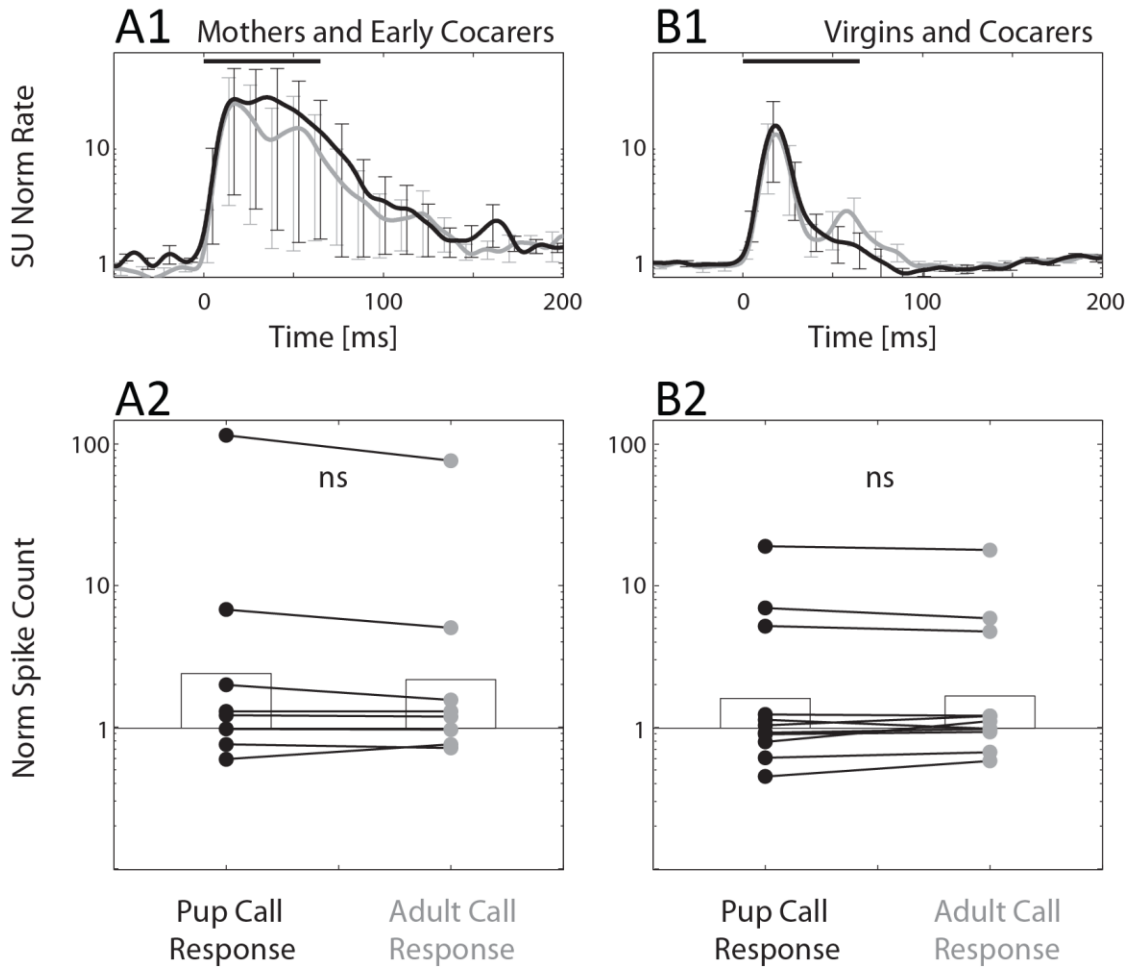


Figure 4.12: For well predicted SUs, animal groups do not differentially respond to pup and adult calls. (A1) Shows the mean PSTH response ($N_{mothers}$ = 6, $N_{early\ cocarers}$ = 2) to the 18 pup calls (black line) and 18 adult calls (gray line). We found no differences in their overall mean PSTH responses, or the normalized spike count (A2). (B1) The mean PSTH response ($N_{virgins}$ = 1, $N_{cocarers}$ = 10) to the 18 pup calls and 18 adult calls. For virgins and cocarers, we found no difference in their responses to pup and adult calls. The normalized spike count was computed by integrating the spikes from 200-300ms based on the mean length of responsiveness in A1 and B1.

features (i.e. frequency modulation). Hence, we asked whether after matching for the simple features (onset frequency and duration), whether these SUs would fire spike patterns that would better separate the response of an adult call from its frequency and duration matched pup call, compared to another frequency and duration matched adult call. We addressed this using the van Rossum metric to compute the distances between two stimulus evoked collapsed spike trains (see Methods 4.2.2). For the 18 calls, we computed a mean pup-adult call distance matched in their onset frequency and duration. As a relative measure of how well each SU differentiated between the two calls, we directly compared this mean distance to the SU's ability to discriminate between two frequency and duration matched adult calls (Fig B.1, call 19 vs. 22, 25 vs. 28, 31 vs. 34 etc.). In total, there were nine matched adult-adult call pairs, and the average distances between these pairs was considered the adult-adult call distance.

For the SUs with Q_{Best}-like characteristics, we found no animal group differences in their pup-adult call distance compared to their adult-adult call distance (Fig. 4.13A1; M/EC: Wilcoxon Sign Rank, W=8, p>0.05; V/C: Wilcoxon Sign Rank, W=31, p>0.05). In addition, we found no differences between these two animal groups in their pup-adult call distance normalized by their adult-adult call distances (Inset, Fig. 4.13A1, Mann-Whitney, U=24, $N_{M/EC}$=8, $N_{V/C}$=11, p>0.05, 2-tailed). This is demonstrated by the example SU in figure 4.13A2, where its spiking responses to the pup calls are similar to its responses to the adult calls. In contrast, for the subset of SUs with Q_{Poor}-like characteristics, we found for the SUs in mothers and early cocarers, they had a significantly greater pup-adult call distance compared to their adult-adult call distance

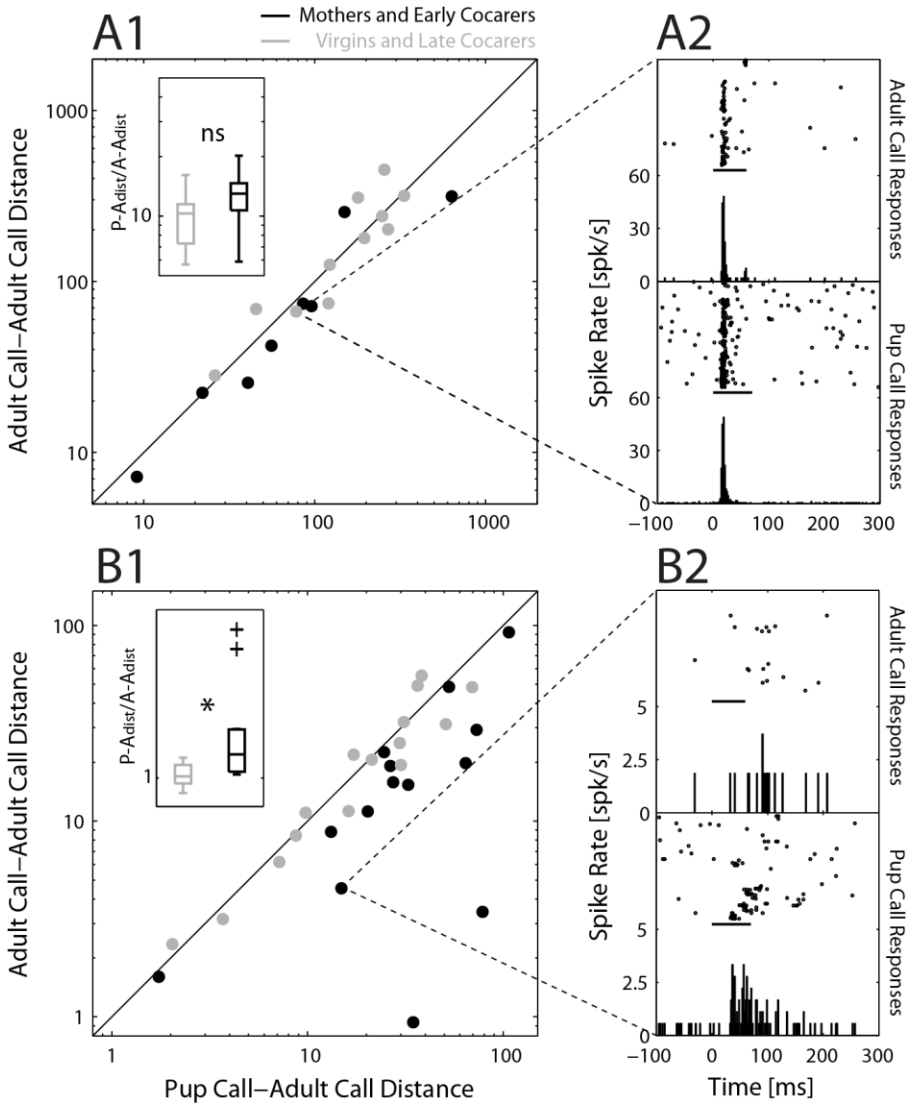


Figure 4.13: SUs with Q_{Poor}, but not Q_{Best}, like characteristics in mothers and early cocarers show unique spike pattern responses to the behaviorally salient pup calls, but not the acoustically matched less salient adult calls. (A1, B1) The van Rossum spike train distances between a pup call and the adult call (x-axis) plotted against an adult call and its frequency and duration matched adult call (y-axis) for the Q_{Best} SUs (A1) and the Q_{Poor} SUs (B1). Notice that in B1 most of the points from mothers and early cocarers lie below the unity line while virgins and cocarers are along the line. This indicates that SUs in mothers and early cocarers show greater spike train differences between pup and adult matched calls. Inset shows the pup-adult call distance divided by the adult-adult call distance. There was a significant difference in this distance value between animal groups. (A2, B2) Figures show examples of SUs in each subset with the lower raster and PSTH plot showing the response to 18 pup calls, and the upper raster and PSTH showing the corresponding response to the 18 adult calls. Black bar indicates the stimulus period.

(Fig. 4.13A1, Wilcoxon Sign Rank, W=0, $p<0.001$). In comparison, the SUs in virgins and cocarers were located closer to the unity line (Wilcoxon Sign Rank, W=47, $p>0.05$). Moreover, mothers and early cocarers had a significantly greater pup-adult call distance normalized by the adult-adult call distances (Inset, Fig. 4.13A1, Mann-Whitney, U=33, $N_{M/EC}=14$, $N_{V/C}=15$, $p<0.01$, 2-tailed). These results further support the idea that unlike the well predicted subset of SUs, the Q_{Poor}-like subset of SUs are more sensitive to the higher order acoustic differences between pup and adult calls, both in their spike patterns and in their strength of response.

These results demonstrates that there is a subset of acoustically faithful cortical thin spiking neurons, and also a subset of thick spiking neurons that seemingly encode the behavioral relevance of pup vocalizations. Importantly, neurons in the latter group in mothers and early cocarers show a preferential response to pup calls, possibly tuned to a more subtle acoustic feature of these calls (Fig. 4.13B). Taken together, we demonstrate that subsets of neurons in core auditory cortex play different roles in representing both the acoustic and perceptually relevant information in natural vocalizations. This further supports the idea that hierarchical processing occurs at the level of core auditory cortex, and that through pup experience it undergoes plasticity in both call-inhibited and –excited neurons, which may functionally enhance an animal's call detection and discrimination.

4.4: Discussion

A main conclusion of this work was that diversity in auditory cortical responses to sound onsets can be meaningfully sorted according to a computational model of peripheral encoding, permitting a principled way of pruning a heterogeneous collection of units down to those that may be more likely to represent the perceptual relevance of a category of natural communication sounds. Using data collected from awake mice, we arrived at this by applying the LIEFTS model of sound pressure integration to predict cortical responses (Fig. 4.3). Cortical SUs whose responses to ultrasonic calls were best predicted by this model were systematically different from poorly predicted SUs (Fig. 4.5): the former responded to more calls with earlier and more precise transient onsets (Figs. 4.8, 4.9), and had greater sensitivity to the acoustic features of natural call onsets (Fig. 4.7). The latter were generally more call-selective, fired later and less precisely (Fig. 4.8, 4.9), and were not consistently sensitive to sound frequency and amplitude envelope (Fig. 4.7). In particular, their first spikes did not closely follow the peripheral mechanism for responding to sound onsets, and these SUs were acoustically responsive since all showed an excitation to the calls as a whole. Their call responsiveness became selectively enhanced for a more salient class of sounds based on experience. This may have arisen from higher nonlinearities created de novo by processing within the central auditory system, which tuned the neurons into more subtle acoustic features that would distinguish this class of communication sounds. Indeed, an obvious difference between pup and adult calls is the more modulated frequency trajectory of adult calls after the initial onset. Hence, our results suggest that hierarchical processing by the core auditory cortex may preserve a peripheral encoding of stimulus onsets within some short latency,

thin spike cortical neurons, and construct a more complex acoustic selectivity within other neurons depending on behavioral relevance (Fig. 4.11, 4.13).

4.4.1: Model caveats

First, we used a functional rather than a detailed model of subcortical circuitry (Dugue et al., 2010), incorporating only effective excitatory input across frequency channels. Inhibition, while not explicitly included, could have contributed as long as it did not abolish the excitatory response, and might explain why some observed latencies were longer than predicted (Fig. 4.5C). Moreover, interactions between frequency channels simultaneously excited by a sound were ignored, since we assumed that only the channel centered at a sound's initial frequency contributed to the cortical neuron's acoustic sensitivities. Our particular class of ultrasonic calls contained single frequencies with little frequency modulation, so this "winner-take-all" approach ensured that the frequency channel that should respond best had the strongest influence. Indeed, an earlier multiunit study in anesthetized mice also using pup calls corroborated that auditory cortical spiking on average does not detect the mild frequency modulation in these calls (Liu et al., 2006). Nevertheless, while our assumption appeared valid for our best predicted SUs, the poorer predictions of some other SUs may have arisen from sensitivity to a call's precise frequency trajectory. Importantly though, this would still mark a distinction between how the acoustics of sound onsets drive spiking in Q_{Poor} vs. Q_{Best} SUs.

Second, our model was relatively simplistic, but had the advantage of only needing to optimize the parameter *k* to be able to predict FSLs that were significantly correlated with experimental FSLs for all SU groups. In implementing the LIEFTS model, we have also tried using a multi-step fitting procedure (Heil et al., 2007) to simultaneously optimize either *k* and L_{min}, or *k*, L_{min} and τ. While more complex, these cases nevertheless produced comparable differences in the physiological properties of segregated SUs, thus providing some assurance that our conclusions were not highly sensitive to the details of model implementation.

Third, although incorporating a frequency dependent *k(f)* accounted for the FSL of Q_{Best} SUs to the ultrasonic whistles, its validity for predicting either subsequent spikes or more complex sound (e.g. noise or multiharmonic calls) responses was not directly evaluated. In principle, the LIEFTS algorithm can model hair cell neurotransmitter release rate throughout a sound and can be combined with a cochlear filter bank (Fishbach et al., 2001; Fishbach et al., 2003). Combinations of central excitation and inhibition could then be fitted, creating cortical discharge patterns that could be matched to data. Importantly though, these steps were unnecessary to draw the conclusions reached here about the distinct, stereotyped properties of Q_{Best} SUs.

4.4.2: Classification of thin and thick spike SUs

Since our Q_{Best} SUs all had thin spike waveforms, it is tempting to speculate that these map onto a functionally distinct subset of cortical neurons. The idea that cortical cell types can be classified at least partly by extracellular spike durations has support.

Theoretically, thin spikes may reflect neuronal expression of particular variants of potassium and sodium voltage-gated ion channels that help minimize energy consumption during rapid spiking (Hasenstaub et al., 2010). Experimentally, electrophysiological and morphological traits of thin and thick spike neurons recorded intracellularly are clearly different. The latter are often regular-spiking pyramidal or spiny stellate neurons that have long after-hyperpolarizations, adapt strongly to depolarizing currents, and are found across cortical layers 2-6 (McCormick et al., 1985). However, regular-spiking *non*pyramidal neurons can also have thicker spikes (Kawaguchi and Kubota, 1993, 1997), indicating that some interneurons may be present along with pyramidal cells in a population of thick spike SUs. Neurons with thin spikes can correspond to either chattering pyramidal cells or fast spiking GABAergic interneurons, both of which tend to lie more in supragranular and granular cortical layers (Gray and McCormick, 1996; Azouz et al., 1997; Nowak et al., 2003). Between them, fast spiking neurons fire more rapidly with very little spike-frequency adaptation during depolarizing current injection (McCormick et al., 1985). Chattering neurons produce bursts that give rise to a clearly bimodal ISI distribution, during both current injection (Nowak et al., 2003) and long duration sensory stimulation (Gray and McCormick, 1996).

Clearly then, intracellular recordings with morphological identifications are needed to unequivocally identify thin spike neurons as fast spiking GABAergic interneurons, even though this is often assumed to hold for *most* thin spiking SUs (Swadlow, 2003; Atencio and Schreiner, 2008). Hence, some degree of error is possible in inferring that our thin spike SUs may be mostly fast spiking interneurons, especially

given the comparatively frequent rate at which we encountered thin spike SUs without directly targeting them (compare (Atencio and Schreiner, 2008) and (Rose and Metherate, 2005) to (Wu et al., 2008)). In fact, in a few instances (4/34 thin spike SUs), we did observe bimodal ISI distributions of the type previously associated with chattering cells (Gray and McCormick, 1996; Nowak et al., 2003), including the SU illustrated in Fig. 4.1B. However, since ISI parameters can depend on the nature of the stimulation (Azouz et al., 1997), and our sounds were themselves very brief, this should not be taken as definitive evidence for chattering neurons.

Table 4.2: Thin and thick spiking SUs have different characteristics. The table shows the number of thin and thick spiking SUs. There were significant differences between the two groups of SUs in the spontaneous rate, tuning width, recording depth, FSL per-call, and the FSL per-SU.

	Thin Spks		Thick Spks
# of SUs	30		21
Spont Rate [spk/s]	10.6 ± 2.0	***	2.2 ± 0.4
Tuning Width [BF/BW]	1.9 ± 0.1	***	2.7 ± 0.1
Depth rel. Surface [um]	484.1 ± 20.5	*	553.4 ± 18.8
FSL per-call [ms]	22.6 ± 0.8	***	41.4 ± 1.6
FSL per-SU [ms]	24.3 ± 2.3	***	45.0 ± 3.2

In favor of the possibility that our thin spike population might nevertheless be dominated by suspected fast-spiking interneurons, we found that the average depth of the recording sites for thin spike SUs was significantly shallower than for thick spike SUs (Table 4.2), consistent with the expected spatial distribution described above.

Moreover, the characteristics of our thin spike SU population (Table 4.2) were in agreement with other reports about extracellularly-identified "suspected interneurons" (Swadlow et al., 1998; Bruno and Simons, 2002; Atencio and Schreiner, 2008): higher spontaneous rate, broader stimulus tuning (to frequency in our case) and earlier latencies relative to the thick spike SUs. Note that these differences remained significant even if the four SUs with bimodal ISIs were removed from the comparisons in Table 4.2 (data not shown). Furthermore, although 3 of these SUs fell into the Q_{Best} group by relative error segregation, these were the longest latency SUs in that group (three black circles in center of Fig. 4.9B). Since none were among the intersected Q_{Best} group, where differences between Q_{Best} and Q_{Poor} SUs actually became more significant, this further supports the possibility that most of our best predicted SUs form one physiologically distinct group that may correspond to suspected fast spiking interneurons.

If the above were true, then we should ask why neurons that more faithfully preserve the peripheral nonlinear mechanism for sound onset encoding might convey inhibition rather than excitation in the cortex. Speculatively, one answer may be simply that purely excitatory scaling with the integrated envelope could be too metabolically costly to encode sounds (Hasenstaub et al., 2010), and might also saturate the dynamic range of neuronal spiking. Indeed, sparse cortical coding has been hypothesized to be enabled by precisely timed inhibition (Wolfe et al., 2010). In addition, work from other sensory systems indicates that fast-spiking interneurons relay a feed-forward inhibition from the thalamus (Gibson et al., 1999). The best evidence comes from the somatosensory cortex of rodents, where fast-spiking interneurons receive strong

*mono*synaptic thalamic input (Porter et al., 2001; Cruikshank et al., 2007) at short latencies (Yamamoto et al., 1988; Agmon and Connors, 1992; Welker et al., 1993). Furthermore, anatomical analysis of visual cortex also suggests that basket cells (thought to be one type of fast-spiking neuron (Kawaguchi et al., 1995)) receive targeted albeit sparse thalamic synaptic input (Freund et al., 1985; Ahmed et al., 1997). In addition, studies in an auditory thalamocortical slice preparation have found that presumed fast-spiking interneurons in layers 1-4 receive relatively short latency monosynaptic input upon thalamic stimulation (Rose and Metherate, 2005; Verbny et al., 2006). Thus, the fact that our Q_{Best} SUs were all thin spiking cells with both shorter and more precise latencies suggests that fast-spiking interneurons could be receiving direct thalamic input (Fig. 4.14A).

Do fast-spiking interneurons then serially inhibit excitatory pyramidal cells in auditory cortex, as implied by the proposed feed-forward circuit in Fig. 4.14A? This may not be the case for all fast-spiking cells. Different types of fast-spiking cells that are GABAergic can form unique connections with excitatory cells. This includes basket cells that form synapses onto the soma and proximal dendrites (Kisvarday et al., 1985; Freund et al., 1986; Freund and Katona, 2007), and chandelier cells that form axo-axonic connections (Howard et al., 2005). In particular, the latter have longer sensory-evoked latencies, suggesting activation by local cortical circuits rather than thalamic afferents (Zhu et al., 2004; Howard et al., 2005). Hence, only a subset of fast-spiking interneurons likely conveys direct feed-forward inhibition, which is consistent with our finding that only a subset of transient onset, thin spike neurons are Q_{Best}. If this direct feed-forward

inhibition exists, one prediction would be that the activity of the post-synaptic targets of these fast-spiking interneurons should reflect this inhibition. Indeed, in the somatosensory cortex, fast-spiking cells generally fire earlier than pyramidal cells (Yamamoto et al., 1988; Agmon and Connors, 1992; Welker et al., 1993), probably because the former receive stronger monosynaptic excitatory postsynaptic potentials (Porter et al., 2001; Cruikshank et al., 2007). However, such a cell-type difference in postsynaptic potentials has not been found in auditory cortex (Rose and Metherate, 2005; Verbny et al., 2006). Consequently, disynaptic inhibition in layer 1-4 auditory cortical pyramidal cells elicited by thalamic stimulation *in vitro* is rarely observed (Rose and Metherate, 2005; Verbny et al., 2006), although it can be seen in layer 5 pyramidal cells (Hefti and Smith, 2000). On the other hand, i*n vivo,* anesthetized, intracellular studies can detect slightly delayed inhibitory inputs that are approximately balanced with excitation (Wehr and Zador, 2003; Zhang et al., 2003), but whether this is disynaptically driven from thalamic input is not known. Hence, the role of direct thalamic feed-forward inhibition in auditory cortex is not so clear. We have on occasion observed a brief inhibition of spiking at sound onset before the excitation of a thick-spike neuron (Fig. 4.14B), demonstrating at least some overt evidence in an awake animal for effective feed-forward inhibition in auditory cortex.

Considering the role of thin and thick spiking neurons more abstractly based on our Q_{Best} and Q_{Poor} results, it may be that the functional role of pyramidal neurons in auditory cortex is not simply to represent acoustic features, but to extract so-called auditory objects (Ulanovsky et al., 2004). In this case, perhaps the activity of

"acoustically-faithful" feedforward inhibition might serve to synchronize different pyramidal neurons encoding higher-level features of auditory objects (Bush and Sejnowski, 1996).

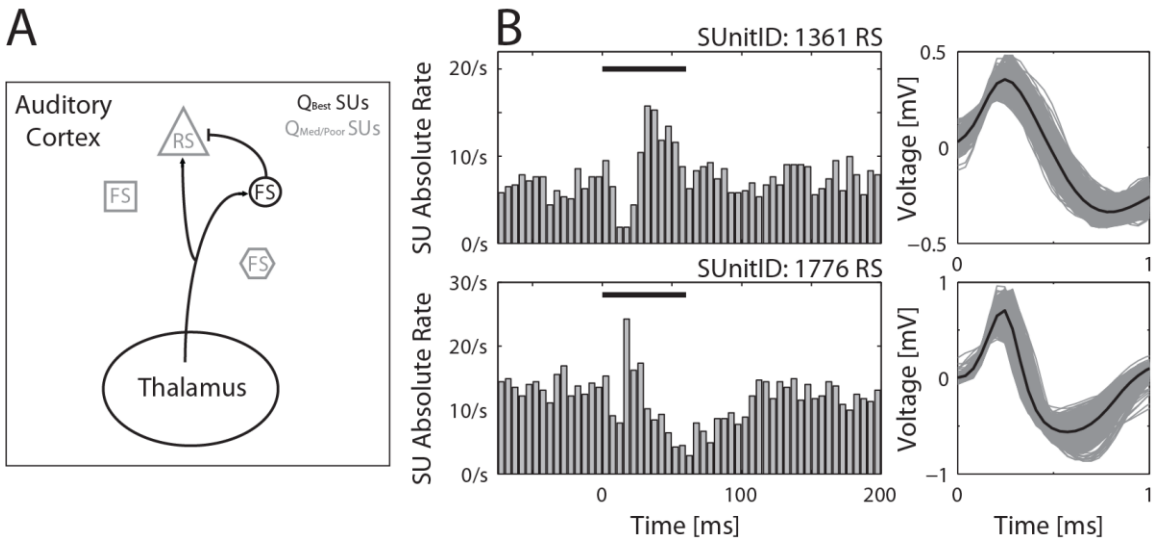


Figure 4.14: The proposed function of Q_{Best} SUs. (A) Schematic for the proposed function of well predicted SUs (black), a subset of fast spiking inhibitory interneurons in the auditory cortex. We hypothesize that these cells are involved in a feed forward thalamocortical inhibition circuit and are disynaptically connected to excitatory thick spiking cells. The excitatory cells along with other fast spiking cells that were part of the Q_{Mid} and Q_{Poor} groups are in gray. Synaptic inhibition is represented by the line, whereas excitation is represented by the arrow. (B) Potential evidence of this hypothesized model is demonstrated by SUs 1361 and 1776's response to pup calls. Both SUs are thick spiking cells based on their peak to peak times and shows early inhibition following the onset of the sound (red bar).

4.4.3: Relation to prior work on first spike coding

Our analysis of first spikes contributes to a growing literature on this particular feature of the spiking code. There is evidence from the visual (Oram and Perrett, 1992; Thorpe et al., 1996) and somatosensory (Johansson and Birznieks, 2004) systems suggesting that these spikes carry perceptually and behaviorally relevant information for

fast sensory decisions (Thorpe et al., 2001). For audition, the cortical LIEFTS model essentially converted the strength of an acoustic stimulus into a first spike timing delay by trading quieter sound pressures for longer latencies in Q_{Best} SUs (Fig. 4.3A and B). Thorpe (1990), proposed exactly this kind of "analog-to-delay" converter as a basis for a "rank-order" spike timing based population code (Thorpe et al., 2001). Notably, to decode such a representation, a feed-forward shunting inhibitory circuit pooling across converging inputs has been suggested (Thorpe, 1990; Thorpe et al., 2001), a role potentially filled by the best predicted, putative fast spiking SUs.

Second, our FSL data for natural calls complemented earlier studies of cortical FSL that used simpler synthetic stimuli (pure or frequency-modulated tones and noise bursts), carried out mainly in anesthetized preparations (Phillips and Hall, 1990; Heil, 1997; Heil and Irvine, 1997; Phillips, 1998; Nelken and Versnel, 2000; Fishbach et al., 2001; Furukawa and Middlebrooks, 2002). While first spikes were generally found to be early and precise in those cases, (Ter-Mikaelian et al., 2007)), recently concluded that cortical FSLs for BF tones become later and less precise in awake animals (but see (Huetz et al., 2009) for an example where overall temporal resolution for discriminating certain sounds can become finer). Our data (Fig. 4.3F) was consistent with Ter-Mikaelian (2007), since our FSL standard deviations were quantitatively similar to their awake cortical data. Interestingly, they found a few neurons (roughly 9/54 = 17%) with short latencies <20 ms and high precision <2 ms. Their population PSTH for sinusoidally amplitude-modulated tones also revealed a strong, distinct spiking contribution in the awake A1 from short-latency cells. Our results suggest that rather than simply representing

variability in cortical responses, these neurons may have corresponded to a systematically separable subpopulation of cortical neurons analogous to our Q_{Best} SUs.

Finally, our work extended prior cortical modeling efforts that used selected data collected from anesthetized animals (Heil, 1997; Fishbach et al., 2001; Fishbach et al., 2003; Heil, 2004). In contrast to their focus on responses with prominent onsets, we did not preselect response types, requiring only an excitatory response from which we could extract first spikes. This led us to conclude that most cortical SUs in awake animals actually have responses to sound onsets that are *not* well described by the nonlinear envelope integration mechanism at work in auditory nerve fiber firing (Heil et al., 2008; Neubauer and Heil, 2008). This could be considered either trivial because of the high number of intervening processing stages, in which case the success for the best predicted SUs is surprising, or unexpected because of this mechanism's success in anesthetized animals (Phillips and Hall, 1990; Heil and Irvine, 1997).

4.4.4: Implications for hierarchical processing

The fact that Q_{Best} SUs in the auditory cortex preserve the peripheral mechanism for sound onset integration suggests that neurons in earlier auditory stations should show a similar encoding of sound onsets. In particular, the presence of strong transient responses has been documented subcortically in both anesthetized (Pfeiffer, 1966; Rhode and Smith, 1986b, a; Blackburn and Sachs, 1989; Syka et al., 2000; Woolley and Casseday, 2004; Zheng and Escabi, 2008) and non-anesthetized preparations (Pollak et al., 1978; Shofner and Young, 1985). However, classifying neurons solely by their onset

PSTH may not be equivalent to identifying Q_{Best} neurons because discharge patterns can change depending on acoustic stimuli (Pfeiffer, 1966; Pollak et al., 1978; Wang et al., 2005). In fact, we found onset components to call responses even among non-Q_{Best} SUs (Fig. 4.9B), as well as sustained components for some stimuli among Q_{Best} SUs (e.g. Fig. 4.3C and 4.6D). Thus, future studies could benefit from classifying auditory neurons by their expected (i.e. modeled) transformation of sounds instead of their stimulus-specific PSTH. This may provide a more consistent taxonomy that reflects the neural sensitivities to acoustic features like sound amplitude.

Functionally, Q_{Best} and Q_{Poor} SUs differed in their sound encoding and in principle might be distinct neuronal groups at the same level of cortical processing (e.g. different pyramidal cells). In particular, Q_{Best} SUs may preserve the peripheral mechanism to sound onsets, whereas Q_{Poor} SUs may represent subsequent acoustic modulations or non-auditory information such as the sound's behavioral relevance. This difference could relate to the stimulus-synchronized and non-stimulus-synchronized neurons reported in the awake marmoset auditory cortex (Lu et al., 2001). However, results from our spike waveform analysis argue strongly against a parallel representation by the same cell type. Instead, by utilizing this forward model, we found a set of Q_{Best} SUs, which were all thin spiking neurons with earlier and more transient first spike responses that may instead be a hierarchically different class of neurons from those in the later-firing, call-selective Q_{Poor} group.

Indeed, by using the call response characteristics of the Q_{Poor} subset as a template for selecting similar neurons from the heterogeneous population recorded, we

demonstrated that thick spiking neurons, which were least faithful to a peripheral sound encoding in mothers and early cocarers, had an increased response to pup calls compared to acoustically matched adult calls. This suggests that acquiring the significance of a class of communication sounds engaged the auditory cortex in a way, which selectively enhanced those sounds' representation. How is this enhancement achieved? Using the van Rossum metric, we found that these neurons were selective for more complex acoustic features which must distinguish pup from adult calls, such as the calls' precise frequency trajectories. This was not the case in virgins and cocarers. This suggests that in core auditory cortex, pup experience has reshaped the encoding of pup calls so that the acoustic features which can better discriminate the more salient sound class (pup calls) from the less salient one (adult calls) are being "learned".

Plasticity is a well-studied phenomenon in the adult auditory cortex. Certainly, by training an animal to either detect or discriminate specific acoustic features, the auditory cortex undergoes changes that correlate with the most behaviorally salient features (Wetzel et al., 1998; Ohl et al., 1999; Beitel et al., 2003; Polley et al., 2006; van Wassenhove and Nagarajan, 2007). Our findings now demonstrate how natural communication experience can change the sensitivity of a distinct subset of single neurons in the auditory cortex to the defining acoustic features that facilitate the recognition of pup calls.

The results here begin to bridge our understanding of plasticity and its role in the processing of natural communication sounds. Species-specific vocalizations are an important class of signals because of its roles in a variety of social interactions. A

number of studies have demonstrated that neurons in the auditory cortex are combination sensitive and can differentially respond to a species-specific call over its time-reversed version (Wang et al., 1995; Wang, 2000; Wang and Kadia, 2001). How these differences arise though is unclear, as they could be innate or the result of auditory experience during development. In addition, it has been argued that this response asymmetry is not evidence for call selectivity, as training ferrets with these vocalizations does not lead to the same results (Schnupp et al., 2006). Instead, it is suggested that call specificity occurs at higher order cortical areas (Rauschecker and Tian, 2000), but how this representation is facilitated by core areas is not yet clear. Our results here provide some answers to these questions. We suggest that neurons in the auditory cortex are not call-specific in their responses, but instead through experience, change to encode the acoustic features that represent the most behaviorally salient parts of the sound. We hypothesize that this change at the level of primary auditory cortex facilitates the downstream encoding of sounds as acoustic objects, and that this subset of neurons are involved in the core to non-core auditory cortical transformation of the acoustic features to its behavioral recognition.

CHAPTER 5

CONCLUSION

5.1: Summary

Our acoustic environment is incredibly complex, providing a diversity of sounds to characterize the world around us. From the hum of the computer, to the ring of an alarm, our auditory system facilitates the processing of these sounds to mediate the appropriate response. Communication is a critical part of this, but how we transform speech sounds from their acoustic structure to their behavioral meaning remains unanswered. The goal of this dissertation was to begin addressing this question by investigating how changes in the behavioral relevance of a communication sound alter the neural representation. We hope that by developing an understanding of these changes, we can then create a framework to explore how the auditory cortex functionally contributes to sound recognition and behavior.

To study the above question, we recorded neural activity from awake-restrained mice and utilized an ultrasonic communication system between mouse pups and adult females. Comparing the SU and LFP responses in animals that either do (mothers) or do not (pup-naïve virgins) recognize pup ultrasounds, we found greater call-evoked inhibition in mothers. Importantly, this difference was most apparent for recording sites whose preferred frequency was lower than the pup calls' high-ultrasonic frequency range.

What then could be the functional consequence of these results? Recall that in section 1.1.2, we discussed that the mouse auditory cortex was spatially distinct in its frequency responsiveness. This implies that neurons with their BFs in the call frequency range may be clustered together, whereas those neurons with much lower BFs may be located in a different region of auditory cortex. Given this, our results suggest that the greater call-evoked inhibition in neurons tuned to frequencies lateral to the pup call range acts to enhance the contrast in the spatial representation of pup calls. This finding of inhibitory plasticity in the "lateral band" is unique in its idea, as past studies of auditory plasticity have mostly demonstrated an enhancement of the excitatory responses to a trained target frequency (Weinberger and Diamond, 1987; Bakin and Weinberger, 1990; Fritz et al., 2003). Thus, in this natural communication system, we hypothesize that the change in the calls' behavioral relevance leads to an enhanced representation in the auditory cortex that facilitates downstream areas in the detection and discrimination of the pup calls.

The results in chapter 2 raised a number of important questions, one of which was how pup experience and/or maternal physiological state would affect inhibitory plasticity. Pregnancy, parturition, and lactation can lead to distinct physiological changes in the mother, and while experience and learning are known to be correlated with auditory cortical plasticity, how the intrinsic state of the animal interacts with this experience is less well understood. To address this, we recorded from cocarers, a group that has had pup experience and recognize pup calls as behaviorally relevant (Ehret, 1982; Ehret et al., 1987). Looking primarily at neurons in the auditory fields with

frequencies lateral to the pup call range (A1 and AAF), we found that cocarers were more similar to virgins in their features of call-evoked inhibition. This might lead one to suppose that pup experience alone does not play a role in inhibitory plasticity. However, two specific studies made us question this conclusion. The first is based on a study demonstrating that while post-weaned cocarers and mothers could behaviorally recognize calls, mothers, but not cocarers continued to show call recognition behavior one month later (Ehret, 1989). The second is the fact that isolated pups stop emitting ultrasound calls by post-parturition day 13, approximately nine days prior to the start of our recordings (Hahn et al., 1998). In particular, the former study suggested that experience induced changes may decay over time in the cocarer. This motivated us to record from a group of early cocarers (post-parturition 9-11) and target SUs in A1 and AAF to look at their lateral band inhibition. From this, we found that early cocarers showed call-inhibited responses more similar to mothers. These results allowed us to revise our previous conclusion to suggest that pup experience indeed could result in cortical plasticity, but that maternal physiological state plays a role in retaining this plasticity long after the pups have stopped vocalizing.

This new finding led us to explore two additional questions: first, what mechanism might help maintain inhibitory plasticity in the mother, and second, whether this plasticity correlated with call recognition behavior. To answer the first, we discovered that unlike any of the virgin animal groups, mothers showed lower spontaneous activity in their call-responsive neurons. Importantly, we did not find these same differences when looking at neurons responsive to synthetic pure tones. This

suggested that the change in spontaneous firing was selective only for those neurons in the mother that responded to behaviorally relevant pup calls, leading us to hypothesize that this may be related to the maintenance of cortical plasticity. In support of this, recent modeling studies in the lateral amygdala demonstrated that low spontaneous rates can help to preserve plasticity through decreased Hebbian synaptic weakening (Li et al., 2009). Thus, our results suggest that the maternal physiological state may promote the long-term maintenance of synaptic plasticity, and that decreasing the spontaneous activity of call-responsive neurons mediates these changes.

These results led to our second question, which was to understand whether this cortical plasticity correlated with call recognition behavior. We addressed this in chapter 3 by using a two-alternative choice test. Here, we designed a novel closed loop feedback behavioral test with two servo-controlled doors. This provided us with the ability to remotely control the position of the animal during sound playback, and the exact time at which playback would stop. During each test, we placed two speakers at opposing ends of our W-maze apparatus, with one playing back pup calls, and the other a neutral tone sequence. Based on the number of approaches towards the pup call speaker and the time spent searching the maze following playback, we found that both mothers and early cocarers showed call recognition behavior, but late cocarers did not. Thus, our cortical inhibitory plasticity results correlated with call recognition behavior.

Given this correlation, we speculate that preserving lateral band inhibitory plasticity has functional benefits for the mother. A number of studies have shown that the physiological changes associated with motherhood contribute to the consolidation

of long-term maternal behavior (Bridges, 1975; Scanlan et al., 2006). In addition, both primiparous (1 litter) and multiparous (>1 litter) mothers show an improvement in memory task performance long after pup weaning, when compared to their virgin counterparts (Kinsley et al., 1999; Gatewood et al., 2005; Love et al., 2005; Lemaire et al., 2006; Pawluski et al., 2006; Macbeth et al., 2008). Therefore, we hypothesize then that the retention of these cortical changes facilitates the rapid induction of future maternal behavior by improving the detection or discrimination of pup calls.

Much of the work in chapters 2 and 3 looked primarily at call-evoked inhibition; however, this is obviously only part of the story. Although we did not find changes in the overall call-excited responses (see Chapter 2), we cannot ignore the possibility that our initial approach may have failed to uncover more subtle changes. In the auditory cortex, the stimulus evoked spiking of excited neurons showed a myriad of different response characteristics. This includes onset, offset, sustained, and any combination of the three. Due to this variability, we began by studying a small piece of this puzzle – how excited neurons encode natural sound onsets in the hopes that systematic differences in this might distinguish different types of neurons.

Auditory cortical neurons can encode different aspects of acoustic information. This varies from the acoustically faithful to the more complex context-dependent representation of a sound. To segregate the diversity in excitatory neural responses, we started with the idea that complexity in the neural representation increases at higher stages of auditory processing (Ahmed et al., 2006; Atencio et al., 2009). However, studies in the anesthetized auditory cortex demonstrated that even at the level of

cortex, there are neurons that encode sound onsets similar to the auditory periphery (Heil and Irvine, 1997). Thus, to understand how natural sound onsets are encoded in the auditory cortex, we utilized a previously described physiological model of the auditory nerve fiber's encoding of acoustic structure.

Applying this model, we identified a group of acoustically faithful (well-predicted) cortical neurons, and another that showed more nonlinear or unpredicted responses to the pup calls (poorly predicted). Interestingly, for the group of well-predicted neurons, the animal groups showed no overall differences in their response to pup and adult calls, two classes of sounds that are acoustically similar but distinct in their behavioral meaning. In contrast, for the most poorly predicted neurons which may be higher in the processing hierarchy, mothers and early cocarers showed distinct differences in their excitatory responses to pup and adult calls compared to cocarers and virgins. Thus, our data suggests two things: first, that there is a subset of neurons that preserve the peripheral encoding of natural sound onsets, and second, that a different subset of neurons distinct in their call response properties can selectively enhance their encoding of a sound class when it gains behavioral relevance.

In this dissertation, we set out to understand how auditory cortical neurons encode behaviorally relevant communication sounds. Using the mouse ultrasonic communication system, this thesis is the first to describe how auditory cortical neurons in the awake-restrained mouse encode pup vocalizations. In doing so, we have created a framework that will allow us to study in more depth the functional purpose of inhibitory

and excitatory plasticity, how this coding is affected by the maternal physiological state, and how it contributes to sound recognition and behavior.

5.2: Future Directions

The work in this dissertation has opened the door to a number of new questions that need to be addressed for us to approach a more complete understanding of how the auditory cortex translates acoustic features into meaning, and how it uses this to promote behavioral action. Specifically, we want to understand exactly how the auditory cortex encodes pup vocalizations that lead to pup retrieval behavior, and how specific maternal hormones affect experience dependent plasticity.

One of the first questions that stems from this work is whether our results are involved in auditory processing during active behavior in a single animal. In order to study this, we need the ability to record from freely behaving mice. I have begun to solve this problem by designing a working prototype for a chronic electrode implant that can record SUs, MUs, or LFPs (see Appendix D). Using the prototype, we tested its long term recording feasibility by implanting a virgin female mouse and recording SU responses for 35 days. Figure 5.1 shows two recorded SUs that have similar tuning curves, but actually respond to the frequency tones in opposing ways. With this implant, we were able to record two different SUs from the same electrode 20 days apart, and up to 30 days after surgery.

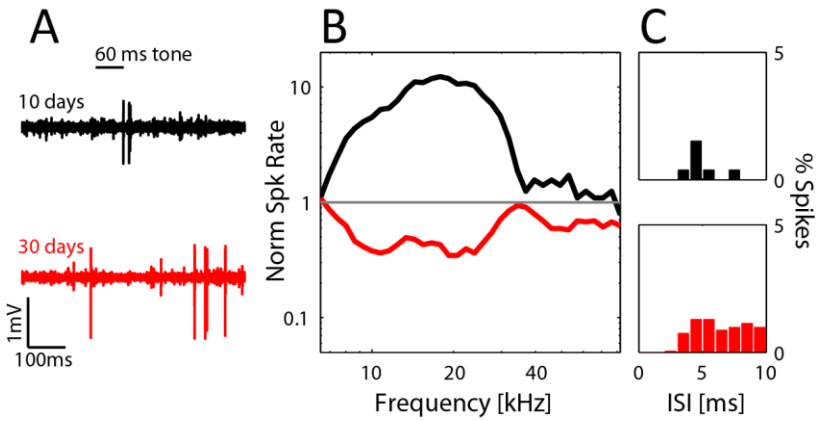


Figure 5.1: SU recordings taken from implanted electrode microdrive system. (A) Two SUs were recorded 20 days apart from the same implanted electrode and the black and red traces show examples of a single spike train response to a frequency tone. (B)The tuning curves for each SU, where the top unit (black trace) was excited by tone frequencies, and the bottom unit (red trace), was inhibited by the tone frequencies. (C) Shows the ISI of the spikes recorded during the tone frequency playback, which included a total of 40 frequencies and 600 trials with each trial having a 600 ms long recording length. Notice that there are no spikes in the 0-2ms bins for both units.

The idea then is to combine the electrode microdrive implant with our design of the closed loop behavioral paradigm in chapter 3 to explore how neurons encode pup calls during recognition behavior. This will inevitably provide us with more insight as to how neurons encode pup calls during passive and active behavior so that we might better understand how the auditory cortex processes sounds at the single neuronal level. A second question that stems from this work is the functional purpose of greater call-evoked cortical inhibition. While we have hypothesized a model of greater call-evoked inhibition to facilitate pup call detection, its functional purpose needs to be tested. The question then is whether call recognition behavior correlates with suppression in A1 and AAF relative to UF. For example, we could approach this by implanting electrodes across UF, A1, and AAF, and then testing the call recognition

behavior of the animal in a background noise. By varying the intensity of the background noise, there is presumably a point at which the mothers and early cocarers begin to perform the call recognition task at chance levels. With the implanted electrodes, we can compare the activity across auditory fields in the low versus high intensity noise task, and correlate the suppression activity across fields with behavioral performance.

A third question we can now address with a working chronic electrode implant is how the cortical plasticity is either retained or lost over time. In chapter 3, we demonstrated that SUs show stronger call-inhibited responses early in their pup experience, but decays to pup-naïve virgin levels following pup weaning. By using the chronic electrode implant, there is the opportunity to place several electrodes in a virgin prior to pup experience to investigate how the cortical responses to calls change over time. This should bring to light when the changes occur and disappear, and by using the behavioral experiment, we can test whether the magnitude of these changes correlate with call recognition performance.

Associated to this, a fourth question is whether changes in cortical inhibitory plasticity in cocarers is a result of its gonadal hormones. To address this, we should perform electrophysiology and behavior experiments in two groups of cocarers: ovariectomized (OVX) and OVX with hormone replacement. Ovariectomy is the removal of the ovaries and results in the loss of gonadal hormones such as estrogen and progesterone. While an OVX cocarer can still perform maternal behavior (Stolzenberg and Rissman, 2011), it is unknown how this might affect the auditory cortical processing of pup calls. Thus, if the OVX cocarers show similar changes in call-evoked inhibition at

the early (P9-11) and late (P21-35) time points, then we can conclude that these cortical changes are a result of only pup experience.

A fifth question deals with understanding how the maternal physiological state contributes to the long-term retention of cortical plasticity. In chapter 3, we had proposed that the dopaminergic system interacts with the maternal physiological state to play a role in this consolidation. To test this hypothesis, one possibility is to implant a cannula over the auditory cortex for the application of dopamine antagonists. In this case, a mother could be given a dopamine antagonist during pup experience and then recorded at post-weaning to test if its strength of call-evoked inhibition is more similar to cocarers. This would help to suggest the role that dopamine plays in the long-term consolidation of auditory cortical plasticity.

A sixth question we should further explore is how sounds are encoded at different stages of the auditory pathway. One idea is that as information progresses along the auditory system, the subcortical structures encode a greater amount of the acoustic information, but higher order areas (e.g. secondary auditory areas) increasingly respond selectively. By recording neurons and their responses to tones and vocalizations, we can ask whether lower areas, such as the inferior colliculus show a greater proportion of acoustically faithful neurons, while higher order areas, such as DP or A2 show a greater proportion of neurons encoding the behavioral relevance. Through this, we can begin to understand how the ascending auditory system encodes sound and how each stage represents and extracts acoustic information. Together, these future studies can help uncover how the auditory cortex changes when the relevance of a

sound changes, and its functional purpose in driving behavior. In addition, it will further reveal how the internal physiological state during sound experience influence this cortical plasticity.

APPENDIX A

A.1: Experimental Animals

Experiments were carried out on CBA/CaJ female mice, all between 14 and 24 weeks old. For both electrophysiological and behavioral experiments, four different groups of female mice were used: virgins, cocarers, early cocarers, and mothers. Virgins are female mice that have had no contact with pups and no breeding experience. In contrast, cocarers are virgin mice that have had the same amount of pup experience as a mother. Both cocarers and mothers are mice that have had 21 days of pup experience and tested within 2 weeks of pup weaning. Early cocarers are animals tested following 6 days of pup experience. Animals were housed under a reversed light cycle (14 hours light/10 hours dark), had access to food and water *ad libitum*, and were tested during their dark cycle, corresponding to the active period for mice. A total of 13 mothers, 14 cocarers, 6 early cocarers, and 14 virgins were used for electrophysiology. A total of 13 mothers, 13 cocarers, and 7 early cocarers were used for behavior experiments.

Table A.1: Animal list used for chapter 2.

Animal	Group	Animal	Group
E06081002A	Mother	E06111301A	Virgin
J07012301A	Mother	E06120301A	Virgin
J07012302A	Mother	E06120303A	Virgin
J07012302B	Mother	E06120304A	Virgin
J07012303A	Mother	E06120304B	Virgin
J07012305C	Mother	E06120305A	Virgin
J07012306C	Mother	E08060804A	Virgin
		J07012303C	Virgin

Table A.2: Animal list used for chapter 3.

Electrophysiology

Animal	Group	Animal	Group
J07012302B	Mother	E09012903A	Early Cocarer
J07012302A	Mother	E09033103A	Early Cocarer
J07012301A	Mother	E09060903A	Early Cocarer
J07012306C	Mother	E09060306A	Early Cocarer
E08053002A	Mother	E09061803A	Early Cocarer
E08091401A	Mother	E09062303A	Early Cocarer
J07012305C	Mother	E06111301A	Virgin
E08060601B	Mother	E06120305A	Virgin
E09081201A	Mother	E06120304A	Virgin
E09081202A	Mother	E06120303A	Virgin
E06081002A	Mother	E06120301A	Virgin
J07012303A	Mother	E06120304B	Virgin
E08042401A	Mother	J07012303C	Virgin
E08021802A	Cocarer	E08060804A	Virgin
E08031702A	Cocarer	E08082801A	Virgin
J07012305A	Cocarer	E08082901B	Virgin
E07112104A	Cocarer	E08082802A	Virgin
J08040802A	Cocarer	E09091901A	Virgin
E07040402A	Cocarer	E09092202A	Virgin
E07041603A	Cocarer	E09092201A	Virgin
E07050701A	Cocarer		
E08031002A	Cocarer		
E08102001A	Cocarer		
E08102003A	Cocarer		
E07112904A	Cocarer		
E08030402A	Cocarer		
E08022702B	Cocarer		

Behavior

Animal	Group
E411010401B	Mother
E311011401A	Mother
E711010702A	Mother
E311011403B	Mother
E311020601A	Mother
E311011501A	Mother
E311030202B	Mother
E411030701A	Mother
E411031503A	Mother
E311031503A	Mother
E411032003B	Mother
E411042401B	Mother
E411050501A	Mother
E711010704A	Cocarer
E411010403B	Cocarer
E311030203B	Cocarer
E311031505A	Cocarer
E411030703A	Cocarer
E411031504A	Cocarer
E411031505A	Cocarer
E311031504A	Cocarer
E411032005B	Cocarer
E411040302A	Cocarer
E411041802B	Cocarer
E411050502A	Cocarer
E411032403A	Early Cocarer
E411040304B	Early Cocarer
E411040702A	Early Cocarer
E411041803A	Early Cocarer
E411041904A	Early Cocarer
E511042104A	Early Cocarer
E411043003A	Early Cocarer

Table A.3: Animal list used for chapter 4.

Animal	Group	Animal	Group	Animal	Group
E07050701A	Cocarer	E08042401A	Mother	E06120303A	Virgin
E08021802A	Cocarer	E08053002A	Mother	E06120304A	Virgin
E08022702B	Cocarer	E08060601B	Mother	E06120304B	Virgin
E08030402A	Cocarer	E08091401A	Mother	E06120305A	Virgin
E08031002A	Cocarer	J07012302A	Mother	E08060804A	Virgin
E08031702A	Cocarer	J07012303A	Mother	E08082801A	Virgin
E08102003A	Cocarer	J07012306C	Mother	E08082802A	Virgin
J07012305A	Cocarer			E08082901B	Virgin
J08040802A	Cocarer			J07012303C	Virgin

A.2: Surgery

Aseptic surgery was performed to stereotaxically define a recording grid over the left auditory cortex and implant a head post. Animals were first anesthetized using Isofluorane (2-5%, with O_2), and then placed on a heating pad (Harvard Apparatus, Holliston, MA) set to 37°C. The animal's head was secured using a sliding nose clamp in a stereotax (Model 900, David Kopf Instruments, Tujunga, CA), and hair on the top of the head was removed with Nair. After applying lidocaine jelly (5%) along the midline of the head, an incision was made and Schwartz vessel clips (World Precision Instruments, Sarasota, FL) were used to expose the skull. Using a periosteal elevator, the left temporal muscle was separated from the skull to access the AC, and then the positions of Bregma and Lambda were stereotaxically measured. The angle of the head was adjusted until both points were at the same height. Using the coordinate positions of Bregma and Lambda, a 5 column by 3 row recording grid (Fig. A.1) was marked onto the skull using a stiff wire mounted on a stereotaxic manipulator. The wire applied India Ink

to the skull to make dots that were ~100um in diameter. The columns were located at 50% to 90% of the Bregma-Lambda distance (~3.8 mm to 4.6 mm), measured from Bregma in 10% steps, while the rows were defined as 1.5, 2.0 and 2.5 mm below Bregma.

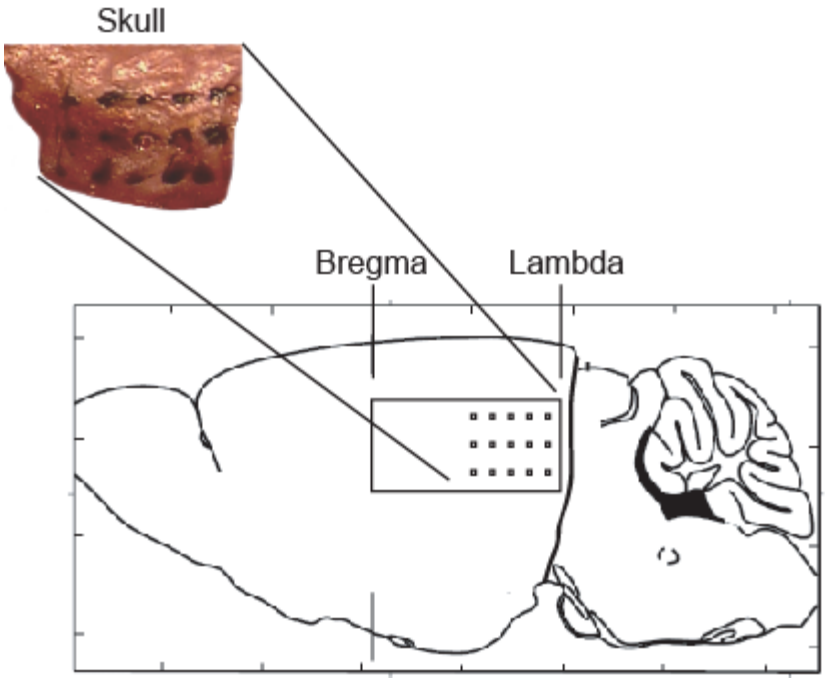


Figure A.1: Auditory Cortex Grid. Figure demonstrates the location of the 5 column x 3 row grid over the left AC relative to Bregma and Lambda.

Following the placement of the recording grid, a flat-head machine screw was inverted and placed on the midline equidistant from Bregma and Lambda, secured using a UV-cured dental cement (MaxCem Kerr, Orange, CA). In addition, two inverted, flat-head bone screws (Small Parts, Miramar, FL) were placed ventral rostral and ventral

caudal to the recording grid. A thin layer of dental cement was then spread over the grid and other exposed areas of skull, and the incision was sutured.

After surgery, the animal's behavior was monitored and Buprenorphine (0.05 mg/kg) was administered once it began to awaken. Electrophysiology recordings began after two days of rest, at which point their weight and activity levels returned to normal. If the animal's weight decreased by more than 15% of its pre-surgery body weight, it was no longer used for recordings.

A.3: Electrophysiology

During electrophysiology, the recording electrode was connected to a RA16AC high impedance headstage, which fed to a RA16PA medusa preamplifier (Tucker Davis Technologies). An 18-pin DIP connector was pre-soldered to connect to the RA16AC headstage connector with channel one connected to the recording electrode, and channels 2-16 soldered together with the ground terminal. Then, the entire DIP connector was covered with silicone to act as an electrical insulator. Both the RA16AC and the connected DIP were attached to a micromanipulator with a hydraulic microdrive prior to each recording session.

On the day of recording, the animal was first re-anesthetized with isofluorane and the head was secured using the stereotax. Holes (approximately 150 um in diameter) were drilled with an insect needle held by a pin vise (Fine Science Tools, Foster City, CA) in places previously marked by the recording grid. In addition, a hole for the ground wire was drilled in the left frontal cortex. Two hours after recovering from

this procedure, the animal was handled for 10 minutes and then placed into a homemade cylindrical (~3 cm diameter) restraint device. This plastic tube restraint was lined with foam in order to secure the animal's body while the head remained exposed (Fig. A.1). The animal was then suspended by rubber bands held by two metal bars on a vibration-isolation table in an anechoic chamber (Industrial Acoustics, New York, NY, USA). The animal's head was immobilized by placing the implanted screw in a restraining post positioned at approximately 45 degrees with its right ear 11 cm from a speaker. Each recording typically lasted 3-4 hours, and excessive movement or signs of stress signaled the end of an experiment.

Electrophysiological recording locations were stereotaxically targeted by a grid of holes over the left auditory cortex, covering mainly UF, A1, AAF (Galindo-Leon et al., 2009). Prior to inserting the recording electrode, a ground wire (stainless steel, 127 um diameter), was placed approximately 0.5-1 mm deep into the left frontal cortex. Then a 6 MΩ Tungsten electrode (FHC, Bowdoin, ME) was secured in a micromanipulator (World Precision Instruments), and positioned orthogonal to the AC. It was first advanced manually to ~200 um from the drilled hole, and then moved further using a hydraulic microdrive (FHC), while monitoring the activity on a computer speaker. The electrode was advanced at 10 um/s until there was change in the background noise level, which occurs when the electrode touches the cortical surface. This point was defined as the reference depth, and then the electrode was advanced quickly to 700 um depth at 500 um/s to minimize cortical dimpling. All recordings were located between 300 and 700 um relative to the reference depth (layers 3, 4 and 5, see supplemental

figure from Rothschild et al., 2010), and the average depth of all SU recordings was 536 um.

At each recording site, SUs and LFPs were co-recorded from the same electrode. Single units were isolated by moving the electrode towards the cortical surface in 5 um steps until the signal to noise level of the action potential was optimized. In BrainWare (Tucker Davis Technologies, Alachua, FL), spikes were detected using positive and negative thresholds, high-pass filtered at 300 Hz, low-pass filtered at 6 kHz and sampled at 24 kS/s. In addition, LFPs were high-pass filtered at 2 Hz, low-pass filtered at 300 Hz, and notch filtered at 60 Hz.

A.3.1: Single neuron extracellular recording

During the recording session, SUs were determined in BrainWare based on a number of characteristics in their action potential waveforms and spike times. Isolation of the SU was based on the absence of spikes during the absolute refractory period (within 1 ms), and on a cluster analyses of its various spike features (first vs. second peak amplitudes, vs. peak-peak times, vs. trigger-trigger times, vs. peak-trigger times, and vs. spike area). Figure 4.1A-D shows four different SUs with different signal to noise levels (4.1A1-D1), action potential shapes (4.1A2-D2), and the corresponding interspike intervals (4.1A3-D3). The insets in figures 4.1A3-D3 demonstrate the absence of spiking during a refractory period. In several cases, multiple SUs were recorded at one location and could be extracted by clustering based on spike features in BrainWare.

A.3.2: Local field potential recording

At each SU location, an LFP was recorded simultaneously. Offline, the signals were then despiked by deleting a -0.5 to 4 ms window around each spike and replacing it with a spline-interpolated signal, decimated (keep every 24th point), and low pass filtered (Parks-McClellan optimal equiripple FIR filter, transition band between 90 and 100 Hz) forward and backward to eliminate traces of action potentials without introducing phase delays (Galindo-Leon and Liu, 2010).

A.4: Single Unit IDs for Q_{Best} and Q_{Poor}-like Neurons

Table A.4: SUnitID list used for section 4.3.4.

Group	SUnitID	SU Group
Mother	1415	Q_{Poor}
Mother	1720	Q_{Poor}
Mother	1727	Q_{Poor}
Mother	1728	Q_{Poor}
Mother	1378	Q_{Poor}
Mother	1498	Q_{Poor}
Mother	1740	Q_{Poor}
Mother	1916	Q_{Poor}
Mother	1370	Q_{Poor}
Mother	1738	Q_{Poor}
Mother	1739	Q_{Poor}
Early Cocarer	1861	Q_{Poor}
Early Cocarer	1856	Q_{Poor}
Early Cocarer	1901	Q_{Poor}
Cocarer	1804	Q_{Poor}
Cocarer	1662	Q_{Poor}
Cocarer	1821	Q_{Poor}
Cocarer	1592	Q_{Poor}
Cocarer	1590	Q_{Poor}
Cocarer	1598	Q_{Poor}
Cocarer	1697	Q_{Poor}
Cocarer	1699	Q_{Poor}
Virgin	1679	Q_{Poor}
Virgin	1772	Q_{Poor}

Group	SUnitID	SU Group
Virgin	1789	Q_{Poor}
Virgin	1760	Q_{Poor}
Virgin	1005	Q_{Poor}
Virgin	1758	Q_{Poor}
Virgin	1687	Q_{Poor}
Mother	1381	Q_{Best}
Mother	1412	Q_{Best}
Mother	1743	Q_{Best}
Mother	1745	Q_{Best}
Mother	1513	Q_{Best}
Mother	1918	Q_{Best}
Early Cocarer	1906	Q_{Best}
Early Cocarer	1886	Q_{Best}
Cocarer	1403	Q_{Best}
Cocarer	1805	Q_{Best}
Cocarer	1695	Q_{Best}
Cocarer	1823	Q_{Best}
Cocarer	1594	Q_{Best}
Cocarer	1636	Q_{Best}
Cocarer	1644	Q_{Best}
Cocarer	1652	Q_{Best}
Cocarer	1698	Q_{Best}
Cocarer	1601	Q_{Best}
Virgin	1773	Q_{Best}

APPENDIX B

B.1: Acoustic Stimuli

During both electrophysiological and behavioral experiments, pure tone frequencies and natural pup vocalizations were played back to the animals. To present sound stimuli, the output from the digital-analog converter (sample rate of 223214.2857 samples/s), was passed through a PA5 programmable attenuator to an SA1 stereo amplifier module. The sound delivery system was calibrated by TDT software using a Brüel and Kjær (B&K, Norcross, GA, USA) 1/4" free-field microphone coupled to a B&K 2669 preamp and 2690 amplifier. Stimuli were generated using Tucker-Davis Technologies (TDT, Alachua, FL, USA) System 3 hardware and software and presented through the BrainWare application via modules programmed in the RPvdsEx environment.

B.1.1: Tones

For the electrophysiological recordings, auditory responses were located using pure tone frequencies. Tones were played at 60 dBSPL for 60 ms and had 10 ms $\cos^2$ onset and offset ramps. To derive frequency response curves, 40 different tones logarithmically spaced ranging from 6.4 to 95 kHz were presented randomly every 600 ms and repeated either 5 or 15 times. If a SU had an excitatory response to tones and a BF could be defined, a tonal rate level function was derived by playing the SUs BF

frequency from 30 to 70 dBSPL. The tones were presented in increments of 10 dBSPL, and repeated 10 times per amplitude in a blocked format.

Tones were also used during ABRs to assess an animal's threshold of hearing. Stimuli were generated using TDT SigGenRP software and presented through a speaker placed 11 cm away from the right ear of the anesthetized animal. Pure tones (20 and 64 kHz) of 3 ms in duration with 1.5 ms rise/fall times were presented at a rate of 21 Hz 500 times in 5 dB steps. In addition, clicks with a duration of 0.5 ms were presented at a rate of 19 Hz. These tone frequencies were chosen to span the previously reported audible and ultrasonic ranges of CBA/CaJ hearing (Liu et al., 2003; Radziwon et al., 2009).

B.1.2: Natural sounds

Eighteen pup and adult vocalizations were drawn for playback (Liu et al., 2003) from a library of natural ultrasonic CBA/CaJ vocalizations (Fig. B.1). Sound snippets were high pass filtered in software (25 kHz corner, 8-order Butterworth filter, butter, MATLAB), spectrally denoised (Liu et al., 2003), and then Hilbert transformed to extract the instantaneous frequency and amplitude envelope. These were used to re-synthesize a clean version of each pup call on a silent background, multiplied by a 0.5 ms $\cos^2$ onset and offset function, and scaled to a target RMS amplitude corresponding to 65 dBSPL. A maximum of fifty trials (600 ms long) of each pup call along with a blank stimulus were presented in random order, with sound onset usually beginning at 200 ms after trial

onset. Occasionally, a SU drifted sufficiently in amplitude that it could no longer be isolated, in which case the call stimuli were terminated with fewer trials.

B.1.3: Behavioral stimuli

For the behavioral experiments, two different stimuli were used in the two alternative choice test. This consisted of the mouse hearing alternating bouts of 2-5 randomized pup calls and randomized tones separated by one second of silence. The pup calls were chosen from four different vocalizations (calls 7, 10, 13 and 16, see figure B.1) with a RMS amplitude of 70 dBSPL measured at the nest depression. These four calls varied in both frequency (65, 71, or 74 kHz), and duration (35 or 55ms). In addition, the opposing speaker played a bout of pure tones (5ms $\cos^2$ onset and offset function, 55 dBSPL at nest depression) that also varied in frequency (19, 20, or 21 kHz), and duration (35 or 55ms) to match the stimulus variability in our pup call stimuli.

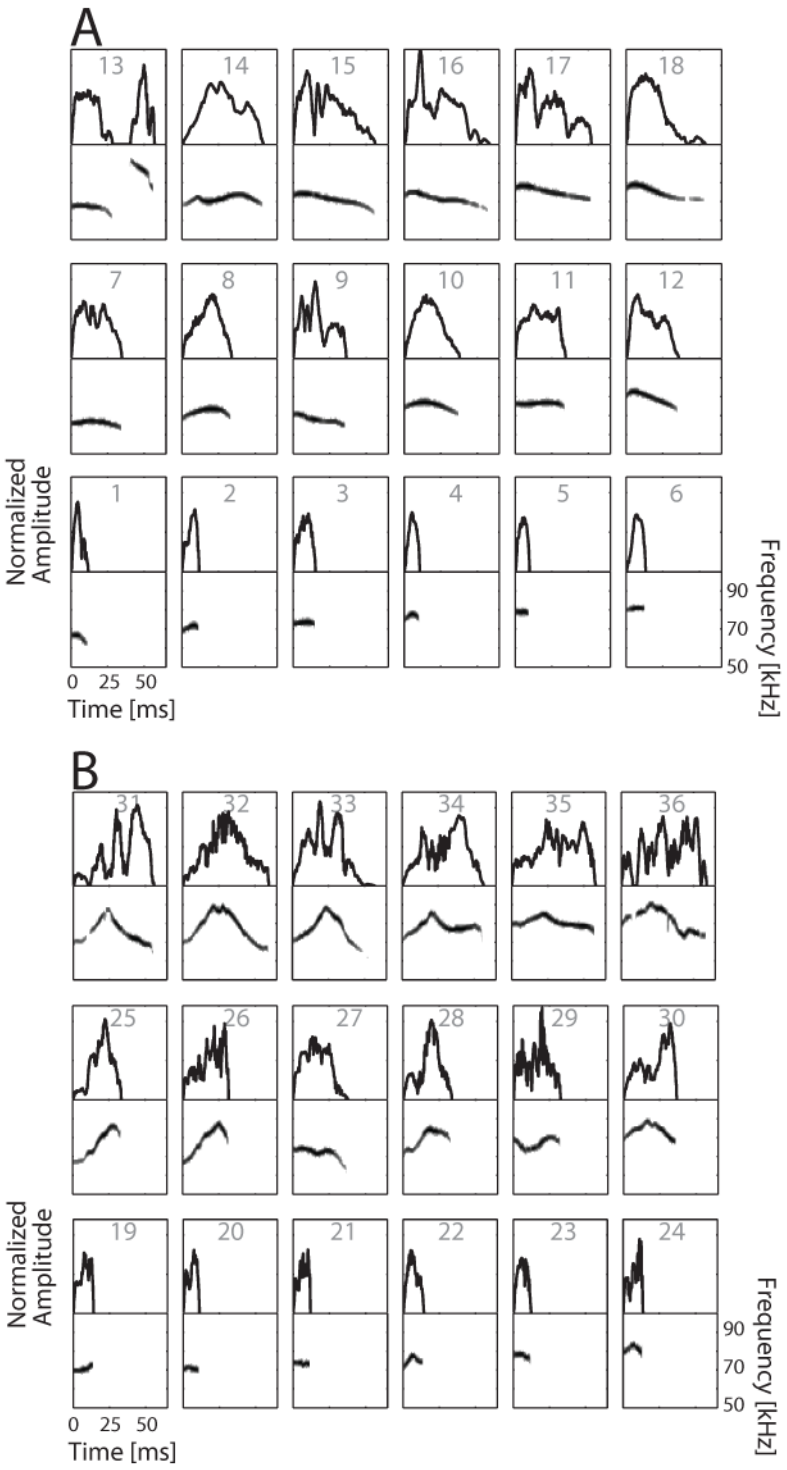


Figure B.1: Acoustic structure of pup and adult calls. (A) Illustration of the pup call stimuli amplitude envelopes (top row), and their frequency spectrograms (bottom row). The calls are ordered with frequency ascending from left to right panel in each row and with duration increasing from bottom to top panel in each column. (B) Illustration of the adult call stimuli with their amplitude envelopes and frequency trajectories.

APPENDIX C

C.1: Statistical Tests

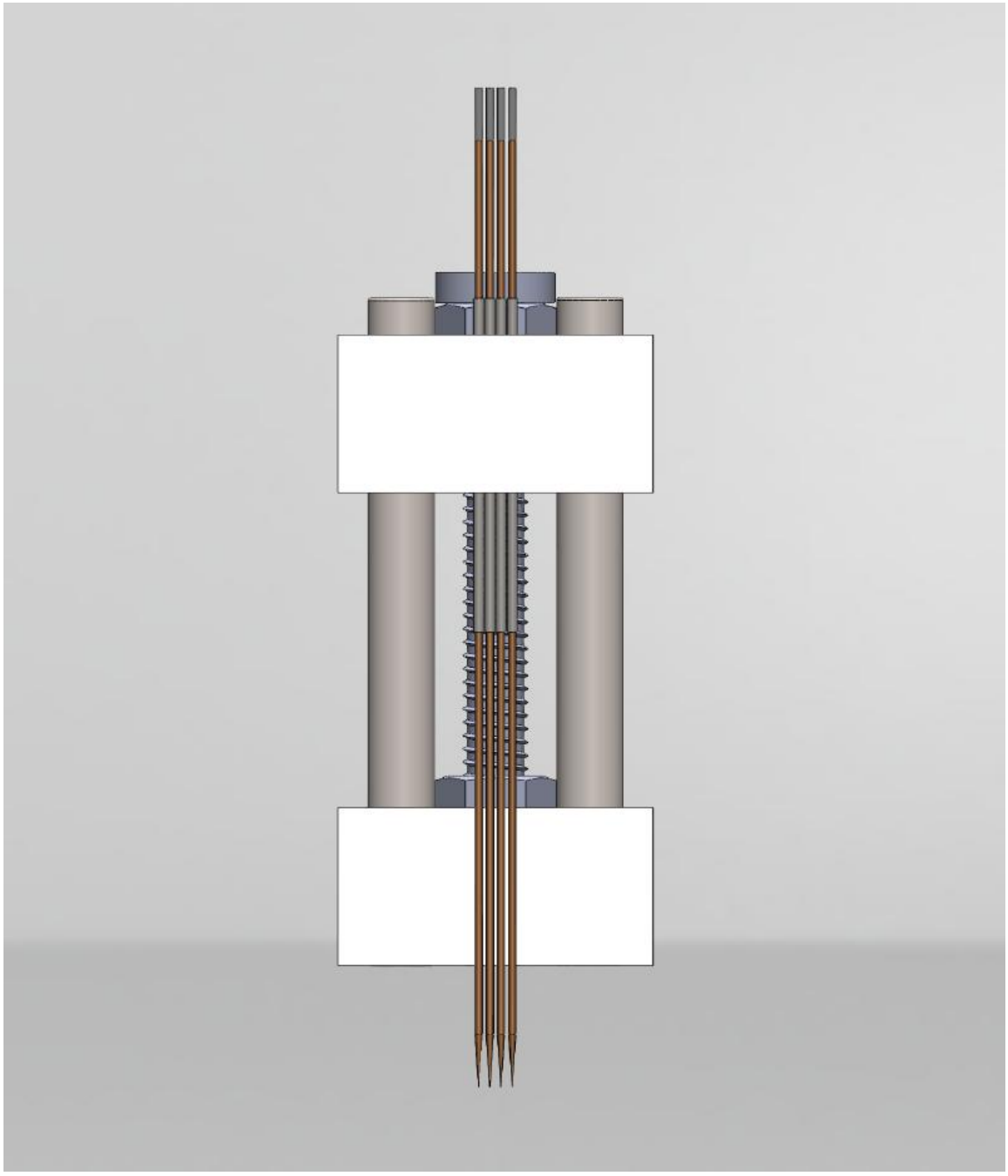
Parametric and non-parametric statistical tests were used to compare group differences. Parametric tests were used only if all groups were normally distributed and did not statistically differ in their variances. Using a significance criterion of $p<0.05$, we used the two-tailed Lilliefors goodness-of-fit test for normality, and a two-tailed F-test for variance. If all groups satisfied both criterion then we proceeded to test for group differences using the analysis of variance (ANOVA) or an unpaired t-test if only two groups were being compared. If any of the groups failed the test for normality or variance, group differences were tested using a Kruskal-Wallis for multiple groups, or the Mann-Whitney U (U reported is the min,(U_1,U_2)), if only two groups were being compared. If either the ANOVA or the Kruskal-Wallis test returned $p<0.05$, we performed group comparisons using Fisher's least significant difference criterion. If however two groups of data show inequality of variances we used the unequal variance test on the original data (if the data is normal, Satterthwaite t-test). This involves differences in the computation of the test statistic t and the degrees of freedom (Ruxton, 2006). When comparing two time traces from different animal groups, we used an N-way analysis of variance followed by multcompare using Fisher's least significant difference method for correcting multiple pairwise t-test comparisons at each time point.

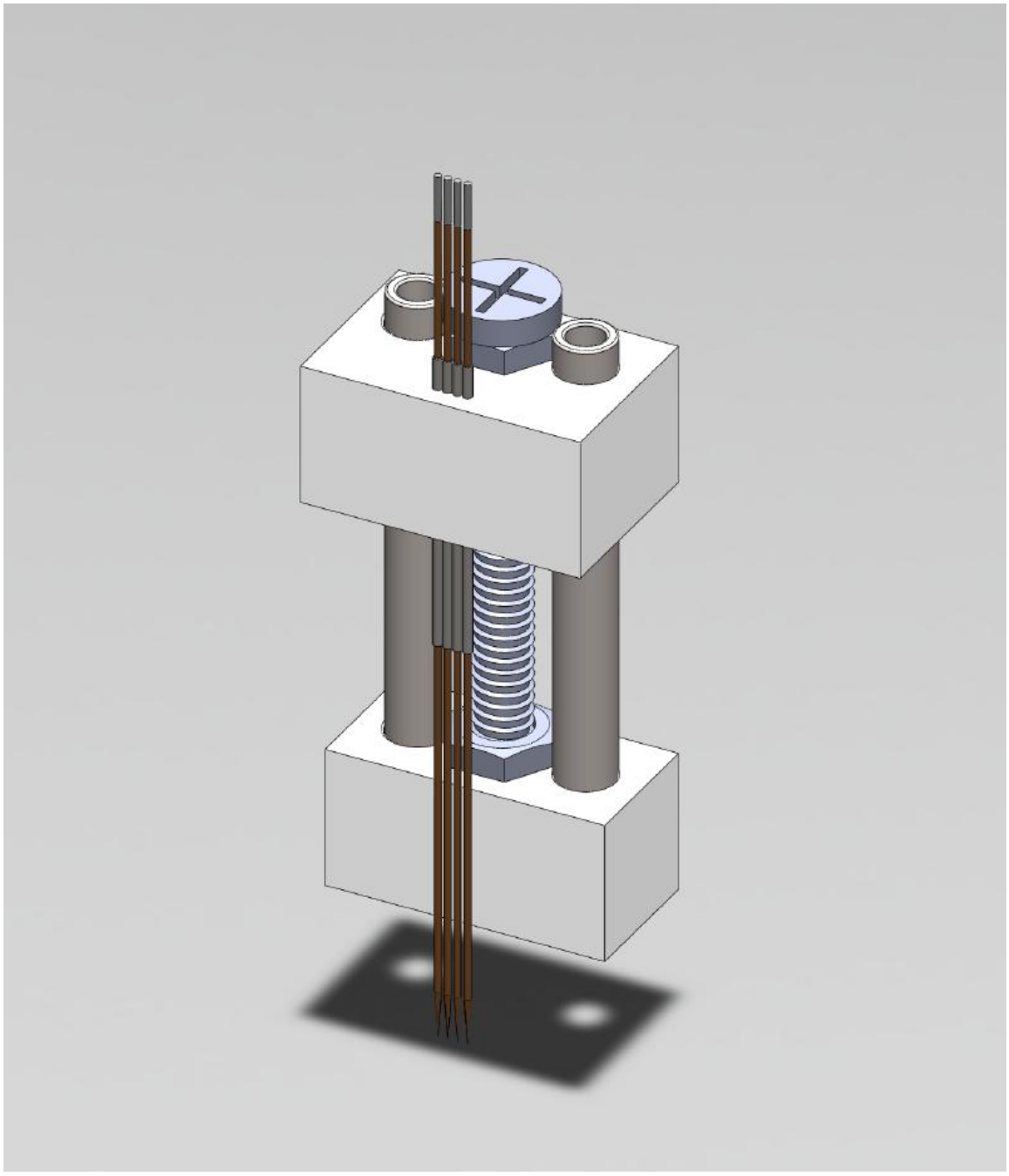
For the two-choice alternative choice test, we used a binomial test to investigate whether the proportion of pup call responses differs from chance level (50%). To test the effects of the call test on the exploration times of the different groups of animals we used only those tests where there was paired data, a post-retrieval and post-call test exploration times. In order to use the repeated measures ANOVA, we transformed the data using logarithms, which resulted in exploration times for the three different animal groups having normally distributed data that also did not violate the equality of variances. If there was a significant interaction effect between time and group we proceeded to perform an ANOVA with a multiple comparisons using Fisher's least significant difference criterion. For testing spontaneous rates, we performed a square root transformation of the data. The square root transformation is one that has often been used to convert neural activity (firing rate) with a Poisson distribution to a normal distribution (Baker et al., 2002; Prince et al., 2002; Ogawa and Komatsu, 2004; Hayden and Gallant, 2005). This transformation is often applied when the variances of the data are proportional to the means, and is often applied to data when the samples are taken from a Poisson distribution (Zar, 1999).

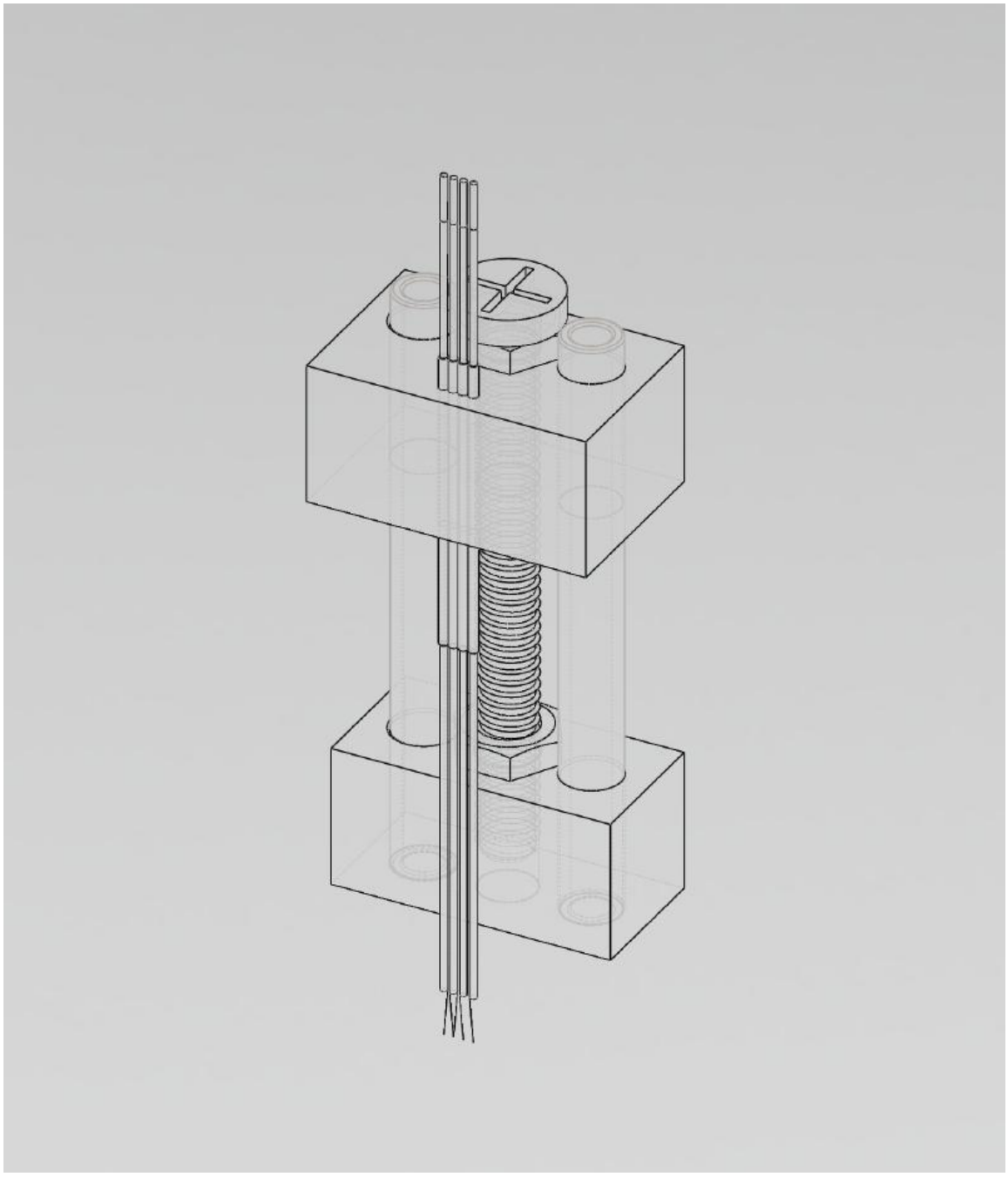
Significance of the phase precision was computed by the Rayleigh statistic (Fisher, 1993), and depended only on the number of trials. For pup calls and responses to tones, at a significance level of $p \leq 0.05$, $R = \sqrt{-\log(p)/N}$ should be larger than 0.058 for N = 900 trials and 0.199 for N = 75 trials, respectively. We discarded the phase precision for an LFP site if it did not exceed this significance level during the stimulus period.

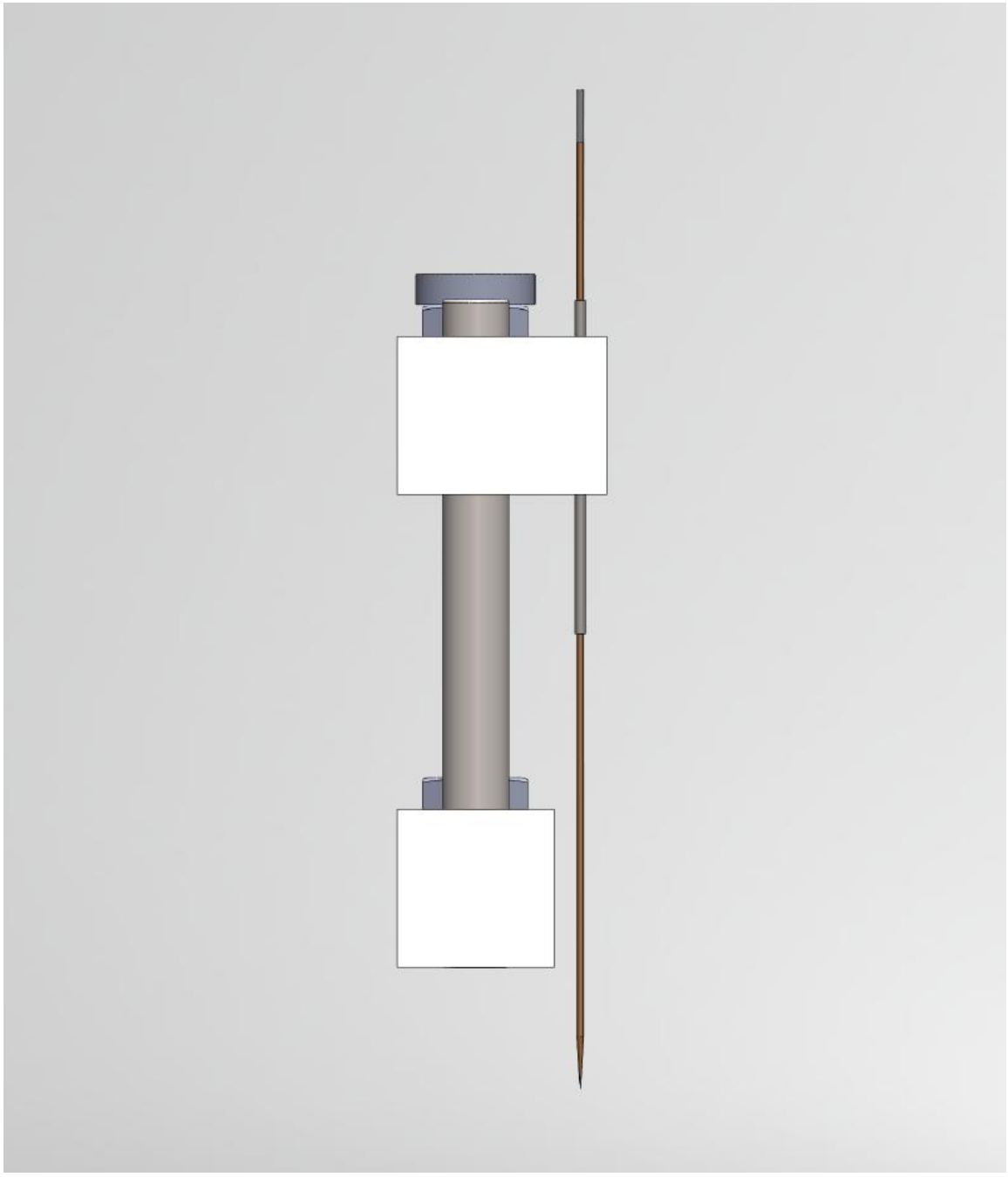
APPENDIX D

D.1: Electrode Microdrive Implant Design









The implant was drawn in Solidworks 4.0 and several views of the prototype design are shown in the figures above. The basic design consists of a number of parts which include: an upper and lower plastic base each with three milled holes (~1.0 mm diameter), two 19 gauge guide tubes, four 27 gauge guide tubes for the electrodes, four Tungsten 6 MΩ (FHC) electrodes, a 00-90 machined screw, and two 00-90 hex nuts. The two outer 19 gauge guide tubes are cemented to the base, whereas the upper white rectangle can freely move in the vertical axis. In addition, the lower 00-90 hex nut is attached to the base, preventing it from moving, but allowing the 00-90 screw to move. With the given thread distances on the 00-90, we can measure the depth of electrode penetration while the implant is in the brain. The only object permanently attached to the upper white rectangle are the four 27 gauge guide tubes and the four electrodes. Therefore, when the 00-90 screw is being turned clockwise (driving downwards), the upper rectangle and the electrodes will move downwards together. This design was implanted in the mouse primary auditory cortex and shown to be able to record SUs from an awake-restrained mouse for up to 30 days (See Fig. 5.1).

Table D.1: Parts list for the electrode implant design.

	Length [mm]	Width [mm]	Height [mm]	Gauge
Lower Base	7.7	2.7	4.0	NA
Upper Base	7.7	5.0	4.0	NA
Electrode Guide Tube	NA	NA	9.0	27.0
Guide Posts	NA	NA	13.5	19.0
Screw (J.I. Morris)	00-90			
Weight	0.5 g			
Electrodes (FHC)	20mm	6MΩ	125um shank	

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