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Experience-dependent Auditory Cortical Map Plasticity Across the Lifespan

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Experience-dependent Auditory Cortical Map Plasticity Across the Lifespan

By

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B.A., Ithaca College, 2008

Advisor: Robert Liu, PhD

An abstract of
a dissertation submitted to the Faculty of the
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Abstract

Experience-dependent Auditory Cortical Map Plasticity Across the Lifespan

By Kathryn N. Shepard

Throughout our lives, the sounds that we engage with sculpt our auditory responses. This is particularly true in auditory cortex (AC), where salient acoustic experiences can reorganize the distributions of response properties that are mapped across its surface. Though such "map plasticity" has been studied across sensory systems since the 1960s, the processes leading to its emergence are still not fully understood. This dissertation constitutes an effort to better understand the circumstances that promote map plasticity in the AC.

I first examine the physiological environment that permits map plasticity during development, when mere exposure to a stimulus can be enough to drive map plasticity. While the mechanisms that render the developing AC so sensitive to its acoustic environment are incompletely understood, studies in the visual system have suggested that the neuromodulator norepinephrine (NE) may be involved. Further, recent work in the AC has connected NE to map plasticity in adult animals. The first aim of this dissertation links these two bodies of research by asking whether NE is required for map plasticity during the critical period for frequency tuning in AC development. Using the dopamine β-hydroxylase knockout mouse, which cannot produce NE, I show that NE is required for the map plasticity that follows from sound exposure during development.

The second aim concerns map plasticity in adulthood. In adulthood, it is accepted that map plasticity only follows from behavioral engagement with sounds; this is generally explored through the use of laboratory conditioning paradigms in which a sound is reinforced with a reward or punishment. Here, I ask whether map plasticity occurs when a sound is made behaviorally relevant through natural experience (specifically, motherhood) rather than laboratory conditioning. In contrast to expectations based on previous work, I found that map plasticity does not occur among maternal females for any feature of their pups' ultrasonic vocalizations (USVs). Rather, more subtle plasticity in USV response magnitude emerges that may serve to suppress background neural activity when pups are calling.

Taken together, this work advances our understanding of AC plasticity by establishing physiological and behavioral prerequisites for map plasticity in development and adulthood, respectively.

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List of Symbols and Abbreviations

cm	Centimeter
dB SPL	Decibel sound pressure level
Hz	Hertz
kg	Kilogram
kHz	Kilohertz
mΩ	Megaohm
mg	Milligram
mm	Millimeter
ms	Millisecond
s	Second
5HT	Serotonin
6-OHDA	6-hydroxydopamine
A1	Primary auditory area
A2	Secondary auditory area
AAF	Anterior auditory field
ABR	Auditory brain stem response
AC	Auditory cortex
ACh	Acetylcholine
BF	Best frequency
Ca^{2+}	Calcium
CF	Characteristic frequency
DA	Dopamine
DBH	Dopamine β-hydroxylase

Dbh -/-	Dopamine β-hydroxylase knockout
DP	Dorsoposterior field
FM	Frequency modulation
FRA	Frequency-response area
HSD	Honestly significant difference
IAF	Insular auditory field
IP	Intraperitoneal
L-DOPS	L-3,4-dihydroxyphenylserine
LTP	Long-term potentiation
MGB	Medial geniculate body
NE	Norepinephrine
P	Postnatal day
PSTH	Peri-stimulus time histogram
UF	Ultrasound field
USV	Ultrasonic vocalization

Chapter 1

Introduction

Throughout their lifetimes, individuals are engaged by sounds that leave lasting impressions. For instance, a ring tone that is at first a meaningless song eventually comes to produce a powerful jolt of recognition and an ensuing behavioral response. Processes like this, in which an auditory cue acquires behavioral relevance upon pairing with a reinforcer (i.e., the social reward that comes from a phone call), have generated a tremendous amount of interest among experimenters. This is particularly true when the behavioral consequences of cue exposure pertain to the public interest: expansive bodies of work focus on the neural mechanisms through which exposure to drug-associated cues can lead to relapse (reviewed in O'Brien et al., 1992; See, 2002), for example, or on the neural correlates of auditory fear memory (reviewed in Maren and Quirk, 2004; Kim and Jung, 2006; Johansen et al., 2011).

Historically, these fields have focused primarily on the changes in cognitive or motivational processes that produce a new behavioral output in response to a formerly irrelevant cue. Substantially fewer investigations have addressed how auditory perception itself can be altered by experience. As a case in point, perusal of a widely cited review on "the neurobiology of Pavlovian fear conditioning" reveals 200+ mentions of the term "amygdala", and only 36 of the term "auditory" (and still fewer for other sensory modalities) (Maren, 2001). Because sensory areas provide input to such higher-order processing stations, the benefits of strengthening our understanding of auditory plasticity are self-evident.

The studies that have focused on plasticity in auditory nuclei have revealed a tremendous capacity for experience-dependent plasticity, particularly in the auditory cortex (AC). Galambos *et al.* (1956) provided the first evidence that neural responses in the AC could be changed by experience when they demonstrated that sound-shock pairing leads to potentiation of the sound-evoked electroencephalogram recorded over the AC. Subsequent work showed this effect to be

persistent and highly replicable: lasting potentiation of the neural response to a newly salient sound has been observed using functional imaging (Morris et al., 1998; Thiel et al., 2002), local field potentials (Galvan and Weinberger, 2002), multi-unit recordings (Bakin and Weinberger, 1990; Kisley and Gerstein, 2001), and single unit recordings (Weinberger et al., 1984; Diamond and Weinberger, 1986; Froemke et al., 2007).

One of the best-documented manifestations of such experience-dependent plasticity appears in the spatial organization of response properties in the AC, which can change to reflect the relative importance of acoustic features that are associated with behaviorally relevant stimuli (Recanzone et al., 1993; Rutkowski and Weinberger, 2005; Polley et al., 2006; Bieszczad and Weinberger, 2010a, b, c). Because the time course of this "map plasticity," as well as the scenarios that elicit it, are similar to those associated with behavioral long-term memories, it has been suggested that map plasticity is a neural correlate of auditory memory (Weinberger, 2004, 2007). However, a number of knowledge gaps and unresolved conflicts cloud the relationship between map plasticity and long-term auditory memories. To resolve the role of auditory map plasticity in memory, it is critical that we develop a more thorough understanding of how the former arises.

This dissertation constitutes an effort to further our understanding of the circumstances under which map plasticity occurs. I first focus on the physiological circumstances that permit plasticity in the developing animal. Recent work has implicated the neuromodulator norepinephrine (NE) in the control of AC plasticity in the adult animal, but because map plasticity tends to be studied separately in adults and neonates, it is not known whether this agent is involved in establishing AC organization during development. I attempt to answer this question in the studies described in chapter 2. Subsequent chapters focus on the contextual circumstances that permit map plasticity in the adult. Virtually all of what we know about map plasticity in the adult was gleaned from studies in which sounds were made relevant to the animal via laboratory training or conditioning paradigms – pairing a tone with foot shock, for instance. Whether map

plasticity results when a sound becomes relevant through natural experience is unknown. In chapters 3 and 4, I search for evidence of map plasticity during motherhood, a natural experience that involves sensitization to new auditory cues.

To provide relevant background, the sections below briefly outline our current understanding of the causes, phenomenology, and consequences of map plasticity. Section 1.1 provides an overview of the pertinent aspects of AC organization, and section 1.2 describes how this organization can be altered by experience, resulting in map plasticity. Section 1.3 concludes with a discussion of the neuromodulatory influences on map plasticity.

1.1 The anatomy of the AC

Although experience-dependent plasticity can be observed in other auditory nuclei, neural activity in the AC stands out as being particularly prone to change with experience. This malleability is likely due to its unique position in the auditory system. Situated at the end of the canonical auditory pathway (Winer, 1992; Malmierca and Hackett, 2010), it occupies a station that can neither be considered wholly "sensory" nor "cognitive" (Naatanen et al., 2001). Feed-forward activity is primarily provided via thalamocortical afferents, though the AC also receives projections from a variety of other cortical, limbic, and modulatory regions; these inputs may provide the contextual information that permits the AC to modulate stimulus representations according to their behavioral relevance. Below, I provide a broad overview of AC anatomy, referencing classic research that was carried out primarily in rats, cats, and primates. I then focus specifically on the mouse AC, which is gaining popularity as a model system, and is employed in the studies described here.

1.1.1 Intrinsic organization of the AC

The cytoarchitecture of the AC features the six-layered structure common to neocortical areas (for review, see Winer, 1992; Winer et al., 2005). Feed-forward input arrives in lower layer III and layer IV primarily from the medial geniculate body (MGB) of the thalamus (Smith and Populin, 2001; Cruikshank et al., 2002). Excitatory thalamorecipient neurons then relay the acoustic signal upward to cells in layers II and III (Matsubara and Phillips, 1988; Barbour and Callaway, 2008), where it is then transmitted to other cortical areas, including ipsilateral and contralateral auditory cortices, multisensory association areas, and higher-order processing centers (Code and Winer, 1985; Budinger and Scheich, 2009). Layers V and VI receive feedback from the upper layers, as well as from many of the same cortical territories to which the upper layers project, and transmit their output back to the auditory thalamus and other subcortical centers (Kelly and Wong, 1981; Games and Winer, 1988; Winer, 2005). Layer I is composed largely of neuropil, in which the dendritic arbors of more deeply situated neurons mingle with long-reaching axons from cortical and neuromodulatory centers, as well as a sparse distribution of inhibitory cells (Winer and Larue, 1989).

The vertical flow of acoustic information through the cortical layers leads neurons situated in the same "cortical column" to share common response properties (Mountcastle, 1997; Jones, 2000). For example, a neuron in layer II/III of AC will likely respond to a frequency range similar to that of a subjacent neuron in layer IV, by virtue of the more superficial neuron's "inheritance" of the layer IV neuron's pattern of thalamically-determined excitation. Intracortical inputs in fact make up most of the synaptic connections present in the AC (Lee and Winer, 2008b; Budinger and Scheich, 2009), although the function of many intracortical transformations is not well understood (Linden and Schreiner, 2003; Horton and Adams, 2005). Approximately 20% of intracortical connections are inhibitory, and these tend to be more local than excitatory projections, as they typically terminate in a column immediately adjacent to, or within, their column of origin (Katzel et al., 2011). Across sensory modalities, lateral inhibition of neighboring

cortical columns is thought to be critical for accurate stimulus perception (Blakemore and Tobin, 1972; Ma and Suga, 2004; Helmstaedter et al., 2009).

Although neural response properties tend to be homogenous within cortical columns, they vary across the cortex in ways that are predictable from a neuron's spatial position. Such topographic organization arises from the stereotyped innervation patterns of feed-forward afferents; the tuning of an AC neuron generally reflects the consensus of the thalamic input it receives. This "inheritance" of thalamic response properties is most evident in the frequency domain. Neurons in the ventral division of the MGB fire most strongly to a particular frequency (known as the best frequency or "BF"), across many sound pressure levels, and BFs are spatially distributed in a smooth gradient across this region. This organizational attribute is preserved in the AC, where neurons inherit their BFs from their thalamic inputs (Hackett et al., 2011). The orderly, topographic distribution of BFs across the surface of particular AC subregions (see section 1.1.2) is known as tonotopy, and can be sampled electrophysiologically by recording responses to a set of pure tones spanning an animal's hearing range (Merzenich et al., 1975; Reale and Imig, 1980; Sally and Kelly, 1988). Overlapping maps of auditory response properties like bandwidth (Schreiner and Mendelson, 1990; Read et al., 2001) and binaurality (Imig and Adrian, 1977; Middlebrooks et al., 1980), and of tuning for other acoustic properties, including rate of frequency modulation (Mendelson et al., 1993) and intensity (Schreiner et al., 1992; Polley et al., 2007), have been described as well. Because the tonotopic map is the most robustly characterized, however, it will serve as the primary focus here.

1.1.2 Core versus non-core AC

Tonotopy is not uniformly present across the AC, but is rather considered to be an attribute of most "core" auditory cortical subregions. Across species, AC response properties and

connectivity have been found to vary systematically in such a way that motivates the definition of distinct subregions or fields. Although the subregional composition of AC varies between species, often in a way that reflects ethological demands, there is a general division between "primary" or "core" and other auditory subregions across mammalian orders. Core areas receive direct, feed-forward thalamic input from the ventral division of the MGB (Roger and Arnault, 1989; Romanski and LeDoux, 1993; Kimura et al., 2003), and their neurons tend to have short onset response latencies and clear frequency tuning. These regions are often tonotopically organized.

Non-core auditory regions have received less attention from auditory researchers than have core fields. Consequently, less is known about these areas, although they have been fairly well characterized in the primate. In the primate auditory pathway, a signal ascends from core to so-called "belt" and then "parabelt" regions. Over the course of this progression, neurons come to prefer increasingly complex stimuli (e.g., noise bursts, frequency-modulated sweeps, or vocalizations instead of pure tones; (Rauschecker et al., 1995; Wessinger et al., 2001; Tian and Rauschecker, 2004). In specific auditory belt regions, the processing stream begins to be segregated into separate pathways specialized for auditory objects and their spatial positions (Romanski et al., 1999; Recanzone et al., 2000; Tian et al., 2001). These pathways are proposed to be akin to the ventral "what" and dorsal "where" streams of visual cortex (Kaas and Hackett, 1999; Rauschecker and Tian, 2000), although the extent to which the existing experimental data support this comparison remains a subject of debate (Recanzone and Cohen, 2010).

Although belt and parabelt regions per se have not been characterized in other species, it is accepted that functionally and anatomically distinct, higher-order AC regions exist in other mammalian species (Eggermont and Kenmochi, 1998; Cruikshank et al., 2001; Polley et al., 2007; Lee and Sherman, 2008; Covic and Sherman, 2011); they are usually referred to as "non-core," "nonprimary," or "secondary" fields. Regardless of nomenclature, these regions receive feed-forward input from core fields in addition to modulatory input from the dorsal and medial

divisions of the MGB (Arnault and Roger, 1990; Lee and Winer, 2008a; Llano and Sherman, 2008), and are usually not tonotopic. Neurons in non-core fields tend to have complex tuning properties, and often respond more robustly to noise or frequency-modulated stimuli than to pure tones (Rutkowski et al., 2003).

1.1.3 The mouse AC

The core/non-core organization of the mammalian AC is exemplified in the mouse; because this model system is employed in the studies described in this dissertation, I briefly highlight its organization here (Figure 1.1), referring to the seminal characterization of mouse AC by Stiebler et al. (1997) unless otherwise noted. Like all mammals, the mouse has a primary auditory field (A1) that is tonotopically organized, with a high- to low-frequency BF gradient running rostrocaudally. Rostral to A1 is a second core region, the anterior auditory field (AAF), which features a tonotopic gradient that runs in the opposite direction as that of A1; a band of sites with high BFs can be observed where these two fields meet (Hackett et al., 2011). In A1 and AAF, BFs typically do not exceed 40 kHz. BFs between 40 and approximately 80 kHz can be found in a third core region, the ultrasound field (UF). Unlike A1 and AAF, UF is non-tonotopic, but it is classified as a core region because its anatomical connectivity to the ventral division of the medial geniculate resembles that of the other core regions (Stiebler, 1987; Hofstetter and Ehret, 1992). Additionally, the mouse AC includes two non-core regions, the dorsoposterior field (DP) and the secondary auditory field (A2), which are situated dorsocaudally and ventrally, respectively (Guo et al., 2012). Neurons in these subregions exhibit the broad frequency tuning and preferential responses to complex stimuli over pure tones that are typical of non-core fields.

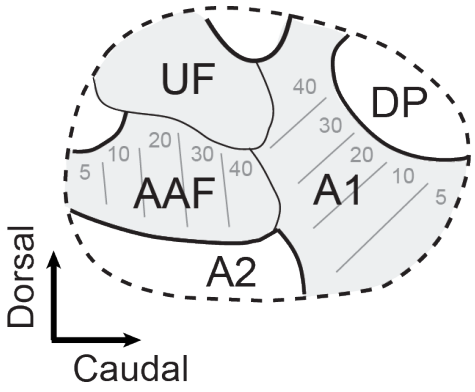

Figure 1.1 Schematic of mouse AC, adapted from Stiebler *et al*. (1997). Core areas AAF, A1, and UF are shaded gray. Lines/labels indicate approximate pattern and frequency ranges of tonotopy.

Although it is accepted that tonotopy is a common feature of many core auditory fields across mammals, the degree of tonotopy in the mouse AC has been a topic of debate. Early studies in which multi-unit electrophysiological responses to pure tones were recorded across a portion of the mouse AC with a resolution of 200-300 μm revealed coarse tonotopy (Willott et al., 1993; Stiebler et al., 1997). Subsequent studies, mapping the entire AC using finer sampling resolutions (25-50 μm, still multi-unit), confirmed the presence of tonotopy in core fields AAF and A1 (Hackett et al., 2011; Guo et al., 2012). Further, by making *in vitro* recordings from AC in slices that preserved thalamocortical connectivity, Hackett *et al.* (2011) were able to determine that tonotopy in A1 is determined by the pattern of projection of tonotopically organized neurons in the ventral division of the medial geniculate body. The advent of functional imaging technologies with single-neuron resolution (e.g., two-photon Ca^{2+} imaging), however, enabled the mapping of frequency responses on still finer scales. With one exception (Issa et al., 2014), these methodologies revealed a tonotopy that was less uniform than had been previously reported: neighboring neurons (i.e., so close that they may belong to the same cortical column) could have BFs that differed by >1 octave (Bandyopadhyay et al., 2010; Rothschild et al., 2010). Because the mouse A1 generally represents 5-6 octaves across its ~1mm extent, this finding suggests a more heterogeneous tonotopy than earlier work had indicated.

The picture painted when these studies are considered together – coarse tonotopy that erodes at finer scales – is believed to reflect the true nature of tonotopy in core fields of the mouse AC. This model of tonotopy suggests that a neuron's BF is heavily influenced by the tuning of thalamocortical afferents but modulated by other factors on a neuron-to-neuron basis (for example, membership to different functional networks within the cortex) to an extent that can de-correlate a cortical BF from that of its thalamic projections (Kanold et al., 2014). Experiment-to-experiment differences in the degree of tonotopy found are believed to stem from differences in recording technique (with coarser sampling more likely to reveal tonotopy), depth of recording (with recordings from thalamorecipient layers more likely to reveal tonotopy), or anesthetic state (with anesthetic depth and agent determining degree of tonotopy). Importantly, this apparent breakdown of map organization in rodent sensory cortex is not unique to AC – in visual cortex, the organized map of orientation tuning found in primates and carnivores was absent in the rat (Ohki et al., 2005) – and may be a byproduct of the small size and rapid development of the rodent brain relative to that of larger mammals. Here, we employ the mouse model despite its poor tonotopy at fine scales, because this species is well suited to genetic manipulation and has been used with success to study experience-dependent plasticity, as detailed below.

1.2 Experience-dependent map plasticity in AC

The pattern of evoked activity across the core AC is dictated to a large extent by the tonotopic distribution of BFs. Nevertheless, exactly how cortical neurons respond to specific sounds depends on more than just sound frequency, and those responses can in fact be remodeled by behaviorally relevant acoustic experiences on a dynamic basis. This remodeling has traditionally been studied at either the neuronal single unit or population level. For example, evoked firing rates in single units are typically potentiated in an enduring, frequency-specific manner after presentation of that frequency is paired with a high-valence stimulus (e.g., foot shock, as in a fear conditioning paradigm, or reward, as in an operant training paradigm) (Bakin

and Weinberger, 1990; Ohl and Scheich, 1996; Gao and Suga, 2000). In some cases, the response to the conditioned frequency comes to exceed the response to the original BF of the single unit, such that the conditioned frequency actually becomes the new BF. When such plasticity occurs, a single unit is said to have been "retuned". At the population level, training in a tone discrimination task can result in a long-lasting increase in the proportion of units that are tuned to the behaviorally relevant frequency range (Recanzone et al., 1993). When analyzed spatially across the surface of an auditory cortical field, this type of plasticity is referred to as tonotopic map expansion. One can intuitively see how these manifestations are related to one another: the combined retuning of a *population* of single units produces map expansion. However, it is important to note that individual single units could undergo tuning curve changes without generating large-scale map plasticity. For example, individual single units in a local population may not all retune to the same final BF; in this case, map plasticity would be absent. Even within a single unit, the response to a salient frequency could be potentiated without "retuning" the neuron's BF, much less changing the BF map. Thus, although map plasticity implies single unit plasticity, single unit plasticity does not necessarily imply map plasticity. When map plasticity is observed, however, it confirms recruitment of a larger population of neurons by a behaviorally relevant stimulus feature.

1.2.1 Map plasticity and auditory memory

Because map plasticity can occur in an associative context, where the events a sound signifies becomes associated with the features of the sound itself, it has been postulated that map expansion may provide a physiological trace for an auditory memory of a relevant frequency (Weinberger, 2004). This hypothesis was initially informed by the observation that the long-term map plasticity that can follow an arousing acoustic experience shares many qualities with long-term memory. First, the plasticity that arises is always frequency-specific: map expansion occurs

only for frequencies at or near the reinforced stimulus. Second, the single unit retuning events that ultimately lead to map plasticity can be induced rapidly. A hallmark of long-term memories is that they can be produced after a single salient experience (McGaugh, 1966), and indeed, the receptive fields of single neurons may shift following five or fewer tone-shock pairings (Edeline et al., 1993). Additionally, AC plasticity can, under some conditions, last indefinitely, as long-term memories do. Though experimental restraints limit the time period over which plasticity can be studied, one study found that frequency tuning shifts observed within a cortical column could endure for up to eight weeks (Weinberger et al., 1993). Finally, some evidence has suggested that receptive field shifts in AC during behavior result from Hebbian synaptic plasticity (Ahissar et al., 1998), the phenomenon that is believed to underlie long-term memory in a number of systems.

While the aforementioned studies establish a number of similarities between AC plasticity and memory, they do not rule out the possibility that map plasticity is an epiphenomenon that accompanies, but is not actually involved in, memory formation. Given that certain attributes of map reorganization correlate with aspects of behavioral memory, however, it seems likely that map plasticity is somehow involved in learning or memory formation. For example, the magnitude of map expansion following operant training with a particular frequency is correlated with the resistance to extinguish the behavioral response to that frequency, indicating that map expansion may directly relate to memory strength (Bieszczad and Weinberger, 2010c). Further, the involvement of the AC in auditory learning is supported by studies showing that the learning strategy an animal uses in an auditory discrimination task determines the resulting degree of map reorganization (Bieszczad and Weinberger, 2010b, a). These examples of co-variation between map plasticity and auditory learning and memory support the view that AC map plasticity and learning are more than parallel phenomena.

Still more convincingly, absolute blockade of plasticity in AC, either through injection of a protein synthesis inhibitor (Kraus et al., 2002) or antagonism of *Arc/Arg3.1*, a key plasticity-related gene (Carpenter-Hyland et al., 2010), prevents animals from attaining typical levels of performance on an auditory learning task. This finding suggests that plasticity in the AC is in fact necessary for auditory learning. Conversely, there is evidence that inducing map plasticity in the absence of behavioral experience can improve performance during learning of a tone discrimination task (Reed et al., 2011). The mechanism by which map plasticity facilitates learning is not yet understood, but one possibility is that when a larger cortical territory responds to a relevant frequency, it more strongly activates downstream areas involved in decision making or motor behavior, ultimately producing the reinforced behavior. Indeed, optogenetic stimulation of corticostriatal neurons in the AC biases decisions toward the reinforced frequency, provided stimulation is taking place at tonotopic positions associated with the reinforced frequency (Znamenskiy and Zador, 2013). Presumably, recruitment of more excitatory neurons via map expansion would produce a similar effect (e.g., a larger volley of activity in downstream sites), assuming map plasticity recruits corticostriatal neurons. Taken together, these studies indicate that AC map plasticity has behavioral consequences, and may be centrally involved in auditory learning.

Nevertheless, questions about the precise role of AC map plasticity in learning and memory linger. First, the finding that performance in auditory learning tasks can improve without apparent map plasticity seems to counter earlier work suggesting an involvement of AC map plasticity in auditory learning. Brown and colleagues (2004) trained cats for 9 to 12 months on a frequency discrimination task and, after mapping BFs across their ACs, found no evidence of map expansion in the behaviorally relevant frequency range. This negative result stands in stark contrast to prior findings from Weinberger *et al.* (1993), who showed that retuning of AC neurons following behavioral conditioning was still apparent up to 8 weeks after the initial training

experience. Reed *et al.* (2011) provided a possible explanation for this disparity: they showed that induction of tonotopic map plasticity helped rats learn a frequency discrimination task, but that this plasticity did not persist with continued training. When animals continued to train after having reached a plateau in behavioral performance, AC organization reverted to its pre-training state. This finding supports map plasticity as a stage of memory formation, rather than the final manifestation of a long-term auditory memory, and offers a potential explanation for why map plasticity was not observed by Brown *et al.* (2004): with 9 to 12 months of training, their auditory task was likely well-learned, and map plasticity may have dissipated by the time of experimentation. Taken together, these results appear to indicate that map expansion is not necessary for the expression of auditory memory, even while it may be involved in learning or memory formation.

A second unresolved question regarding map plasticity concerns its role in "real-life" learning scenarios. In natural acoustic environments, hundreds or thousands of sounds, many of which vary across multiple acoustic dimensions simultaneously, may hold particular relevance to an individual. If expanding the representation of salient stimulus features across the cortical surface were required for auditory memory formation or storage, the finite dimensions of the AC would presumably become overtaxed by the formation of a mere few auditory memories. Current methods of studying map plasticity have precluded exploration of this apparent conflict: In most studies, only one stimulus at a time is rendered behaviorally relevant. Further, the reinforcers that imbue these stimuli with relevance, and the schedules on which reinforcement occurs, differ from the circumstances under which real-life auditory learning occurs (Bennur et al., 2013). This incongruity motivates the use of more naturalistic models of auditory learning to further elucidate the relationship between map plasticity and auditory memory.

A third and final unresolved question rests on map plasticity in non-core AC fields. All the studies discussed above concern plasticity occurring in core subregions, principally A1. The

wealth of evidence for plasticity in core regions does not imply a dearth of plasticity in non-core regions – if anything, the higher-order nature of non-core fields suggests that they may be even more susceptible to experience-dependent plasticity than core regions. Limited experimental evidence seems to confirm this hypothesis (Diamond and Weinberger, 1984; Weinberger et al., 1984; Polley et al., 2006; Puckett et al., 2007), although in general non-core regions have received less attention from researchers than have core regions. This lack of attention to non-core regions is perhaps because plasticity there manifests in ways that are more difficult to index than map expansion, or because a lack of agreement on the homologs of non-core areas and their spatial positioning between species limits the perceived generalizability of any findings. Nevertheless, understanding how non-core regions are involved in experience-dependent plasticity will likely be critical to understanding how the auditory system achieves higher-order tasks such as categorization or object recognition, as it is clear that the participation of core fields in these functions is limited.

1.2.2 Map plasticity during the developmental critical period in AC

The aforementioned studies make a strong case that AC map plasticity is a product of learning following active engagement with sounds (e.g., learning an auditory discrimination task). Under certain circumstances, however, map plasticity can also occur following mere exposure to sounds, in the absence of behavioral reinforcement. This exposure-induced plasticity has been studied at the greatest depth in the developing animal, during the auditory cortical critical period.

Across systems, developmental critical periods serve to sensitize the brain to the statistically common features of the sensory environment. Although thalamic afferents and intracortical projections innervate the mammalian sensory cortex early in life (Rubel, 1978), it is believed that during a "critical period," enhanced impressionability of responses to sensory stimulation allows the animal to customize its neural connectivity according to the type of

environment in which it finds itself (Knudsen, 2004). The seminal example of critical period plasticity was characterized in visual cortex by Hubel and Wiesel in the 1960s: they found that the visual cortices of kittens reared with one eye occluded became insensitive to stimulation of that eye upon its uncovering, as long as the occlusion occurred during a circumscribed critical period (Hubel and Wiesel, 1970). Importantly, cortical blindness to stimulation of the occluded eye lasted into adulthood, and was not apparent when monocular occlusion occurred outside the sensitive period. In other words, during a period of heightened sensitivity to the environment, cortical processing space was diverted from inputs that carried no information and reallocated to richer sources of visual input. This mechanism provides a way for an individual to adapt to the environment it is born into on a much faster timescale than evolutionary mechanisms would allow.

Analogous to the classic studies in visual cortex that exploited its ocular dominance organization to uncover plasticity from monocular deprivation, many studies in AC have taken advantage of its tonotopy and used long-term sound exposure to drive map expansion in developing rodents. By rearing rat pups and their mothers in the presence of pulsed tones of a particular frequency at a moderate intensity (60-70 dB SPL) for 10 to 16 hours per day, Zhang *et al.* (2001) demonstrated that the experience was reflected in the cortices of adult animals who had received stimulation as pups. Specifically, the amount of A1 dedicated to processing the frequency of exposure was approximately double what it was in naïve animals. This doubling only occurred in the animals that had been exposed as pups; importantly, the mothers of exposed pups, which had received the stimulation in adulthood, did not show evidence of map expansion. Later work replicated these findings and delineated specific critical periods for tone exposure-induced map plasticity in both rats and mice as 3-4 day periods beginning around the onset of hearing (de Villers-Sidani et al., 2007; Han et al., 2007; Barkat et al., 2011).

While the critical period may be defined in terms of an animal's age, it is not age but rather experience that determines how maturation of auditory cortical responses occurs. In particular, it appears that patterned inputs are required for acquisition of normal auditory cortical responses during the critical period. Removing patterned input by exposing animals to a continuous, unmodulated tone rather than pulsed tones delays fails to produce frequency-specific map plasticity (Zhou et al., 2008). Further, this intervention actually delays closure of the auditory sensitive period, as does exposure to continuous white noise, which lacks both spectral and temporal structure (Chang and Merzenich, 2003). That exposure to continuous stimuli delays critical period closure is evident when the cortical organization of a tone-exposed pup is compared to that of an animal reared in continuous noise into early adulthood, *then* moved to an environment in which they were exposed to pure tones. The frequency of exposure is overrepresented in the ACs of both groups, suggesting that though the noise-exposed animals were mature at the time of tone exposure, their ACs were as plastic as those of pups. Additional support for the idea that temporal regularity is a key determinant of critical period timing can be found when animals are reared in the presence of white noise pulses – this manipulation does not alter the time course of critical period plasticity (Zhang et al., 2002; Zhou and Merzenich, 2008), even while it does alter tuning properties of AC neurons. Thus, temporal patterning appears to be critical for driving frequency-specific plasticity during the critical period, as well as regulating its closure.

Dorrn *et al.* (2010) have begun to explore the cellular basis of the requirement for temporal patterning. Using *in vivo* intracellular electrophysiology, they first established that a key difference between the adult and developing ACs concerns the balance of excitatory and inhibitory tuning. Specifically, while the amplitude and specificity of excitatory and inhibitory frequency tuning are closely matched in adulthood, inhibitory tuning is markedly less frequency-specific in developing animals. Hypothesizing that patterned input drives the inhibitory-excitatory

matching that characterizes the mature AC, the authors measured inhibitory and excitatory tuning curves before and after presenting a bout of pulsed tones. Indeed, patterned stimulation strengthened the correlation between inhibitory and excitatory conductances, inching the profile of cortical activity closer to that of the mature animal. This work suggests that progress through the critical period is not simply a result of aging, but rather the incremental fulfillment of a "quota" of exposure to temporally patterned input.

1.2.3 Developmental versus adulthood map plasticity

The persistent absence of plasticity in adults following the same interventions that produce plasticity in neonates initially led researchers to conclude that map plasticity cannot arise in adults following passive exposure to sounds. This hypothesis has been contested, however; a series of studies have shown that long-term, passive exposure to tones spanning a range of frequencies induces robust plasticity in the adult cat AC (Norena et al., 2006; Pienkowski and Eggermont, 2009, 2010). Given that this plasticity is observed after >12 hours of stimulation per day, for 6 to 12 consecutive weeks, one might assume that the duration of passive exposure required to produce plasticity is simply greater for adults. However, the observation that this plasticity actually manifests as a suppression, rather than a potentiation, of the core AC response to the frequencies of exposure suggests that the mechanisms guiding exposure-induced plasticity in adults may differ from those at play in the developing brain.

Indeed, map plasticity following active engagement of sounds in adulthood actually bears a stronger resemblance to exposure-induced developmental plasticity than does exposure-induced plasticity in adulthood: both manipulations lead to an increase in the proportionate area of AC tuned to the relevant frequency band. This similarity has led to the hypothesis that the same proximate neural mechanisms generate AC plasticity, but that these mechanisms are differently engaged in the developing versus the adult brain (Shepard et al., 2012). Specifically, there is a

general belief that the threshold for inducing map plasticity is simply higher in the adult, and as a result plasticity is only induced when other systems (e.g., attentional, motivational) are recruited during auditory experience (Seitz and Dinse, 2007). The relationship between activation of these systems and AC plasticity is likely mediated by neuromodulators, a variety of which are released widely throughout sensory cortex (Gu, 2002). While parallel studies of plasticity in developing and adult animals are extremely rare, what experimental evidence exists tentatively supports this hypothesis. Chun *et al.* (2013) showed that long-term potentiation (LTP) can be induced in thalamocortical synapses in AC slices taken from neonates and adults alike, but that LTP induction in adults, but not neonates, requires local acetylcholine (ACh) release. This parallel suggests that map plasticity in adulthood and development may originate from common mechanisms, and that ACh could serve as a gating mechanism for plasticity in the adult AC (Blundon and Zakharenko, 2013).

Given the resemblance between developmental exposure-induced plasticity and adulthood plasticity following associative learning, in terms of both phenomenology and potential mechanism, it is perhaps surprising that the behavioral outcomes of these processes do not match. Though few studies have described the behavioral consequences of map expansion, the data that do exist indicate that when map plasticity is induced in adulthood, auditory discrimination for the target frequency is improved (Reed et al., 2011), whereas when plasticity is induced via exposure in development, discrimination at the target frequency worsens (Han et al., 2007). Taken together, these results seem to suggest that while the resulting map plasticity is similar between these groups, as-yet hidden neural differences exist that bias perception and/or behavior in divergent ways. The overall dearth of studies comparing map plasticity between development and adulthood, however, motivates a careful re-examination of this apparent discrepancy. Particularly because these studies were executed in different laboratories, it could be that subtle differences in experimental procedure led to the observed differences.

Indeed, common experimental practices have precluded a clear understanding of how

experience-dependent plasticity in development and in adulthood relate to one another. Few studies directly compare the AC maps of animals that have experienced the same acoustic manipulation during either development or adulthood. This gap is partially owing to the challenges that accompany behavioral conditioning of developing animals. Though it is more straightforward to perform a sound exposure manipulation on both adult and developing animals, this is rarely done. A cohesive understanding of the mechanisms of map plasticity, and of its perceptual consequences, will only be achieved once the segregated bodies of work based on developing and adult animals are united.

1.3 Neuromodulation of map plasticity

A key to creating a unified understanding of map plasticity -- one that spans development and adulthood -- involves understanding the contributions of neuromodulators. Neuromodulatory tone varies with age, and could explain why plasticity is more easily achieved in development than in adulthood (Herlenius and Lagercrantz, 2004; Li et al., 2006). It also varies on shorter timescales, changing with arousal, mood, attention, and motivation (Lee and Dan, 2012). These short-term fluctuations may, among other things, permit plasticity under behavioral circumstances where memory formation would be advantageous (Gu, 2002). Given the likely connection between AC map plasticity and memory, it is perhaps not surprising, then, that the AC is richly interconnected with these systems, and that they have been linked to experience-dependent AC plasticity to various degrees.

1.3.1 Acetylcholine

ACh is, by far, the most extensively studied neuromodulator with respect to map plasticity. Release of ACh into AC appears to be the proximate mechanism enabling learning-

induced plasticity, as cortical blockade of cholinergic muscarinic receptors prevents retuning under circumstances that otherwise permit it (Bakin and Weinberger, 1996; Ji et al., 2001; Miasnikov et al., 2001; Ji and Suga, 2003; Ji et al., 2005). In addition to being necessary for expression of auditory cortical plasticity, release of ACh into the AC is sufficient for induction of map plasticity when paired with tone presentation: tonotopic map expansion can be induced by pairing tone presentation with stimulation of the nucleus basalis, which provides the forebrain with ACh (Boutelle et al., 1990; Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998; Dimyan and Weinberger, 1999). Recently, technological advances have enabled a more detailed understanding of how ACh enables the retuning events associated with map plasticity: ACh release, triggered by salient events such as footshock, activates layer I inhibitory neurons in the AC. This, in turn, disinhibits layer II/III pyramidal neurons by inhibiting local inhibitory interneurons, and allows for the excitatory events that lead to long-term potentiation and, ultimately, map plasticity (Letzkus et al., 2011; Chun et al., 2013). Indeed, the link between ACh and AC plasticity is so strong, it is often referred to as the final common pathway for induction of plasticity, and it has been exploited to enable study of plasticity-related phenomena (Kilgard et al., 2001b; Miasnikov et al., 2011).

The idea that cholinergic signaling facilitates the formation of auditory associations is consistent with broad hypotheses about the role of ACh in learning and memory. Nicotine, an agonist of the nicotinic ACh receptor, has long been known to enhance learning in adults (Garg, 1969; Mangan, 1983). Antagonism of muscarinic ACh receptors also disrupts learning and memory processes (Hagan et al., 1987; Taffe et al., 2002). It has been proposed that these behavioral effects are mediated by the action of ACh on local cortical networks, specifically via the suppression of activity in intracortical neurons without altering input from ascending afferents (Hasselmo et al., 1992; Hasselmo and Bower, 1992). The result is enhanced receptivity to afferent stimulation without interference from ongoing intracortical firing patterns.

1.3.2 Dopamine

Because of its close involvement in reward signaling, a number of studies have explored dopaminergic influences on auditory cortical plasticity. Stimulation of the dopaminergic ventral tegmental area (VTA) is known to produce a pleasurable feeling in animals, to the extent that it can serve as a reward in operant learning paradigms. Auditory system researchers have taken advantage of this, yoking a particular behavioral contingency to VTA stimulation in some auditory learning tasks (Hui et al., 2009). Indeed, in animals that learned the association between the conditioned stimulus and the VTA stimulation reward, an increase in cortical area devoted to the reinforced frequency occurred. Others have shown that simply pairing VTA stimulation with tone presentation, without a behavioral contingency, also results in retuning in primary and secondary auditory cortices (Bao et al., 2001; Kisley and Gerstein, 2001). Since the VTA is a heterogeneous nucleus consisting of ~50% dopaminergic cells as well as some GABAergic and glutamatergic neurons (Steffensen et al., 1998; Margolis et al., 2006; Yamaguchi et al., 2007), additional work is needed to clarify whether dopaminergic signaling is specifically involved in subsequent plasticity, and whether VTA activation is a direct contributor to AC retuning.

1.3.3 Serotonin

Partly because of a lack of attention from researchers, the role of serotonin (5HT) in plasticity has been hard to define in the AC. There, 5HT appears to enable behaviorally-induced tuning shifts that would otherwise be subthreshold at low doses (D'Amato et al., 1987). High doses of 5HT, on the other hand, impeded the development of plasticity. These somewhat contradictory effects are presumably mediated by differential effects on the various 5HT receptors. Indeed, ritanserin, a 5HT-2A receptor antagonist, always facilitated tuning curve shifts when applied to the AC in the same study, suggesting activation of those receptors ordinarily opposes plasticity induction. In addition to its direct action on the AC, 5HT may also facilitate plasticity in earlier auditory processing centers, which would impact subsequent AC responses. In

the inferior colliculus, which is positioned two stations upstream of the AC in the auditory pathway, 5HT exerts variable functions on neural response properties depending on which receptor populations it activates (Hurley and Pollak, 2001; Ramsey et al., 2010). Through feedforward connections, 5HT -modulated plasticity in lower areas may impact AC activity, or potentially even impact the likelihood of plasticity in cortical areas by biasing of feedforward responses.

1.3.4 Norepinephrine

NE release from the locus ceruleus is widely understood to occur following experience with an arousing or salient stimulus (Foote et al., 1980; Aston-Jones et al., 1991; Sara, 2009). Since the locus ceruleus releases NE widely throughout the forebrain, including the AC (Harper and Wallace, 1995), as well as regions involved in associative memory and plasticity that project to the AC, such as the cholinergic nucleus basalis (Haring and Wang, 1986; Espana and Berridge, 2006) and the basolateral amygdala (Radwanska et al., 2010), NE is well positioned to trigger AC plasticity following a salient acoustic experience. Indeed, pairing tones with pulsed microinfusion of NE leads to enduring changes in frequency tuning in auditory cortical neurons (Manunta and Edeline, 2004). Follow-up work replacing exogenous NE infusion with stimulation of the locus ceruleus led to mixed results: in one lab's hands, this pairing consistently led to frequency-specific enhancements of the AC response (Martins and Froemke, 2013), whereas others found that frequency-specific enhancement occurred about as often as frequency-specific suppression (Edeline et al., 2010). Interestingly, the former study also found that after pairing, frequency-specific responses also appeared in the locus ceruleus itself, and that these changes could be observed before retuning emerged in the AC. This study raises the intriguing possibility that not just activity, but *plasticity* in neuromodulatory centers may influence plasticity in sensory cortex.

1.3.5 Unanswered questions regarding the neuromodulation of AC plasticity

Taken together, this work supports the hypothesis that experience-dependent cortical map plasticity is at least partially under the control of neuromodulators. In the AC, this is particularly true of the cholinergic system, although the contributions of other neuromodulators have not been clearly defined. It is known, for instance, that ACh, NE, dopamine (DA), and 5HT are all able to facilitate retuning in the AC; it is less clear what, if anything, differentiates plasticity induced by disparate neuromodulators. It may be that these agents act on a common substrate and truly do not differ in terms of the plasticity they effect, but are simply released under different circumstances. Alternatively, they may act on different substrates to enact plasticity (for instance, facilitating retuning by acting on intracortical versus thalamocortical synapses), but the resolution of current techniques does not permit experimenters to make such a distinction. Additional research will be required to address these possibilities. Another question involves the combined effects of multiple neuromodulators: given that many "real world" contexts likely activate a number of neuromodulatory systems simultaneously (Carrasco and Van de Kar, 2003), how do their interactions impact the induction of plasticity? To achieve a holistic understanding of neuromodulator-dependent plasticity, the study of neuromodulators in isolation should eventually give way to studies of how they operate in concert.

1.4: Summary and objectives

In this dissertation I will attempt to answer a subset of the questions raised above, focusing on the circumstances that are conducive to the emergence of map plasticity and treating developmental and adulthood plasticity separately. I begin in chapter 2 by seeking support for the hypothesis that differences in neuromodulatory tone across the lifespan may inform changes in the threshold for plasticity that occur with age. Specifically, I ask whether NE, which is associated with AC plasticity in the adult animal (see section 1.3.4), is also involved in regulation of plasticity during the critical period for frequency tuning in the AC. In chapters 3 and 4 I turn to

adulthood, supplementing what has been gleaned about AC plasticity from laboratory conditioning studies with a characterization of the consequences of natural maternal experience with pup vocal cues. Chapter 3 focuses on changes in frequency tuning throughout motherhood. I find that, in contrast to what was inferred from prior studies, BF map plasticity does not occur with auditory experience in a more ethological paradigm. Though the BF map is apparently stable throughout motherhood, I do observe changes in the *strength* of the response to behaviorally relevant frequencies across the AC such that detection of these cues could be improved in downstream regions. In chapter 4, I analyze responses to pup cues themselves. My findings again show that the spatial map of responses to the behaviorally relevant cue does not change with experience. Instead, I observe an apparent change in sensitivity to acoustic variation in pup cues that manifests specifically in a non-core subregion of AC. Finally, chapter 5 concludes with a discussion of the broader implications of this work, and provides recommendations for follow-up work.

Chapter 2

Norepinephrine and developmental plasticity in the auditory cortical map

As described in section 1.3, the neuromodulatory mechanisms contributing to experience-dependent map plasticity are incompletely understood in AC. This knowledge gap is perhaps more obvious for exposure-induced plasticity in development than it is for adulthood map plasticity that accompanies learning: In the latter case, at least, a strong argument has been made for the involvement of the cholinergic system in regulating plasticity (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998; Letzkus et al., 2011). The work in this chapter was motivated by a desire to identify a physiological determinant of map plasticity in the developing animal. Drawing from the considerable literature on the developing visual cortex, I assess whether NE, which has been linked to the critical period there, is required for experience-dependent plasticity during the auditory cortical critical period.

2.1 Introduction

In section 1.2.2, I described the critical period for tonotopy in core AC: exposure to a biased acoustic environment (e.g., though repeated playback of pure tones) during this time leads to a persistent overrepresentation of the frequency of exposure in the tonotopic map in A1 (de Villers-Sidani et al., 2007; Han et al., 2007; Barkat et al., 2011). That this effect is not observed in adult animals following an identical exposure regimen (Zhang et al., 2001) begs the question of what conditions, present in development but not in adulthood, permit such plasticity.

One hypothesis states that the neuromodulatory environment during development facilitates plasticity, but changes in neuromodulatory tone that accompany maturation raise the threshold for induction of plasticity (Herlenius and Lagercrantz, 2004; Blundon and Zakharenko, 2013). Thus, the time course of neuromodulator availability may determine the course of critical period plasticity. Consistent with this hypothesis, work in the visual cortex in the 1970s and ‘80s

found that ocular dominance column plasticity was inhibited by NE depletion (Kasamatsu and Pettigrew, 1976) and restored with its replacement (Pettigrew and Kasamatsu, 1978; Kasamatsu et al., 1979, 1981), indicating that NE is necessary for ocular dominance column plasticity in that region. However, subsequent work called this finding into question (Bear and Daniels, 1983), reporting instead that only loss of *both* NE and ACh would prevent ocular dominance column plasticity, and that with respect to the critical period in visual cortex, these neuromodulators may be functionally redundant (Bear and Singer, 1986). While this work, taken together, has lent support to the neuromodulatory hypothesis of critical period plasticity, it has painted a muddled picture of the particular role played by NE in this phenomenon.

Though the links between NE and developmental plasticity may be tenuous, recent work implicating NE in experience-dependent plasticity in the adult AC motivates an investigation of NE's involvement in the auditory cortical critical period. Pairing of pure tones with pulsed application of NE into AC leads to lasting shifts in frequency tuning that are not apparent when NE or tones are presented on their own (Manunta and Edeline, 2004). The same effect can be achieved when NE infusion is replaced with stimulation of the locus ceruleus, which supplies the forebrain with NE (Edeline et al., 2010). On top of being sufficient to induce plasticity when paired with tone presentation, NE from the locus ceruleus is apparently required to cement tuning shifts following pairing, as blockade of noradrenergic receptors after pairing renders these changes temporary (Martins and Froemke, 2013). Interestingly, the locus ceruleus is actually more easily excited in development than in adulthood, and its activation is longer lasting, as well (Nakamura et al., 1987), due to an immature pattern of expression of inhibitory autoreceptors, which serve to limit the period of excitation in adults (Nakamura et al., 1988). The heightened sensitivity of the developing locus ceruleus parallels the lower threshold for induction of map plasticity in development, raising the question of whether maturation of the locus ceruleus is directly involved in establishing that threshold.

Given the mounting evidence linking NE to experience-dependent plasticity in the adult AC, I sought here to assess whether it may also be involved in experience-dependent plasticity in the developing AC. I addressed this question using the dopamine β-hydroxylase knockout mouse (*Dbh -/-*), which is NE-deficient from birth onward. I hypothesized that, consistent with its role in adulthood, NE is required for experience-dependent plasticity during the critical period, and that *Dbh -/-* mice, but not their NE-competent littermates, would fail to show evidence of experience-dependent plasticity following a sound exposure manipulation known to drive tonotopic map reorganization in intact animals.

2.2 Method

Subjects

To determine how the presence of NE influenced experience-dependent plasticity during development, I employed a 2x2 experimental design (Figure 2.1) in which groups of NE-competent or NE-deficient animals were either reared under standard colony conditions, or in the presence of a stimulus known to drive plasticity in intact animals.

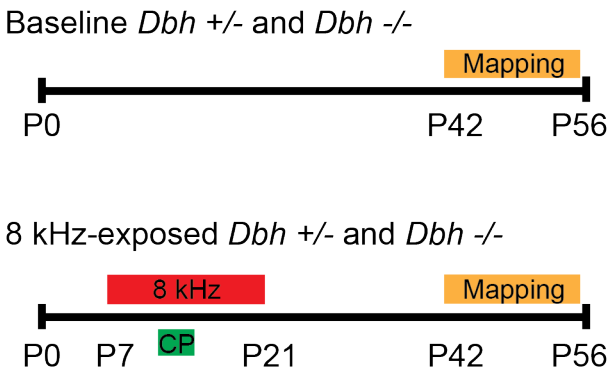

Figure 2.1 Experimental design. Lines illustrate experimental timeline for baseline groups (receiving no developmental sound exposure) and 8 kHz-exposed groups (receiving repetitions of an 8 kHz tone for 14 days encompassing the AC critical period [“CP”]).

NE-deficient animals were 6- to 8-week-old female *Dbh* -/- mice bred on a mixed *C57BL6/J* and *129SvEv* background. These mice lack the enzyme dopamine β-hydroxylase, and therefore cannot synthesize NE from DA. Because NE deficiency is fatal to embryonic *Dbh* -/- mice (Thomas et al., 1995), adrenergic function was transiently restored during fetal development via administration of phenylephrine (20 mcg/ml), isoproterenol (20 mcg/ml), and ascorbic acid (2 mg/ml) through maternal drinking water between E9.5 and E14.5, and the NE precursor L-3,4-dihydroxyphenylserine (L-DOPS; 2 mg/ml) and ascorbic acid (2 mg/ml) from E14.5 until birth. This intervention allows *Dbh* -/- embryos to synthesize enough NE to enable normal fetal development (Thomas et al., 1998). Once born, *Dbh* -/- mice do not require pharmacological intervention for survival despite complete NE deficiency. Furthermore, other than lacking NE, their noradrenergic system is similar to that of a NE-competent animal: projections from the locus ceruleus develop normally, and adrenergic receptors and transporters are observed in quantities comparable to wild-type animals (Weinshenker et al., 2002; Jin et al., 2004; Sanders et al., 2006). *Dbh* +/- littermates, which are heterozygous for the dopamine β-hydroxylase gene, have NE levels comparable to wild-type (*Dbh* +/+) mice (Thomas et al., 1998; Bourdelat-Parks et al., 2005), were used as NE-competent controls. Additionally, a small group of *Dbh* +/+ mice (n = 3) were used to confirm that some phenotypes observed in the *Dbh* +/- mice were attributable to the background strain, and not partial loss of the *Dbh* gene.

Developmental sound exposure

Animals of each genotype were exposed to one of two acoustic environments during development. “Baseline” animals were housed in a standard colony environment until electrophysiological recording between postnatal days 42 and 56 (P42-P56). “8 kHz-exposed” animals of both genotypes were housed in a standard colony environment except during sound

exposure, which occurred in a sound-attenuating chamber (IAC Acoustics, Bronx, NY, USA) for 16-18 hours/day from P7-P21, a time period which encompasses the P12-P15 critical period for frequency tuning, as defined in the *C57BL/6J* mouse (Barkat et al., 2011). Stimuli consisted of 8 kHz pure tones (80 dB SPL, 100 ms duration, presented in 1s trains at 5 Hz, alternated with 2 s silence). A 4-watt white LED light bulb and an automatic timer were used to maintain the same light cycle as the colony room.

Auditory brain stem responses

Previous research indicated that *Dbh* -/- mice are particularly susceptible to hearing loss due to higher incidence of middle ear infection (Maison et al., 2010). To ensure the experimental cohort had adequate hearing, peripheral hearing thresholds were assessed for all animals by taking auditory brainstem responses (ABRs) prior to AC electrophysiology. As described in Miranda et al. (2014), needle electrodes were inserted subdermally into the fatty region ventrolateral to each external pinna, and at the vertex of the skull. Using TDT System 3 hardware, signals were sampled at 24 kilosamples/s, amplified 200,000x, and bandpass filtered between 100-3000 Hz. ABRs were assessed for clicks and tone pips at 8, 16, 24, and 32 kHz (3 ms duration with 1.5 ms rise/fall times, presented at 21 Hz), presented through a free-field speaker (HiVi RT1.3 Planar Isodynamic Tweeter) positioned 20 cm from and 45° anterolateral to the right ear. Stimuli were presented at decreasing sound intensities (5 dB interval) until sound-evoked peaks were no longer apparent in the ABR signal. Each stimulus was repeated 500x, such that each ABR waveform represented an average response.

To truly assess the *peripheral* auditory response, ABR thresholds were assessed with respect to the first peak, which represents activity in the auditory nerve (Figure 2.2) (Henry, 1979). Only mice showing clear peaks in response to clicks, 8 kHz tones, and 16 kHz tones went

on to cortical electrophysiology. For animals that met this criterion, definitive thresholds were determined offline by an observer blinded to genotype and rearing environment.

Electrophysiology and data processing

Craniotomy, electrophysiological recording, and data processing were conducted as described in appendix A. Experimental stimuli consisted of pure tones (30 frequencies log-spaced 2-32 kHz, 7 sound intensities in 10 dB steps from 5-65 dB SPL, 60 ms duration). Tones of a

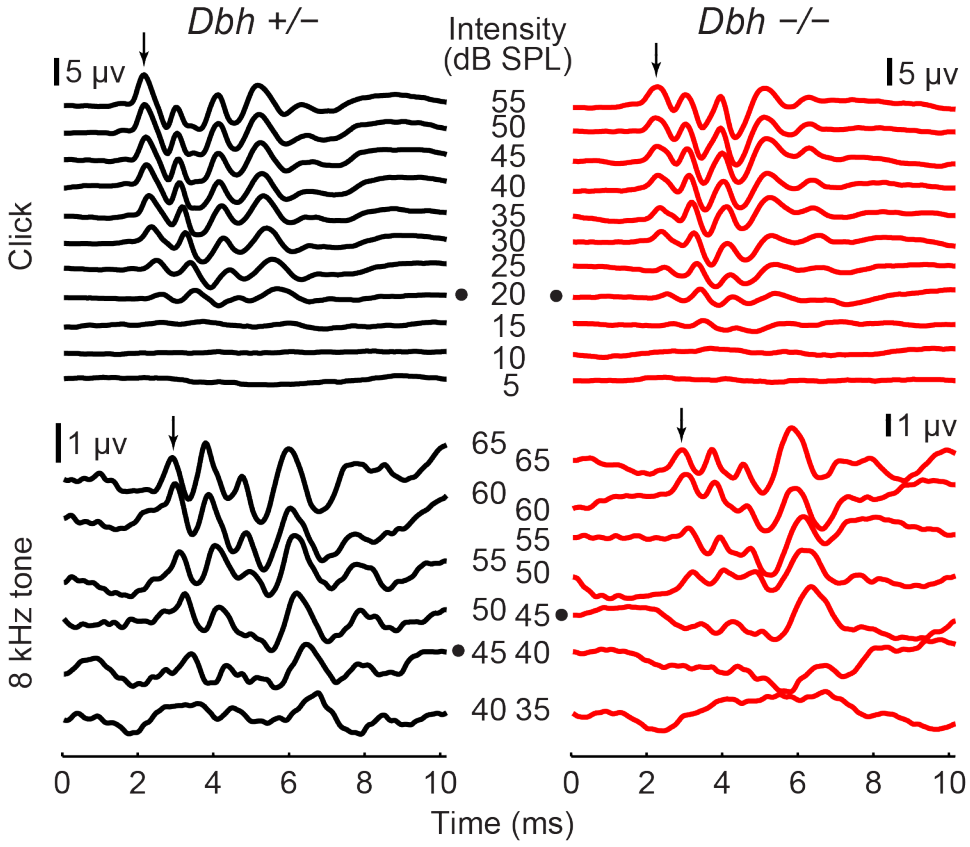

Figure 2.2 Example click- (top) and tone-evoked (bottom) ABR traces from individual *Dbh -/-* (right) and *Dbh +/-* (left) mice. Each trace shows the ABR evoked by a 3-ms click with onset at time 0. As sound intensities grow quieter, peaks become less apparent. Peak 1 is identified with an arrow. The quietest sound intensity at which peak 1 is reliably apparent is the threshold (labeled with black dot).

given frequency and intensity were repeated 5 times and presented in pseudorandom order. Stimuli were presented through a free-field speaker (HiVi RT1.3 Planar Isodynamic Tweeter) positioned 20 cm from and 45° anterolateral to the right ear. Although some attempted mappings could not be completed for technical reasons, only complete maps were included in the analyses presented here.

Statistical analysis

Lilliefors tests were conducted on data sets to confirm that they were normally distributed. When they met this criterion, data were analyzed with a two-way ANOVA with genotype (*Dbh* -/- vs. *Dbh* +/-) and rearing experience (baseline vs. 8 kHz-exposed) as factors. When this analysis yielded a significant effect ($p < 0.05$), Tukey's honestly significant difference (HSD) was calculated for pairwise, post-hoc comparisons. To compare BF distributions, a two-sample Kolmogorov-Smirnov test was used. The significance level was set to 0.001 to correct for this test's high power, and for the artificially inflated sample size we incurred by pooling multi-units by group.

2.3 Results

2.3.1 Comparable peripheral auditory responses in NE-deficient and -competent mice

Because *Dbh* -/- mice experience higher rates of deafness relative to NE-competent controls (Maison et al., 2010), we performed ABRs on all experimental animals to assess their hearing thresholds prior to cortical electrophysiology. To ensure that differences in hearing thresholds did not give rise to any observed cortical map plasticity, only mice that showed clear ABR peaks in response to clicks, 8 kHz tones, and 16 kHz tones went on to cortical mapping. Consistent with prior research, more *Dbh* +/- mice (baseline: 9/11; 8 kHz-exposed: 12/18) than *Dbh* -/- mice (baseline: 14/22 8 kHz-exposed: 13/26) met these criteria. Among animals that were

included, no group differences in ABR threshold emerged (Figure 2.3). There was no effect of genotype on ABR threshold for peak 1 (2x2 ANOVAs: clicks: $F_{genotype}[1,27] = 0.12$, $p = 0.73$; 8 kHz: $F_{genotype}[1,28] = 0.63$, $p = 0.43$; 16 kHz: $F_{genotype}[1,28] = 1.2$, $p = 0.28$), which reflects activity in the auditory nerve, for clicks, 8 kHz tones, or 16 kHz tones. Animals that underwent developmental sound exposure had thresholds comparable to baseline (2x2 ANOVAs: clicks: $F_{experience}[1,27] = 0.84$, $p = 0.37$; 8 kHz: $F_{experience}[1,28] = 1.79$, $p = 0.19$; 16 kHz: $F_{experience}[1,28] = 1.92$, $p = 0.18$). Further, no significant interactions between genotype and experience were observed (2x2 ANOVAs: clicks: $F_{interaction}[1,27] = 4.18$, $p = 0.05$; 8 kHz: $F_{interaction}[1,28] = 2.96$, $p = 0.10$; 16 kHz: $F_{interaction}[1,28] = 2.6$, $p = 0.12$).

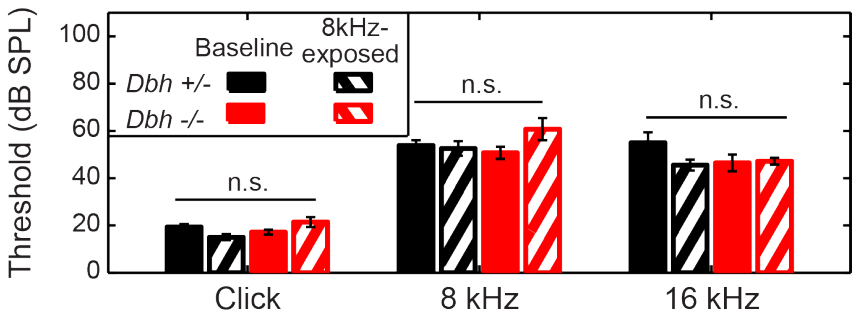

Figure 2.3 *Dbh +/-* and *Dbh -/-* mice share comparable ABR thresholds in response to clicks and tones. Bars illustrate group means ± standard error of the mean (SEM). "n.s." indicates the main effects and interaction for a genotype x experience ANOVA are not significant ($p > 0.05$).

24 kHz and 32 kHz ABR stimuli were played to these animals, although most animals of both genotypes were deaf to these frequencies at the sound intensities tested (up to 75 dB SPL). Poor high-frequency hearing was also apparent in *Dbh +/+* mice (Figure 2.4), suggesting that strain background rather than dopamine β-hydroxylase deficiency is responsible for these animals' limited hearing range. This finding implies that the mixed *C57BL6/J* and *129SvEv* background features a lower high-frequency hearing limit than that observed in most mouse

strains (Zheng et al., 1999). Nevertheless, this mutant may still be used to assess the capacity for AC plasticity, provided that plasticity-inducing sound exposure occurs at a frequency within its limited hearing range.

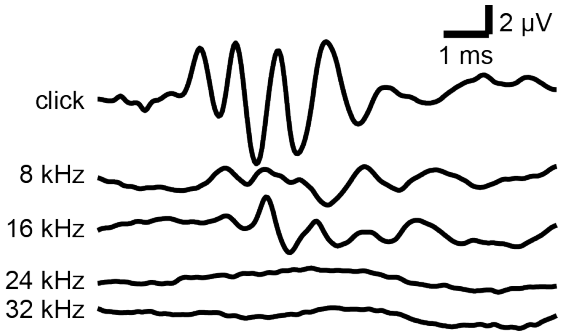

Figure 2.4 Representative ABRs from a *Dbh* +/+ mouse reveal a hearing range comparable to that observed in *Dbh* +/- and *Dbh* -/- mice. Traces show the response to the given stimulus at the loudest intensity presented (clicks: 55 dB SPL; 8 kHz tones: 75 dB SPL; 16 kHz tones: 60 dB SPL; 24 kHz tones: 75 dB SPL; 32 kHz tones: 75 dB SPL). Stimuli were 3 ms in duration, with onset at time = 0 (left extent of trace).

2.3.2 Comparable baseline AC physiology in NE-deficient and -competent mice

We first characterized AC responses in a cohort of "baseline" mice reared in a quiet colony environment. Tone-locked responses (Figure 2.5A) were obtained from animals in each group, and frequency-response areas (FRAs), which display response strength as a function of stimulus frequency and intensity, showed classic V-shaped tuning in *Dbh* +/- and *Dbh* -/- animals alike (Figure 2.5B). BFs were extracted from these FRAs by identifying the frequency that elicits the strongest firing rate response over all supra-threshold sound intensities, and complete BF maps (i.e., maps where a perimeter of nonresponsive sites was established around the tuned area) were constructed for the left ACs of 8 *Dbh* +/- (284 total multi-units) and 7 *Dbh* -/- (220 total multi-units) mice.

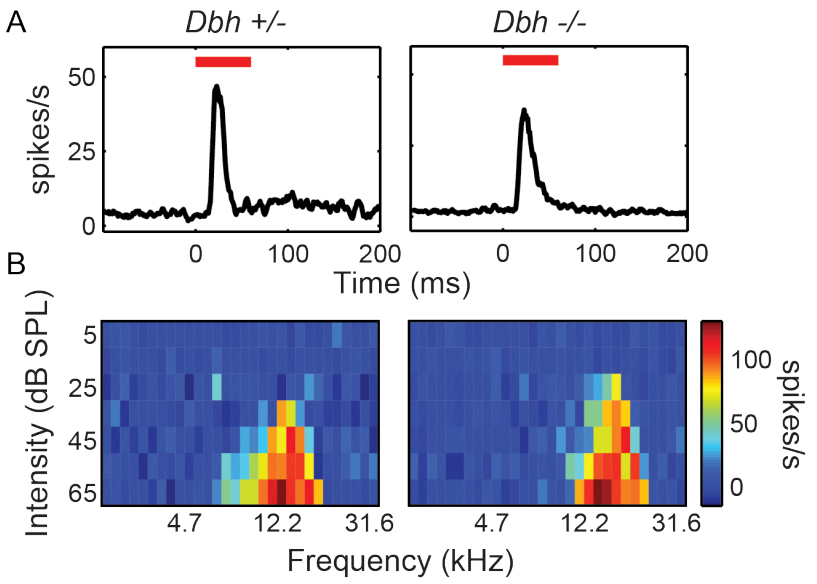

Figure 2.5 Example PSTHs and FRAs from individual *Dbh* +/- (left) and *Dbh* -/- mice (right). **A** Pooled PSTHs combining all trials (i.e., all frequencies and intensities) from a given recording. Red bar indicates timing of stimulus playback. **B** FRA from same multi-unit as PSTH.

Consistent with their limited hearing range, AC maps were often dominated by sites tuned to 13-16 kHz, and BFs above 22 kHz were uncommon (see examples in Figure 2.6A and B). Area UF, which typically encompasses neurons tuned to ultrasound frequencies above ~40 kHz, was consequently absent in these mice. Additionally, few multi-units exhibited response properties characteristic of non-core fields DP or A2, though those that did were excluded for the purposes of this study, which focuses on plasticity in core AC.

In core areas A1 and AAF, the spatial organization of BFs was heterogeneous, but in most cases a coarse tonotopic frequency gradient could be discerned. By averaging all the maps for a particular group together (Figure 2.6D and E), the expected tonotopic gradient became more apparent for *Dbh* +/- and *Dbh* -/- mice. Therefore, whether or not NE is present, AC organization is non-uniform but coarsely tonotopic for animals with this strain background. Mapping a wild-type *Dbh* +/+ control (Figure 2.6C) confirmed that the strain background, and not full or partial loss of dopamine β-hydroxylase, is responsible for the cortical topography we observed.

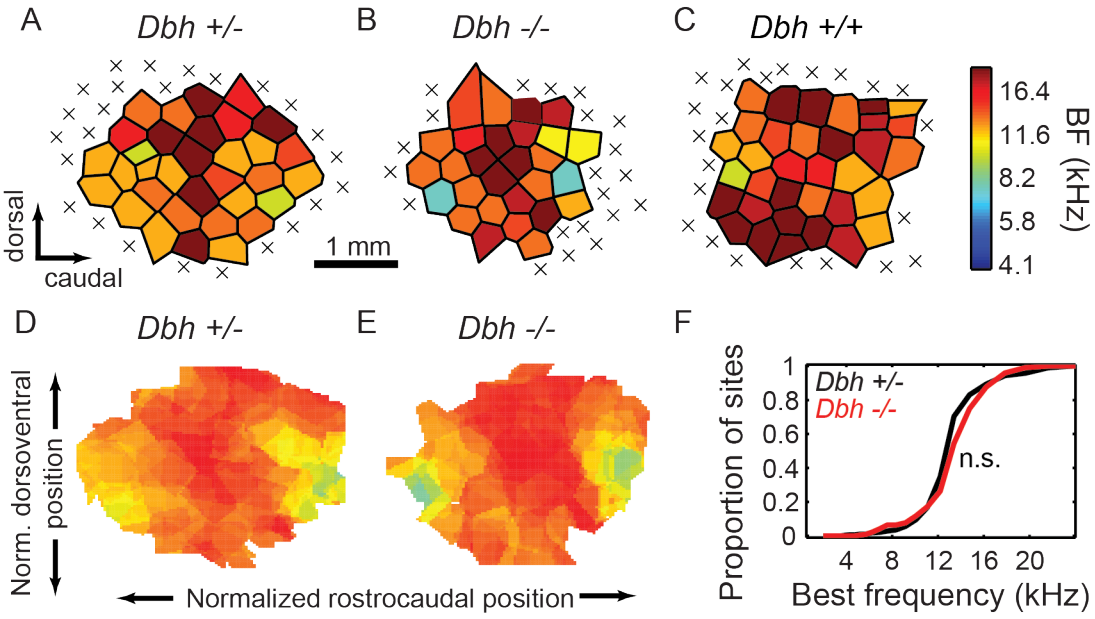

Figure 2.6 Comparable BF distributions in baseline *Dbh* +/- and *Dbh* -/- ACs. **A-C** Example BF maps from the core ACs of individual *Dbh* +/-, *Dbh* -/-, and *Dbh* +/+ mice, respectively. All maps are plotted on the same spatial and color scales. X indicates a recording site outside the AC. **D-E** Average BF maps for pooled *Dbh* +/- and *Dbh* -/- groups. Each cell reflects the average BF at that spatial position, normalized to the rostrocaudal and dorsoventral boundaries of each individual map. **F** Cumulative distribution of BFs, pooled by group. "n.s." indicates non-significant Kolmogorov-Smirnov test.

Table 2.1 Group mean response properties of *Dbh* +/- and *Dbh* -/- mice. T-statistics and p-values were calculated using a two-sample T-test in all cases except for the comparison of AC size, in which a Mann-Whitney U-test was used.

Parameter	*Dbh* +/-	*Dbh* -/-	T	p
Response latency (ms)	21 ± 0.9	21 ± 0.8	0.07	0.94
Response duration (ms)	66 ± 4.6	71 ± 6.5	0.65	0.53
Threshold (dB SPL)	33.5 ± 2.7	34.6 ± 1.0	0.38	0.71
Bandwidth at threshold + 20 dB (octave)	1.08 ± 0.06	0.99 ± 0.07	1.05	0.31
Evoked firing rate at BF (spikes/s)	90.2 ± 6.8	81.7 ± 3.5	1.06	0.31
Spontaneous firing rates (spikes/s)	7.4 ± 1.1	7.3 ± 0.9	0.12	0.91
AC size (mm^2)	2.93	2.73	18 (U)	0.28

Consistent with these qualitative impressions, quantitative comparisons of baseline *Dbh* +/- and *Dbh* -/- multi-unit response properties were similar. The proportion of multi-units tuned to each frequency tested did not differ between groups (Figure 2.6E; $D[502] = 0.16$, $p > 0.001$). Further, the total size of AC, and unit response properties including response latency, response duration, threshold, bandwidth at 20 dB above threshold, and evoked and spontaneous firing rates, were not significantly different between groups (Table 2.1).

These data show that, when reared in a quiet colony environment, loss of NE does not appreciably affect the organization of core AC. While this result suggests that NE is not required for the initial patterning of auditory circuitry and its maintenance in an unperturbed environment, it does not preclude the possibility that NE might be involved in adaptation of the AC to an environmental manipulation during the critical period.

2.3.3 Critical period plasticity is impeded by NE deficiency

We next attempted to drive developmental plasticity directly by altering the acoustic environment during the critical period for frequency tuning in the mouse. Cohorts of mixed *Dbh* +/- and *Dbh* -/- littermates were exposed to repeated presentations of 8 kHz tones between P7 and P21. The 8 kHz frequency of exposure was selected because all animals mapped in our baseline study showed BFs at or around 8 kHz, but in no animal was 8 kHz the most prominent frequency. Thus, there would be cortical territory for an 8 kHz exposure frequency to expand into given the proper conditions, yet the possibility that the consequence of exposure would not be apparent due to pre-existing saturation at that frequency was unlikely.

Complete BF maps were obtained from 10 *Dbh* +/- (356 total multi-units) and 7 *Dbh* -/- mice (214 total multi-units). Consistent with prior work, exposure to 8 kHz tones during development drove an approximate doubling of the core cortical area devoted to that frequency in

NE-competent animals. This effect was apparent at the level of individual cortical maps in many cases (Figure 2.7A-B), as well as in the group average maps (Figure 2.7C-D). In contrast, there was no evidence of such an expansion in NE-deficient animals, as the proportion of cortex tuned to 8 kHz ± 0.35 octaves was not significantly different between exposed and unexposed cohorts of *Dbh* -/- mice. Thus, there was a significant interaction between genotype and experience for

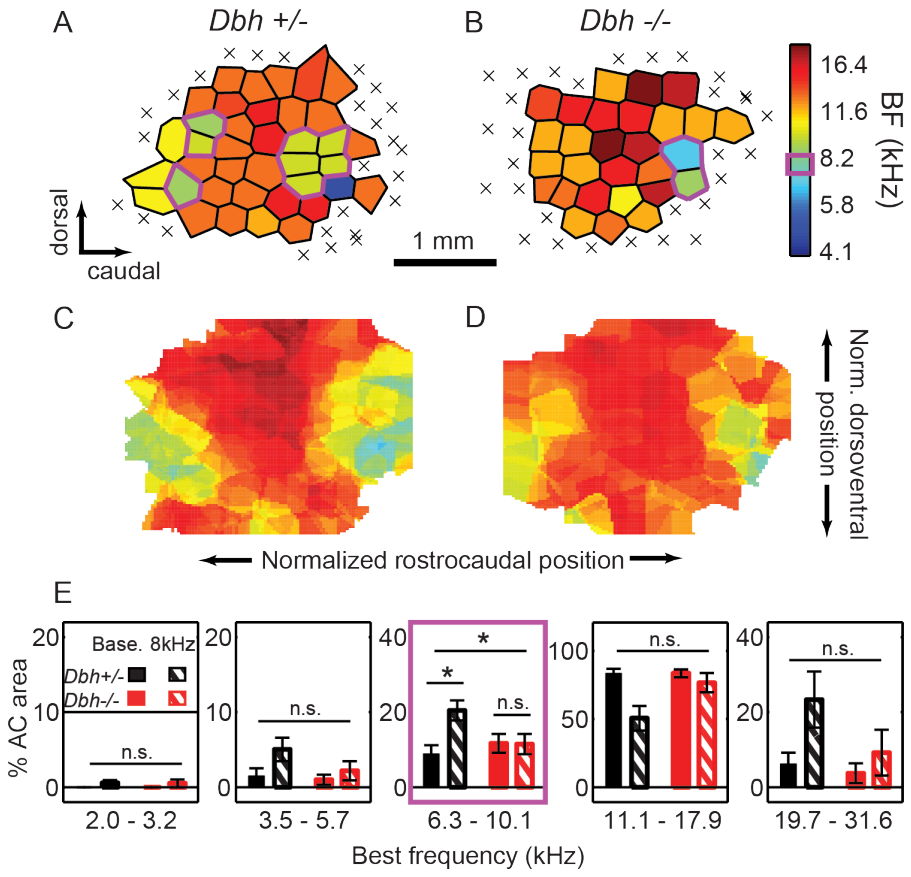

Figure 2.7 *Dbh* -/- mice (right) do not show BF map plasticity, whereas their *Dbh* +/- counterparts (left) do. **A-B** Example BF maps from 8 kHz-exposed mice, as constructed in Figure 2.5A-C. Sites with BFs falling at or near the 8 kHz frequency of developmental sound exposure are highlighted in magenta. **C-D** Average BF maps, as constructed in Figure 2.5D-E. The blue-green color corresponding to the 8 kHz frequency of exposure is more abundant in the *Dbh* +/- than the *Dbh* -/- map. **E** Proportionate area of AC tuned to each of 5 frequency bins spanning the range tested. Only for the frequency bin centered on the 8 kHz frequency of exposure is there a significant interaction between genotype and rearing (* over line spanning all bars indicates $p < 0.05$). * over line spanning same-genotype bars indicates significant Tukey's HSD only for the *Dbh* +/- group ($p < 0.05$). "n.s." indicates non-significant interaction for a 2x2 ANOVA ($p > 0.05$). Bars illustrate group means ± SEM

the 8 kHz band (Figure 2.7E; $F[1,28] = 5.09$, $p = 0.03$). Proportionate area devoted to any frequency band other than the 8 kHz band of interest did not differ significantly between exposed and unexposed cohorts. That is, in no other case was a significant interaction observed for genotype and rearing experience (2x2 ANOVAs: 2-3.39 kHz band: $F[1,28] = 0.01$, $p = 0.93$; 3.4-5.99 kHz band: $F[1,28] = 0.84$, $p = 0.37$; 10.5-18.73 kHz band: $F[1,28] = 3.52$, $p = 0.07$; $F[1,28] = 1.01$, $p = 0.32$). The specificity of this effect for the band of exposure implicates NE as a critical regulator of experience-dependent map plasticity during the critical period.

2.4 Discussion

In this study, I employed *Dbh* -/- mice with genetic lesions of the noradrenergic system to assess the role of NE in experience-dependent plasticity during the critical period in AC. I first observed that, consistent with previous research, *Dbh* -/- mice show an increased susceptibility to deafness. For *Dbh* -/- mice with adequate hearing, I found that baseline organization of the AC was comparable to that of NE-competent, *Dbh* +/- mice. In these NE-deficient mice, however, I did not observe a redistribution of BFs following exposure to a biased acoustic environment during development; this manipulation *was* sufficient to drive such a reorganization in *Dbh* +/- mice. From these results, I conclude that NE is required for plasticity during the critical period for frequency tuning in the AC.

2.4.1 NE loss affects AC organization in sound-exposed, but not baseline groups

While NE was required for plasticity stemming from sound exposure during the critical period for frequency tuning in the AC, the baseline organization of the AC following rearing in a normal colony environment was no different in *Dbh* +/- and *Dbh* -/- mice. Since one would

expect critical period plasticity to shape AC organization even in the absence of an overt acoustic manipulation, it may be unusual that a phenotype associated with NE loss was not observed in the cohort of baseline animals. The most likely explanation for why no such phenotype was apparent is that the acoustic features of the colony environment "match" the intrinsic organization of AC that is determined genetically and wired prior to the onset of acoustic experience. That is, the tuning properties of the ACs of colony-reared animals may closely overlap the acoustic properties of the colony environment, such that little to no re-tuning occurs during the critical period. Indeed, a previous study in which AC topography was mapped in separate groups of young mice at critical period onset (P12) and offset (P15) showed no difference in cortical topography between these groups when these animals were colony-reared, but dramatic plasticity when they were raised among pure tone repetitions (Barkat et al., 2011).

In the present study, I also observed an expansion in the area of AC tuned to the frequency of exposure (8 kHz in this case). Although the statistical comparison was only significant for this frequency band (i.e., the interaction between genotype and rearing experience was significant), some non-significant trends were observable for other frequency ranges. For instance, in the frequency band lateral to and above 8 kHz (spanning approximately 11 to 18 kHz), *Dbh +/-* mice, but not *Dbh -/-* mice, showed a decrease in proportionate AC area following sound exposure. This effect is not necessarily surprising, since for an individual animal, the proportionate area of AC tuned to all frequency ranges must sum to 100%. Thus, increasing the area of the 8 kHz range necessitates a reduction in the area occupied by other frequency ranges.

Another non-significant trend was apparent in the highest frequency bin (~20 to 32 kHz), where sound exposed groups (though particularly the sound-exposed *Dbh +/-* mice) tended to have more cortical area devoted to this band than did baseline groups. Unlike the trend in the 11 to 18 kHz band, this trend is not readily understood in relation to the significant effect at the 8 kHz frequency of exposure. Instead, it appears to be caused by increased variability in the high-

frequency hearing of sound-exposed animals. While the proportion of cortex tuned between 19.7 and 32.6 kHz does appear to increase following sound exposure in *Dbh* +/- mice, variability increases as well, such that the standard deviation for 8 kHz-exposed *Dbh* +/- mice reaches 24% (compare this to the 8% standard deviation observed for the 6.3 to 10.1 kHz bin in the same group). This increase in variation prevents even a t-test between the baseline *Dbh* +/- and 8 kHz-exposed *Dbh* +/- groups for this high-frequency bin from reaching significance. Closer inspection of the data revealed that approximately half of the animals in the 8 kHz-exposed *Dbh* +/- group (all of which had non-measurable hearing thresholds at 24 and 32 kHz) had very small proportions of their cortices tuned to this frequency range, and the other half of this group (all of which had measurable hearing thresholds at 24 kHz) had much larger proportions of their cortices tuned to this frequency range. Because of this correlation between high-frequency hearing range and proportion of cortex tuned to the highest frequency range, I surmise that the apparent change in high-frequency tuning in the AC is determined by increased variability in the high-frequency hearing of the 8 kHz-exposed *Dbh* +/- mice. The cause of this increased variability in hearing range is not clear, but could be related to the greater amount of time this group spent in the anechoic chamber during postnatal development. Importantly, this apparent increase in the proportion of AC tuned to higher frequencies would have been expected to reduce the proportion of AC tuned to lower frequency bins, including the bin centered on the 8 kHz frequency of exposure. However, I still observed a significant *increase* in the proportion of AC tuned to the 6.3 to 10.1 kHz bin among the *Dbh* +/- group, indicating that the developmental sound exposure regimen had its intended effect.

2.4.2 Achieving NE deficiency using genetic versus neurotoxic lesions

The finding that NE was required for critical period plasticity conflicts with a number of prior studies from visual cortex, which argued against the necessity of NE for critical period plasticity there (Bear and Daniels, 1983; Adrien et al., 1985; Bear and Singer, 1986). One possible reason for this discrepancy is the use of different methodologies to deplete NE. Earlier studies relied on neurotoxins, particularly 6-hydroxydopamine (6-OHDA), to destroy noradrenergic neurons. 6-OHDA is relatively nonspecific, as it acts indiscriminately on catecholaminergic axon terminals (Uretsky and Iversen, 1970; Walker et al., 2013), and can therefore lesion dopaminergic projections unless it is delivered alongside a DA transporter inhibitor. Furthermore, 6-OHDA acutely compromises cholinergic signaling via muscarinic receptors (Bear and Singer, 1986). Given the availability of ACh in cortex (Wenk et al., 1980; Eckenstein et al., 1988), and this system's established role in synaptic plasticity (Metherate and Ashe, 1991; Rasmusson, 2000; Metherate and Hsieh, 2004), this is potentially a serious confound. Finally, depending on the route of administration and dosage, a 6-OHDA lesion may not include all the noradrenergic projections in the area of interest. The *Dbh -/-* mouse does not carry these risks of nonspecificity and incompletion, and its use here represents an opportunity to validate and update the foundational work implicating NE in experience-dependent developmental plasticity.

Use of the *Dbh -/-* mouse bypasses many of the problems associated with neurotoxic lesions, though it introduces a few of its own. First, this model lacks the regional specificity afforded by some methods of neurotoxin delivery. The DBH knockout is systemic, so when an effect of NE depletion is observed, it can be difficult to determine where and how NE is acting. Its actions here may be direct, as NE receptors can be found throughout the cortex (Young and Kuhar, 1980; Rainbow et al., 1984; Domyancic and Morilak, 1997), or indirect, owing to the effects of NE on sites that ultimately project to the AC. Because NE is released in the periphery as part of the stress response through the sympathetic adrenal medullary axis, there is even a

chance it acts there to regulate critical period plasticity, though this possibility is unlikely as no study has yet established that stress or arousal play a necessary part in sensory development. The lingering question of where NE acts may be resolved by future studies in which noradrenergic tone is restored to NE-deficient animals during the critical period, in a site-specific manner. The most precise way to achieve this would be through use of a site-specific *Dbh* knockout mouse in which *Dbh* expression is limited only within the forebrain, leaving NE production mechanisms in the brain stem and periphery intact.

Second, given the long time course of NE deficiency, there is a large time window during which a secondary effect could intervene to either mask an effect of NE depletion, or to produce an effect of its own. Since loss of NE through the *Dbh* mutation also lowers extracellular DA levels in some brain areas (Schank et al., 2006), for example, one may be concerned that a secondary, dopaminergic effect, rather than the primary loss of NE, led to the effect we observed. However, the alterations in dopaminergic function described by Schank et al. were local to subcortical regions including the striatum, and cortical DA was largely normal, making it unlikely that the dopaminergic physiology of the *Dbh* -/- knockout directly explains the plasticity I observed here. Furthermore, other research has shown that stimulation of the dopaminergic VTA can lead to map plasticity in AC (Bao et al., 2001), potentially through the direct actions of DA in AC. Thus, extracellular DA would likely promote, rather than inhibit, plasticity if it were more abundant in the ACs of the *Dbh* -/- cohort.

An additional concern relating to the lack of temporal specificity of NE deficiency in the *Dbh* -/- mouse pertains to the possibility that NE loss prevented the *expression of*, rather than the *induction of* map plasticity. That is, it could be that plasticity occurred as expected following sound exposure in the *Dbh* -/- mice, but the absence of NE at the time of electrophysiological recording prevented the observation of plasticity. This possibility is unlikely, however, since it would require that the mechanism through which BF map plasticity is masked be frequency-

specific. That is, such a mechanism would have to spuriously elevate firing rates in response to frequencies outside the 8 kHz range and diminish them around the 8 kHz frequency of exposure, leading the experimenter to falsely assign BFs outside the 8 kHz range, to produce the appearance that plasticity did not occur following developmental sound exposure. Nevertheless, additional experiments in which NE is transiently restored at the time of recording, perhaps via administration of the NE precursor L-DOPS immediately prior to electrophysiology, would protect against this possibility.

Third, the presence of gross developmental delays in the *Dbh -/-* mouse (Thomas et al., 1995) introduces the possibility that the critical period window is shifted in this population, and therefore that sound exposure failed to cause plasticity not because these animals' brains are inherently less malleable, but because stimulation simply occurred outside the critical period window. While this is a possibility, the long duration of sound exposure was designed to protect against any such changes in critical period timing. The developmental exposure protocol employed here terminates nearly a week after the close of the critical period for frequency tuning in the mouse AC (Barkat et al., 2011), and will therefore accommodate critical period shifts of up to about one week. Little is known about the time course of brain development in these mice, but given that their trajectory of body mass gain incurs a delay on the order of 1-2 weeks (Thomas et al., 1995), the exposure regimen used here seems sufficient to cover developmental delays in the likely range.

Finally, the *Dbh* mutation was bred on a background strain (mixed *C57BL/6* and *129SvEv*) that is apparently not well suited for auditory research. Under baseline conditions, *Dbh +/-* and *Dbh -/-* mice both showed comparably poor hearing thresholds for frequencies at or above approximately 24 kHz. Furthermore, the organization of core AC fields in both groups was somewhat unusual, with an overabundance of BFs in the 13-16 kHz range, and some scrambling of the expected tonotopic gradients. This pattern was not believed to stem from complete or

partial NE loss, as NE levels are comparable to wild-type in *Dbh* +/- mice (Thomas et al., 1998; Bourdelat-Parks et al., 2005). Nevertheless, I measured hearing thresholds and conducted mapping experiments in a small cohort of *Dbh* +/+ mice, and these experiments confirmed that the strain background, and not NE loss, was to blame. While these auditory phenotypes do not preclude an investigation of experience-dependent plasticity, provided stimulation occurs within the hearing range, they do limit the use of this mutant in future investigations of AC, particularly if the stimuli of interest include natural sounds such as ultrasonic vocalizations. Because *Dbh -/-* mice exhibit some social behavior deficiencies (Thomas and Palmiter, 1997; Marino et al., 2005), exploring the role of NE in processing such communication sounds is potentially of interest, and backcrossing the *Dbh* mutation onto a strain with better high-frequency hearing could be worthwhile.

2.4.3 Possible targets of NE

This study, combined with recent work implicating NE in experience-dependent plasticity in the adult AC (Edeline et al., 2010; Martins and Froemke, 2013), will inevitably lead to the question of what cellular substrates NE acts upon to facilitate retuning. Answering these questions with respect to the auditory cortical critical period for frequency tuning specifically will require a deeper understanding of local critical period mechanisms than the one we currently have. In visual cortex, for example, critical period opening and closure is thought to rely on maturation of local inhibitory circuitry and solidification of these neurons' synaptic contacts via remodeling of the extracellular matrix, respectively (Hensch, 2005). Once these foundational processes have been elucidated, it is easier to propose new hypotheses about how critical neuromodulators interact with them to enable experience-dependent plasticity.

In comparison with the visual cortex, less is known about the mechanisms underlying

critical period plasticity in the AC. What is known was gleaned from functional studies that, while enlightening, do not identify key molecular players. Nevertheless, the existing evidence implies that changes in thalamocortical synapse strength underlie map plasticity during the critical period. Using brain slices taken from developing mice, Barkat *et al.* (2011) measured AC responses to electrical stimulation of loci along the tonotopic axis of the medial geniculate nucleus, and found that the stimulation/response relationship was altered by acoustic experience in a way that mirrored the changes observed in traditional mapping studies that make use of acoustic, rather than electric, stimuli. That is, after exposing animals to 7-kHz pure tone repetitions during (but not after) the critical period, the AC response evoked by stimulation of a thalamic locus known to be tuned to the 7 kHz range was potentiated relative to the response recorded from colony-reared mice. This critical period for thalamocortical connectivity may underlie the critical period for tonotopic map plasticity during development. Thus, an intriguing follow-up study could assess whether NE is similarly required for this parallel window of AC plasticity.

Although the critical period for thalamocortical connectivity could be caused by processes unrelated to plasticity at thalamocortical synapses, Barkat *et al.* also showed that morphological changes in dendritic spines in layer 4 (a large portion of which are expected to be thalamorecipient) that parallel the observed functional changes, and accelerated the critical period by selectively inhibiting a negative regulator of dendritic spine maturation in pyramidal neurons in the cortex, including the thalamorecipient pyramids in layer IV. The involvement of thalamocortical synapses in critical period plasticity is further supported by the finding that in slices, long-term potentiation of thalamocortical synapses can be induced by tetanic stimulation in neonates and adults alike, though intracortical disinhibition and cholinergic signaling are additionally required for plasticity to develop in adults (Chun et al., 2013). The presence of additional requirements for plasticity induction in adults echoes the higher threshold for plasticity observed in mapping studies following sound exposure: exposure alone is sufficient to drive

plasticity in developing animals, whereas behavioral reinforcement is required for map plasticity in adults (Zhang et al., 2001).

If reweighting of thalamocortical synapses determines retuning during the critical period for tonotopic map plasticity, there are a number of potential routes through which NE could act to permit plasticity. NE has long been known to facilitate long-term potentiation in the CA1 region (Izumi and Zorumski, 1999; Gelinas and Nguyen, 2005) and dentate gyrus (Stanton and Sarvey, 1987; Dahl and Sarvey, 1989; Walling and Harley, 2004) of the hippocampus, likely through β-adrenergic receptor-dependent activation of adenylyl cyclase. In other systems, NE directly enables long-term potentiation by participating in AMPA receptor trafficking (Hu et al., 2007; Joiner et al., 2010; Liu et al., 2010), again via β-adrenergic receptors, and by altering neurotransmitter release probabilities through a α_2-receptor-dependent mechanism (Carey and Regehr, 2009). There is also evidence that NE can indirectly regulate plasticity via its actions on GABA circuitry. In the amygdala, NE permits long-term potentiation in thalamorecipient neurons by reducing the inhibition of these cells by GABAergic interneurons (Tully et al., 2007). This effect is at least partially dependent on the activation of β-adrenergic receptors. This potential mechanism is especially appealing in the AC, as disinhibition of thalamorecipient neurons there must occur in order for plasticity to be expressed in the adult (Chun et al., 2013). It may be that greater noradrenergic tone during development (Nakamura et al., 1987; Nakamura et al., 1988) constitutively disinhibits thalamorecipient neurons throughout the critical period, maintaining a low threshold for plasticity during this time.

While it is clear that NE is capable of permitting plasticity through a variety of mechanisms, central to most of them is activation of β-adrenergic receptors. Thus, when one is considering whether NE mediates critical period plasticity in the AC directly or indirectly, it would be helpful to know whether β-adrenergic receptors are expressed on likely cellular targets within the AC. While β-adrenergic receptors are present in the AC (Sutin and Minneman, 1985), a description of adrenergic receptor expression across AC layers, let alone across specific cellular

subtypes, does not currently exist. However, comprehensive surveys of β-adrenergic receptor distribution across the rodent brain have shown that β-adrenergic receptors can be found throughout the neocortex, specifically in layers I, IV, and VI (Bylund and Snyder, 1976; Rainbow et al., 1984). Additional research is required to determine whether this pattern of receptor expression across layers holds in the AC and if so, whether the cells expressing β-adrenergic receptors include the thalamorecipient layer IV neurons that likely undergo plasticity during the critical period for frequency tuning.

2.4.4 Summary

In conclusion, I employed an alternative model of NE deficiency here to re-examine the question of whether NE is required for critical period plasticity in sensory cortex. I showed that while NE does not apparently affect AC organization if animals are reared in a quiet colony environment, NE loss inhibits plasticity following a manipulation of the acoustic environment. Though the systemic nature of this model limits what can be said about how NE permits plasticity, future investigations may restore NE to the AC in a site-specific manner, enabling a clearer understanding of the pathways NE acts on to achieve its effects.

Chapter 3

Tonotopic map plasticity in the maternal adult

In chapter 2, I used the NE-deficient *Dbh -/-* mouse to clarify that neuromodulator's necessity for experience-dependent plasticity in the developing tonotopic map. As described in section 1.2.3, map plasticity occurring during development and during adulthood differ in terms of the conditions under which they are evoked and in their phenomenology. In this chapter, I turn to map plasticity in adulthood, with the intent of better understanding the conditions under which it arises. Using an ethological model of auditory learning, I ask whether map plasticity is necessary to support learning when a sound is made relevant under more natural circumstances than those usually employed in the laboratory.

3.1 Introduction

As detailed in section 1.2.1, most studies of map plasticity in the adult AC make use of laboratory conditioning paradigms to imbue a sound with relevance. These types of manipulations can produce robust plasticity in the tonotopic map (e.g., a doubling in the cortical area tuned to the frequency of interest), but may not adequately represent the manner in which "real-life" auditory learning takes place. The animals used in these studies are most often inbred laboratory mice or rats, which have been reared in relatively impoverished sensory environments compared to those encountered by wild-caught animals. With this basis of experience, a strong positive reinforcer such as footshock constitutes an extremely salient event. Compare such reinforcement with the circumstances under which sounds are typically learned in everyday experience: sounds are often reinforced in a subtler way. In my second study, I was interested in exploring whether map plasticity would be observable if sounds were reinforced under more natural circumstances.

Maternal mice constitute an ideal population in which this question can be addressed, owing to the acoustic communication system they share with their pups. Maternal females rear their pups in a nest until they are old enough to ambulate and feed independently. Pups that are separated from the nest during this time emit stereotyped ultrasonic vocalizations (USVs) that elicit maternal search and retrieval behaviors (Sewell, 1968; Sewell, 1970; Smith, 1976; Ehret and Haack, 1981). Search and retrieval is likely guided by sensory cues in multiple modalities, though maternal animals display a preference for pup USVs that pup-naïve females do not: Given the choice to approach pup USVs or low-frequency tones without particular relevance, maternal females more often approach the vocalizations, whereas pup-naïve females approach either stimulus without preference (Ehret et al., 1987; Lin et al., 2013)

Although increased maternal responsiveness to pup USVs could be caused exclusively by an increased motivation to approach pup-related stimuli, a growing body of evidence suggests maternal and non-maternal females may actually process these USVs differently. These sensory changes may support maternal behavior. Recent work focused on the AC has shown that the timing of responses to pup USVs is more reliable in mothers compared to pup-naïve females (Liu et al., 2006), and that maternal spike trains are better at discriminating and detecting pup USVs (Liu and Schreiner, 2007). This improved detection may stem from enhanced pup USV-evoked inhibition of activity in neurons tuned outside the USV frequency range (Galindo-Leon et al., 2009). Finally, maternal AC responses to pup USVs are apparently facilitated by the presence of pup odors, identifying the AC as a site of multisensory integration of pup stimuli (Cohen et al., 2011). Together, this work has established that sensory changes accompany pup sensitization, and may contribute to the induction and maintenance of maternal behavior.

These studies have also established pup USV processing in the maternal mouse as an ethological model of auditory plasticity. Because tonotopic map plasticity is a widely observed correlate of auditory memory for behaviorally relevant acoustic frequencies, one might expect that pup USV sensitization would drive map plasticity for frequencies characteristic of pup USVs.

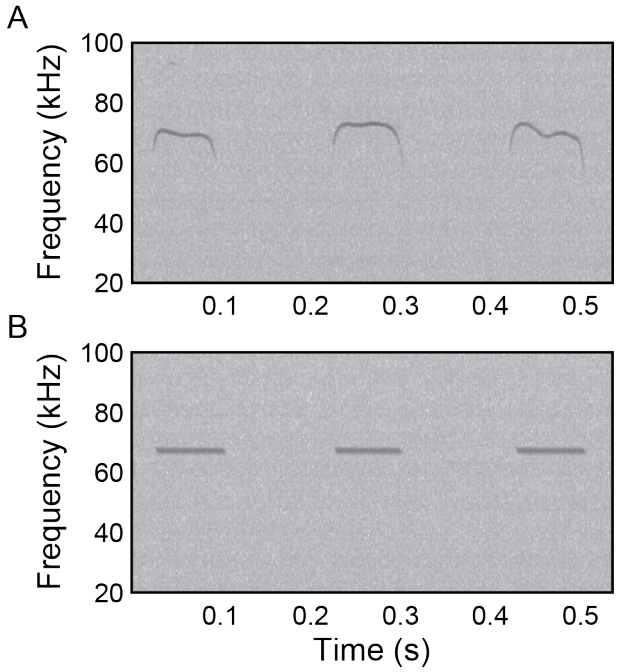

Figure 3.1 Acoustic structures of pup USVs and ultrasonic tones are comparable. **A** Spectrogram of a bout of USVs recorded from a pup on P6. Note the flat frequency trajectory. **B** Simulated spectrogram of a train of 65-kHz tones.

The spectrotemporal structure of pup USVs is, after all, comparable to that of pure tones (Figure 3.1), which have been used to drive tonotopic map plasticity when reinforced through laboratory conditioning methods (Recanzone et al., 1993; Bieszczad and Weinberger, 2010c). Under the right behavioral conditions, then, stimuli similar to pup USVs are capable of driving map plasticity. Further, the maternal context has proven to be an adequate "behavioral condition" for map plasticity induction in somatosensory cortex, where representation of the ventral areas from which pups nurse is expanded in mothers (Xerri et al., 1994). Thus, if map plasticity necessarily accompanies auditory memory formation, we might expect that a similar phenomenon can be observed for pup USV frequencies in the maternal AC following pup sensitization. Nevertheless, there is evidence that the mode through which stimuli are reinforced influences the plasticity that develops: In one study where different reinforcement paradigms were used to make a simulated vocalization relevant to two groups of animals, the group that learned those sounds through natural experience showed evidence of left-hemisphere specialization for recognition of those

sounds, whereas the group that underwent conditioning did not (Ehret, 1987). This finding suggests that social reinforcers may enact plasticity through different mechanisms than do laboratory rewards or punishments. Depending on the degree to which map plasticity is sensitive to such mechanistic differences, we may or may not observe plasticity here. I test this hypothesis, and describe my results here.

3.2 Method

Subjects

Electrophysiological recordings of pure tone responses were taken across the ACs of pup-naïve virgin females (n = 8) and three cohorts of dams. Because map plasticity may be a persistent engram of auditory memory or a transient stage of auditory memory formation (see section 1.2.1; (Reed et al., 2011), separate groups of dams were mapped at three postnatal time points: 3-4 days after parturition (n = 7), 9-10 days after parturition (n = 8), or immediately following pup weaning (approximately 21 days after parturition; n = 8), in an effort to capture

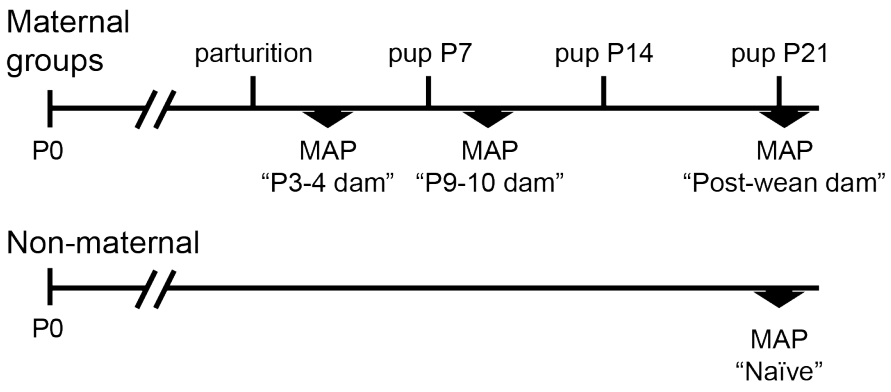

Figure 3.2 Experimental design. On timelines for both maternal (top) and non-maternal (bottom) groups, animal age is represented below, and (for maternal groups), pup age is specified above.

any transient peak in map plasticity (Figure 3.2). All subjects were *CBA/CaJ* mice aged 11-17 weeks at the time of recording.

All maternal animals had natural home cage experience with their own pups prior to electrophysiological recording (duration of experience depended on which maternal group an animal was assigned to). A subset of dams spanning all maternal groups underwent 10-minute pup retrieval trials on postnatal days 3 and/or 7 in which 3 pups were scattered from the nest, and maternal retrieval behavior was scored. Dams were not separated from their litters during this trial, or at any time prior to electrophysiological recording. On the morning of recording, dams were removed from their litters at the time of anesthetic induction while pups were killed with an overdose of isoflurane.

Electrophysiology and data processing

Craniotomy, electrophysiological recording, and data processing were conducted as described in appendix A. Experimental stimuli consisted of pure tones (30 frequencies log-spaced 4-80 kHz, 7 sound intensities in 10 dB steps from 5-65 dB SPL, 60 ms duration) and USVs; this chapter focuses only on responses to pure tones (see Chapter 4 for analysis of USV responses). Tones of a given frequency and intensity were repeated 5 times and presented in pseudorandom order. Stimuli were presented through a free-field speaker (EMIT high-energy speaker, Infinity Systems, Stamford, CT) with a flat frequency response up to 100 kHz, positioned 11 cm lateral to the right ear.

In some cases, complete maps could not be obtained from experimental animals due to experimental difficulties. These partial maps (n = 9: 3 naïve, 4 P3-4 dams, 1 P9-10 dam, 1 post-wean dam) were not included in area-based analyses (e.g., proportionate area of AC tuned to a given frequency range). However, multi-unit recordings from these partial maps *were* included in non-area-based analyses (e.g., average response properties associated with particular subregions) if enough of the AC had been mapped to permit confident assignment of units to AC subregions.

In addition to being assessed manually (as described in appendix A), thresholds and characteristic frequencies (CFs) were determined in an automated fashion, using methods described elsewhere (Kim and Bao, 2013). Briefly, FRAs were smoothed with a 3x3 median filter, then thresholded at 50% of the maximum response within the FRA. If, after this smoothing and thresholding, there were non-contiguous regions of the receptive field, the largest one was chosen for analysis. The threshold was defined as the quietest intensity at which an evoked response (at least 50% of the maximal firing rate for the FRA) was observed. The CF was defined as the average frequency eliciting a response at the threshold intensity (i.e., the tip of the classic, V-shaped tuning curve).

Response strength was calculated by taking the firing rate over either a uniform 100-ms time window, or a custom time window fitted to the onset and offset of the individual unit's excitatory response. The latter was used in descriptive reports of response properties by subregion (as in section 3.3.1) and to display firing rates in FRA cells, whereas the former was used to compare firing rates evoked by ultrasonic tones and USVs (see section 3.3.3).

Statistical analysis

Lilliefors tests were conducted on data sets to confirm that they were normally distributed. If they met this criterion, data were analyzed with a one-way ANOVA to compare means across naïve animals and the three maternal groups. If data were not normally distributed, a Kruskal-Wallis H test of ranks was employed instead. When analyses yielded a significant effect ($p < 0.05$), Tukey's honestly significant difference was calculated for pairwise, post-hoc comparisons. For comparisons of distributions, Kolmogorov-Smirnov tests were run between each maternal group and the naïve group.

3.3 Results

3.3.1 AC organization in CBA/CaJ mice

1484 multi-unit recordings were taken from a total of 40 mice spanning all 4 maternal groups (for a breakdown of multi-unit sample sizes by group and AC subregion, see Table 3.1). These multi-units were used to construct complete maps for 31 animals (Figure 3.3). As specified in appendix A, multi-units were assigned into AC subregions based on their spatial location, BF, response latency, tuning bandwidth, responsiveness to USVs, and tendency to habituate to repeated presentation of a stimulus. Response profiles typical of the 5 AC subregions described in Stiebler *et al.* (1997) were apparent. Tonotopic organization and BFs ranging from approximately 4 to 50 kHz characterized core fields A1 and AAF, which met in a band of high-frequency-tuned sites designated as "AAF/A1." High-ultrasonic BFs clustered in the rostrodorsal portion of the map, above AAF. Although it has been argued that this region is simply an extension of the tonotopy observed in AAF and A1, and is not a separate auditory field (Guo et al., 2012), these sites were not incorporated into a clear tonotopic gradient in this study, and as such were

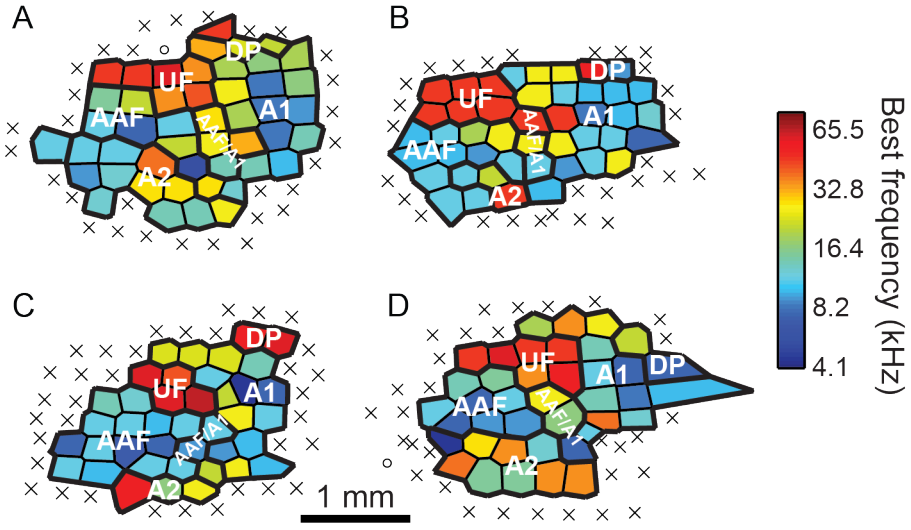

Figure 3.3 Example BF maps from individuals representing each group (A: Naïve, B: P3-4 dam, C: P9-10 dam, D: Post-wean dam). Maps were constructed as in Figure 2.6A-C, with Voronoi polygons surrounding each auditory recording site, color-coded by BF. Xs mark non-auditory sites, and Os mark sites belonging to an unknown subregion.

considered to be a third core subregion, UF. Non-core areas A2 and DP were also apparent in the ventral and dorsoposterior AC, respectively.

I first pooled recordings without respect to maternal status in order to characterize the properties of the different AC subregions, as designated based on my field assignment criteria. As one would expect, significant subregional differences arose for the response properties employed as field assignment criteria. BF and CF distributions were distinct to subregion (Figure 3.4A), and were highly correlated with one another on a per-unit basis (Figure 3.4B). As one would expect, they tended to skew high in UF (median BF = 47.7 kHz; median CF = 43.0 kHz), which by definition contains sites with BFs in the high ultrasound range. Core areas A1 and AAF shared similar BF distributions, with peaks in the teens (A1: median = 12.5 kHz, P_{25} = 10.1 kHz, P_{75} = 18.8 kHz; AAF: median = 11.2 kHz, P_{25} = 10.1 kHz, P_{75} = 13.8 kHz), while AAF/A1 BFs were slightly higher (AAF/A1: median = 20.9 kHz, P_{25} = 12.5 kHz, P_{75} = 31.6 kHz). Non-core regions

Table 3.1 Number of multi-units recorded from each maternal group and AC subregion. In each cell, top line lists number of multi-units recorded from all experiments, including animals from which only partial maps were constructed. Bottom line lists only the number of units belonging to complete maps (i.e., maps in which a complete perimeter of non-responsive units encircles the AC).

Region		Naïve	P3-4 dam	P9-10 dam	Post-wean dam	All animals
All AC	All recorded	416	384	336	348	1484
	In complete maps	326	278	316	322	1242
UF	All recorded	53	52	50	38	193
	In complete maps	37	37	44	34	152
AAF/A1	All recorded	39	26	18	24	107
	In complete maps	22	15	14	22	73
AAF	All recorded	140	141	102	106	489
	In complete maps	99	93	92	95	379
A1	All recorded	122	117	97	111	447
	In complete maps	101	92	96	103	392
A2	All recorded	69	41	52	52	214
	In complete maps	60	37	52	50	199
DP	All recorded	19	16	22	20	77
	In complete maps	17	15	19	19	70
Unknown	All recorded	11	13	12	18	54
	In complete maps	10	2	12	18	42

A2 and DP contained a wider range of BFs (A2: median = 18.8 kHz, P_{25} = 12.5 kHz, P_{75} = 35.0 kHz; DP: median = 25.7 kHz, P_{25} = 16.6 kHz, P_{75} = 43.0 kHz); these results match what others have shown (Stiebler et al., 1997; Joachimsthaler et al., 2014). Overall, these BF distributions are consistent with other reports in which the mouse auditory system is found to be most responsive to frequencies between 10 and 20 kHz.

Aside from BF distribution, onset latencies differed predictably by subregion (Figure 3.5A; one-way Kruskal-Wallis test: χ^2 [5] = 308.39, p = $1.6*10^{-64}$), though a clean separation between core and non-core areas was not observed (i.e., for no response property were core regions all comparable to one another, and all different from non-core regions). Latencies were longest in A2 (median = 18 ms, P_{25} = 15 ms, P_{75} = 22 ms) and shortest in UF (median = 10 ms, P_{25} = 9 ms, P_{75} = 13 ms) and AAF (median = 11 ms, P_{25} = 9 ms, P_{75} = 14 ms), as one would expect for non-core and core areas, respectively. As others have demonstrated in the anesthetized mouse, latencies were significantly longer in core field A1 (median = 17 ms, P_{25} = 11 ms, P_{75} = 23 ms) versus AAF (Linden et al., 2003; Guo et al., 2012). Latencies were shorter in DP (median = 13 ms, P_{25} = 10 ms, P_{75} = 18 ms) compared to A2, even though these are both non-core fields. This pattern of results, in which core fields (with the exception of A1) have shorter latencies than A2, with DP latencies falling intermediate, is consistent with reports from the awake mouse, showing that latency differences between subregions persist under anesthesia (Joachimsthaler et al., 2014). Bandwidths also helped dictate subregion assignment, and differed accordingly by field (Figure 3.5B; one-way Kruskal-Wallis test: χ^2 [5] = 128.73, p = $4.4*10^{-26}$). They are reported here as unitless Q20s, which are calculated by dividing the CF by the bandwidth at 20 dB above threshold, such that lower Q20s indicate wider tuning bandwidths. Consistent with prior work (Stiebler et al., 1997; Issa et al., 2014; Joachimsthaler et al., 2014), Q20s were lowest for area A2 (median = 1.05, P_{25} = 0.73, P_{75} = 1.51) and higher for core areas (A1: median = 1.59, P_{25} = 1.18,

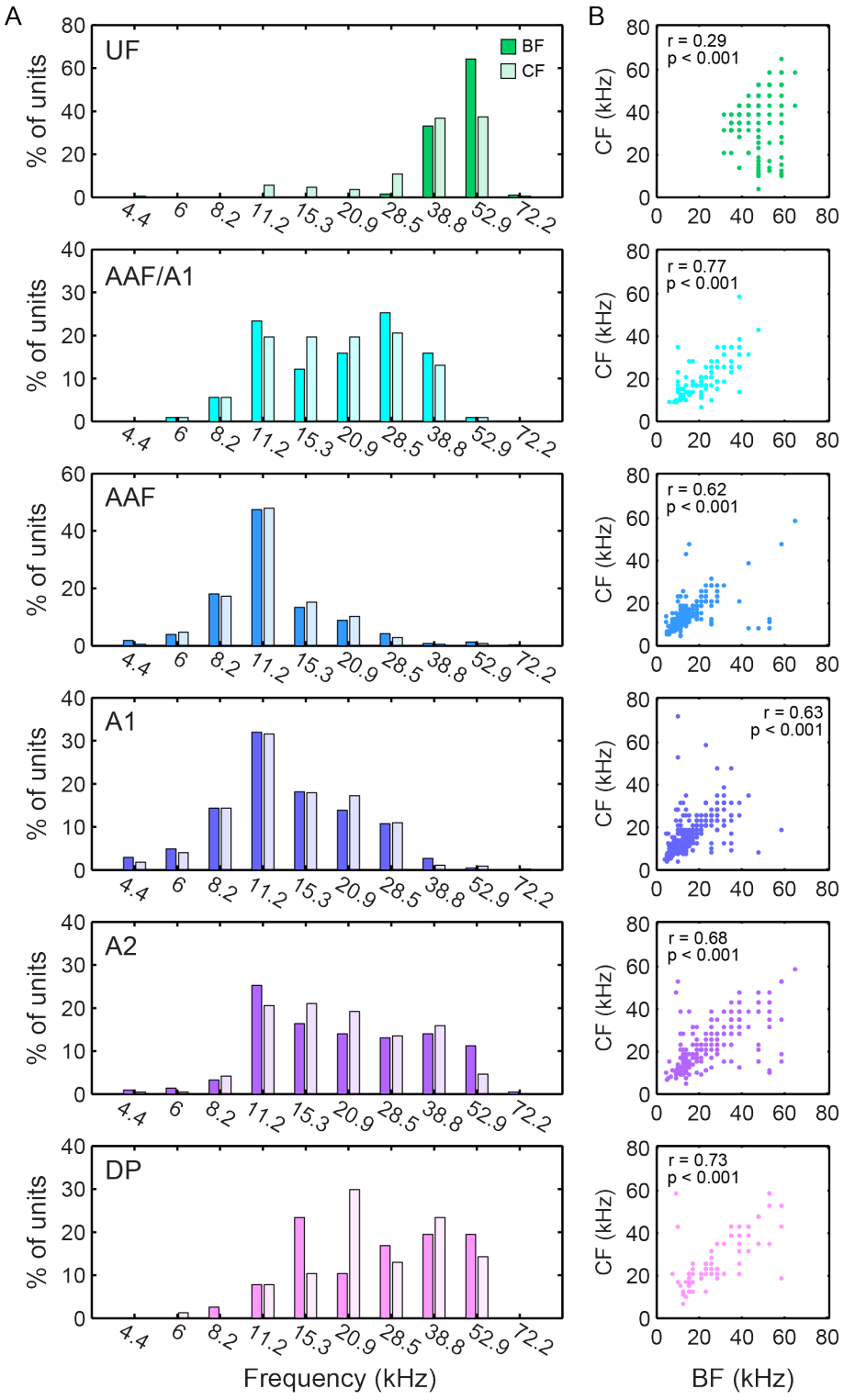

Figure 3.4 Frequency tuning by AC subregion. **A** BF (bold color) and CF (pastel) distributions for each AC subregion. Multi-units corresponding to each subregion were pooled regardless of maternal status of the animal they were recorded from, and expressed as a percent of all units assigned to the same subregion. **B** Scatter plots show each unit's BF plotted against its BF.

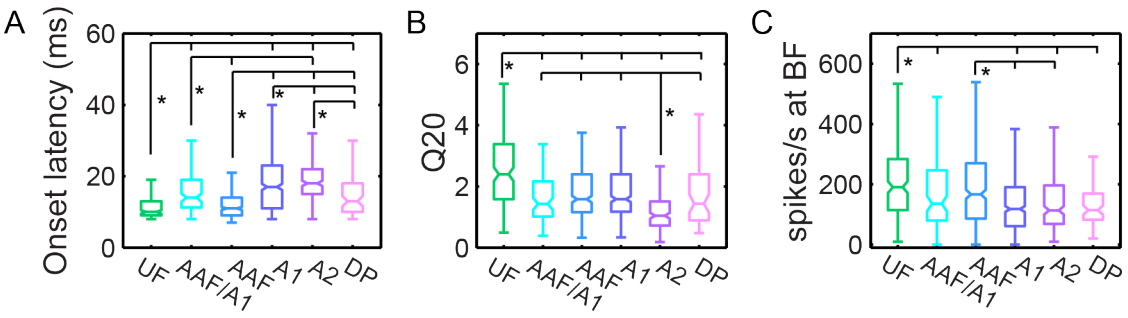

Figure 3.5 Median response properties for parameters factoring into subregion assignment. For all plots, boxes illustrate median ± 25th and 75th percentiles, and whiskers illustrate range of data, excepting statistical outliers. One-way Kruskal-Wallis tests were significant for all 3 parameters, and * indicates significant Tukey's HSD for underlying subregion, compared to others indicated with brackets. **A** Onset latency, as determined manually by a blinded observer. **B** Q20 (tuning bandwidth at 20 dB above threshold [kHz]/CF [kHz]). Lower Q20 indicates wider bandwidth. **C** Firing rate evoked by a pure tone at the unit's BF, calculated over the manually-determined time window encompassing the excitatory response.

P_{75} = 2.40; AAF: median = 1.59, P_{25} = 1.16, P_{75} = 2.40). They were highest for area UF (median = 2.40, P_{25} = 1.58, P_{75} = 3.38), indicating that high-frequency-tuned sites tend to have the narrowest tuning bandwidths. Units belonging to non-core area DP represented a wide range of Q20s, with a median intermediate to A2 and the core subregions (median = 1.44, P_{25} = 0.89, P_{75} = 2.40).

Finally, tone-evoked firing rates can provide an indirect window into the degree to which an area can be excited by simple stimuli. Because non-core regions are thought to be more effectively excited by more complex stimuli with some frequency modulation, one might expect to see lower tone-evoked firing rates in non-core as opposed to core subregions. Consistent with that, evoked firing rates varied by field (Figure 3.5C; one-way Kruskal-Wallis test: χ^2 [5] = 69.72, p = $1.2*10^{-13}$), with non-core areas tending to show the lowest firing rates in response to tones presented at each unit's BF (A2: median = 114 spikes/s, P_{25} = 69 spikes/s, P_{75} = 197 spikes/s; DP: median = 114 spikes/s, P_{25} = 83 spikes/s, P_{75} = 169 spikes/s). The highest firing rates were observed in core areas UF (median = 190 spikes/s, P_{25} = 114 spikes/s, P_{75} = 283 spikes/s) and AAF (median = 167 spikes/s, P_{25} = 87 spikes/s, P_{75} = 269 spikes/s).

Other acoustic parameters differed reliably by AC field, even though they were not factored into subregion assignment. Thresholds were lowest in A1 (median = 25 dB SPL, P_{25} = 5

dB SPL, P_{75} = 45 dB SPL) and AAF (median = 25 dB SPL, P_{25} = 15 dB SPL, P_{75} = 45 dB SPL), while non-core fields (A2: median = 35 dB SPL, P_{25} = 15 dB SPL, P_{75} = 45 dB SPL; DP: median = 35 dB SPL, P_{25} = 25 dB SPL, P_{75} = 45 dB SPL) had higher thresholds (Figure 3.6A; one-way Kruskal-Wallis test: χ^2 [5] = 41.73, p = $6.7*10^{-8}$). The higher thresholds observed in UF (median = 35 dB SPL, P_{25} = 25 dB SPL, P_{75} = 45 dB SPL) are consistent with higher behavioral thresholds at frequencies near the limit of hearing.

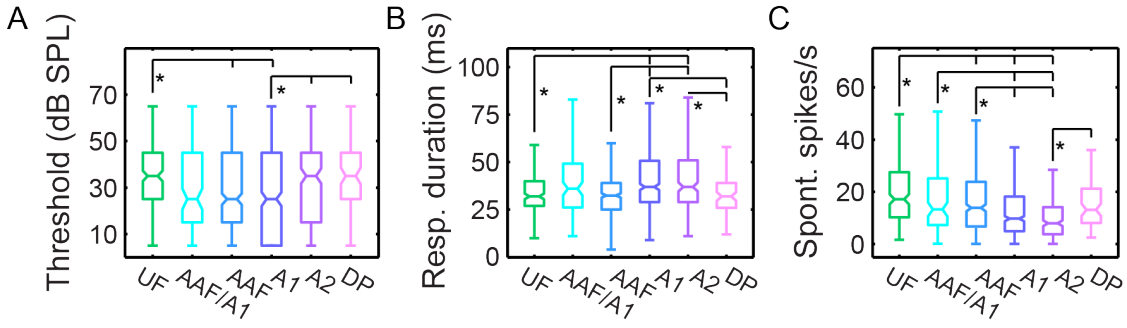

Figure 3.6 Median response properties for parameters not factoring into subregion assignment. As in Figure 3.5, boxes illustrate median ± 25th and 75th percentiles, and whiskers illustrate range of data, excepting statistical outliers. One-way Kruskal-Wallis tests were significant for all 3 parameters, and * indicates significant Tukey's HSD for underlying subregion, compared to others indicated with brackets. **A** Threshold, defined as quietest intensity at which an auditory response is evoked. **B** Response duration, as determined manually by a blinded observer. **C** Spontaneous rate, captured in a 100-ms time window beginning 200 ms prior to stimulus onset, and averaged over all trials from a given recording.

Response durations differed by subregion in an unsystematic way (Figure 3.6B; one-way Kruskal-Wallis test: χ^2 [5] = 62.06, p = $4.5*10^{-12}$), with core area A1 and non-core area A2 sharing the longest durations (A1: median = 37 ms, P_{25} = 29 ms, P_{75} = 51 ms; A2: median = 37 ms, P_{25} = 29 ms, P_{75} = 51 ms). The shortest durations were observed in UF (median = 32 ms, P_{25} = 27 ms, P_{75} = 40 ms), AAF (median = 33 ms, P_{25} = 25 ms, P_{75} = 39 ms), and DP (median = 32 ms, P_{25} = 26 ms, P_{75} = 39 ms).

Finally, spontaneous firing rates were lowest in A1 (median = 10 spikes/s, P_{25} = 5 spikes/s, P_{75} = 18 spikes/s) and A2 (median = 8 spikes/s, P_{25} = 4 spikes/s, P_{75} = 14 spikes/s), with the remaining core areas (AAF: median = 14 spikes/s, P_{25} = 7 spikes/s, P_{75} = 24 spikes/s; UF:

median = 17 spikes/s, P_{25} = 10 spikes/s, P_{75} = 28 spikes/s) showing the highest spontaneous rates (Figure 3.6C; one-way Kruskal-Wallis test: χ^2 [5] = 102.62, p = $1.5*10^{-20}$). This pattern of results was somewhat reminiscent of the trend observed for evoked firing rates, in which core areas tended to have higher rates than non-core areas. Unlike for evoked firing rates however, area DP showed a higher spontaneous rate (median = 13 spikes/s, P_{25} = 8 spikes/s, P_{75} = 21 spikes/s) than non-core region A2. This effect may be caused by the more bursty baseline activity characteristic of DP (Stiebler et al., 1997).

Table 3.2 Average sizes of AC subregions in the *CBA/CaJ* mouse, expressed in absolute mm^2 and as a proportion of total AC area.

Region	**Absolute area (mm^2, ± STD)**	**Proportionate area (% of all AC, ± STD)**
All AC	3.74 ± 0.54	100
UF	0.45 ± 0.14	12.1 ± 3.9
AAF/A1	0.22 ± 0.11	5.9 ± 2.8
AAF	0.92 ± 0.29	24.8 ± 7.5
A1	1.17 ± 0.24	31.3 ± 5.3
A2	0.62 ± 0.26	16.2 ± 6.1
DP	0.22 ± 0.13	5.8 ± 3.5

Finally, the area of each subregion, as a percentage of the entire AC area, was calculated by summing the areas of the Voronoi polygons assigned to each subregion, for a given animal (Table 3.2). The average size of the entire AC was 3.74 ± 0.54 mm^2. This area is slightly smaller than the AC size reported in Stiebler *et al.* (1997), potentially because of a strain difference, as that study used the outbred NMRI mouse rather than the *CBA/CaJ* employed here. Despite this disparity, the proportionate sizes of core fields are consistent with those described by Stiebler and colleagues: A1 was the largest subregion, followed by AAF, and these regions together comprised more than 50% of the total AC area. UF was smaller, occupying approximately 12%

of the total area of AC. The sizes of non-core fields, on the other hand, differ from those previously reported. Whereas the left DP and A2 were approximately the same size in (Stiebler et al., 1997), most animals in the present study had a small or absent DPs, and an A2 that was larger than expected. Interestingly though, the sum total area of non-core fields in the present study was comparable to that observed by Stiebler and colleagues, possibly suggesting compensatory changes in non-core subregion sizes.

3.3.2 Tonotopic map in AC is stable throughout motherhood

Having characterized the average AC organization in the *CBA/CaJ* mouse, I next sought to address my initial hypothesis, asking whether tonotopic map plasticity might be observed in maternal animals following sensitization to pups. Since sites with BFs in the ultrasound range in which pups call are generally found in the core field UF, and UF is functionally (rather than anatomically) defined, I first asked whether UF may increase in size in maternal mice. The proportionate size of UF, however, did not differ with maternal experience (Figure 3.7B, left; one-way ANOVA: $F[3,27] = 1.59$, $p = 0.22$). Further, there was no overall difference in AC size over the course of motherhood (Figure 3.7A; one-way ANOVA: $F[3,27] = 0.39$, $p = 0.76$), nor were there any apparent changes in size for any other subregion (Figure 3.7B; one-way ANOVAs: AAF/A1: $F[3,27] = 1.82$, $p = 0.17$; AAF: $F[3,27] = 1.04$, $p = 0.39$; A1: $F[3,27] = 0.23$, $p = 0.88$; A2: $F[3,27] = 0.98$, $p = 0.42$; DP: $F[3,27] = 0.1$, $p = 0.96$). Thus, experience with pup USVs did not appear to alter the subregional organization of AC throughout motherhood.

Though area UF did not expand at any of the maternal time points tested, the possibility remained that the maternal AC was reorganized to over-represent the behaviorally relevant ultrasound frequencies, but that the retuned sites were not spatially contiguous with UF and were

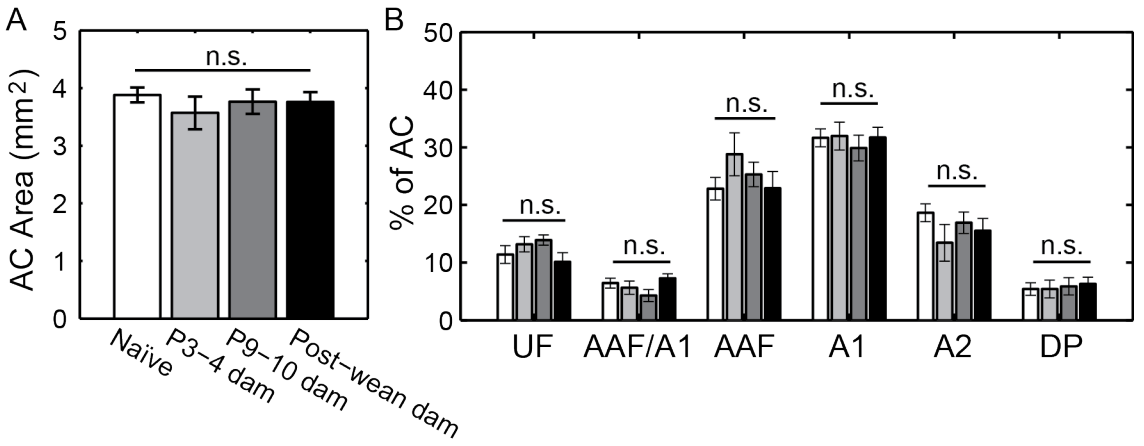

Figure 3.7 Maternal status does not affect size of AC or its subregions. **A** Total size of AC. Bars illustrate group means ± SEM. **B** Proportionate size of each subregion. "n.s." indicates non-significant one-way ANOVA ($p > 0.05$).

therefore not included in the UF area measurement. To address this possibility, I next assessed the proportionate area of AC tuned to each of 6 frequency ranges (as in Figure 2.7E). This analysis, too, provided no evidence for map plasticity, as motherhood was not associated with changes in proportionate area of AC tuned to any of the BF ranges tested (Figure 3.8A; one-way ANOVAs: 4-6 kHz band: $F[3,27] = 1.65$, $p = 0.20$; 6.1-10.1 kHz band: $F[3,27] = 1.07$, $p = 0.38$; 10.2-17 kHz band: $F[3.27] = 0.56$, $p = 0.65$; 17.1-28.5 kHz band: $F[3,27] = 2.52$, $p = 0.08$; 28.6-47.7 kHz band: $F[3,27] = 0.32$, $p = 0.81$; 47.8-80 kHz band: $F[3,27] = 0.76$, $p = 0.52$). Further, the BF distributions of each AC subregion did not differ throughout motherhood, indicating that local map plasticity, restricted to an individual subregion, does not occur (Figure 3.8B-G).

Taken together, these analyses fail to support the hypothesis that BF map plasticity occurs for frequencies in the ultrasound range following the experience with pup USVs that accompanies motherhood. Instead, they depict a map of the AC that is stable throughout motherhood, at least with respect to its subregional organization and the spatial organization of its frequency tuning.

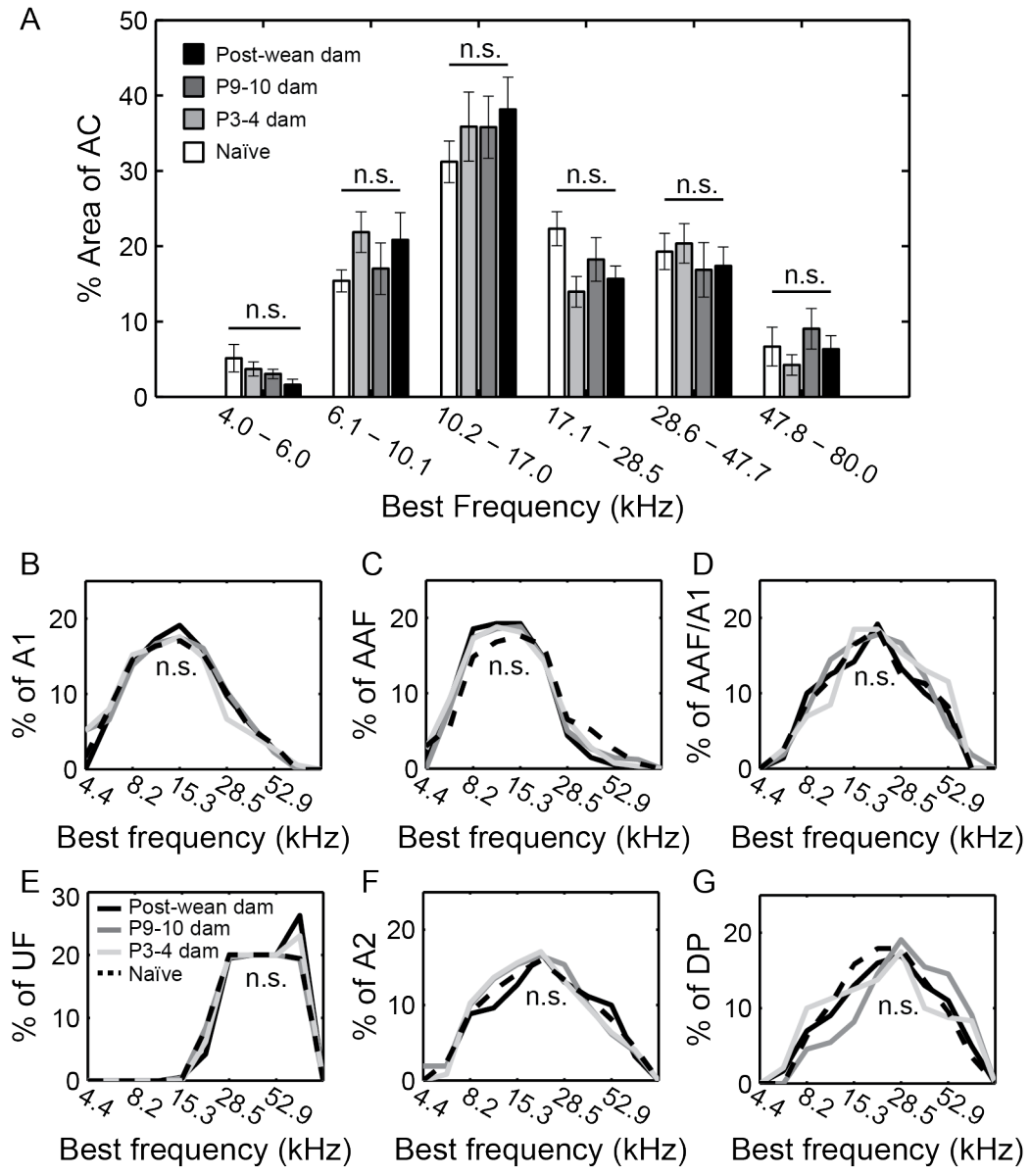

Figure 3.8 Distribution of BFs is stable throughout motherhood. **A** Area tuned to each of 6 frequency bands, taken as a proportion of the entire AC. Bars illustrate group means ± SEM. "n.s." indicates non-significant one-way ANOVA ($p > 0.05$). **B-G** Smoothed BF histograms for each subregion. Bar plots as in A were not constructed due to small sample sizes for some subregions. "n.s." indicates all non-significant Kolmogorov-Smirnov tests between each maternal group and the naïve group ($p > 0.05$).

3.3.3 Maternal suppression of ultrasound responses in core sites representing a lateral frequency band

Though BF distributions do not apparently change in the maternal AC, there exists the possibility that systematic changes in tuning do alter responses in the behaviorally relevant

ultrasound range, without shifting BFs. Indeed, prior work in our lab has found that in core AC, ultrasound-evoked inhibition of single neurons tuned to low frequencies (< 40 kHz) is stronger in mothers than in pup-naïve females, while the strength of inhibition in neurons with high BFs (> 40 kHz) is comparable between groups (Galindo-Leon et al., 2009). Ultrasound-evoked overall excitation in single units showed no such modulation by maternal status. This observation has led to the hypothesis that, upon ultrasound presentation, net population activity (excitation – inhibition) in low-frequency-tuned regions of core AC (i.e., A1 and AAF), but not in area UF, would be suppressed to a greater degree in mothers compared to naïve females (Figure 3.9). The result would produce, in mothers, greater contrast in activity between high- and low-frequency-tuned regions of core AC, possibly supporting better detection of ultrasounds at downstream sites.

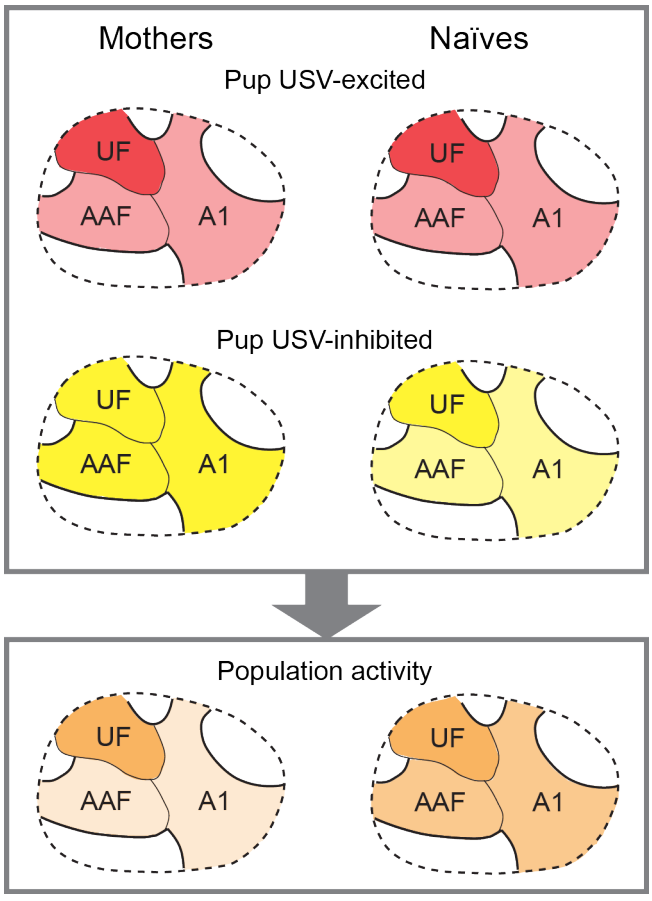

Figure 3.9 Hypothesized suppression of ultrasound-evoked activity in AAF and A1 (bottom box), stemming from known effects of motherhood on strength of excitation and inhibition among core single units (top box). Depth of shading corresponds to relative strength of activity. Adapted from Banerjee and Liu (2013).

My dataset provided an opportunity to test this hypothesis, and potentially clarify when, over the course of motherhood, any such plasticity arises. To that end, I averaged the firing rate responses to a subset of ultrasonic tones (65-80 kHz, 55-65 dB SPL) over a 100-ms time window beginning at stimulus onset. For each multi-unit, responses were normalized by subtracting the spontaneous rate. Averaging together the normalized firing rates of all AAF, A1, and AAF/A1 units per group revealed a suppression of ultrasound responses (Figure 3.10A; one-way ANOVA: $F[3,944] = 6.76$, $p = 0.0002$). Ultrasound-evoked firing rates in these "lateral-band" fields were near zero in post-wean dams and P9-10 dams; both of these groups' evoked firing rates were significantly lower than those of naïve animals. Firing rates observed in P3-4 dams were not lower than those of naïves, but were statistically greater than those of P9-10 dams. Consistent with our hypothesis, no such suppression occurred for UF sites (one-way ANOVA: $F[3,186] = 1.9$, $p = 0.13$). Similar results were observed when core units were grouped by BF rather than subregion. Firing rate responses of core multi-units with BFs below 40 kHz in P9-10 and post-wean dams were significantly lower than those observed in naïve mice (Figure 3.10B; one-way ANOVA: $F[3,973] = 5.86$, $p = 0.0006$), but no significant suppression was observed among core units with BFs above 40 kHz (one-way ANOVA: $F[3,157] = 1.18$, $p = 0.32$).

To further validate this hypothesis, I also examined firing rates evoked by a single pup USV exemplar (Figure 4.1, USV 1). Again, suppression of responses to this ultrasonic stimulus was observed in AAF, A1, and AAF/A1 multi-units in mothers, relative to naïve animals (Figure 3.10C; one-way ANOVA: $F[3,914] = 5.16$, $p = 0.002$). As in the ultrasonic tone analysis, post-wean dams exhibited a significant suppression of the evoked response to USVs relative to naïve mice. In contrast to what was observed using tones though, significant suppression was observed in P3-4 dams, but *not* in P9-10 dams, relative to naïves. Among UF multi-units, suppression of ultrasound responses was not observed for any maternal group compared to the naïve group,

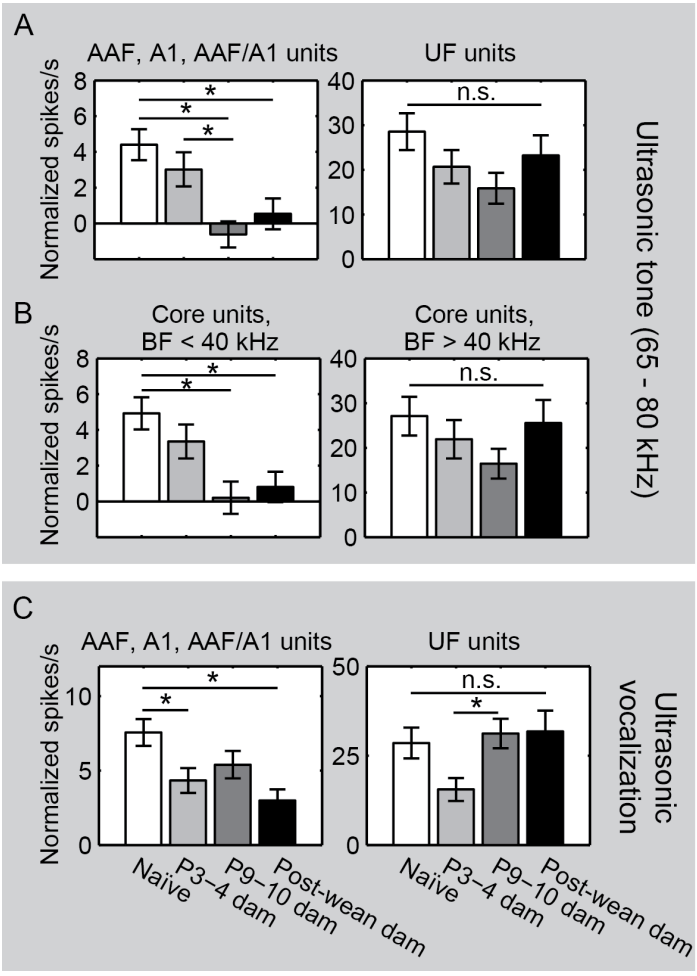

Figure 3.10 Maternal suppression of ultrasound responses in core sites with low BFs. **A** Firing rate response (evoked – spontaneous spikes/s) in core fields AAF, A1 and AAF/A1 (left) vs. UF (right) to tones between 65 and 80 kHz, as a function of maternal status. **B** Firing rates calculated as in A, grouping units by BF. **C** Firing rates, calculated as in A, evoked by a single USV exemplar. Units are grouped by subregion, as in A. Bars illustrate means ± SEM. * indicates significant Tukey's HSD following a significant one-way ANOVA ($p < 0.05$). "n.s." indicates non-significant one-way ANOVA ($p > 0.05$).

though the response magnitude among P3-4 dams was significantly lower than that observed in P9-10 dams (one-way ANOVA: $F[3,187] = 3.25$, $p = 0.02$).

These analyses indicate that while gross reorganization of the frequency tuning map does not occur at the maternal time points analyzed here, the spatial representation of ultrasounds across core AC (including units with BFs outside the ultrasound range) is changed more subtly by motherhood. Specifically, ultrasound-evoked activity is suppressed among low-frequency-tuned units in maternal mice, while response strength in high-frequency-tuned areas is not changed by

maternal experience. This pattern of activity produces greater contrast between the low- and high-frequency-tuned populations in core AC.

3.4 Discussion

To my knowledge, this study constitutes the first attempt to characterize AC map plasticity in adulthood following learning that occurs in an ethological context, rather than a laboratory conditioning paradigm. By mapping the ACs of four groups of female mice - spanning the maternal timeline from pup-naïve through post-weaning - I showed that map expansion for ultrasound frequencies does not apparently occur. Rather, the map of BFs across the AC appears to remain stable throughout pup rearing. Despite this map stability, a change in the population response to ultrasonic frequencies was observed, wherein motherhood led to a suppression of ultrasound-evoked firing rates that was restricted to core AC units with BFs below 40 kHz (largely AAF, A1, and AAF/A1 sites). The ultrasound response was not changed in units with BFs above 40 kHz. This pattern of results supports a hypothesis formulated from previous work in our lab, showing an enhancement of pup USV-evoked inhibition in low-frequency-tuned sites in core AC.

3.4.1 Gross organization of the CBA/CaJ mouse AC

Multi-units recorded across the AC were assigned to subregions based on how closely their response properties matched those associated with specific auditory fields according to previous research. Though only a handful of studies have attempted to characterize the auditory fields of the mouse in a comprehensive way, there is some consensus about the response properties typical of certain subregions. Since they are situated higher up in the auditory pathway, non-core regions A2 and DP, for instance, have longer response latencies than do core regions (Guo et al., 2012; Joachimsthaler et al., 2014). Non-core fields are also thought to have more complex receptive fields, since they incorporate input from neurons in core AC and the auditory

thalamus that have simpler receptive fields; this complexity is often apparent in the form of broad or multi-peaked tuning curves (Stiebler et al., 1997; Issa et al., 2014; Joachimsthaler et al., 2014). The most informative response property in many cases, however, is BF – as in most mammalian ACs, tonotopy is readily apparent in A1 and its sister field, AAF (Stiebler et al., 1997; Linden et al., 2003; Guo et al., 2012; Issa et al., 2014; Joachimsthaler et al., 2014), and departures from tonotopic organization are helpful in delineating adjacent non-core fields.

Nevertheless, there remains a high degree of disagreement regarding which properties best define a subregion. For instance, while AAF has been shown to have shorter response latencies than A1 by three separate groups (Linden et al., 2003; Sawatari et al., 2011; Guo et al., 2012), a recent study in awake mice failed to find any latency difference between these two regions (Joachimsthaler et al., 2014). There is even some disagreement on which subregions should be defined as discrete auditory fields. Though Stiebler *et al.* describe units tuned to ultrasonic frequencies as residing in a unique ultrasound field UF, Guo *et al.* recognize only two core subregions, suggesting instead that area UF is actually the apex of the high-frequency band at which A1 meets AAF. Additionally, Sawatari *et al.* and Guo *et al.* describe a novel non-core auditory field, the insular auditory field (IAF), situated rostral and ventral to AAF. This region is identified largely by its BFs, which do not fit the tonotopic gradient of adjacent area AAF. Though they do not characterize it as a separate subregion, Stiebler *et al.* did report the appearance of a similar cluster of units that did not apparently fit into any known field, though it was not seen in all subjects. Here, any such units were classified as belonging to an "unknown" subregion and were not included in subsequent analyses.

The data presented here agree most closely with the AC organization described in Stiebler *et al.* (1997), though this is perhaps not surprising given that the recording methodology and AC subregion assignment criteria employed here best match the methods used in that study (i.e., animals were anesthetized, stimuli were similar, electrode spacing was comparable, and similar response properties were used to delineate subregions). Here, I describe the same AC

fields, though the high-frequency reversal band where A1 meets AAF was classified as "AAF/A1" to avoid improper classification of ambiguous sites. Further, the subregions described here are approximately the same size as those reported in Stiebler *et al.* (1997), save for DP and A2, which are slightly smaller and larger, respectively, than Stiebler *et al.* described. This disparity could be owing to a strain difference, as that study made use of *NMRI* mice, whereas the *CBA/CaJ* mouse was under study here.

3.4.2 Absence of map plasticity for ultrasounds in the maternal AC

Tracking AC organization over the course of motherhood, from a pup-naïve time point through pup weaning, I did not observe evidence of map plasticity for behaviorally relevant ultrasound frequencies. This finding counters the theory that map expansion is necessary for memory formation or storage, whether as a transient stage of learning or as the final engram of an auditory memory. However, though unlikely, it is possible that experimental factors, rather than a true physiological absence of plasticity, prevented me from observing map reorganization. First, given concerns about the clarity of tonotopy in the mouse AC, it may be that map plasticity cannot be observed in this species. However, prior studies, including the work described in chapter 2 of this dissertation, challenge this idea by demonstrating tonotopic map plasticity in the mouse AC (Barkat et al., 2011). Thus, it seems likely that I would have observed map plasticity in maternal mice, were the circumstances conducive to its development.

Second, it may be that a transient peak in map plasticity occurred very shortly after parturition, and had already dissipated before the first maternal time point was recorded at P3-4. This hypothesis could explain the absence of plasticity observed here, but seems unlikely given the very short time scale on which map plasticity would then have to emerge and dissipate. Prior work involving laboratory conditioning to tones has reported map plasticity after anywhere from 6 to 37 hours' worth of non-consecutive training sessions (Polley et al., 2006; Hui et al., 2009; Bieszczad and Weinberger, 2010c). Since these training sessions involve continuous engagement

with sounds, whereas the maternal context presumably involves more sporadic engagement with USVs depending the pups' behavior, it is difficult to estimate how this time frame maps onto the one used in this experiment. Nevertheless, if map plasticity did not emerge and dissipate in those prior studies, after up to 1.5 days' worth of continuous acoustic reinforcement, one can reasonably assume that such a transient peak in map plasticity would not have occurred prior to the earliest maternal time point measured here (P3-4), as reinforcement is considerably more intermittent in the maternal context. Moreover, while map expansion was not evident at any time point, the suppression of the ultrasound response among units with lower BFs emerged between P3-4 and P9-10. Thus, this time scale was sufficient to capture plasticity in the ultrasound response; one might presume that if such suppression results from mechanisms shared by map plasticity, map changes would have been observed here as well, had they occurred.

Third, it is possible that the sampling resolution used to construct maps in this study was not sufficiently fine to detect map plasticity. Spacing between electrodes was approximately 300 μm, and while map plasticity has been detected before using comparably spaced electrodes (chapter 2; Bieszczad and Weinberger, 2010c), it is possible that more naturalistic experiences, like motherhood, produce more subtle plasticity that is only detectable with finer resolution. Addressing this possibility by further decreasing the interelectrode spacing, though, would increase the likelihood of recording overlapping neural populations through adjacent electrodes. The spacing used here was intended to reduce the incidence of such redundancy, though it does introduce the possibility that a slight map expansion was missed. While that cannot be ruled out, it can be said that map plasticity on the scale of what has been observed following behavioral conditioning (i.e., a doubling of the representation of the behaviorally relevant frequency range), was not seen here following natural experience with pups and their USVs.

Finally, it could be argued that this study overestimates the relevance of pup USVs to maternal mice. Because USVs are emitted as distress signals upon isolation from the nest, and the animals under study here were reared in standard mouse cages where the dam can never be more

than a few inches from the nest, one might assume that a mouse pup rarely has occasion to emit isolation calls. In the experience of our lab however, sporadic USVs *can* be heard when ambient sounds are recorded from an undisturbed litter and its dam. These USVs are likely produced by pups at the periphery of the nest. I nevertheless conducted pup retrieval trials on a subset of dams in this study to ensure that they gained experience with pup USVs. Though an effect of this experience was not apparent here, such a "training" intervention is regularly employed in our lab and has led to the observation of plasticity in the past.

Even with these caveats in mind, it is clear that map plasticity on the order of what others have found following conditioning with pure tones (i.e., a two-fold expansion of the representation of the behaviorally relevant frequency in the BF map) does not occur in maternal mice following natural experience with pup USVs. This finding argues against the idea that persistent map reorganization serves as the enduring memory trace of an acoustic experience. The notion that map plasticity occurs as a stage of learning has somewhat more face validity (Reed et al., 2011), but the present study opposes this hypothesis as well. It has been suggested that transient map plasticity represents the retuning of a large subset of AC neurons, a mere subset of which maintain this tuning shift over time; these neurons that maintain their tuning shifts may serve as the neural basis of memory. If this were the case, an auditory memory could conceivably develop without map plasticity occurring at all. Indeed, in an ideal system, only the neurons that ultimately retain their tuning shifts would ever undergo plasticity in the first place. The mechanisms underlying auditory memory formation in the real world may not operate quite so efficiently, but nevertheless, physiological systems have an incentive to minimize needless reorganization. The result of such an incentive may come to light in the more ethological model employed here. While "map" plasticity does not occur at these maternal time points, our lab has provided abundant evidence for experience-dependent plasticity in the maternal mouse, much of it only apparent in single unit recordings (Shepard et al.; Galindo-Leon et al., 2009; Lin et al., 2013).

3.4.3 Relationship to prior work

Like the present study, the earliest work from our lab made use of multi-unit electrophysiology in anesthetized mice, though the recording strategy prevented the construction of complete AC maps. Instead, this work revealed that motherhood brought changes in the neural entrainment to call onsets within a train of pup USVs, as the maternal AC was better able to "keep up" with USV bouts with repetition rates near the mean natural call rate of 5 Hz (Liu et al., 2006). Liu and colleagues also showed, using mutual information-based analyses, that multi-unit recordings taken from mothers were better able to detect and discriminate among different pup USV exemplars (Liu and Schreiner, 2007). Interestingly, the latter study also described response onset latencies to pup USVs that were shifted earlier in mothers as compared to pup-naïve females. Though I do not report onset latencies to USVs specifically in this dissertation, this result was not borne out in my data set. This disparity may be due to a slight difference in the recording methodology, specifically the use of an electrode with higher impedance than the one used in Liu's early work (Robinson, 1968; Humphrey and Schmidt, 1990). Higher impedance will bias the experimenter toward recording from neurons that have larger extracellular fields, which in layer IV of the AC are primarily pyramidal neurons. Pyramidal neurons have different response dynamics than the neurons a lower-impedance electrode would capture (at this location, more inhibitory interneurons), and may be differentially susceptible to experience-dependent plasticity.

More recent work from our lab has focused on changes in single unit, rather than multi-unit, responses in awake mice. We previously showed that pup USV-inhibited single units are inhibited more deeply and to a greater degree in mothers than in pup-naïve females (Galindo-Leon et al., 2009; Lin et al., 2013). This effect was carried by single units with BFs below 40 kHz, leading to the hypothesis that inhibition of this lateral band leads to greater contrast in the activity of high- versus low-frequency-tuned regions upon pup USV presentation. This hypothesis

is supported by the present study. Subsequent research replicated this lateral band inhibition in co-caring virgin females recently removed from active pup care (1-5 days from pup care experience), but not in co-caring females tested after pup weaning, suggesting that the physiological changes that accompany maternity help reinforce experience-dependent changes arising from pup experience (Lin et al., 2013).

Other researchers have successfully applied the maternal mouse model of auditory learning to show that the AC integrates multimodal pup cues to enhance the evoked response to pup USVs (Cohen et al., 2011), and that network dynamics in the maternal AC appear to be fundamentally altered compared to that of the pup-naïve mouse (Rothschild et al., 2013). In the latter study, the authors used Ca^{2+} imaging with single-neuron resolution to establish that across a local population of neurons, sound-evoked and spontaneous activity were more correlated in mothers than naïve mice. While the authors did not examine whether this effect was apparent in other cortical areas, their finding that the activity correlations persisted in the absence of acoustic stimulation suggests a mechanism related to physiological state, rather than auditory experience. Maternal animals experience dramatic changes in sex hormone levels during pregnancy and throughout lactation, and such hormones can have broad effects on the functioning of neural networks (McEwen, 1991; McEwen et al., 1991; Woolley, 2007). Such hormone fluctuations raise the question of whether observed changes are truly attributable to acoustic experience, or are rather a byproduct of changes in physiological state. While the answerability of such a question varies with experimental design, effects that are frequency-specific (such as the lateral band suppression observed here) are unlikely to stem from global changes in physiology, and are more likely to be informed by acoustic experience.

3.4.4 Summary

Here, I sought evidence for the hypothesis that map plasticity favoring ultrasound frequencies occurs in maternal mice following experience with pup USVs. In contrast to

expectations based on prior work, I failed to observe a change in the representation of ultrasonic frequencies across the BF map. This result was somewhat surprising, since our lab has characterized the maternal AC as a rich site of experience-dependent plasticity for pup stimuli, and since map plasticity has been widely reported following experience with salient acoustic stimuli. Since pup USVs are complex stimuli that vary across many dimensions, it remains possible that map plasticity occurs in mothers, but is driven by a feature or features other than frequency. In the next chapter, I continue to search for evidence of map plasticity across the AC, this time using pup USVs themselves rather than only tones as acoustic stimuli.

Chapter 4

Plasticity for pup USV responses in the maternal adult

In chapter 3, I showed that experience with pups does not lead to large-scale reorganization of the tonotopic map to reflect the enhanced salience of the ultrasound frequency band in which isolation USVs are emitted. Pup USVs, however, are complex acoustic signals, and it is possible that a feature other than frequency or, still more likely, a combination of such features, drives experience-dependent plasticity across the AC map. In this chapter, I address this possibility by directly examining how pup USVs, which by their nature contain all acoustic features characteristic of that class of vocalizations, are represented across the AC.

4.1 Introduction

Pup USVs are, as described in section 3.1, single-frequency whistles bearing a strong resemblance to pure tones. As such, they are ideal for use in studies of auditory plasticity: they resemble stimuli that are commonly used in studies that employ laboratory conditioning paradigms to imbue a sound with relevance. However, pup USVs are used in ethological research on auditory learning precisely because of how they differ from manufactured sounds like pure tones: they vary naturally across multiple acoustic dimensions, in ways that modulate their communicative significance. How the auditory system handles such variation is one of the most compelling mysteries in the field.

Some of the dimensions over which pup USVs vary are predictive of membership to that vocalization class. The most obvious example is frequency: pup USVs are often defined by their ultrasonic frequencies and indeed, this observation motivated the work described in chapter 3. However, pup USVs are also associated with a particular range of durations (Figure 4.1A) and degrees of frequency modulation (FM) at onset (Figure 4.1B), and also share higher-order features like their overall frequency trajectories, which tend to be flatter than other USV classes

(Figure 4.1C). Chapter 3 showed that map plasticity for the ultrasound frequencies of pup USVs does not occur. The possibility remains, however, that plasticity might arise for one of the other features associated with the pup USV class. Indeed, while the literature on map plasticity is dominated by studies of the tonotopic map, experience can also alter maps of intensity tuning (Polley et al., 2006) and bandwidth (Kilgard et al., 2001a). Thus, it is possible that by focusing on frequency tuning, the study presented in chapter 3 missed plasticity occurring in a different feature map in AC.

To test this possibility, I mapped responses to pup USVs themselves over the course of motherhood. Rather than using a single pup USV exemplar, or a randomly selected subset of them, I made use of a stimulus set developed in our lab that systematically sampled USV exemplars across the range of frequencies and durations they are likely to possess (Figure 4.1). This stimulus set provided the additional opportunity to assess how prototypicality – that is, how representative an exemplar was of its entire USV class – impacted the neural response. USVs 1 and 2, for instance, are pup USVs that share frequencies and durations that are very characteristic of that USV class (i.e., reside in the space with highest USV probability density in the brown probability cloud), and can therefore be considered more prototypical pup USVs. USVs 5 and 6, in contrast, have frequencies and durations that are dissimilar to those of the most common pup USVs, and are actually more similar to those that are characteristic of adult USVs (Figure 4.1A, orange probability cloud). These would therefore be described as less prototypical. Since maternal females likely hear the acoustic combinations that define prototypical pup USVs most frequently by definition, one might expect that map expansion could emerge specifically for those parameters or combinations of parameters associated with the most prototypical pup USVs. This stimulus set allowed me to test that possibility.

As a control stimulus, I used a set of "courtship" USVs emitted by adult male mice (Holy and Guo, 2005). These USVs do not carry sustained salience among females (Hammerschmidt et al., 2009; Shepard and Liu, 2011), whereas pup USVs retain their interest to mothers (but not

pup-naïve females) even after pup care experience has concluded (Ehret et al., 1987; Lin et al., 2013). Adult USVs are similar to, yet tend to be acoustically distinguishable from, pup USVs in terms of their characteristic durations, frequencies, degrees of FM at onset (orange contour plots in Figure 4.1A and B), and higher-order features like their frequency trajectories (compare spectrograms in Figure 4.1C). Using these adult USVs as control stimuli can provide a window into whether any observed plasticity is attributable to maternal experience with the behaviorally relevant pup USV class. That is, if maternity-dependent changes are observed among responses to pup USVs with different acoustic features, assessing whether they are also apparent among the responses to a set of adult USVs matched for those acoustic features will clarify whether those

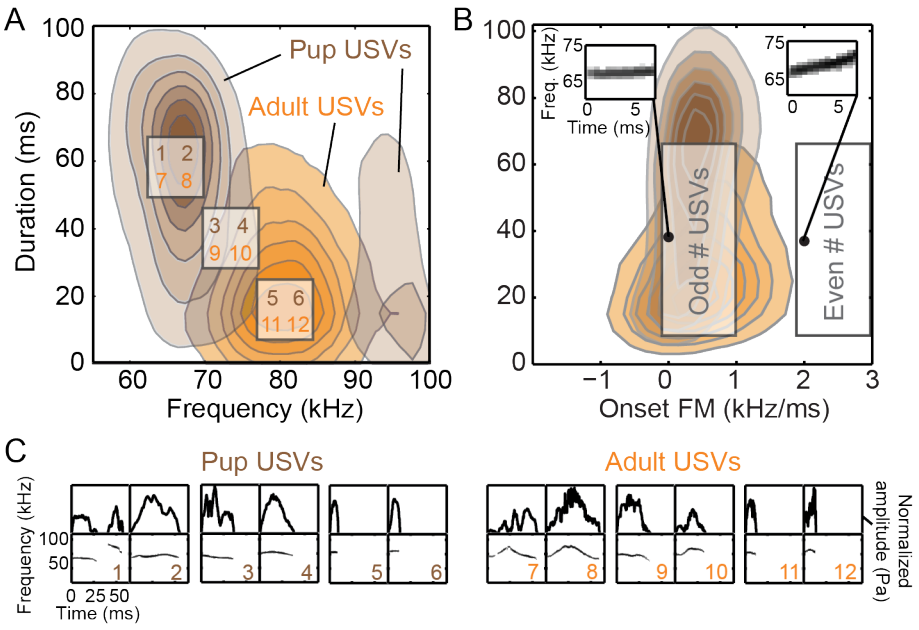

Figure 4.1 Distribution of pup and adult USVs across frequency-duration space. **A** Contour plots represent the probability distributions of pup (brown; n = 51,954 USVs) and adult (orange; n = 11,248) USVs across frequency and duration space. Cells indicate regions of frequency-duration space that USV exemplars used in this study were taken from, and span acoustic space from most pup-USV-like (left, top) to most adult-USV-like (bottom, right). Adapted from Liu *et al.* (2003). **B** Contour plots represent the probability distributions of pup and adult USVs across FM and duration space. USVs of the same class within the same cell in A differ in their degree of frequency modulation at onset, with odd-numbered calls showing little to no frequency modulation (0 to 0.97 kHz/ms), and even-numbered calls showing frequency modulation between 2.97 and 3.97 kHz/ms. Insets illustrate a flat USV onset and a USV onset with more FM. Adapted from (Liu, 2006). **C** Amplitude envelopes and spectrograms of all USVs used in this study. Spectrogram labels correspond to labeled cells in A.

changes are owing to pup experience or some other aspect of motherhood (Miranda and Liu, 2009).

4.2 Method

Subjects

The same experimental cohort described in section 3.2 was used here. To reiterate, multi-unit electrophysiological recordings were taken across the ACs of pup-naïve females ("Naïve"), dams at pup age P3-4 ("P3-4 dam"), dams at pup age P9-10 (P9-10 dam"), and dams that just weaned their first litter ("Post-wean dam"). Mice were *CBA/CaJ* females aged 11 to 17 weeks at the time of recording.

Electrophysiology and data processing

Craniotomy, electrophysiological recording, and data processing were conducted as described in appendix A and section 3.2. Experimental stimuli consisted of pure tones (see section 3.2) and USVs. Vocalization stimuli were a subset of natural pup and adult USVs (6 of each class, see Figure 4.1) taken from a library of 36 USVs selected by our lab (Liu et al., 2003) to systematically sample pup- and adult-USV probability space (probability clouds in Figure 4.1). Each pup USV was matched to an adult USV in the frequency and duration dimensions, as well as in the degree of frequency modulation it exhibited at onset, such that USV 1 was matched to USV 7, 2 to 8, 3 to 9, and so on. The frequency and duration of each USV are apparent from the cell it occupies in Figure 4.1A. Each frequency-duration cell contains one pup and one adult USV with a flat frequency onset (frequency modulation ranging from 0 to 0.97 kHz/ms), and one pup and one adult USV with frequency modulation at onset (2.97 to 3.97 kHz/ms).

Pup USVs were recorded from pups at P7, and adult USVs were recorded from adult males presented with an adult female. These USVs were high-pass filtered in MATLAB (25 kHz

corner, 8-order Butterworth filter), denoised, and Hilbert transformed to extract the frequency and amplitude envelopes. These envelopes were used to re-synthesize a clean version of each USV against a silent background. These stimuli were convolved with a 0.5 ms $\cos^2$ onset/offset function, and scaled to a maximum amplitude of 65 dB SPL. Stimuli were repeated 15 times each in pseudorandom order through a free-field speaker (EMIT high-energy speaker, Infinity Systems, Stamford, CT) positioned 11 cm lateral to the right ear.

Offline, units were judged responsive or non-responsive to each USV exemplar through the use of a simple algorithm. Custom MATLAB software compared the distribution of average firing rates evoked by an exemplar (over a 100-ms time window beginning at stimulus onset) to the distribution of spontaneous firing rates (calculated over a 100-ms window beginning 200 ms prior to stimulus onset) using a 2-sample t-test. USVs were judged to have elicited a response if the t-test returned a p value < 0.001 (to account for the large number of comparisons).

Statistical analysis

Lilliefors tests were conducted on data sets to confirm that they were normally distributed. If they met this criterion, data were analyzed with a one- or two-way ANOVA, depending on the design of the comparison. When this analysis yielded a significant effect ($p < 0.05$), Tukey's HSD was calculated for pairwise, post-hoc comparisons. Chi-square tests were used to compare responsive and non-responsive proportions of subregions.

4.3 Results

4.3.1 Auditory cortical map of USV responses is stable across motherhood

When executing electrophysiological mapping experiments, it became evident that there was not a simple correlation between a multi-unit's frequency tuning and its responsiveness to USVs. Sites with BFs well outside the ultrasound range could respond robustly to USVs, and

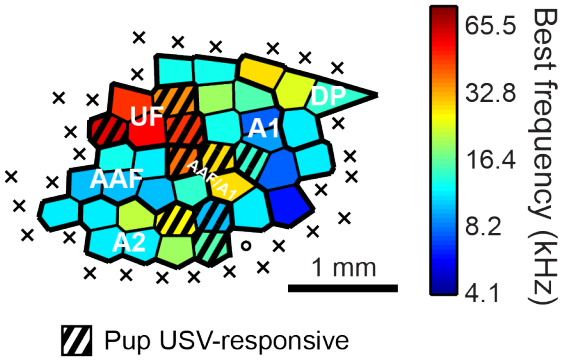

Figure 4.2 Example map of BFs and pup USV responses from a representative P3-4 dam. Map plotted as in Figure 3.3, with Voronoi polygons surrounding each auditory recording site, color-coded by BF. Xs mark non-auditory sites, and Os mark sites belonging to an unknown subregion. Note that some sites with ultrasonic BFs are not pup USV-responsive (hatched polygons), and some sites with non-ultrasonic BFs are pup USV-responsive.

sites with BFs in the ultrasound range could be unresponsive to USVs (Figure 4.2). Thus, while maternal plasticity was absent in the tonotopic map, it could still occur in an overlapping map of pup USV responses, perhaps driven by enhanced sensitivity to feature combinations present in behaviorally relevant USVs.

I first sought to characterize the relative responsiveness of the AC and each of its subregions to pup USVs and, as a point of comparison, adult USVs. Pooling the maps belonging to all experimental groups together, it became apparent that nearly a third of the AC was responsive to at least one pup USV (Figure 4.3A). The AC subregions contributed unequally to this figure, as a 2x6 ANOVA revealed a clear separation between the areas more and less likely to respond to USVs (Figure 4.3B; F_{field} [5,30] = 10.74, p = $7.7*10^{-9}$). The non-core fields A2 and DP, as well as core field UF, were most likely to respond to USVs, whereas core fields AAF, A1, and AAF/A1 were comparatively less USV-responsive. This separation was almost binary; the pup USV-responsive proportions of UF, A2, and DP fell within 20% of one another, without any statistical difference among these subregions, and the same was true of AAF, A1, and AAF/A1.

Interestingly, pup USVs excited an approximately twofold greater extent of the AC than did adult USVs (Figure 4.3A; 2-sample t-test: t[30] = 6.86, p = $1.3*10^{-7}$). The greater tendency to

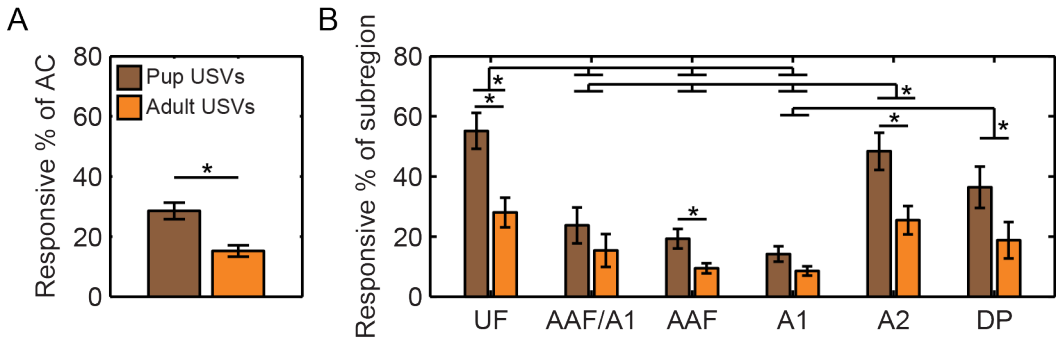

Figure 4.3 Pup and adult USV-evoked excitation across AC subregions, pooling across groups. **A** Proportionate area of AC responding to at least one pup or adult USV. Bars illustrate means ± SEM. * indicates significant 2-sample t-test ($p < 0.05$). **B** Proportionate area of each subregion responding to at least one pup or adult USV. Bars illustrate means ± SEM. * indicates significant Tukey's HSD ($p < 0.05$) following significant 2x6 ANOVA.

respond to pup versus adult USVs was borne out in each of the subregions (Figure 4.3B; 2x6 ANOVA: $F_{USV\ type}[1,30] = 35.61$, $p = 1.5*10^{-6}$). Since the pup and adult USVs presented here were matched for basic acoustic features such as median frequency, duration, and degree of onset frequency modulation, this pattern of results could be owing to differences in innate excitability to higher-order acoustic properties such as frequency trajectory, which do differ between USV classes. Alternatively, it could reflect differential sensitivity to these vocalization classes among the maternal groups, which then bias the pooled averages presented in Figure 4.3.

To examine this possibility, I charted the proportionate area of the AC and its subregions responding to at least one pup or adult USV exemplar. Consistent with the map plasticity results presented in section 3.3.2 though, the proportion of AC responding to pup USVs did not vary with maternal status (Figure 4.4A; one-way ANOVA: $F[3,27] = 0.69$, $p = 0.57$). This result held true for all individual AC subregions, indicating that no region was specialized to over-represent the behaviorally relevant pup USV class (Figure 4.4B; one-way ANOVAs: UF: $F[3,27] = 0.86$, $p = 0.47$; AAF/A1: $F[3,27] = 0.39$, $p = 0.76$; AAF: $F[3,27] = 1.75$, $p = 0.18$; A1: $F[3,27] = 0.37$, $p = 0.77$; A2: $F[3,27] = 0.37$, $p = 0.78$; DP: $F[3,24] = 1.21$, $p = 0.33$). Not surprisingly, there was also no evidence for plasticity in the map of adult USV responses, whether in the AC at large (Figure

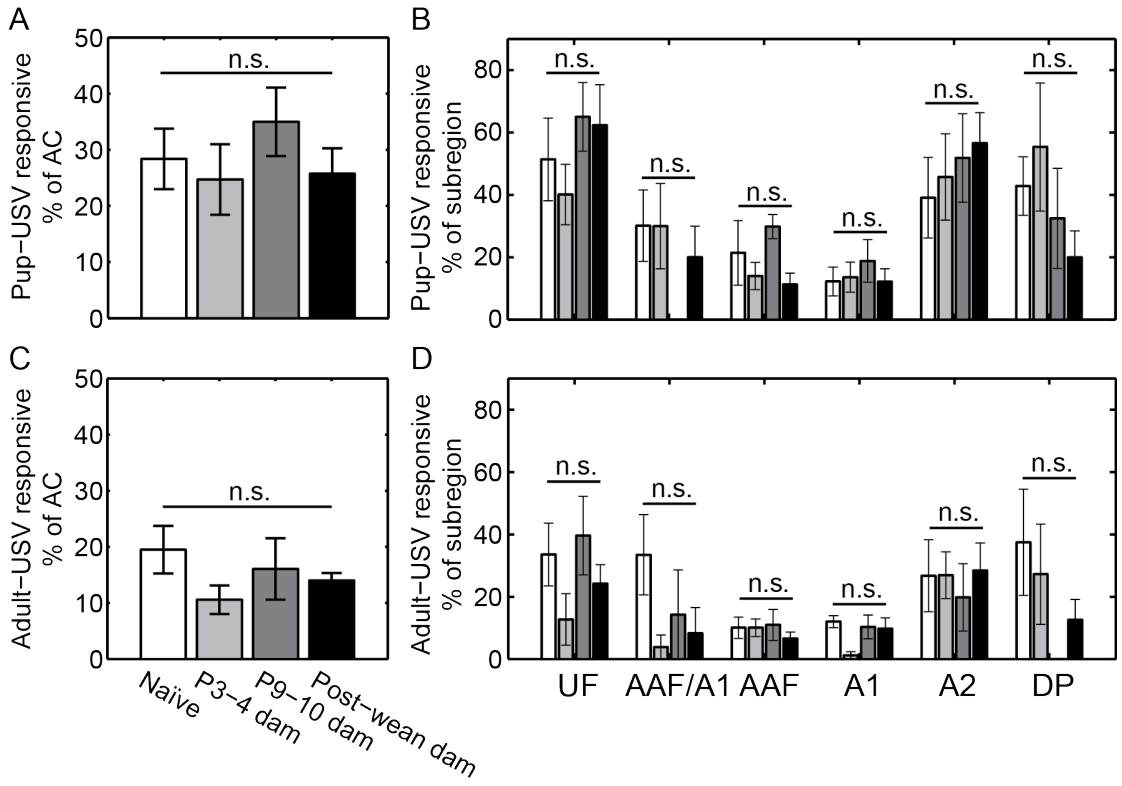

Figure 4.4 Motherhood is not accompanied by plasticity in map of USV responses. **A** Proportionate area of entire AC responding to at least one pup USV. **B** Proportionate area of each subregion responding to at least one pup USV. The color scheme from A is applied here. **C** Proportionate area of entire AC responding to at least one adult USV. **D** Proportionate area of each subregion responding to at least one adult USV. The color scheme from C is applied here. In all cases, bars illustrate means ± SEM. "n.s." indicates non-significant one-way ANOVA ($p > 0.05$).

4.4C; one-way ANOVA: $F[3,27] = 0.92$, $p = 0.44$), or in individual subregions (Figure 4.4D; one-way ANOVAs: UF: $F[3,27] = 1.42$, $p = 0.26$; AAF/A1: $F[3,26] = 1.53$, $p = 0.23$; AAF: $F[3,27] = 0.3$, $p = 0.82$; A1: $F[3,27] = 2.69$, $p = 0.07$; A2: $F[3,27] = 0.15$, $p = 0.93$; DP: $F[3,24] = 2.03$, $p = 0.14$). Thus, in keeping with the findings presented in section 3.3.2, I observed no evidence of plasticity in the map of USV responses in the AC, suggesting that neither frequency nor any other pup USV feature drives map plasticity in dams. Interestingly though, this finding signifies that pup USVs are inherently more likely to evoke excitation than adult USVs, even in naïve female mice to whom neither class of vocalizations carries relevance.

4.3.2 Different vocalization exemplars evoke markedly different response profiles

By measuring the cortical area responding to at least one USV exemplar of either class, the analyses conducted here have considered all USV exemplars within a class identically. The acoustic features of the USVs in this stimulus set, however, vary systematically in their frequencies, durations, and degrees of onset FM. To assess whether sensitivity to any of these dimensions was altered by motherhood, I compared the proportionate area of the AC responsive to each individual pup and adult USV exemplar.

While there was no effect of maternal status on the map of responsiveness to any individual USV (Figure 4.5A; 4x12 ANOVA: F_{group} [3,27] = 0.59, p = 0.63), it was clear that

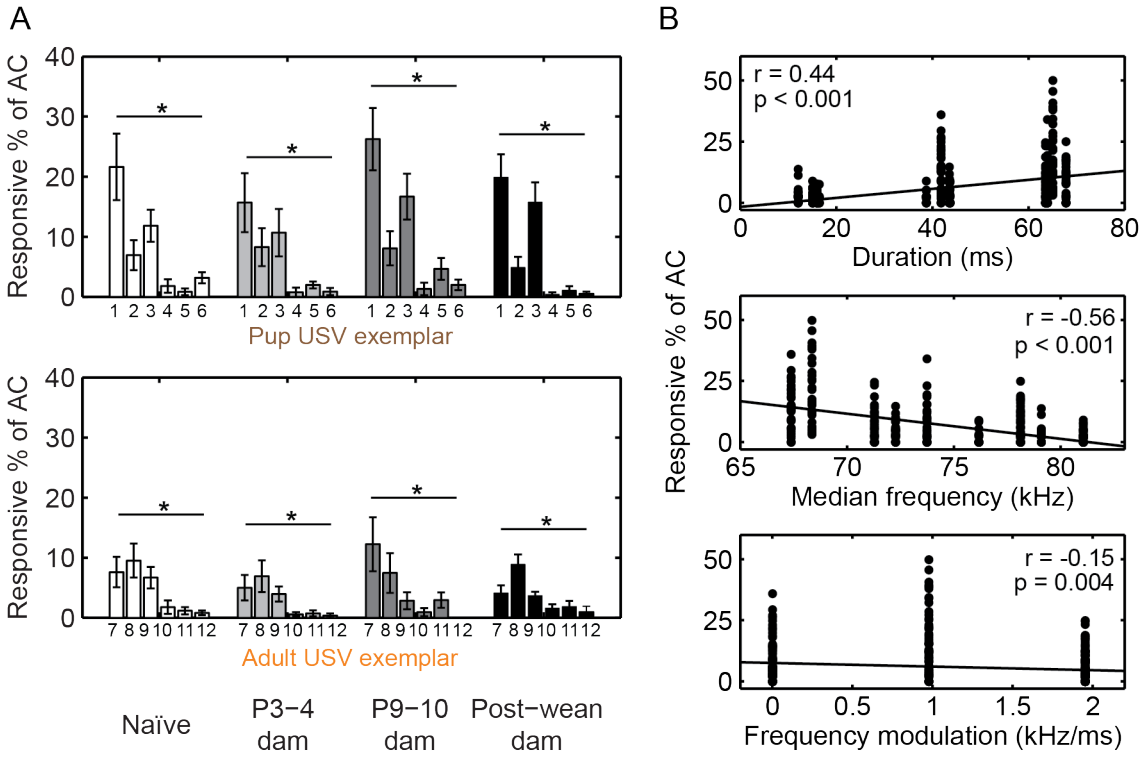

Figure 4.5 Response to each USV differs by exemplar and correlates with USV acoustics. **A** Proportion of AC responding to each pup (top) and adult (bottom) USV exemplar. Exemplar identifiers correspond to spectrograms shown in Figure 4.1. In all cases, bars illustrate means ± SEM. * over bracket indicates significant within-group one-way ANOVA following significant 4x12 ANOVA. **B** Scatter plots showing the proportionate area of AC responding to a given USV, as a function of that USV's duration (top), median frequency (middle), and degree of onset frequency modulation (bottom). Points represent measurements from a single animal, for a single USV exemplar. All maternal groups were pooled, as there was no main effect of maternal status on USV responses. Plots are overlaid with best-fit lines. r and p refer to correlation coefficients and p-values for correlations.

across groups, certain USV exemplars elicited more excitation than did others (4x12 ANOVA: $F_{exemplar}$ [11,27] = 41.15, p = $2.2*10^{-55}$). Nearly half of the exemplars in each class (pup USVs 4, 5, and 6 and adult USVs 10, 11, and 12) elicited almost no excitation across the AC. Among these were all four USVs (exemplars 5, 6, 11, and 12) belonging to the frequency-duration cell (see Figure 4.1) with the highest median frequency (~80 kHz) and shortest duration (~10 ms). Because this cell includes the USVs of extremely short duration, with frequencies approaching the hearing limit of the mouse (Heffner and Heffner, 2007), it is perhaps not surprising that these USVs do not evoke a strong profile of excitation. The remaining half of the USVs that do evoke clear responses include the four USVs in the frequency-duration cell with the longest duration and lowest frequency (exemplars 1, 2, 7, and 8). The acoustic features of calls in this cell are most characteristic of pup USVs, but responses to both pup and adult USVs with such features were apparent here. The remaining two exemplars generating responses were the pup and adult USVs from the intermediate frequency-duration cell that feature no FM at onset (exemplars 3 and 9). Interestingly, though the other two USVs occupying this cell (exemplars 4 and 10) share a common frequency and duration, and differ only in that they are frequency-modulated at onset, they evoke a negligible response. This example illustrates a trend that was apparent across frequency-duration cells. Comparing calls within the same class and the same cell, the exemplar featuring FM at onset tended to elicit a weaker response than the exemplar with no FM at onset, and this was especially true for pup USVs.

Taken together, these observations suggested that the acoustic features of a USV determine the degree of excitation it will evoke across cortex. To confirm this hypothesis, I correlated the duration, frequency, and degree of FM at onset of each USV with the proportionate area of AC it excited for each subject. Subjects were pooled between all groups, since there was no significant effect of maternal status on responsiveness to the various USV exemplars. Indeed, USV duration was significantly positively correlated with the responsive proportion of AC (Figure 4.5B, top; r = 0.44, p = $6.6*10^{-19}$), and USV frequency and degree of FM at onset were

each significantly negatively correlated with the responsive proportion of AC (Figure 4.5B, middle, bottom; frequency: $r = -0.56$, $p = 4.5*10^{-32}$; degree of FM at onset: $r = -0.15$, $p = 0.004$).

To summarize, this analysis showed that different USV exemplars differ in the degree to which they excite the AC, and they do so in a way that depends systematically on their acoustic features. Specifically, USVs with longer durations, lower frequencies, and less FM at onset are more likely to evoke a response, whereas short-duration, high-frequency USVs are less likely to promote excitation. This trend seems to hold regardless of USV class, as the same trends in responsiveness were observed for pup and adult USVs. That maternal experience does not alter the map of responses for any USV implies that these exemplar-to-exemplar differences are a result of their acoustics and not their semantic value to the receiver.

4.3.3 The maternal A2 loses sensitivity to acoustic differences in pup USV exemplars

I showed above that overall AC responsiveness to individual USV exemplars differs with a call's acoustic features – namely the frequency, duration, and onset FM parameters controlled for in this stimulus set. As the probability maps in Figure 4.1 show, these features are predictive of USV class membership. While one might expect that motherhood brings about changes in sensitivity to the features that predict USV class membership, perhaps in such a way that biases responses toward the behaviorally relevant pup USV category, such a change was not observed in AC on the whole. Nevertheless, it is possible that individual subregions may express such changes in sensitivity. Indeed, this sort of modulation of auditory responses by semantic value is thought to occur increasingly as one ascends the auditory processing hierarchy, and may therefore be more likely to arise in higher-order stations, potentially including the non-core fields of the AC. Therefore, I next tested whether AC subregions would display experience-dependent changes in the degree to which they respond to USV exemplars with acoustic parameters that are common to the relevant USV category. I chose to focus on UF and A2, as these subregions represented the core and non-core AC, respectively, and they were each abundantly responsive to

USVs. DP was excluded from this analysis because it was unusually small in this experimental cohort (see section 3.3.1), and not present in all subjects. In these subregions, I contrasted the population response to calls with acoustic features more and less predictive of USV class, focusing on the frequency and duration parameters, and pooling USVs with different onset FM to increase power. I also increased power by reporting data as proportions of all A2 or UF multi-units, pooled by group, rather than proportionate area of AC per subject, since in a single AC map, the number of recording sites responsive to any single USV exemplar was generally low.

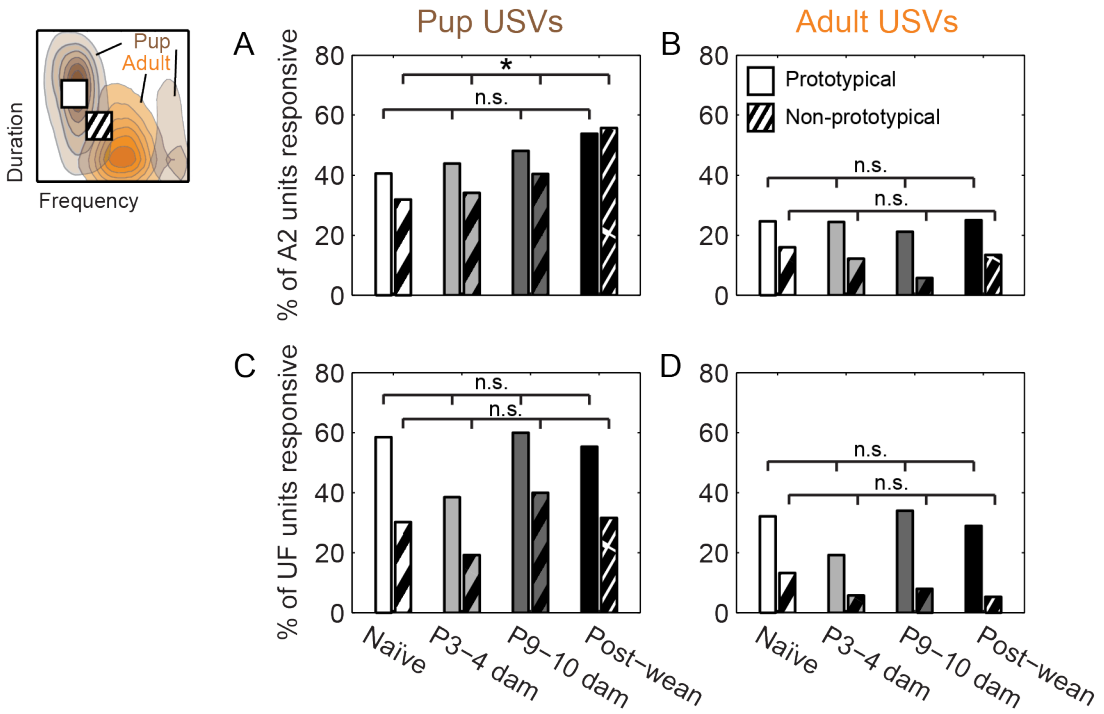

Figure 4.6 Increased responsiveness to pup USVs in the maternal A2, but not UF. **A** Proportions of A2 units excited by pup USVs with prototypical (i.e., characteristic of pup USV category; solid bars) and non-prototypical (hatched bars) durations and frequencies increase with motherhood, though only the change in the non-prototypical pup USV response is significant. **B** Proportions of A2 units excited by adult USVs with prototypically pup-like and non-prototypical acoustic features do not change with motherhood. **C** Proportions of UF units excited by pup USVs with prototypical and non-prototypical acoustic features do not change with motherhood. **D** Proportions of UF units excited by adult USVs with prototypically pup-like and non-prototypical acoustic features do not change with motherhood. **Inset** reproduces pup and adult USV probability clouds from figure 4.1 to make clear why "prototypical" and "non-prototypical" frequency-duration cells were labeled as such. * indicates significant Chi-square for groups indicated with bracket ($p < 0.05$). "n.s." indicates non-significant Chi-square for bracketed groups ($p > 0.05$).

Plotting the proportion of A2 responding to pup USVs with more and less prototypical acoustic features revealed that as motherhood progressed, this region became more responsive to each type of pup USV (Figure 4.6A). This increase was significant when non-prototypical pup USV exemplars were considered (χ^2 [3, n = 214] = 7.86, p = 0.049), but not for the prototypical pup USV responses (χ^2 [3, n = 214] = 2.26, p = 0.52). Consistent with the finding that shorter durations and higher frequencies produce less robust responses (Figure 4.5), the less prototypical pup USVs that share these features reliably evoked a smaller response across A2, at least for naïve mice and P3-4 dams. This difference appeared to dissipate by the post-weaning time point. In that group, the proportionate areas excited by pup USVs from either cell appeared comparable.

This effect was not apparent when adult USVs were considered ($\chi^2_{\text{prototypical}}$ [3, n = 214] = 0.28, p = 0.96; $\chi^2_{\text{non-prototypical}}$ [3, n = 214] = 3.00, p = 0.39). Across all maternal time points, adult USVs with frequencies and durations most typical of pup USVs elicited a stronger response than did exemplars from the intermediate frequency-duration cell (Figure 4.6B). This trend is comparable to that seen for pup USVs at the naïve time point. A change in responsiveness across motherhood was not observed in the core region UF for pup or adult USVs (Figure 4.6C-D). There, the characteristic pattern of greater excitability to prototypical (pup: χ^2[3, n = 193] = 6.11, p = 0.11; adult: χ^2[3, n = 193] = 3.27, p = 0.35) as compared to non-prototypical (pup: χ^2[3, n = 193] = 5.29, p = 0.15; adult: χ^2[3, n = 193] = 2.58, p = 0.46) USV exemplars held for all groups, and was not altered by motherhood. Thus, only in A2 did maternal status alter the population response in a way that was specific to pup USVs. The increase in the pup USV-excited proportion of A2 was not accompanied by any parallel increase in response magnitude (Figure 4.7). When A2 multi-units were pooled by group and evoked firing rates were compared, maternal status did not have an overall effect on firing rates (2x4 ANOVA: F_{group}[3,207] = 0.15, p = 0.93), nor did it interact with exemplar type (prototypical vs. non-prototypical; 2x4 ANOVA: $F_{\text{interaction}}$ [3,207] = 0.44, p = 0.72).

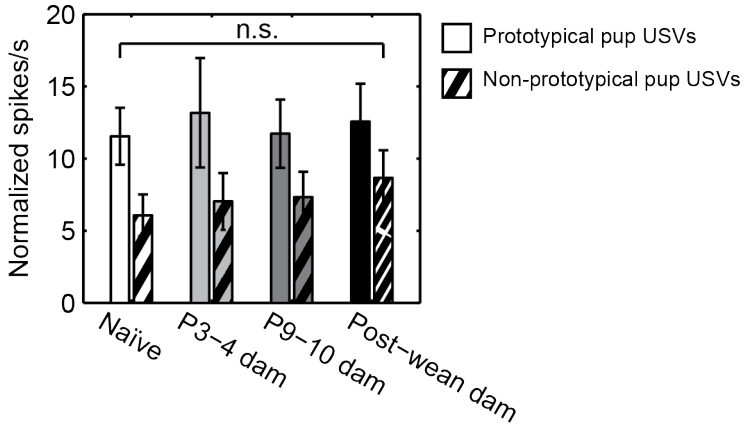

Figure 4.7 Pup USV-evoked firing rates in A2 do not vary with maternal status. Bars illustrate means ± SEM. "n.s." indicates non-significant interaction in 2x4 ANOVA ($p > 0.05$).

Interestingly, among A2 multi-units, the proportion responding to the non-prototypical pup USVs increased to a greater extent than did the proportion responding to the prototypical pup USVs, such that at the post-weaning time point, there was little apparent difference in the proportion of A2 multi-units excited by pup USVs with one set of acoustic features or the other. This observation raised the question of whether these units belong to overlapping or non-overlapping populations. If the same units are responding to prototypical and non-prototypical pup USVs in motherhood, that might suggest an increased tolerance of variation among exemplars belonging to the same class of behaviorally relevant stimuli, and a subsequent loss of sensitivity to acoustic differences between those individual USVs. Alternatively, the finding that different units respond to prototypical and non-prototypical USVs would indicate the recruitment of a new pool of neurons by a set of behaviorally relevant stimuli that remain distinguishable from others in their class by way of subtle differences in their acoustic properties.

To answer this question, I measured the proportion of A2 units responding to at least one prototypical *and* one non-prototypical pup USV. I found that the proportion of A2 multi-units responding to both prototypical and non-prototypical pup USVs increases with motherhood (Figure 4.8, left; $\chi^2[3] = 7.78$, $p = 0.05$), suggesting that the increase in responsiveness to non-

prototypical pup USVs is attributable to units that are already responding to prototypical pup USVs. Indeed, the proportion of prototypical pup USV-responsive units also responding to at least one non-prototypical pup USV increases with maternal experience as well ($\chi^2[3] = 8.67$, $p = 0.03$), such that in post-weaning mothers, nearly all of the multi-units that respond to at least one prototypical pup USV also respond to at least one non-prototypical USV (24/26 units). No such change was observed for adult USVs (Figure 4.8, right; $\chi^2[3] = 1.53$, $p = 0.68$). These results imply a widening of the receptive fields in the maternal A2 in such a way that renders them more responsive to non-prototypical USVs that nevertheless belong to the behaviorally relevant pup USV class.

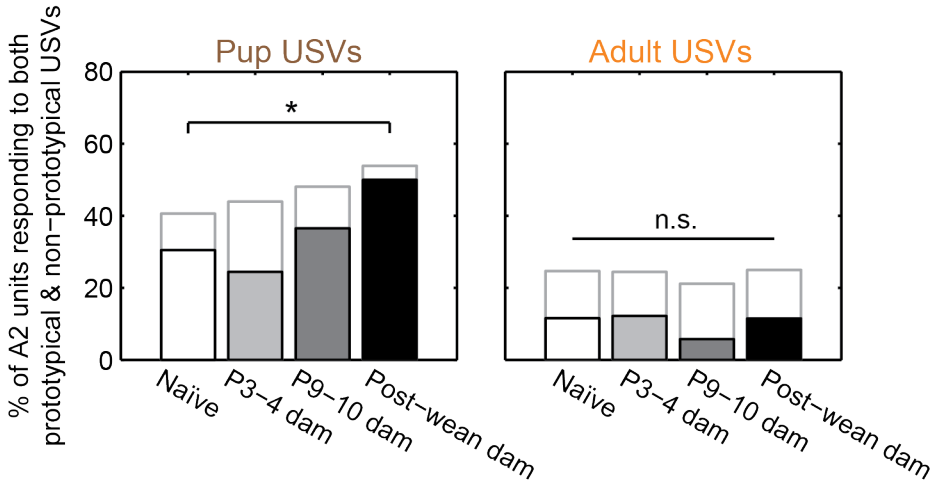

Figure 4.8 Same-unit responsiveness to multiple pup USV exemplars increases with motherhood in A2. Solid bars illustrate mean proportion of A2 units, pooled by group, responding to at least one prototypical *and* one non-prototypical pup (left) or adult (right) USV (illustrated in figure 4.6 inset). Open bars show proportion of A2 units responding to at least one prototypical USV. * indicates significant post-hoc z-test between bracketed groups (solid bars) following significant Chi-square ($p < 0.05$). "n.s." indicates non-significant Chi-square.

4.4 Discussion

In this chapter, I used recorded vocalization stimuli to confirm and extend the results presented in chapter 3, wherein I showed that map plasticity did not occur for the ultrasonic

frequencies associated with pup USVs. I showed here that, indeed, there was no experience-dependent change in responsiveness to USVs on the whole, or to any specific USV exemplar. Instead, I found a pattern of responses specific to the non-core field A2: While multi-units in other regions responded to USV exemplars in a way that correlated with those calls' acoustic features, multi-units in this region displayed less sensitivity to the acoustic differences between different pup USV exemplars. This effect was observed in the absence of any changes in pup USV-evoked firing rates, suggesting that while the total *amount* of pup USV-evoked excitation across the AC does not change with maternal experience, the pattern of *how* a particular AC subregion responds to variation in pup USVs may reflect those calls' behavioral relevance.

4.4.1 Absence of plasticity in map of USV responses

The absence of map plasticity observed here, while not surprising in light of the results presented in chapter 3, reinforces the finding that experience with pup USVs does not drive map plasticity in maternal females. Though the ultrasonic frequencies that characterize pup USVs were the focus of chapter 3, the use of pup USVs themselves as stimuli in the present chapter would permit the observation of plasticity for any of the features that define pup USVs. That plasticity was not observed in the map of pup USV responses on the whole indicates that no single USV feature or combination of features induces widespread map plasticity across the entire AC.

That such an effect was not observed over the AC on the whole may not be surprising, since over-representing every stimulus or stimulus class that gains behavioral relevance would likely overwhelm the finite boundaries of the AC. However, the failure to observe an increase in overall responsiveness to pooled pup USVs in non-core regions (Figure 4.4), particularly A2, is somewhat unexpected in light of previous research. Others have shown, using the maternal mouse model, that pup experience leads to an increase in the number of c-fos-expressing neurons in A2 following playback of pup USVs (Fichtel and Ehret, 1999). Though c-fos expression does not

directly translate to an increase in neural firing, this finding might cause one to expect a change in overall proportion of multi-units responsive to pup USVs.

Nevertheless, the fact that I did not observe any gross increases in the population response to pooled pup USVs is consistent with other work from our lab showing more subtle effects of motherhood on auditory processing. In no previous study, for instance, has motherhood led to a uniform increase in pup USV-evoked firing rates across the AC population. Plasticity tends to manifest instead in the timing of firing (Liu et al., 2006), the informational content of spike trains (Liu and Schreiner, 2007), or the response dynamics of distinct subsets of single units (Shepard et al.; Galindo-Leon et al., 2009; Lin et al., 2013). Thus, the absence of straightforward map plasticity is somewhat in line with prior work carried out in our lab.

4.4.2 USV processing in core versus non-core AC

Though map plasticity was not evident here, this data set provided an opportunity to compare USV responses in core and non-core auditory subregions. Quality recordings of USV-evoked responses in non-core AC subregions are somewhat rare, due to the difficulty of targeting areas DP and A2. DP is small, if present at all, in the *CBA/CaJ* strain, and A2 is less accessible than other subregions, given its position at the ventral extreme of the AC. In the present experiment, I was successful in taking a large number of recordings that could be assigned to area A2 with high confidence. I was consequently able to perform a detailed analysis of USV responses in this subregion, and compare my results to responses from area UF, a core subregion that is, like A2, highly responsive to USVs.

Neither of these regions displayed an experience-dependent change in the proportion of units responsive to pooled pup or adult USVs, but the pattern of excitation produced by specific exemplars was apparently affected by maternal experience. In core area UF, as well as the naïve A2, USV exemplars with longer durations and lower frequencies (relative to other USVs in the stimulus set) reliably excited a larger proportion of multi-units than did short-duration, high-

frequency exemplars. The combination of long duration and low ultrasonic frequency is typical of the pup USV category, but the fact that such exemplars excited a greater proportion of UF and A2 multi-units even in naïve animals suggests that this effect is informed by the calls' acoustic features rather than their semantic value. This modulation of response by acoustic features was apparent across maternal groups in core field UF, but slowly disappeared with maternal experience in non-core field A2. In that field, the proportion of multi-units responding to the short-duration, high-frequency exemplars significantly increased with maternal experience, such that by the post-weaning time point, similar proportions of units responded to both the long-duration, lower-frequency exemplars prototypical of the pup USV category and the less prototypical USVs with higher frequency and shorter duration. This growing similarity in the response profile evoked by acoustic combinations characteristic of prototypical and non-prototypical of pup USVs was likely caused by increased responsiveness to non-prototypical duration-frequency combinations among units that were already responsive to prototypical duration-frequency combinations. This finding seems to suggest that the maternal A2 undergoes an experience-dependent change in sensitivity to acoustic variation among the behaviorally relevant pup USV category.

Interestingly, this change in sensitivity to variation in frequency and duration was not observed for adult USVs. This disparity between pup and adult call response profiles demonstrates that the maternal A2 did not simply lose overall sensitivity to the subtle differences in frequency and duration that separate USV exemplars belonging to different duration-frequency cells (Figure 4.1A). Their loss of sensitivity to these properties is instead specific to the behaviorally relevant pup USV category. This effect may be somewhat surprising, given that frequency and duration actually predict membership to this relevant stimulus category. In other systems, neurons in higher-order stations actually become *more* sensitive to variation along stimulus dimensions that correlate with category membership (Sigala and Logothetis, 2002), which is what one might expect if downstream regions are using this information to judge

stimulus category. One possible explanation for this apparent discrepancy could be that while frequency and duration do vary with USV category membership, other acoustic properties that are not parameterized in this stimulus set also vary with category, and those, rather than frequency and duration, are being used to designate category in the maternal brain.

This result differs somewhat from our lab's previous work, which showed, using mutual information-based analyses of multi-unit spike trains, enhanced discrimination of different pup USV exemplars in the maternal compared to the non-maternal AC (Liu and Schreiner, 2007). Since that study, like the present work, used a set of USV exemplars varying systematically in duration, frequency, and degree of FM at onset, an *enhancement*, rather than a diminishment, in sensitivity to acoustic variation within a category, would be implied. A number of experimental differences between these studies could be responsible for this apparent discrepancy. First, the present study simply quantified the number of responsive multi-units to assess changes in sensitivity, whereas Liu *et al.* took the structure of the USV-evoked spike trains into account. It is possible that both phenomena occur in parallel, though how this would influence downstream processing is unclear. Follow-up work in which the mutual information-based approach taken by Liu *et al.* is applied to the multi-units recorded in the present study may shed some light on whether these parallel processes occur. Second, in the present study, the changes I observed were restricted to non-core region A2, whereas Liu *et al.* recorded from multi-units throughout AC, with the largest proportion of recording sites residing in core regions A1 and AAF. Given that the effect I observed was quite specific to A2, it may not be surprising that Liu *et al.* did not observe the same loss of sensitivity. Finally, different electrode properties could bias the specific cell types that constitute our multi-unit recordings, and these cell types could be differently susceptible to experience-dependent plasticity (see section 3.4.3).

Follow-up investigations could improve upon the present study through two straightforward modifications of the experimental methodology. First, the change in A2 sensitivity to acoustic differences that separate prototypical from non-prototypical pup USVs was

only observed when all units belonging to that subregion were pooled by group, essentially creating one "super A2" meant to represent each maternal category. This result would be more powerful if it held true when A2 responses were averaged within a group, rather than pooled. Adding additional subjects to the experimental cohort would enable such averaging. Second, I observed that the USVs with the longest durations and lowest ultrasound frequencies tended to excite a greater proportion of multi-units, regardless of subregion (except in the maternal A2, Figure 4.6). Because these acoustic properties correlated with the proportion of AC they excited (Figure 4.5B), and because this bias was apparent even in naïve animals to whom no USV class carries particular relevance, I concluded that this greater excitation was attributable to those USVs' apparently more favorable acoustic properties. Nevertheless, the acoustic properties and degrees of prototypicality for the exemplars employed here were simultaneously varied, so the contributions of these two attributes cannot be cleanly separated. In a subsequent study, using a stimulus set that allows one to hold one acoustic property constant while varying another in such a way that spans pup USV probability space (i.e., the brown contour plot in Figure 4.1A) would permit the experimenter to separate the contributions of acoustic parameters from those of higher-order properties such as prototypicality.

4.4.3 A2 in auditory categorization

This comparison provides valuable insight into the roles of core and non-core AC in auditory processing. Though the transformation of a sensory signal is relatively well understood in, for example, visual cortex, the tasks carried out by different processing stations within AC (and particularly the rodent AC) have not been clearly defined. In primate AC, auditory information flows first through "core" and then "belt" fields, during which time the sensory representation shifts from a readout of the sum of the stimulus' acoustic features to a more holistic representation of an "auditory object" (Kaas and Hackett, 2000; Bizley and Cohen, 2013). For instance, one is more likely to see category-selective responses in belt regions, which are

situated downstream of core regions. Still further along the auditory pathway, responses that are selective for conspecific vocalizations and even the voices of specific individuals can be observed (Petkov et al., 2008). The homologues of such higher-order regions in the rodent AC have not been precisely mapped out, if they exist at all. However, it is generally believed that non-core fields, like the primate belt areas, will exhibit a greater degree of nonlinearity in their responses. That is, the neural responses in non-core AC will be less predictable from the basic acoustic features of a stimulus, and will more likely integrate information about the semantic value of the stimulus. The apparent modulation of pup USV responses by maternal status across the non-core region A2 would seem to support this.

Perceptual invariance to subtle differences in exemplars belonging to the same category is commonly observed for behaviorally relevant stimulus categories. Such invariance has been demonstrated repeatedly using behavioral methods, and is exemplified by a classic linguistics study that found that humans perceive sounds belonging to the same categories as more indistinguishable than sounds belonging to different categories, even when the acoustic differences between exemplars are held constant (Liberman et al., 1957). Building on that, Kuhl (1991) showed that this invariance was even stronger when one of the exemplars was prototypical of its category. She describes this effect as “perceptual magnetism”, wherein a perceptual “field” of reduced discriminability surrounds a prototypical stimulus, leading more irregular exemplars within that category to be perceived as more prototypical. The extent to which these phenomena are innate or developed through experience is debated, though experimental data has established that experience with categories can enhance or diminish sensitivity to variation within a category (Kluender et al., 1987; Goldstone, 1994).

Whether the plasticity I observed in A2 underlies such a refinement of the pup USV category among maternal mice is not known, and would be difficult to determine without executing behavioral experiments. Nevertheless, the overall character of the maternal plasticity I observed in A2 bore some resemblance to the perceptual phenomena associated with auditory

categorization. Parallel to the decrease in sensitivity to acoustic differences incurred when comparing exemplars within a category, I showed that only among experienced mothers, the proportion of A2 units responding to different pup USVs was not modulated by those exemplars' frequencies and durations (Figure 4.6A). That this effect was apparently caused by an increase in responsiveness to non-prototypical combinations of frequency and duration among units recalls the perceptual magnetism phenomenon described above. This argument is strengthened by the fact that these changes were specific to the behaviorally relevant category. While interesting, it is important to underscore that these similarities merely hint at the perceptual consequences of the plasticity I observed in A2. Additional exploration of USV responses in A2, ideally alongside behavioral assays meant to elucidate perceptual sensitivity to acoustic variation within USV classes, will be required before a clearer understanding of its role in categorization is reached.

4.4.4 Summary

Here, I confirmed that map plasticity was not present in the maternal AC, this time using USVs themselves to demonstrate that no combination of features drives map expansion during motherhood. I did, however, observe a decreased sensitivity to acoustic variation in pup USVs that developed over motherhood and was restricted to the non-core subregion A2. Taken together, these results indicate that where natural stimuli such as vocalizations are concerned, *how* the AC responds, in terms of which subpopulations are activated by which individual stimuli, is likely more critical than *how much* it responds. Indeed, this observation is consistent with prior work in the maternal model that failed to find changes in broad responsiveness to vocalization categories on the whole, but found rich plasticity in the pattern of spiking responses (Liu et al., 2006; Liu and Schreiner, 2007), and in the effects within specific neural subpopulations (Galindo-Leon et al., 2009).

Chapter 5

Conclusion

Sensory cortical responses are reshaped by experience on a continuous basis throughout the lifetime. Decades of research have characterized these changes on scales small and large, from changes in the receptive fields of individual neurons to the massive reorganizations of cortical topography known as map plasticity. This dissertation focused on large-scale map plasticity in the AC following acoustic experience in both development and adulthood. Using a sound exposure manipulation during development, I showed that NE is required for experience-dependent map plasticity in the AC. In adult animals, I used a natural learning context – motherhood – to establish that map plasticity does not necessarily follow from a salient acoustic experience during adulthood. Instead, other forms of plasticity, none of which alter BF map organization, take place to support the learning of behaviorally relevant stimuli. The implications of these findings are discussed below.

5.1 The mouse model of cortical plasticity

All of the work described in this dissertation was carried out in the mouse AC. Unlike the cat or rat, the mouse is not a classic system in which to study experience-dependent sensory cortical plasticity. Nevertheless, researchers are increasingly turning to the mouse to address questions related to sensory plasticity due to the large genetic toolkit the mouse affords (e.g., the *Dbh* mutant employed here in chapter 2), as well as its ease of use in a laboratory setting. In recent years, for example, use of the mouse in studies of visual cortex has led to powerful new research on the modulation of visual cortical activity by natural locomotion behavior (Niell and Stryker, 2008), and on ocular dominance column plasticity in adult animals (Sawtell et al., 2003; Hofer et al., 2006). This latter line of work was particularly surprising, given that the existing research on ocular dominance column plasticity that had been carried out largely in cats

suggested that ocular dominance columns are static in adult animals. Due in part to research like this, mouse has now supplanted the cat as the preferred model system in which to study ocular dominance column plasticity (reviewed in Gavornik and Bear, 2014).

Among auditory researchers specifically, the mouse is gaining popularity partly because of its natural vocal communication system (e.g., between pups and dams, as described in chapters 3 and 4). Limiting the use of mice in auditory research, however is the ongoing debate on the degree of tonotopy apparent in its core AC fields (Kanold et al., 2014). While one can study synaptic plasticity in a non-tonotopic AC, non-tonotopic organization complicates the study of map plasticity, as it is more difficult to visualize map expansion when it occurs among non-contiguous sites. There may also be some uncertainty regarding how findings can be generalized between models that do and do not share this fundamental organizational principle.

In the studies described here, tonotopy was present to varying degrees. In chapter 2, individual AC maps of *Dbh* +/- and -/- mice exhibited weak tonotopy, partially as a result of the preponderance of units tuned to the 13-16 kHz range. The overabundance of sites in this frequency range was likely a result of compression of the upper limit of the hearing range in these mice. Nevertheless, averaging together the maps of animals belonging to the same group revealed the expected tonotopic gradients. In chapter 3, tonotopy was readily apparent in the core ACs of individual *CBA/CaJ* mice, which have better high-frequency hearing than the *Dbh* mouse (Zheng et al., 1999).

Together, these studies provide additional evidence that the core fields of the mouse AC, like those of most other mammals, are tonotopic. While studies employing high-resolution imaging techniques have argued against tonotopy in the mouse AC (see section 1.1.3), the present studies are consistent with others using multi-unit recording to show tonotopy in core fields AAF and A1 (Barkat et al., 2011; Hackett et al., 2011; Guo et al., 2012). Importantly, tonotopy was evident in the present studies even though the sampling density of the multielectrode array was notably sparser than that employed in prior work by Polley and colleagues. This result indicates

that tonotopy (and even map plasticity, as in chapter 2) can be observed in the mouse even when sampling resolution is relatively coarse.

5.2 Future use of mapping in AC research

The studies here each made use of electrophysiological mapping to chart AC topography. While this method has been in wide use for decades, and has been especially helpful in the investigation of plasticity in sensory cortex, the development and refinement of high-resolution neural manipulation and recording technologies have enabled the study of the mechanisms underlying plasticity at an unprecedented level of detail. For example, the application of *in vivo* two-photon Ca^{2+} imaging and optogenetic methods in one recent paper elucidated how ACh acts on a specific AC microcircuit to trigger associative plasticity (Letzkus et al., 2011). In light of the increasing use of these methods, one may wonder whether mapping studies will fall into disuse, given that the resolution they afford is inherently limited.

An obvious argument for the persistence of mapping studies, at least in the AC, is that they permit the experimenter to assign recording sites to AC subregions with high confidence. While stereotaxic coordinates for cytoarchitecturally-determined primary AC fields and auditory association areas have been established, individual variation in AC topography has precluded the identification of anatomical markers that correspond to the classic, functionally defined AC subregions, at least in the mouse (Stiebler et al., 1997). Response properties within these subregions are distinctive, although similarities between some fields (e.g., A1 and AAF) render them difficult to distinguish unless one gains a sense of the spatial position of a recording site relative to others in the AC. Because one gains the most detailed sense of the spatial distribution of response properties through mapping, it remains the best method if one needs to classify recording sites to specific subregions with a high degree of accuracy.

Mapping studies are also essential to reach a complete understanding of the relationship between map plasticity and auditory learning and memory. So long as this question remains, there

will be a reason to map neural properties across the AC (or a portion thereof), although the specific recording method may change. Ca^{2+} imaging (Bandyopadhyay et al., 2010) or voltage-sensitive dyes (Broicher et al., 2010), for instance, also permit the experimenter to simultaneously record neural activity over a local population of neurons, and the higher throughput that these methods offer as compared to electrophysiological mapping may in fact provide an incentive to use them. Nevertheless, any of these recording methods yield response property maps that can be used to answer fundamental questions about the importance of map plasticity to learning and memory.

Further, the robustness of BF map plasticity (following sound exposure or conditioning in development or adulthood, respectively), positions it as a system in which potential mechanisms of plasticity might be tested. A candidate contributor to plasticity can be added to or removed from the AC prior to an experiential manipulation known to drive map plasticity; deviation from the expected pattern of resulting plasticity will signal the involvement of the candidate agent. This approach has been used with success both here (for NE in chapter 2) and elsewhere (to demonstrate the sufficiency of ACh in generating map plasticity when paired with a sound, see Kilgard & Merzenich). While it may not provide an exceptional level of detail, such a screening approach is useful for identifying candidate mechanisms that can later be studied at greater depth with higher-resolution methods.

Finally, while population-based recording methods cannot generally be used to answer questions about the neural coding of complex sounds, since this typically requires single-unit resolution, chapter 4 of this thesis demonstrates that fairly sophisticated questions regarding the processing of complex stimuli can be answered using multi-unit data from mapping experiments. By selecting stimuli that were varied in controlled ways, I was able to draw conclusions about how sensitivity to acoustic features varied with experience with an acoustic category. My finding that responses in the non-core AC field A2, but not in the core field UF, become less sensitive to acoustic variation within a behaviorally relevant category parallels the invariant responses

observed in higher-order visual cortex following behavioral training with visual categories (Sigala and Logothetis, 2002). Interestingly, however, these findings in the visual cortical literature were obtained through the use of single unit recordings, rather than the multi-unit recordings I employed here. Although single unit recordings from A2 may have permitted exploration of this phenomenon in greater detail, the plasticity that occurred there for pup USVs was apparently robust enough to appear even in multi-unit recordings. Thus, when stimuli are selected thoughtfully, data from mapping studies can be used to address questions other than those simply regarding the distribution of tuning properties across AC.

5.3 The physiological relevance of map plasticity

The absence of map plasticity in the ethological context I employed here (chapters 3 and 4) may bring its relevance to learning and memory under scrutiny. Given its ubiquity in two decades' worth of research on auditory memory, map plasticity in adult animals was widely presumed to underlie auditory memory, or represent a stage of auditory memory formation. Instead, its apparent disappearance when learning occurs outside of a laboratory conditioning context suggests that map plasticity may be the result of a supraphysiological reinforcement scheme, and may not play a key role in natural sound learning. If this hypothesis proves true, it should motivate a redistribution of research efforts away from map plasticity, and toward plasticity phenomena that have been more tightly linked to behavioral memory.

The link between map plasticity and behavioral memory has been difficult to pin down. Blockade of plasticity in AC inhibits auditory learning (Kraus et al., 2002), but such a manipulation will prevent all synaptic plasticity, not just map expansion. Induction of map plasticity facilitates auditory discrimination learning (Reed et al., 2011), but whether map reorganization is required to achieve a facilitatory effect is unknown. It could be that just a subset of the retuning events required to achieve map plasticity are responsible for the behavioral outcome. Whether this is the case is a question that might be answered by studies in which map

plasticity *and* finer-scale plasticity are monitored simultaneously. Although such a design is likely to be technically challenging, the advent of *in vivo* functional imaging technologies may permit such an experiment in the future.

In contrast to its role in adulthood, the importance of map plasticity during the critical period is less disputed. This lack of disagreement is partially owing to the wealth of research on critical periods in other systems, especially the critical period for ocular dominance column plasticity in visual cortex. In that system, monocular deprivation during the critical period induces a reduction in the area of primary visual cortex that is sensitive to the formerly closed eye (Wiesel and Hubel, 1963; LeVay et al., 1980), and also a decrease in visual acuity through that eye (Dews and Wiesel, 1970). The former is presumed to cause the latter. This foundational work prompted clinicians to ensure treatment of ocular abnormalities occurs prior to closure of the relevant critical periods (Daw, 1998; Fawcett et al., 2005). That the early studies of visual cortical critical periods went on to inform clinical practice reinforces the importance of map plasticity in sensory cortex. Further, there is an innate logic in the idea that a system should be tuned, in an experience-dependent fashion, to the features of the immediate sensory environment, since a genetically determined sensory map may devote more space than necessary to uncommon stimulus features or, conversely, may not allot enough cortical space to commonly encountered stimulus parameters.

How plasticity during the critical period for frequency tuning in AC impacts auditory perception and subsequent behavior is not well understood, although the importance of critical periods in other systems suggests the consequences for behavior are potentially great. One of the few studies that has addressed the perceptual consequences of critical period plasticity in the AC has shown that after developmental exposure to trains of pure tones, discrimination is improved in bands flanking the frequency of exposure, but diminished at the frequency of exposure itself (Han et al., 2007). How this phenomenon plays out in a natural environment, when an individual is exposed to a broader range of frequencies, is currently unknown. However, to the extent that

map plasticity is involved in the adaptation of the developing animal to its acoustic environment, the work presented here in chapter 2 implicates NE as a critical regulator of that process.

5.4 NE in AC plasticity during adulthood

A key question that this thesis raises concerns the role of NE in the adulthood plasticity described in chapters 3 and 4. Chapter 2 argued that NE is required for experience-dependent plasticity during AC development – would the same be true of experience-dependent plasticity in the AC during motherhood? Because the strain background on which the *Dbh* mutation was bred shows poor high-frequency hearing, and pup USVs occur exclusively in the ultrasound range, it is not currently possible to address this question (at least using the *Dbh* mouse model of NE deficiency). An effort to breed the *Dbh* mutation onto the well-hearing *CBA/CaJ* background strain is underway, and should enable the study of USV responses in NE-deprived dams.

This dissertation characterized two experience-dependent plasticity processes in maternal animals. The first, a suppression of ultrasound responses among low-frequency-tuned neurons in core AC, will be straightforward to assess in *Dbh -/-* dams. The second, a change in sensitivity to acoustic variation in pup USVs in non-core AC, may be less so. This is because in my initial characterization of the *Dbh -/-* AC (see section 2.3.2), units showing a response profile characteristic of non-core AC were not readily apparent. Because this was the case for *Dbh +/-* and *Dbh -/-* mice alike, the dearth of non-core responses is likely owing to the mixed *C57BL6/J* and *129SvEv* strain background on which the *Dbh* mutation is bred, which is sub-optimal for auditory research due to its poor hearing range. However, future research will need to test this assumption: a new baseline characterization of the *Dbh* +/- and -/- ACs will need to be conducted to confirm the presence of the expected core and non-core subregions before subsequent maternal studies can test for plasticity.

The results of such a study would be of interest to researchers in auditory processing as well as those in the field of maternal behavior. The role of NE in adult auditory cortical plasticity

is of recent interest (Martins and Froemke, 2013), and although the relationship between NE and maternal behavior is not under active study, it was established through use of the *Dbh -/-* mouse that NE-deficient dams show profound deficits in maternal care (Thomas & Palmiter, 1997). Specifically, *Dbh -/-* dams fail to gather and nurse pups; as a result, the pups generally die within a few days of birth. No follow-up work has established the neural basis of this deficit. While one might assume this defect stems from a lack of motivation on the part of the dam, our lab's work hints at an alternative hypothesis, in which a failure of sensory plasticity prevents maternal sensitization to pup cues. While the behavioral consequence of blocking maternal sensory plasticity is unknown, the overall effect could be a reduction in maternal responsiveness to pups. Exploring whether maternal plasticity in AC is prevented through a lesion of the noradrenergic system will be a first step toward understanding the relationship between NE, sensory plasticity in the adult AC, and maternal behavior.

A study of maternal plasticity in the *Dbh -/-* mouse would not only bring this thesis full-circle, but would also help the field work toward two broader goals. First, it would help us understand the relationship between findings gleaned from more ethological and more artificial learning paradigms. An overarching question in AC research is whether principles elucidated by studies of behavioral conditioning with auditory cues still hold in more natural learning environments. Establishing the role of NE in the more ethological maternal context, and comparing it to the hypothesized role for NE based on prior work using laboratory conditioning methods (Edeline et al., 2010; Martins and Froemke, 2013), will help researchers understand the extent to which findings from such conditioning studies can be generalized to "real-life" learning scenarios.

Second, studying AC plasticity in the *Dbh -/-* adult would move us closer to reaching a unified understanding of AC plasticity throughout the lifespan. As it stands, the mechanisms of plasticity are usually investigated either in adult or developing animals, and the result is a splintered portrait of how age impacts the regulation of plasticity. For example, a sizeable body of

work has linked ACh to plasticity in the adult, to the extent that the microcircuit through which ACh enables plasticity has been identified. In contrast, no study to date has assessed the involvement of ACh in experience-dependent plasticity during AC development. Consequently, little is known about the function of ACh during AC development. Even the particular synapses that are most likely to be altered by experience are unknown; only now is there an emerging consensus that thalamocortical synapses are likely to be a critical locus of experience-dependent plasticity in adult and developing animals (Blundon & Zakharenko, 2013; Liu, Basavaraj, Krishnan & Yan, 2011). This fractured understanding of plasticity across the lifespan can only be remedied by the parallel study of adults and juveniles, ideally within the same labs. Toward that end, a study of maternal plasticity in the *Dbh -/-* mouse would help to complete the portrait of NE that chapter 2 of this dissertation began to paint, especially when paired with existing research implicating NE in adulthood plasticity. A complete understanding of the necessity and sufficiency of NE in AC plasticity will enable us to ask deeper questions, such as which networks NE acts through to have its effects, and whether any age-dependent changes in plasticity can be attributed to shifting dynamics within the noradrenergic system.

Appendix A

Common methods

A.1 Surgery and electrophysiological recording

Animals undergoing electrophysiological mapping were anesthetically induced with a ketamine (100 mg/kg) and xylazine (5 mg/kg) cocktail (6:1) delivered intraperitoneally (IP). Maintenance doses of ketamine (30 mg/kg) and xylazine (1 mg/kg) were delivered via an IP cannula when a toe pinch reflex was observed (usually every 20-30 minutes). Throughout the surgery and recording, body temperature was maintained at approximately 26.2°C via a homeostatic heating pad (DC Temperature Controller, FHC Inc., Bowdoin, ME).

Animals' heads were secured in a stereotax with bite bar (Model 900, David Kopf Instruments, Tujunga, CA) and 2% oxygen was delivered through tubing attached to a sliding nose clamp. Fur on top of the head was trimmed and an incision was made down the midline of the scalp. Schwartz vessel clips (World Precision Instruments, Sarasota, FL) were applied along the edges of the incision to further open the wound and reveal the underlying skull. A periosteal elevator was used to detach the left temporal muscle from the skull until the zygomatic arch became visible. This portion of the temporal muscle was then cut away to facilitate access to the skull directly overlying the temporal cortex.

Craniotomy boundaries were labeled with a non-toxic, water-resistant marker (Figure A.1A). The rostral boundary was placed at 30% of the distance between bregma and lambda, the caudal boundary was placed at 90% of the distance between bregma and lambda, the ventral boundary was placed immediately above the zygomatic arch, and the dorsal boundary was placed approximately 1 mm dorsal to the ridge separating the temporal and dorsal surfaces of the skull. After these boundaries were delineated, an inverted flat-head machine screw (0.19" head diameter x 0.47" length) was placed immediately behind bregma and secured in place with dental cement (MaxCem, Kerr, Orange, CA); this screw served as a headpost to maintain head position

throughout the recording. Another flat-head machine screw (0.054" head diameter x 0.09" length) was driven into the skull overlying the frontal lobe to serve as an electrical ground.

Prior to the craniotomy, animals were removed from the stereotax and their headposts were anchored in a mount. The craniotomy was performed with either a hand-held Dremel tool with #1/4 carbide bur, or a scalpel with #11 blade; the tool used for the craniotomy did not appear to alter cortical responses. Upon completion of the craniotomy, silicon oil was applied to the exposed cortex to prevent drying. A high-resolution photo was taken of the cortical surface, to be used later to reference electrode penetration sites (Figure A.1B).

Multi-unit electrophysiological recordings were taken across the left AC using a 4 MΩ 3x1 tungsten matrix microelectrode (Figure A.1C; FHC Inc, Bowdoin, ME) with 305 μm interelectrode spacing. The electrode was driven into thalamorecipient layer 4 (~400 μm) by a micromanipulator with hydraulic microdrive (FHC Inc., Bowdoin, ME), and after a short adaptation period, recording and stimulus playback were initiated. Stimuli were generated using Tucker-Davis Technologies (TDT, Alachua, FL) System 3 hardware, and presented at a rate of 223214.2857 samples/s through a free-field speaker, which was fed by a PA5 programmable attenuator and SA1 stereo amplifier module. Further details about the experimental stimuli vary by study and can be found in the chapters describing each experiment. Stimulus playback and data acquisition were coordinated by TDT System 3 hardware controlled by the BrainWare application using modules programmed in the RPvdsEx environment. Electrophysiological signals were sampled at 24 kilosamples/s, passed through a RA16AC high impedance headstage, amplified with a RA16PA Medusa preamplifier (TDT, Alachua, FL), and filtered above 300 Hz and below 6 kHz, with a notch at 60 Hz. Spikes were detected using a negative threshold set by the experimenter (Figure A.1D). In setting the threshold, effort was made to isolate clearly defined spikes and exclude the increased noise that often accompanies evoked firing in multi-unit recordings. While these criteria were qualitative, post-hoc analysis of a subset of recordings

indicates that the threshold was set such that it exceeded the average root mean square of the signal during spontaneous activity by a factor of 5, on average.

Electrode penetration sites were labeled on the high-resolution photo of the AC by cross-referencing local vascular landmarks between the photo and the live microscope image.

Upon termination of electrophysiological recording, animals were given an overdose of the ketamine/xylazine anesthetic cocktail, and in some cases, a perfusion was performed and the brain was collected. In cases where the brain was not collected, cervical dislocation was performed prior to carcass disposal.

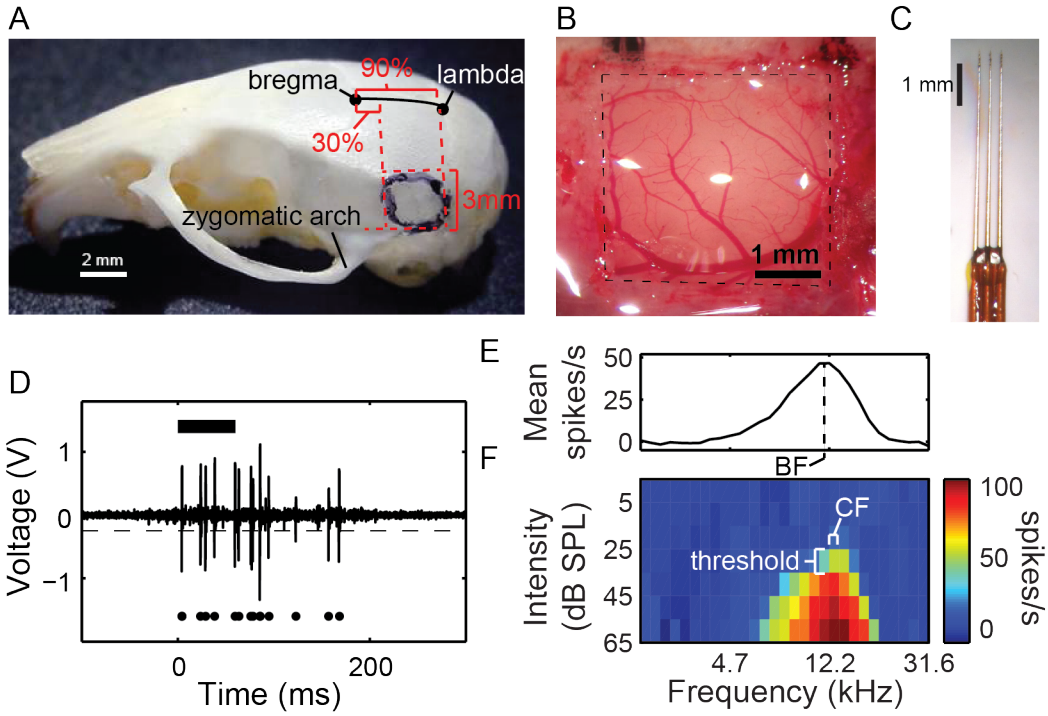

Figure A.1 Methodology of craniotomy and electrophysiological mapping. **A** Mouse skull with craniotomy boundaries delineated. **B** Exposed AC, depicted immediately prior to recording. Dashed lines illustrate craniotomy boundaries. **C** 3x1 matrix multi-electrode used for electrophysiological recording. **D** Sample electrophysiological recording in response to presentation of a single tone (black bar). Dashed lines indicate manually-set thresholds; a spike is counted (dots at bottom of plot) each time the voltage trace crosses the threshold. Signal is amplified 15,000x. **E** Sample tuning curve from a single multi-unit recording, showing the average firing rate as a function of frequency, averaging over all sound intensities presented. **F** Sample FRA from the multi-unit recording shown in E. Firing rate in spikes/s is plotted as a function of tone frequency and intensity. Some response properties are labeled: threshold is defined as the quietest sound intensity at which an evoked response is observed; BF is the frequency eliciting the strongest average firing rate response over all supra-threshold intensities; CF is the frequency that elicits the strongest response at the threshold intensity.

A.2 Data processing

Offline, peri-stimulus time histograms (PSTHs) were created by pooling spikes evoked by all trials (i.e., tones of all frequency/intensity combinations) from a given recording. A blinded experimenter manually determined the temporal boundaries of the excitatory phase of the evoked response. FRAs were constructed for each multi-unit by plotting the magnitude of the response (in spikes/s), integrated over this time window, against the frequency and amplitude of the stimulus (Figure A.1F). A blinded observer referenced each unit's FRA to manually determine its threshold. BFs were extracted in MATLAB (The Math Works, Natick, MA) and defined as the frequency that generated the highest average spike rate over all the intensities equal to or greater than the threshold intensity (Figure A.1E).

In all experiments, efforts were made to surround auditory-responsive sites with a perimeter of non-responsive sites, ensuring that the complete spatial extent of AC was captured. In these cases, BF maps were constructed by performing Voronoi tessellations on all recording sites for a given animal. These maps were used to provide a qualitative impression of auditory cortical organization, and to enable area-based measurements of characteristics of interest (e.g., area of cortex responsive to a given frequency). To construct a BF map, the X and Y coordinates of all recording sites were first extracted from the high-resolution AC photo using open-source software (DataThief). These coordinates were processed using the voronoin function in MATLAB, which returned estimated boundaries for each recording site. These boundaries define the polygons that represent recording sites in displayed maps. Area-based measurements were calculated by summing the areas of the polygons associated with recording sites exhibiting the characteristic of interest. A fraction of the recording sites corresponded to polygons with infinite or spuriously large areas because for technical reasons we were unable to 'enclose' that area with

a perimeter of non-auditory sites. To ensure that these areas did not bias our analyses, we reassigned the median area measurement to the 5% of sites with the largest areas.

In some cases, average BF maps were constructed to give a qualitative impression of tonotopic organization within groups. To generate these, individual animals' BF maps were normalized such that the rostrocaudal and dorsoventral extents of the AC fit a 100x100 grid, and each grid square was colored depending on which Voronoi polygon its center would fall within. These 'pixelated' maps for each animal within a group were 'stacked' and the BFs averaged for each cell in the z-direction. Finally, a mask was fitted over the final average map to exclude cells for which fewer than 6 maps contributed a BF measurement.

A.3 Recording site classification

Recording sites were assigned to one of the 5 subregions of the mouse AC using classification criteria outlined in Stiebler *et al.* (1997). Briefly, subregion designations were based on a recording site's 1) spatial position within AC, 2) BF, 3) response latency, 4) tuning bandwidth, 5) responsiveness to USVs (if USVs were presented), and 6) tendency to habituate to repeated presentations of a stimulus. Core auditory subregions (A1, AAF, and UF) can be characterized by their neurons' short response latencies (< 15 ms), sharp tuning bandwidths, and reliable, non-fatiguing onset responses to repeated presentations of a stimulus. Non-core subregions (A2 and DP) have longer response latencies (> 15 ms), wide or multi-peaked tuning curves, and a tendency to habituate to repeated presentations of a stimulus. These sites also tend to be more responsive to frequency-modulated sounds (like USVs), relative to pure tones with flat frequency trajectories. These criteria helped classify recording sites as core or non-core; frequency tuning and spatial position within the map helped further determine the precise subregion. A core recording site with an ultrasonic BF (> 40 kHz) was determined to be part of area UF so long as it was located rostro-dorsally. Core sites with BFs below 40 kHz were assigned to either AAF or A1, depending on their position relative to the high-frequency band

that separates AAF from A1. Specifically, sites rostral or caudal to that band were designated AAF or A1 sites, respectively. Non-core sites positioned caudo-dorsally relative to core AC were assigned to area DP, whereas those appearing ventrally were assigned to area A2. Subregions were assumed to be spatially contiguous. An example BF map with sample PSTHs and FRAs from each subregion is shown in Figure A.2.

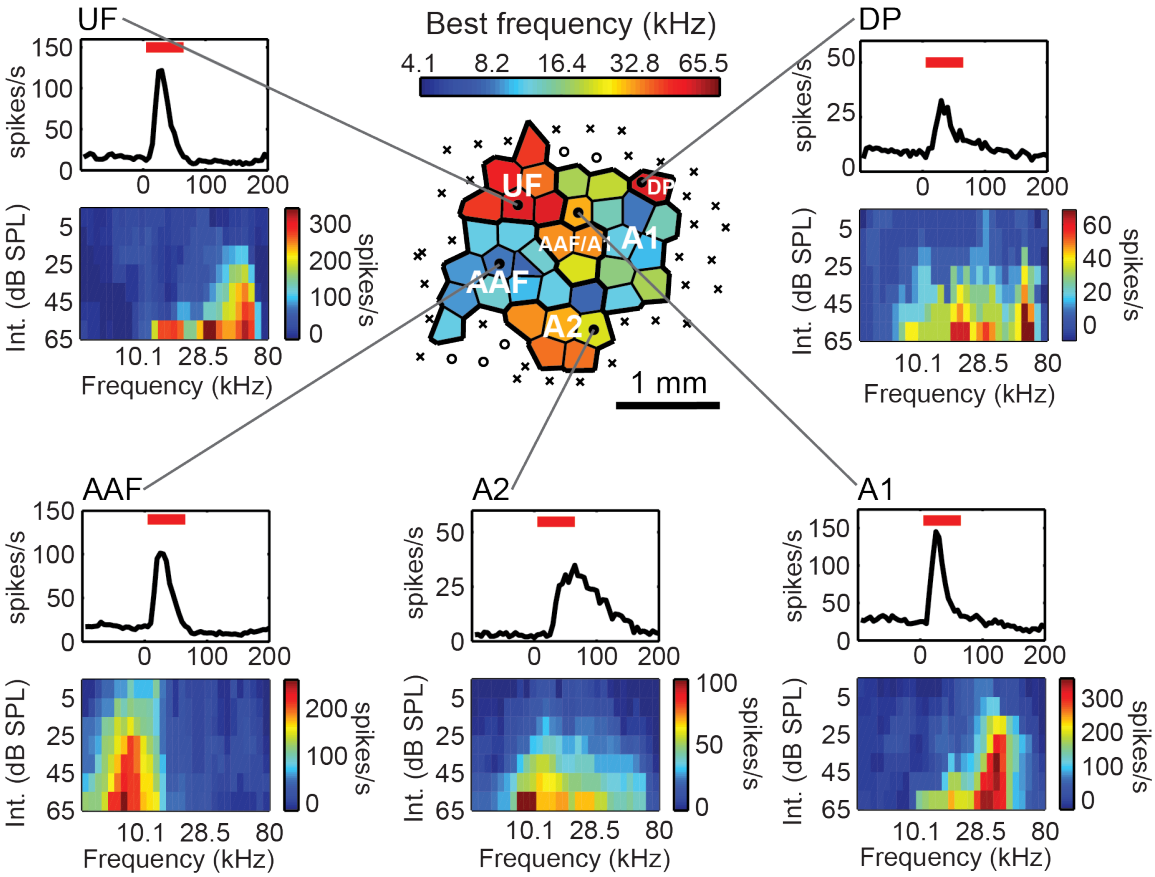

Figure A.2 Sample BF map with example multi-units exemplifying each auditory field. On sample map, Voronoi polygons are drawn around all AC recording sites and color-coded by BF. Thick borders define subregions. Xs indicate non-auditory recording sites. Os indicate auditory sites belonging to unknown subregion. For sample multi-units, PSTHs (above) show pooled average firing rate response to a set of tones (time course illustrated with red bar). FRAs show response strength to tones as a function of their intensity (on y-axis) and frequency (on x-axis).

When these criteria conflicted in a way that precluded an obvious subregion assignment (e.g., a site's spatial position was consistent with an assignment to subregion UF, but its BF was

below 40 kHz), the site's subregion was designated "unknown." Additionally, the actual subregion of sites located in the high-frequency band that separates A1 and AAF was ambiguous. As such, these sites were assigned to a transitional region "AAF/A1." These sites, like those in A1, AAF, and UF, were included in any analyses restricted to core AC.

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