PLASTICITY IN THE AUDITORY CORTEX AND CHANGES IN PERCEPTUAL DISCRIMINATION AFTER NUCLEUS BASALIS STIMULATION IN RATS

by

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by

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PREFACE

This dissertation was produced in accordance with guidelines which permit the inclusion as part of the dissertation the text of an original paper or papers submitted for publication. The dissertation must still conform to all other requirements explained in the "Guide for the Preparation of Master's Theses and Doctoral Dissertations at The University of Texas at Dallas." It must include a comprehensive abstract, a full introduction and literature review and a final overall conclusion. Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported.

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PLASTICITY IN THE AUDITORY CORTEX AND CHANGES IN PERCEPTUAL DISCRIMINATION AFTER NUCLEUS BASALIS STIMULATION IN RATS

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Humans and many other species have the capacity to learn and change their behavioral responses when they repeatedly practice a discrimination task. This change in behavior must be caused by changes in response properties of the nervous system. Understanding the relationship between learning and changes in neural responses has been an important field of study for the past twenty years. Numerous papers have observed correlations between plasticity in primary cortical areas and improved perceptual discrimination abilities, implying that this plasticity is the underlying cause of improved performance. However, a causal relationship cannot be proven unless plasticity is induced outside of a behavioral context. In the following dissertation I document the perceptual consequences of plasticity induced using stimulation of the nucleus basalis paired with auditory stimuli. The nucleus basalis is a deep-brain structure which releases acetylcholine onto the neocortex during behaviorally important events. Damage to this structure has been shown to impair both learning and plasticity, and stimulation during presentation of sensory stimuli produces plasticity which mimics the effects observed after behavioral training. We

demonstrate for the first time that pairing nucleus basailis stimulation with a tone can alter learning and performance of a frequency discrimination task. We also document a pattern of plasticity after discrimination training and nucleus basalis stimulation which indicates that cortical plasticity in primary sensory areas may be important for learning but not performance of a discrimination task. Finally, we report a further possible source of cortical plasticity and behavioral improvement by showing that nucleus basalis-stimulation pairing can cause stimulus-specific plasticity in both primary and secondary cortical areas. The results of these studies reveal that cortical plasticity contributes to sensory discrimination and perceptual learning, and provide new insights about the relationship between cortical plasticity and continued performance of well-learned behavioral tasks.

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CHAPTER 1

INTRODUCTION

The capacity of our nervous system to change its response properties is called plasticity. This capacity is most recognizable when we watch young children learn to walk and talk. In infants, the nervous system is constantly plastic as the brain adjusts to new environmental stimuli and develops the circuitry that will be necessary to communicate with others and interpret and interact with their surroundings. Once we reach adulthood, much of our basic neural circuitry is intact, but we still acquire new skills throughout our lifetime. Plasticity occurs as parents learn to interpret their young child's first attempts at speech, as athletes practice and perfect their golf-swings, and as new college students learn how to find their classes on a unfamiliar college campus. Even more importantly, these people are able to learn and acquire these skills without sacrificing other abilities that they posses. This capacity to acquire plasticity in a balanced way shows that plasticity is tightly controlled by both what we experience (stimulus inputs) and how much we respond to these inputs (attention/behavioral engagement).

Plasticity in adults is observed when stimulus inputs have been permanently altered or when stimuli are behaviorally relevant. Peripheral nerve injuries can lead to a complete and permanent disruption in the pattern of inputs that a sensory cortex receives. For example, after amputation of a finger, the area of cortex that had represented that finger would lose its primary source of input activity. However, rather than simply becoming a 'silent' area of cortex that does not respond to any inputs, this cortical area reorganizes its receptive field properties to begin to respond in a similar fashion as neighboring cortex (Merzenich et al. 1984). Similarly, central

nervous system injuries such as stroke or traumatic brain injury can lead to a situation in which peripheral nerve inputs are no longer represented within sensory cortex. Surrounding areas may then take over the functional role of the lesioned cortex. This plasticity can lead to improvements in sensation or movement abilities, and is hypothesized to be one of the primary sources of stroke recovery (Cramer and Riley 2008). However, in addition to plasticity after traumatic peripheral and central nervous system injuries, the adult brain experiences plasticity during normal learning situations. For example, monkeys who were trained to perform a simple frequency discrimination task showed changes in their primary auditory cortex so that more auditory cortex neurons were responsive to the behaviorally relevant stimuli (Recanzone et al. 1993). This finding has been reproduced across several species, including rats and humans (Menning et al. 2000; Polley et al. 2006; Rutkowski and Weinberger 2005). The type of plasticity that is observed after training seems to be dependent on the task parameters that were most behaviorally important. For example, rats and monkeys that were trained to perform a temporal discrimination task showed improved temporal processing in primary cortical areas (Bao et al. 2004; Recanzone et al. 1992). Training on an intensity discrimination task leads to changes in the intensity response profiles of primary auditory cortex neurons (Polley et al. 2006). Plasticity is also dependent on behavioral relevance, in that stimuli that are not behaviorally relevant do not lead to changes in receptive field properties (Bao et al. 2004; Recanzone et al. 1993; Zhang et al. 2001).

In adults, plasticity during learning is primarily controlled by attention/arousal, and therefore by neuromodulator release. Behaviorally important events lead to activation of the limbic system, which in turn results in the activation of several subcortical structures that project to the cortex and release neuromodulators. Several neurotransmitters such as acetylcholine,

norepinephrine, dopamine and serotonin are released during behaviorally important events (Myhrer 2003). The gating of plasticity through the release of these neuromodulators prevents plasticity in response to behaviorally irrelevant stimuli.

The cholinergic system seems to be particularly important for learning and plasticity. The entire neocortex receives cholinergic inputs from the nucleus basalis, which is active during both positive and negative behavioral events (Richardson and DeLong 1991). Disruption of these cholinergic fibers impairs learning and cortical plasticity associated with skilled reaching tasks (Conner et al. 2005; Conner et al. 2003). It has also been shown that stimulation of the nucleus basalis (NB-stimulation) causes cortical plasticity that resembles the changes that occur after behavioral training (Bakin and Weinberger 1996; Kilgard and Merzenich 1998). Therefore, activation of the cholinergic system appears to be necessary for normal plasticity and for improvements caused after learning to take place. Just as in behavioral studies, the stimulus parameters that are paired with NB-stimulation pairing control the form that cortical plasticity takes. For example, pairing NB-stimulation with a simple tone results in enlarged representation of that tone in primary auditory cortex(Kilgard and Merzenich 1998), while pairing NB-stimulation with temporally modulated stimuli leads to changes in temporal response properties of auditory cortex neurons(Kilgard et al. 2001). The similarity between plasticity after NB-stimulation and the plasticity that occurs after behavior implies that these two techniques employ similar plasticity mechanisms.

If plasticity is the basis of perceptual discrimination improvement, then any techniques that lead to plasticity should also lead to changes in perceptual abilities. For example, subjects commonly report improved discrimination thresholds for stimuli that have an expanded representation because they are at the edge of peripheral lesions (i.e., frequencies that are

adjacent to a hearing loss due to cochlear damage) (Irvine et al. 2001; McDermott et al. 1998). Plasticity induction techniques can also change perceptual discrimination performance. A study in the auditory system looking for changes in frequency discrimination after intracortical microstimulation did not show alterations in frequency discrimination performance after stimulation (Talwar and Gerstein 2001). However, Dinse and colleagues used a tactile coactivation protocol to produce temporary receptive field reorganization in the primary somatosensory system, and found that subjects had improved two-point discrimination performance while receptive field reorganization persisted (Dinse et al. 2003; Godde et al. 2000). These two studies together indicate that short-term plasticity may induce altered perceptual abilities if this plasticity is of sufficient magnitude. The plasticity created after intracortical microstimulation and after tactile coactivation is smaller and more transient in nature than the plasticity observed after nucleus basalis stimulation. We predict that a technique such as NB-stimulation that causes extensive long-term plasticity will cause long-lasting alterations in discrimination abilities. This hypothesis is tested in Chapter 2 of this dissertation.

Many studies that examine plasticity after behavioral training or other plasticity techniques focus on a single station within the auditory cortex. However, plasticity after behavioral training or nucleus basalis stimulation can affect the response properties of many subcortical and cortical stations simultaneously. Studies of classical conditioning have found evidence of plasticity in subcortical stations such as the thalamus and inferior colliculus (Edeline 2003; Edeline and Weinberger 1992, 1991a, b; Ji et al. 2001), and have also found evidence of plasticity in both primary and secondary cortical areas (Diamond and Weinberger 1986, 1984). If plasticity after NB-stimulation is mechanistically similar to the plasticitiy observed after behavioral training, we would expect to see a similar pattern plasticity across multiple auditory stations after NB-

stimulation pairing. Pairing NB-stimulation with a single tone stimulus causes receptive field reorganization of primary auditory cortex. This reorganization in A1 then causes receptive field reorganization in the auditory thalamus and in the inferior colliculus via top-down connections that alter response properties in both of these subcortical areas(Ma and Suga 2005; Zhang and Yan 2008). The purpose of Chapter 3 of this dissertation was to verify that both primary and secondary cortical areas show stimulus-specific plasticity after NB-stimulation pairing.

The rest of this dissertation contains three chapters and two appendix sections. Chapter 2 addresses the main aim of this dissertation, which was to determine the perceptual consequences of cortical map plasticity induced by NB-stimulation pairing. The two appendix sections provide supplementary data and a more detailed description one of the analysis techniques employed in this paper. Chapter 3 of this dissertation reports on plasticity in secondary cortical areas after NB-stimulation pairing. Chapter 4 discusses the interpretation and clinical relevance of the primary findings of this dissertation.

CHAPTER 2

PERCEPTUAL CONSEQUENCES OF CORTICAL RECEPTIVE FIELD PLASTICITY

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ABSTRACT

A correlation between behavioral improvement and cortical plasticity has been observed over many studies in the past twenty years. However, a causal relationship between cortical plasticity and perceptual abilities has been difficult to establish. In the current study, we used nucleus basalis stimulation pairing to induce receptive field plasticity in the primary auditory cortex and examined how this plasticity altered frequency discrimination abilities. We found that receptive field plasticity induced outside of a behavioral context is sufficient to alter frequency discrimination performance. We also observed that cortical plasticity is not maintained in highly-trained animals and thus is not necessary for frequency discrimination performance. These results highlight the complexity of the relationship between cortical plasticity and perceptual discrimination.

INTRODUCTION

Learning must be accompanied by changes in brain responses. In the field of sensory learning, it has often been assumed that improvements in discrimination performance are the result of altered representations of stimuli within a particular sensory station. However, the plasticity which enables improved discrimination performance may be labile in and of itself, and may not always be representated in the same way within sensory areas. Discrimination performance improves and representation of task-relevant stimuli is increased in the visual cortex during early learning, but after a few weeks plasticity fades while discrimination performance remains the same (Yotsumoto et al. 2008). A similarly complex relationship exists in the auditory system – an increase in the representation of behaviorally relevant stimuli within primary auditory cortex has been correlated with improved discrimination performance (Polley et al. 2006; Recanzone et al. 1993; Rutkowski and Weinberger 2005) but this increased representation is not always present after discrimination training which results in perceptual improvement(Brown et al. 2004). These contradictions in the literature may be resolved by the hypothesis that receptive-field plasticity in sensory systems is important for learning of a discrimination task, but that sensory plasticity is not necessary for the performance of learned discriminations.

If plasticity is important for discrimination learning, then plasticity induced outside of a behavioral context should have behavioral consequences. In order to significantly impact discrimination learning or performance, plasticity would have to induce long-lasting stimulus-

specific plasticity within sensory systems. Plasticity could improve behavior if it mimics changes usually observed during learning. Plasticity could worsen behavior if it leads to contrary shifts in receptive field plasticity.

Long-term, stimulus specific plasticity can be induced in the auditory system by stimulating the cholinergic nucleus basalis (NB) concurrently with auditory stimulus presentation (20 daily sessions of 300-320 tone/stimulation pairings in each session). Previous studies have demonstrated that NB-tone pairing leads to receptive field plasticity in the primary auditory cortex which mimics the changes observed after behavioral training (Kilgard and Merzenich 1998a). These changes are also observed in secondary cortical areas such as the posterior auditory field(Puckett et al. 2007), and subcortical stations of the auditory system, such as the inferior colliculus and thalamus (Ma and Suga 2005). This indicates that NB-tone pairing is an effective technique to induce widespread, stimulus-specific plasticity in the auditory system apart from a behavioral context.

To determine if plasticity in the auditory system is important for learning and performance of auditory discrimination tasks, we used NB-tone pairing to induce auditory system plasticity and examined performance on a low-frequency discrimination task.

RESULTS

We predicted that low-frequency receptive field plasticity induced by NB-tone pairing would improve learning of a low-frequency discrimination task because animals would enter training with distinct neural representations of low-frequency tones. Fifteen rats were trained to perform an easy noise-discrimination task so they would be familiar with the procedural aspects of the go/no-go task before moving on to one month of tone exposure (Figure 2.1A, and see Appendix 2 for noise discrimination behavior data). During tone exposure, all groups heard both low (2 kHz) and high (19 kHz) tones. This balanced design was possible because none of the animals were familiar with tones before NB-tone pairing and would not have formed any behavioral associations other than those induced by NB-stimulation. The low tone was paired with NB stimulation for the Task-Naïve Low group (n=5), while the high tone was paired with NB stimulation for the Task-Naïve High group (n=5). A passive control group (Task-Naïve Passive, n=5) heard both tones but received no NB-stimulation pairing. After tone exposure, all rats were trained to perform a low frequency tone discrimination task (1.8 kHz target vs. distracters 0.5, 1.0, and 2.4 octaves above the target).

The Task-Naïve Low group learned to perform the low frequency discrimination task more quickly than the Task Naïve High or Task-Naïve Passive groups (Figure 2.1B). By the end of the easy discrimination period, the Task-Naïve Low group performed the discrimination task significantly better than the other two groups (*d'* discrimination of all three distracter tones, $F(2,14) = 4.9399$, $p = 0.0272$, repeated measures ANOVA). While the Task-Naïve Low group was able to discriminate all three distracter stimuli from the target (Figure 2.1C), the Task-Naïve

High group was only able to perform the easiest discrimination task (Figure 2.1D). After seven days of testing the Task-Naïve Passive group was not able to perform any of the discriminations above chance (Figure 2.1E). These results indicate that NB-tone pairing enhances tone frequency discrimination learning most when the paired tone is in a frequency range that is relevant to the task.

If cortical plasticity is a substrate of discrimination performance, then well-trained animals should also be susceptible to changes in discrimination performance after NB-tone pairing, especially pairing protocols which might cause plasticity for tones that were completely unrelated to the discrimination task. In particular, we predicted that NB-tone pairing with high-frequency tones would impair performance of a low-frequency discrimination task in well-trained animals. Three groups of animals were trained to perform the low-frequency discrimination before exposure to NB-tone pairing (Figure 2.2A), and showed similar performance of the low-frequency discrimination task before NB-tone pairing (Figure 2.3B, *d'* discrimination of 0.38 to 1.0 octave distractors, $F(2,16) = 0.0572$, $p = 0.9446$, repeated measures ANOVA). The Pretrained Low frequency group (n=7) heard only 2kHz tones during NB-stimulation pairing, and the Pretrained High frequency group (n =6) heard only 19 kHz tones during NB-stimulation pairing. Although the 2 kHz tone played during NB-stimulation had different temporal characteristics than the sounds which rats heard during behavior training (the tone during NB-tone pairing was a single 250 ms long stimulus, while the target stimulus during behavior was a train of tone pips), we were concerned that even simple exposure to the 2 kHz tone might interfere with behavior, either by causing additional plasticity as animals recalled the behavior task, or by causing habituation to the tones which would decrease behavioral

performance after Tone Exposure. Therefore, a Pretrained Passive group (n=5) heard the 2 kHz tone with no NB-stimulation.

Since all three groups had reached asymptotic task performance, we expected that NB stimulation paired with the high frequency tone would impair discrimination of the low frequency tones. The Pretrained High group was significantly impaired compared with the Pretrained Low or Pretrained Passive groups during the first three days after tone exposure/NB-tone pairing (Figure 2.2C, *d'* discrimination of 0.3 to 1.0 octave distracters, $F(2,16) = 3.6454$, $p = 0.0496$, repeated measures ANOVA). Therefore, Experiments 1 and 2 confirm our hypothesis that receptive field plasticity is important for learning and discrimination, and confirm that plasticity induced outside of a behavioral context can improve learning or worsen discrimination performance.

Although the behavioral results from Experiment 1 and 2 confirm that NB-tone pairing can significantly improve discrimination learning, these results do not definitively prove that plasticity in primary cortical areas is necessary for maintenance of behavioral performance. Previous literature has demonstrated that plasticity in specific areas can increase during learning, but fade after steady discrimination performance has been reached (Yotsumoto et al. 2008). Therefore, we examined receptive field plasticity in the primary auditory cortex (A1) after a two-period of difficult discrimination testing in all groups of animals in Experiments 1 and 2. We predicted that plasticity would be most prevalent in the animals in Experiment 1, especially those who had shown recent learning of the discrimination task (Task Naïve High and Task Naïve Passive animals, Figure 2.3A). On the other hand, we did not expect to see plasticity in animals from Experiment 2, who had been trained extensively on the low-frequency discrimination (Figure 2.3B). We recorded multiunit responses from the right primary auditory cortex of all

Task-Naïve and Pretrained groups and compared their responses to naïve controls (*n*=9) . Previous studies of plasticity have found that behavioral training commonly results in increased representation and refined receptive fields of target stimuli (Polley et al. 2006; Recanzone et al. 1993). Both the Task-Naïve High and Task-Naïve Passive groups showed low-frequency receptive field plasticity which was similar to that observed in previous studies of plasticity. These groups had an increase in the relative percentage of cortex which responded to low-frequency tones compared to naïve controls (Figure 2.4E, Naïve controls vs. Task-Naïve High, p = 0.0259; Naïve controls vs. Task-Naïve Passive, p = 0.0285, t-tests). Sites which were responsive to the target stimulus were narrowed in both groups, (Figure 2.4F, BW30 for Naïve controls vs. Task-Naïve High, p = 0.0177, Naïve controls vs. Task-Naïve Passive, p = 0.0016, t-tests), but sites which did not respond to the target stimulus were unaltered for both the Task-Naïve High and Passive groups (see Appendix 1 for supplementary data). These results support our hypothesis that recent learning of a discrimination task is most likely to generate stimulus-specific plasticity. The Task-Naïve Low group, who showed the greatest initial behavioral improvement and fastest learning of the discrimination task, did not show evidence of receptive field plasticity, either in terms of increased preference for low-frequency tones (Figure 2.4E, Naïve controls vs. Task-Naïve group, p = 0.2715, t-test), or narrowed receptive fields in target responsive sites (Figure 2.4 F, Naïve controls vs. Task-Naïve group, p 0.09506, t-test). These results imply that the NB-stimulation paired with low-frequency tones which the Task-Naïve Low group experienced served as a surrogate for behavioral training in terms of improving discrimination performance and causing plasticity to shift back to a normal state.

All of the Pretrained groups (Experiment 2) had learned to perform the discrimination task and shown steady discrimination performance before the NB-stimulation or Tone Exposure.

Because these animals had been steadily performing the discrimination task for several weeks, we predicted that stimulus-specific plasticity in these groups might not be present even though discrimination performance had remained high. Although the Pretrained High group showed an initial deficit in frequency discrimination immediately after NB-tone pairing, there was no significant difference in performance between the three Pretrained Groups by the end of training (Figure 2.4G, discrimination of 0.38 to 1.0 octave disctracters, $F(2,16) = 0.5499$, $p = 0.5875$, repeated measures ANOVA). We found no evidence of receptive field reorganization in any of the Pretrained groups (Figure 2.4H, BW30 for Naïve controls vs. Pretrained Low, High and Passive respectively, $p = 0.2452$, $p = 0.7912$, $p = 0.3244$, t-test).

Our hypothesis predicts that the Pretrained groups of animals should have shown receptive field plasticity earlier during their training, immediately after they had learned to perform the low-frequency discrimination task. In order to confirm this, we conducted a high density microelectrode mapping study in A1 immediately after a group of six rats were trained to perform the low frequency tone discrimination task (Behavior Only group, *n*=6, Figure 2.4A). These animals went through the same pretraining procedure as animals in the Pretrained Groups. Animals in this group showed an increase in the percentage of A1 responded to low-frequency (trained) tones vs. high frequency (untrained) tones compared to naïve controls (Figure 2.4B, Naïve controls (n=9) vs. Behavior Only (n=6), $p = 0.0186$, t-test). In addition, A1 sites which were responsive to the target behavioral stimulus (1.7818 kHz) had narrower receptive fields than naïve controls (Figure 2.4C, BW30 of target stimulus responsive sites, $p < 10^{-3}$, student's t-test). Receptive fields of sites which did not respond to the target frequency did not change (see Appendix 2 for supplementary data). Taken together, these results indicate that a short course of frequency discrimination training was sufficient to induce receptive field reorganization toward

the behaviorally relevant stimulus and confirm previous studies (Polley et al. 2006; Recanzone et al. 1993). In addition, long periods of behavior training and NB-tone pairing seemed to encourage a renormalization of map plasticity so that the receptive organization of primary auditory cortex resembled that of naïve controls.

Similar to behavioral training, we observed that the plasticity induced by NB-stimulation lasts for a number of days, but then fades. We examined the time course of plasticity in A1 after NB-tone pairing in five grounps of animals who experienced NB-stimulation paired with a 19 kHz tone. One group of animals was mapped twenty four hours after NB-tone pairing (1 day group), and three other groups were housed in standard laboratory conditions for 10, 20 and 100 days before mapping. The 1, 10 and 20 day groups showed an increase in the percentage of A1 neurons which responded to the paired tone compared to naïve controls (Figure 2.5, control(n=6) vs. 1-day group(n=5): $p = 0.0368$; 10 day group (n=5): $p = 0.0114$; 20 day group (n=5): $p = 0.0071$, one-tailed t-tests). However, receptive field plasticity after NB-tone pairing was not permanent – the 100 day group showed no evidence of receptive field shifts (Figure 2.5, n=6, $p = 0.4341$, one-tailed t-test). Our results indicate that prolonged NB-stimulation/tone pairing is sufficient to produce long-lasting but not permanent plasticity in A1.

The plasticity results from experiment 1 and 2 implied that the duration of plasticity is likely to be influenced by behaviorally relevant stimuli such as those experienced during behavioral training. Although plasticity was relatively stable when animals were housed in a standard animal care environment, enrichment hastened the restoration of A1 to a normal state (Figure 2.5). The percent of cortex which responded to the paired tone was only slightly larger than normal controls after 20 days of housing in an enriched environment (n=5, $p = 0.1930$, one-tailed

t-test). Therefore, receptive field plasticity in primary auditory cortex can be eliminated by either behavioral training or environmental enrichment.

DISCUSSION

We confirm here that learning-induced plasticity is not a permanent feature in sensory systems, and that plasticity can develop and then fade while task-performance remains the same. We believe that early in the learning process, stimulus-specific plasticity in sensory pathways clarifies the representation of target and distracter stimuli and allows for better discrimination learning. As learning progresses, the increased representation of behaviorally important stimuli may become less important for accurate task performance because the animals already have clear categorical representations of the target and distracter stimuli. Sufficient amount of behavioral training, especially training in which animals are adequately performing the discrimination task may then trigger a renormalization of the gross features of the sensory pathway back to a normal state. This renormalization without a loss of discrimination ability would be important for animals in natural environments, in which they would have to learn to perform a variety of perceptual discriminations in order to survive.

We examined plasticity only within the primary auditory cortex, because the majority of studies examining learning-induced plasticity have focused on sensory cortex. Many studies of short-term plasticity after NB-stimulation pairing and after classical conditioning paradigms have indicated that plasticity can occur in several sensory stations at once (Bakin and Weinberger 1996; Diamond and Weinberger 1986, 1984; Edeline and Weinberger 1992, 1991a, b; Ji et al. 2001; Zhang and Suga 2000). However, this same pattern of plasticity may not hold during longer-time courses of training. Future studies should examine whether other sensory stations

follow a similar time course of plasticity and identify which brain regions in highly trained animals respond differently than naïve animals.

Although plasticity is not strictly necessary for task performance, inducing stimulus-specific plasticity in sensory systems alters both learning and discrimination performance. Therefore, treatments which can induce long-lasting plasticity within sensory systems should induce long-lasting improvements in the recovery of patient populations. Using a technique such as NB-stimulation which pairs precise neuromodulator release with sensory input might afford greater functional improvement than therapies which rely on sensory stimulation alone or medications which are unable to create temporally or spatially specific effects.

METHODS

Behavior Training

All rats were trained to perform a simple go/no-go stimulus recognition task. Target or distracter stimuli were presented approximately every ten seconds, and animals were required to press a lever within three seconds of target presentation and refrain from hitting after presentation of a distracter. Rats received a 45-mg sugar pellet when they pressed three seconds after a target stimulus presentation, but pressing the lever after a distracter or during silent periods between sound presentations resulted in a timeout period in which all lights in the cage were extinguished and further sound presentations were delayed for 6-8 seconds.

Experiment 1(Figure 2.1A): For the easy noise-discrimination task before the tone exposure period, the target stimulus was a train of six white noise bursts (25 ms duration, 60 dB intensity, 1-32kHz frequency range) presented at a rate of 5 Hz, while the distracter stimulus was a complex noise stimulus with irregular temporal and spectral features which had the same duration and overall intensity as the target noise-burst train (1025 ms duration, 60 dB intensity, 1-48kHz frequency spectrum). The Task Naïve animals spend 15 days learning to reliably respond immediately after presentation of the target noise stimulus and then spent 3 days learning to discriminate between the target and distracter noise stimuli before moving on to tone exposure. For the low frequency discrimination tasks, the target stimulus was always a train of six tone pips (25 ms duration, 60 dB intensity, 1.7818 kHz carrier frequency) presented at a rate of 5 Hz, while the distracter stimuli varied only in carrier frequency (from 1.9 to 9.5 kHz, or 0.1 to 2.4 octaves above the CS+ stimulus). During the easy discrimination stage for Task Naïve

groups, the distracter stimuli were 0.5, 1.0 and 2.4 octaves above the target stimulus, and during the Difficult Tone Task, the distracter stimuli wee 0.1, 0.26, 0.38, 0.5, 0.75, 1, 1.5 and 2.4 octaves above the target stimulus.

Experiment 2 (Figure 2.2A): The Pretrained groups learned to perform a frequency discrimination task before tone exposure. The target stimulus for this group was identical to the frequency target for the Task Naïve groups (1.78 kHz tone train) and distracter stimuli ranged from 0.1 to 1.0 octaves above the target stimulus. During the pretraining Easy Tone Task, Pretrained rats spent 20 days learning to reliably respond after presentation of the target stimulus, and then spent 10 days learning to respond to target stimulus and ignore a distracter 1.0 octaves above the target stimulus. During the difficult discrimination task, the distracter stimuli were 0.1, 0.2, 0.25, 0.32, 0.38, 0.44, 0.5, 0.75 and 1.0 octaves above the target stimulus.

Discrimination performance was measured using the signal detection theory measure d-prime during all stages of training (Klein 2001). We determined that all groups could reliably discriminate distracter stimuli which were at least 0.38 octaves above the target stimulus, and so used discrimination performance on those stimuli to measure changes in discrimination performance after tone exposure. Statistical comparisons between three or more groups were done using repeated measure ANOVAs. Statistical comparisons between only two groups or relative to zero were done using t-tests.

NB-stimulation pairing

NB-stimulation pairing was conducted using the same methods as in previous publications (Kilgard and Merzenich 1998a, 2002, 1998b; Kilgard et al. 2001a; Kilgard et al. 2001b; Puckett et al. 2007). All NB-stimulated animals and the Pretrained Passive group underwent an implantation surgery 2-3 weeks before training began. A platinum bipolar

stimulating electrode was lowered 7 mm below the cortical surface from a location 2.3 mm posterior and 3.3 mm lateral to bregma in the right hemisphere. Bone screws located approximately 5 mm posterior to the implant and above the cerebellum were used to monitor EEG activity.

During NB-stimulation pairing the paired stimulus was presented approximately every ten seconds 275-350 times per day for a period of 20 days. Silent intervals (and unpaired stimuli for the Task Naïve Groups) were inserted at random to prevent habituation, and each pairing session lasted approximately three and a half hours. Paired stimuli were either a 2 kHz or 19 kHz tone, 250 ms duration, presented at 50 dB SPL. Each tone presentation was accompanied by a short burst of current delivered to the bipolar stimulating electrode (20 biphasic pulses, 0.1 ms duration at 100 Hz) 50 ms after tone onset. The current amplitude ranged from 120-200 μamps for each animal, and was selected to reliably elicit brief EEG desynchronization for 1-3 seconds whenever the animal was in slow wave sleep. Passive exposure animals were trained the same booths and heard the same acoustic stimuli, but were not connected to the stimulators.

Physiology

Physiological experiments used similar methods as reported in previous publications (Kilgard and Merzenich 1998a, 2002; Kilgard et al. 2001a; Kilgard et al. 2001b; Puckett et al. 2007). Physiological recordings in took place under pentobarbital anesthesia (50 mg/kg). Multiunit responses were recorded using two bipolar parylene-coated tungsten electrodes (250 μm separation, 2 MOhm at 1 kHz, FHC Inc., Bowdoinham, ME) which were lowered ~550 μm below the cortical surface (layer IV/V). At each site, a tuning curve consisting of 81 frequencies spanning from 1 to 32 kHz at 16 intensities spanning from 0 to 75 dB SPL was presented (1,296

tones, 25 ms duration, 5 ms rise and fall time, 1 repetition of each). In total, we recorded from 6414 sites in 77 animals.

All sites from control and experimental rats were analyzed using an automated tuning curve analysis program (see Appendix 3 for detailed description). A post-stimulus time histogram (PSTH) was constructed from all of the responses to tone-intensity combinations within the receptive field using 1 ms width bins. The receptive field area was then calculated using image analysis techniques from a grid of the responses to each frequency-intensity combination during the driven response period (from onset to end of peak latency). Several receptive field characteristics were then calculated based on the identified area of driven activity. The lowest intensity that evoked a reliable neural response was defined as the threshold, and the frequency at which this response occurred defined the characteristic frequency (CF). Four bandwidths (BW10-BW40) were calculated as the range of frequencies (measured in octaves) which evoked reliable responses at 10, 20, 30 and 40 dB above threshold.

Voronoi tessellation was used to transform the discretely sampled surface into a continuous map using the assumption that each point on the map has the response characteristics of the nearest recording site. Since regions with above average sampling density have smaller tessellations, they do not bias estimates of the cortical response. A1 sites were identified on the basis of latency and topography. The percent of the cortical area of A1 responding to each tone was estimated as the sum of the areas of all tessellations from sites in A1 with receptive fields that included the tone divided by the total area of the field.

For all behaviorally trained animals, we reported changes in the representation of behaviorally relevant tones by reporting the ratio of the percent of cortex which responded to a 2kHz, 60 dB SPL tone divided by the percentage of cortex which responded to a 19kHz, 60 dB

SPL tone. In behaviorally trained animals, we commonly observe both a shift in tuning towards behaviorally relevant tones and a decrease in receptive field sizes. The net effect of this plasticity is to cause the cortical response to behaviorally-irrelevant tones to decrease while the response to behaviorally-relevant tones is only slightly increased or unchanged. Therefore, a ratio measure provides a clear representation of shifts in frequency organization of A1 which is not influenced by the shift in receptive field sizes. For the time course study in which animals were mapped after NB-stimulation pairing alone, we chose to use the percentage of A1 cortex which responded to a 19kHz, 60d dB SPL tone as our plasticity measure.

T-tests were used for all statistical comparisons between two groups. ANOVA was used when the response properties of three or more groups were compared.

APPENDIX 1

FIGURES

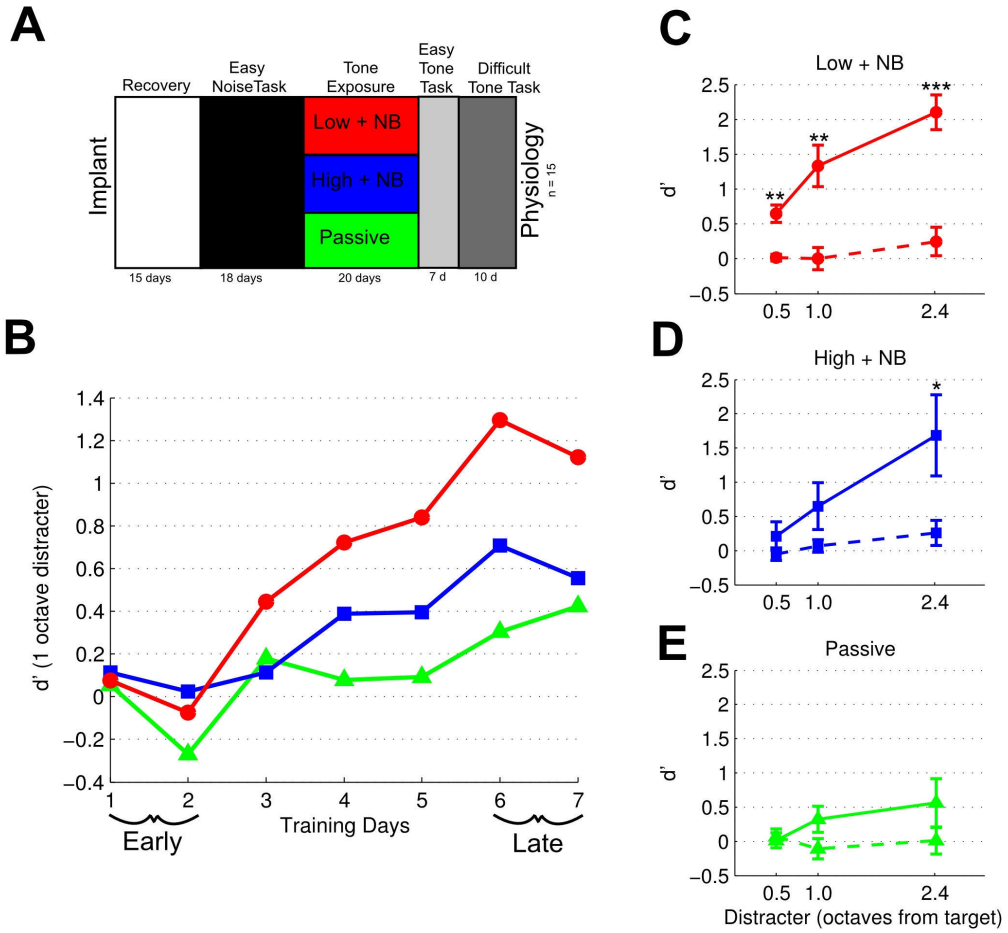

Figure 2. 1. A. Training timeline for Untrained Groups of animals. Animals did not discriminate tone sequences before tone exposure/NB-stimulation pairing began. B. Mean performance of each group during easy tone discrimination training. C,D,E. Mean ± s.e.m. discrimination performance for each group during the first 2 days (Early) and last 2 days (Late) of the easy frequency discrimination period. *, p<0.05; **, p<0.01; ***, p<0.001; all stars indicate statistical results of a t-test of whether discrimination performance was significantly above chance (d-prime of zero).

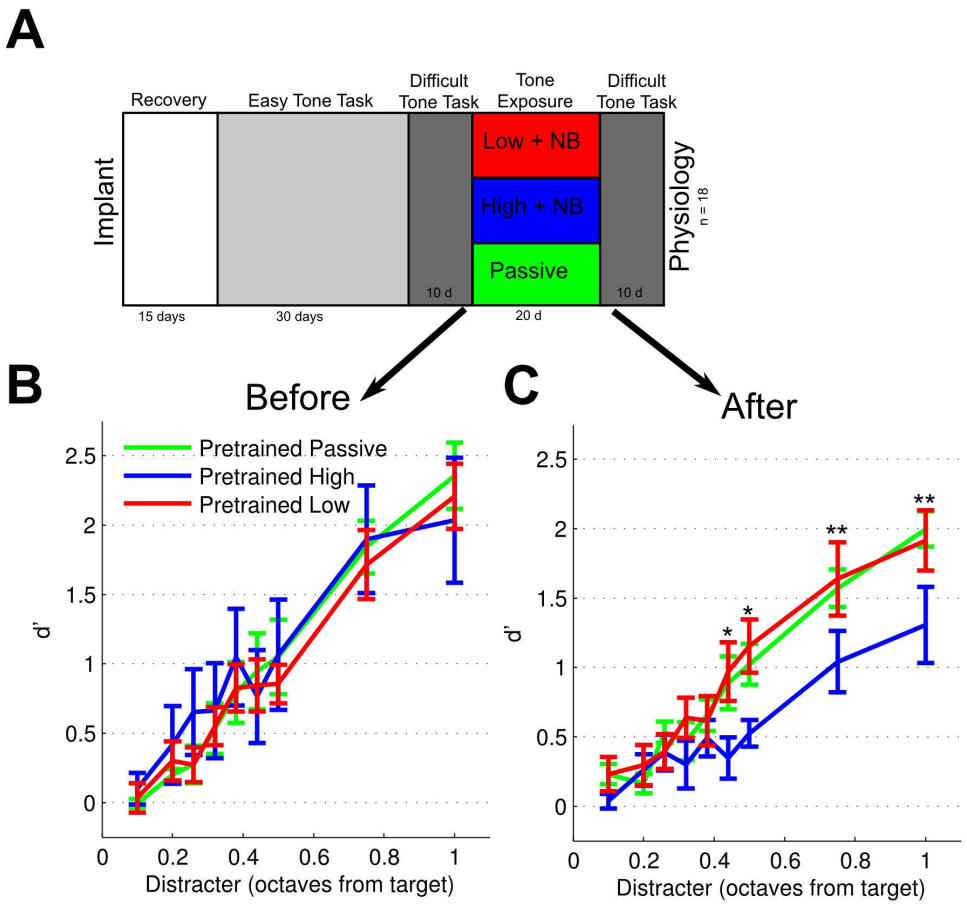

Figure 2. 2. Discrimination performance for the Pretrained groups A. Training timeline for Pretrained Groups of animals. All animals learned to perform the low-frequency discrimination task before NB-stimulation pairing began. B. Mean ± s.e.m. of discrimination performance in all three groups four days before tone exposure and NB-stimulation. C. Mean ± s.e.m. of discrimination performance in all three groups four days after tone exposure and NB-stimulation. *, High group performance was significantly different from Passive group, $p<0.05$. **, High group performance was significantly different from both Pretrained Passive and Pretrained Low group, $p<0.05$.

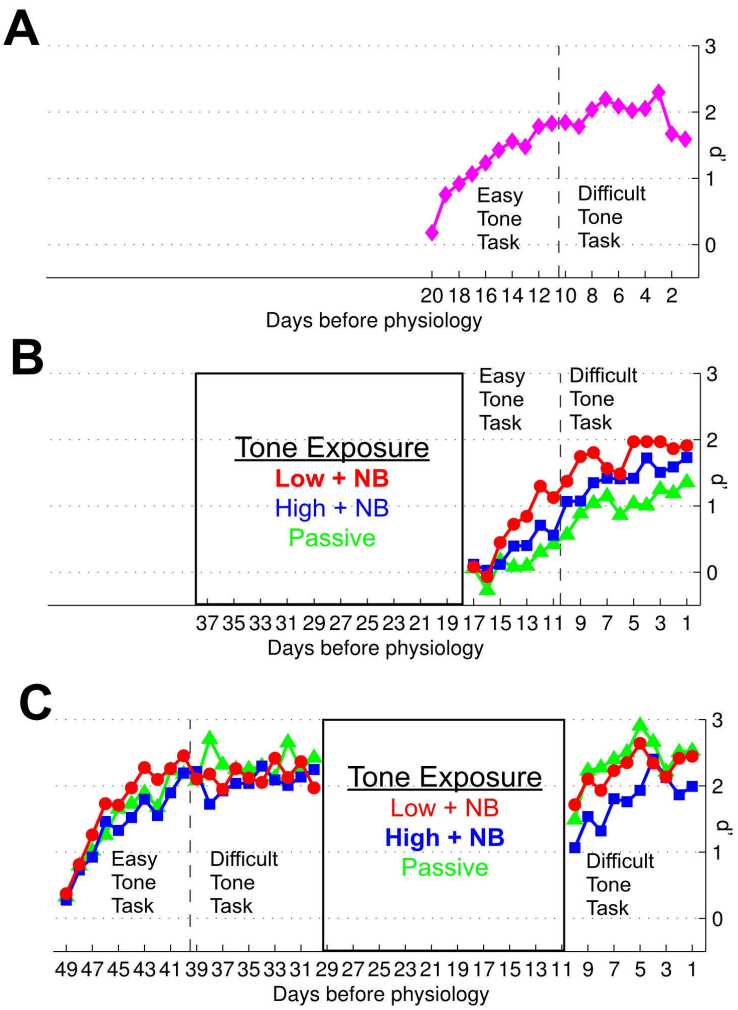

Figure 2. 3. Graphs show mean discrimination performance on the 1 octave discrimination task for each group. The plasticity schematic indicates the amount of map plasticity measured in each group at the end of training. A. Behavior only groups were mapped immediately after behavioral training B. Pretrained Groups mastered the low-frequency discrimination task before tone exposure and NB-stimulation pairing began. C. Untrained Groups were naïve to the low-frequency discrimination task until after tone exposure and NB-stimulation.

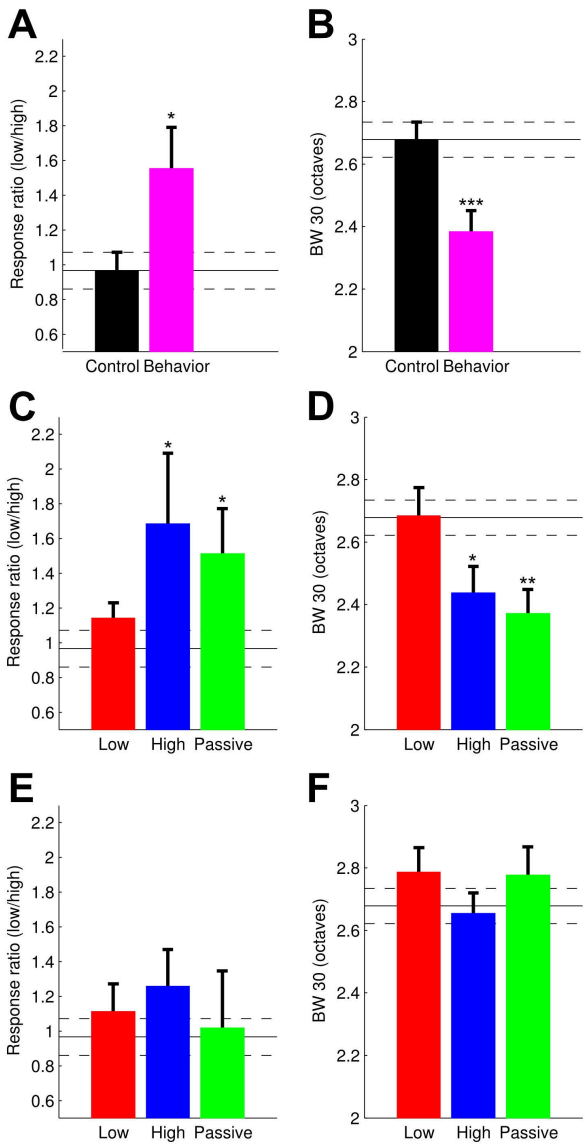

Figure 2. 4. A,C,E. Receptive field plasticity after behavior training (A), and NB-stimulation pairing in Experiment 1 (C), and Experiment 2 (E). Cortical plasticity is measured as the ratio of percentage of cortical neurons which respond to low tones over the neurons which respond to high tones. A value of 1 indicates equal area of response to low and high tones, and all values over 1 indicate an increased relative response to low-frequency tones. The solid and dotted lines in each figure indicate the mean ± s.e.m. values for naïve controls. B,D,F. Receptive field size measured at 30dB above threshold. Receptive field sizes are measured in octaves, so that smaller values indicate smaller receptive fields (more selective tuning). The solid and dotted lines in each figure indicate the mean ± s.e.m. values for naïve controls. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; all stars indicate statistical results of a t-test between an experimental group and naïve controls.

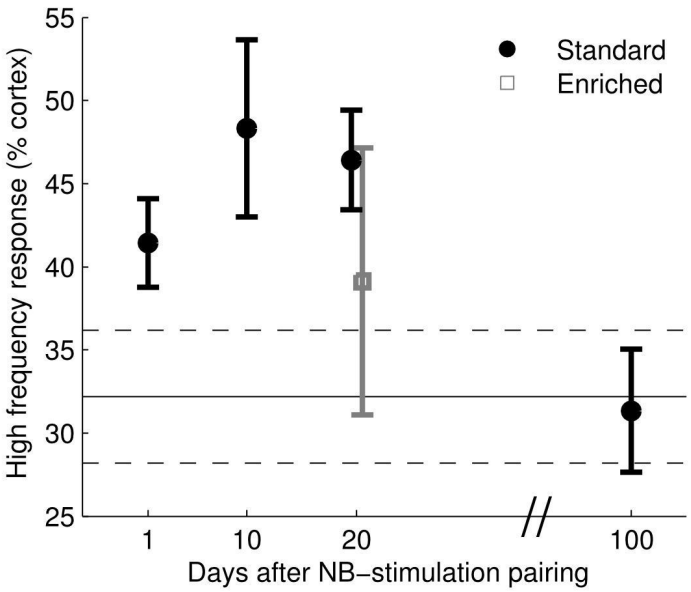

Figure 2. 5. Percent of A1 cortex which responds to high frequency tones (19kHz at 20 dB SPL, mean ± s.e.m.) at various time points after NB-stimulation pairing. The solid and dashed horizontal lines indicate the mean and s.e.m. of cortical response to the high frequency tone in naïve animals, Results of one-tailed t-tests of each experimental group vs. naïve controls: *, P<0.05; **, P<0.01;

APPENDIX 2

SUPPLEMENTAL DATA

Behavioral Discrimination during early periods of training

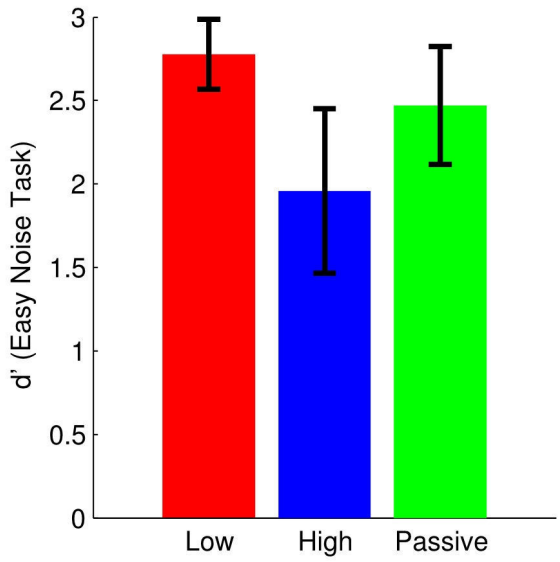

Figure A2. 1. Mean ± mean discrimination performance of the Task-Naïve groups (Low – red, High – blue, Passive – green) on the easy noise discrimination task for the two days before tone exposure began.

It was important for this study to ensure that the discrimination abilities for all experimental groups were not different before tone exposure/NB-stimulation pairing began. The task naïve groups showed no significant differences in discrimination between the noise target and distracter before tone exposure began (Figure A1.1, $F(2,12) = 1.46$, $p = 0.2701$). For the pretrained groups, there were no significant differences in discrimination performance on the frequency task on the first 3 days of training ($F(2,16) = 0.2421$, $p = 0.7878$) or on the last 3 days of training before tone exposure/NB-stimulation pairing (see main text).

Receptive field sizes of sites which were not responsive to target behavior stimulus

We examined receptive field sizes in sites which did not respond to target stimulus (ie, 1.7818 kHz at 60 dB). In general, these sites had narrower tuning that the target stimulus responsive sites.

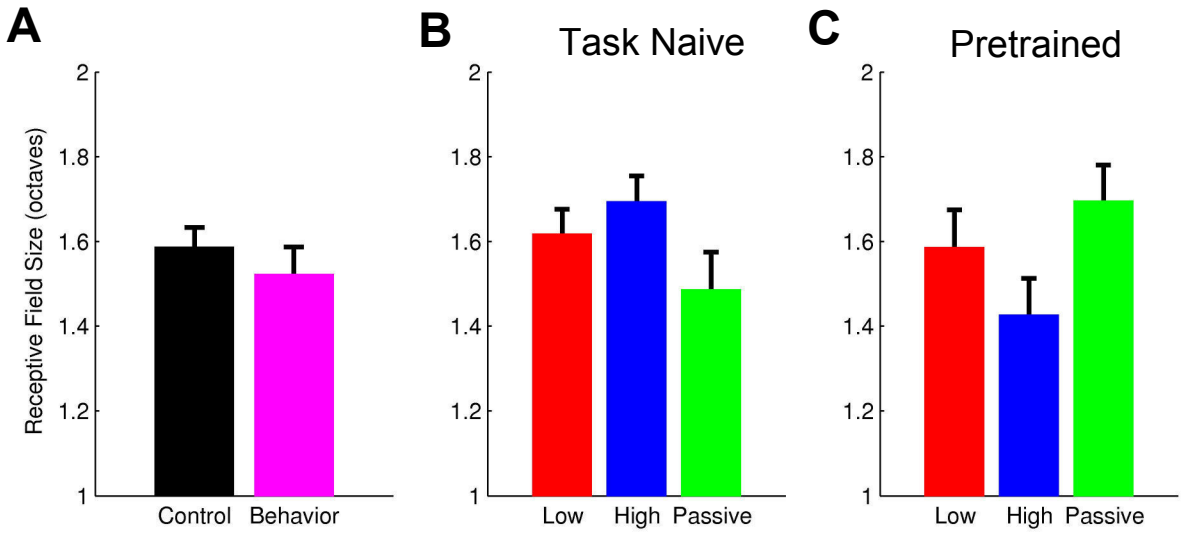

Figure A2. 2. Mean ± s.e.m. BW30 for all experimental groups and naïve controls.

However, there was no significant difference between any experimental groups and controls for BW's 20-40 (Figure A1.2 shows BW30 only). Therefore, the receptive field narrowing which we observed in some of the experimental groups was stimulus-specific and likely to be due to behavioral training.

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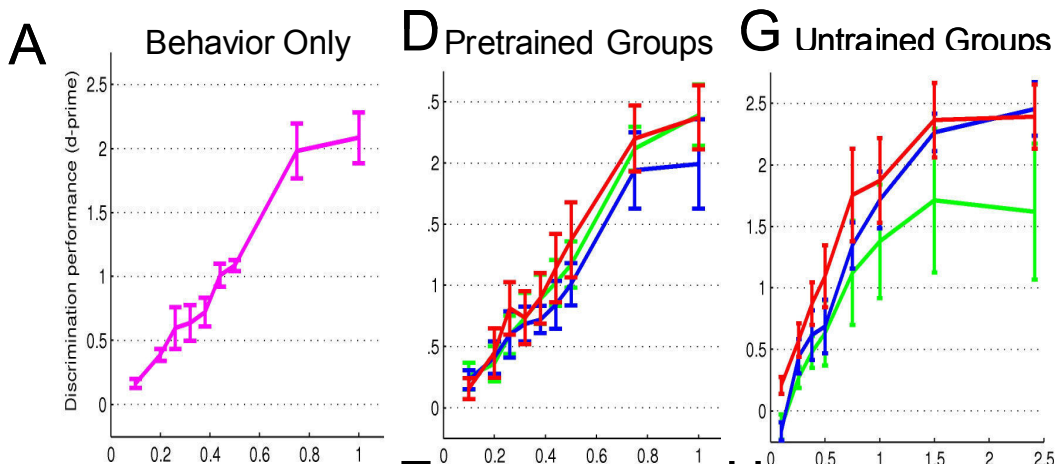

Figure A2. 3. Mean ±s.e.m. of discrimination performance on the last three days of discrimination training

There were no significant differences in the discrimination performance of the behavior only, Pretrained, and Untrained groups of animalson the last three days before mapping.

APPENDIX 3

AUTOMATIC TUNING CURVE ANALYSIS

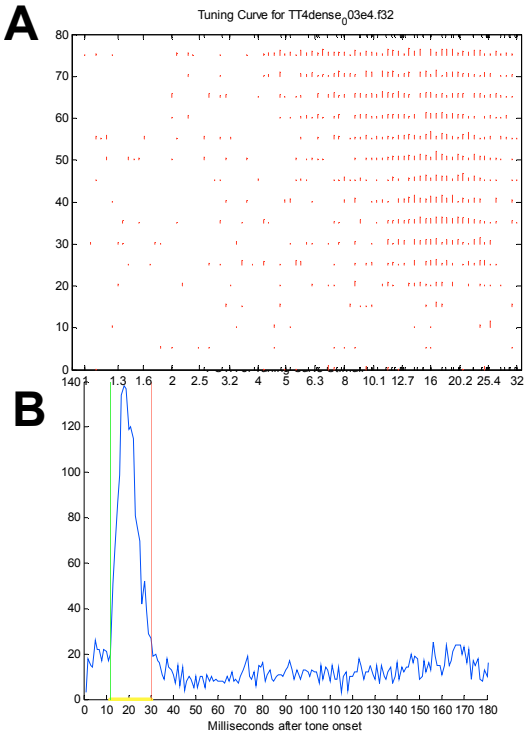

Figure A3. 1. A) Raw tuning curve data for an example site. Only spiking data from within the driven response time period is shown. The length of each red line indicates the strength of response evoked by each frequency/intensity combination. B) PSTH data from the example site. The vertical lines indicate the minimum (green) and end of peak (red) latencies which were chosen by the automated tuning curve program.

To assess receptive field and basic latency properties, a tuning curve consisting of 1296 tone pips (25 ms duration, 5dB steps from 0-75 dB, 1/16th octave steps from 1-32 kHz) were presented at all recording sites. Spiking data was recorded for 400 ms after tone presentation. An automatic tuning curve analysis program was used to extract latency and receptive field information from all files. Using an automated program allowed for fast analysis with less noise due to human error, and also removed the possibility of experimenter bias. Figure A2.1 shows an example of PSTH and tuning curve data.

The automated tuning curve analysis program comprised three basic steps. First, the driven response time period was defined based on the PSTH (post-stimulus time histogram) of each site. Second, a receptive field area was defined using image analysis techniques. Finally, latency information was recalculated using

only frequency/intensity combinations within the defined receptive field area, and then the receptive field was redefined using spike information from the updated driven response time period.

Definition of driven response time period

The initial driven response time period was defined by calculating a minimum and end of peak latency for the PSTH. First, the average and standard deviation of the spontaneous firing rate was calculated as the average and standard deviation of the number of spikes evoked in each 1 ms bin during the first 9 milliseconds after each presentation of the 1296 frequency/intensity combinations. (average spontaneous firing rate was set to 0.00001 spikes/ms if the spontaneous spiking rate was zero). The peak latency was defined as the time point in the PSTH which elicited the greatest response. The minimum latency is the latest time point before the peak latency when the PSTH reached a firing rate that was more than two standard deviations above the spontaneous firing rate for two consecutive milliseconds. The maximum latency was defined as the latest time point after the peak latency when the PSTH response was still greater than two standard deviations above the spontaneous firing rate for two consecutive milliseconds. Figure A2.1B shows the minimum and end of peak latency defined for the example site.

Definition of initial receptive field area

A grid of activity during the driven response time period evoked by each frequency/intensity combination was used to extract receptive field information (Figure A2.2A). The receptive field for each site was defined as a contiguous area with response strength above a preset threshold for driven activity. This threshold was calculated as the spontaneous activity plus 20% of the approximate driven response. The approximate driven response was calculated as 10^{th} percentile of spiking activity of all points in the grid (this response was close to the

maximal response but avoided outliers which would cause the threshold to be inappropriately high). The spontaneous activity for the grid was calculated as average spiking activity evoked by zero dB tones.

A top row of threshold activity was added to the grid of spiking activity to connect discrete 'blobs' responses to loud tones. Finally, the grid of spike data was smoothed by running each point through a convolution matrix so that each point was smoothed with 4 concentric circles of neighbors (weighted with ratios of 1, 0.333, 0.15 and 0.0825, respectively) (Figure A2.2B). The receptive field area was calculated using a sequential algorithm to detect the blob of activity within the grid which was greater than the threshold for driven activity. A starting point was identified as the intensity with the largest response within the frequency band with the highest average activity. First the spiking activity in the starting point was verified as being above the threshold for driven activity. This point is then noted as being within the receptive field. Next all eight possible neighbors (points above, below and at each diagonal) of the starting point were checked. If any of these points had spiking activity above threshold, they were also included within the receptive field. This iterative process continued as the neighbors of each point within the receptive field were checked and all points with sufficient response strength were included within the receptive field. To save time computationally, points which had previously been examined were not reexamined during subsequent iterations. Receptive field identification was complete when all possible neighbors of points within the receptive field did no meet the spiking activity threshold, ie, when a border of below threshold activity was identified (Figure A2.2C).

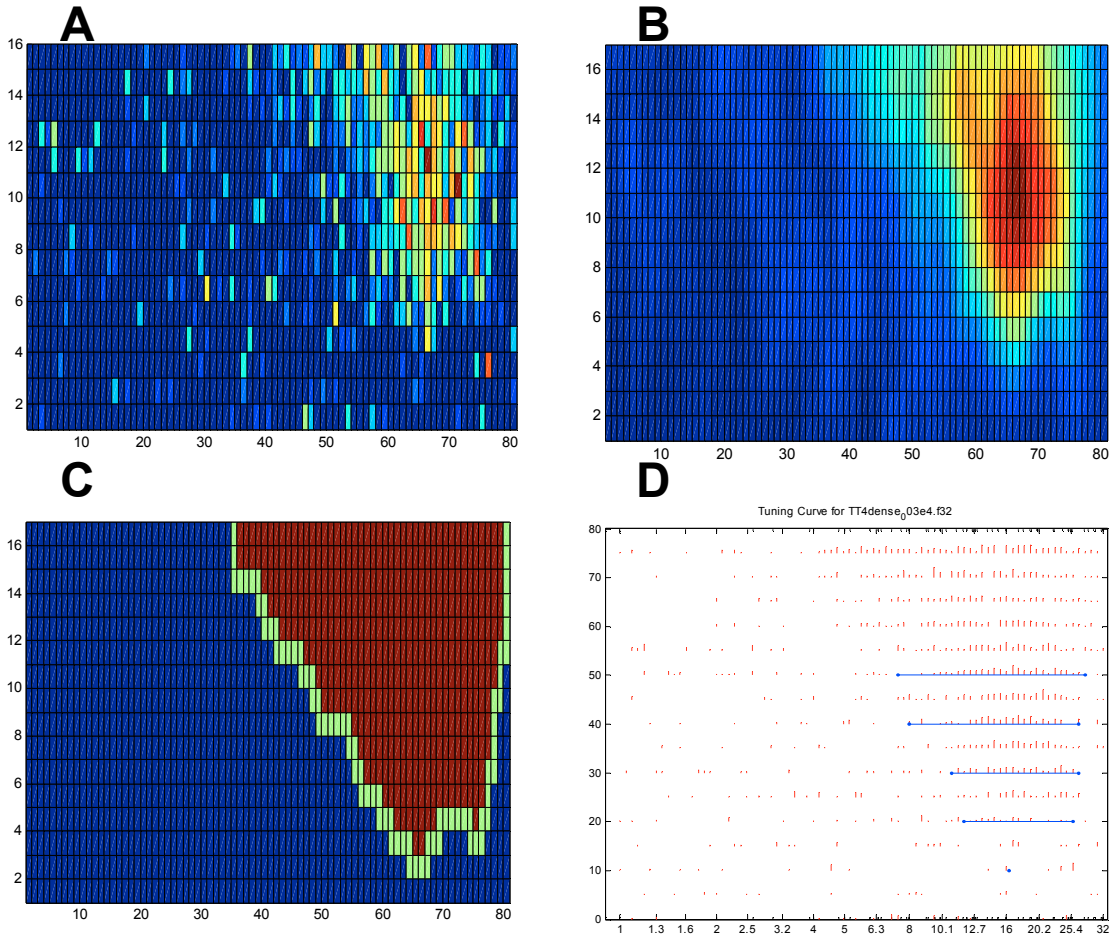

Figure A3. 2 A) Color-coded grid of raw tuning curve data for the example site. Only spiking data from within the driven response time period is shown. B) Color-coded grid of tuning curve data after smoothing. C) Final receptive field (red pixels) surrounded by a frequency/intensity combinations which were checked but did not meet threshold requirements (green pixels). D) Tuning curve characteristics which were extracted from the final receptive field. The blue dot indicates the location of the threshold and CF, and horizontal lines indicate the bandwidths at 10, 20, 30 and 40 dB above threshold.

Refinement of latency and receptive field information

After estimating an initial receptive field area, the driven response time period (minimum and maximum latency) was redefined using only responses to frequency/intensity combinations which were included in the initial receptive field area. This step helped to identify a precise latency measure in sites which had large amounts of spontaneous activity. Finally, the receptive

field area was redefined using the refined driven response time period. The procedure was identical to those used to define the initial receptive field area.

Calculation of Latency and Receptive Field Properites

Basic tuning curve properties were calculated after the final receptive field and driven response time period had been defined. The receptive field threshold is the lowest intensity at which a driven response within the receptive occurred. The characteristic frequency (CF) is the frequency at which a threshold response occurred. If threshold responses occurred at multiple frequencies, the CF was defined as the median of all the frequencies with threshold responses. Bandwidths (BW10, 20, 30 and 40) were calculated as the width of the receptive field (in octave space) at 10, 20, 30 and 40 dB above threshold (Figure A2.2d).

The minimum and end of peak latencies were the earliest and latest time points in which a driven response occurred (Figure A2.1b). The peak latency is the time point of the strongest response and the peak firing rate was the spike rate which occurred at this time point. The spontaneous firing rate is the average number of spikes evoked during the first 9 ms after tone presentation. The signal to noise ratio is the peak firing rate divided by the spontaneous firing rate. The evoked response strength is the average activity elicited by all frequency/intensity combinations within the receptive field over the entire driven response period.

Error correcton and experimenter intervention

The automated receptive field and latency properties of each site were examined by an experience observer. A few sites had to be reanalyzed because the automated program selected an incorrect latency window, such as focusing on a second peak rather than the initial peak response. In these problem sites, the driven response time period was hand-selected by the

experimenter and spiking activity from this time window was used to recalculate the receptive field just as in the automated analysis.

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CHAPTER 3

PLASTICITY IN THE RAT POSTERIOR AUDITORY FIELD FOLLOWING NUCLEUS BASALIS STIMULATION

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ABSTRACT

Classical conditioning paradigms have been shown to cause frequency-specific plasticity in both primary and secondary cortical areas. Previous research demonstrated that repeated pairing of nucleus basalis (NB) stimulation with a tone results in plasticity in primary auditory cortex (A1), mimicking the changes observed after classical conditioning. However, few studies have documented the effects of similar paradigms in secondary cortical areas. The purpose of this study was to quantify plasticity in the posterior auditory field (PAF) of the rat after NB stimulation paired with a high frequency tone. NB-tone pairing increased the frequency selectivity of PAF sites which were activated by the paired tone. This site-specific receptive field decrease led to a reorganization of PAF such that responses to low- and mid-frequency tones were reduced by 40%. Plasticity in A1 was consistent with previous studies - pairing a high frequency tone with NB stimulation expanded the high frequency region of the frequency map. Receptive field sizes in A1 were not altered after NB-tone pairing. These results demonstrate that experience-dependent plasticity can take different forms in primary and secondary auditory cortex.

INTRODUCTION

Neural plasticity in both primary and secondary sensory cortex has been associated with learning. After long-term operant training in primates and rats, large frequency map expansions can develop in response to trained tone frequencies in primary auditory cortex (Brown et al. 2004; Buonomano and Merzenich 1998; Polley et al. 2006; Recanzone et al. 1993; Rutkowski and Weinberger 2005), although such expansion is not always present (Brown et al. 2004). Long-term operant training can also cause frequency-specific plasticity in secondary cortical areas (Polley et al. 2006). Classical conditioning shifts receptive fields in both primary and secondary auditory cortices towards the conditioned stimulus (Diamond and Weinberger 1986, 1984; Weinberger et al. 1984). A greater proportion of neurons in secondary cortical areas are altered by classical conditioning compared to A1 (Diamond and Weinberger 1984; Weinberger et al. 1984). Receptive field plasticity has also been observed in the somatosensory and visual systems, indicating that learning-induced plasticity is a general cortical phenomenon (Buonomano and Merzenich 1998; Ghose 2004; Gilbert 1996; Recanzone et al. 1992a; Recanzone et al. 1992b). In all of these cases, experience-dependent plasticity is specific to the stimuli which were attended to during behavior, and passive exposure does not cause enduring changes in neuronal responses (Bjordahl et al. 1998; Recanzone et al. 1993; Weinberger 1998). These results indicate that stimulus-specific plasticity occurs during learning and significantly alters sensory information processing in multiple cortical areas.

Many studies have demonstrated that attention or the presence of a neuromodulator such as acetylcholine is necessary to generate lasting cortical plasticity. Nucleus basalis (NB), which has

cholinergic and GABAergic projections to all areas of the neocortex, is active when animals are learning new tasks (Butt and Hodge 1997; Richardson and DeLong 1991). Lesions of cholinergic NB neurons or pharmacological blockage of acetylcholine prevent both learning and plasticity in the motor cortex (Conner et al. 2003) and in the primary auditory cortex(Ji et al. 2001). Direct stimulation of NB paired with sensory inputs is sufficient to create stimulus-specific cortical plasticity in A1 (Bakin and Weinberger 1996; Kilgard and Merzenich 1998a). For example, NB stimulation paired with a single tone results in an increased representation of the paired frequency in primary auditory cortex (Kilgard and Merzenich 1998a). The plasticity observed in A1 after NB-stimulation pairing is similar to plasticity induced by classical conditioning and through operant training protocols. Therefore, we expect that repeated stimulation of NB paired with a tone will cause receptive field plasticity in secondary auditory fields that enhances the representation of the paired frequency over other frequencies.

Posterior auditory field (PAF) shows less tonotopic organization, longer latencies and larger receptive fields than A1, and is a well characterized secondary cortical field in the rat (Bao et al. 2001; Doron et al. 2002; Pandya et al. in review; Rutkowski et al. 2003). In this study, we implanted rats with NB stimulating electrodes, paired a high frequency tone with NB stimulation, and measured differences in cortical frequency representation and multi-unit response properties in PAF in experimental and control rats.

METHODS

Six adult female Sprague Dawley rats (250-350g) were implanted with platinum bipolar stimulating electrodes and then exposed to a 19 kHz tone paired with NB stimulation for a period of 20-25 days. Twenty-four hours later, animals were anesthetized and the right auditory cortex was densely mapped by recording tuning curves. Receptive field properties and topographic organization of both primary (A1) and posterior (PAF) auditory fields were quantified. Data from naïve controls (n=14) served as comparisons for all the NB-stimulated animals. Additionally, mapping data from an animal that experienced a 9 kHz tone paired with NB-stimulation during a previous study was analyzed to ensure that plasticity in PAF is frequency-specific. The A1 map for this animal is depicted in Figure 1C of (Kilgard and Merzenich 1998a). Analysis of thresholds and frequency range of neural responses indicate every rat had normal hearing (data not shown). The implantation, stimulation and mapping methods described here are identical to those used in previous studies (Kilgard and Merzenich 1998a; Kilgard et al. 2001; Moucha et al. 2005; Pandya et al. 2005). All procedures were approved by the University of Texas at Dallas Animal Care and Use Committee.

Implantation with NB Stimulating Electrode

Each experimental animal was implanted with a bipolar stimulating electrode (SNE-200, Rhodes Medical Instruments, Woodland Hills, CA) which was lowered 7 mm below the cortical surface from a location 2.3 mm posterior and 3.3 lateral to bregma in the right hemisphere. This stereotaxic location has been used in previous studies of plasticity after NB-stimulation pairing (Bakin and Weinberger 1996; Dimyan and Weinberger 1999; Kilgard and Merzenich 1998a,

2002, 1998b; Kilgard et al. 2001; Moucha et al. 2005; Pandya et al. 2005), and was not histologically confirmed in the current study. A bone screw was placed approximately 5 mm posterior to the stimulating electrode and another screw placed above the cerebellum to record a an EEG. A four-channel connector was used to monitor EEG and deliver current to the stimulating electrode. The entire implant assembly was stabilized with additional bone screws and held in place with dental acrylic. In addition to the pentobarbital anesthetic (50 mg/kg), all animals were given a dose of ceftriaxone antibiotic (20 mg/kg) to prevent infection and atropine (1 mg/kg) and dexamethazone (4 mg/kg) to reduce fluid accumulations in the lungs immediately following anesthetization and after completion of the surgery. All animals were allowed to recover for 1-2 weeks before beginning NB stimulation. Animals were singly housed in wire cages in the animal care facility after the NB-stimulation implant procedure and between NB stimulation sessions.

NB Stimulation Procedure

NB stimulation took place in a 25x25x25cm wire cage located inside of a 50x60x70 cm chamber lined with acoustic insulating foam. Sounds were presented from a speaker hanging above the wire cage. The paired stimulus was a 19 kHz tone (50 dB SPL, 250 ms duration, 5 ms rise-fall time) that was presented approximately every ten seconds 275-350 times per day for a period of 20-25 days. Silent intervals were inserted at random to prevent habituation, and each pairing session lasted approximately three and a half hours. Each tone presentation was accompanied by a short burst of current delivered to the bipolar stimulating electrode (20 biphasic pulses, 0.1 ms duration at 100 Hz) approximately 50 ms after tone onset. The current amplitude ranged from 120-200 μamps for each animal, and was selected to be the level that would reliably elicit brief EEG desynchronization while the animal was in slow wave sleep.

Dense Electrode Mapping of Auditory Cortex

Approximately 24 hours after the tone-NB pairing phase ended, animals were anesthetized with pentobarbital (50 mg/kg), and the right auditory cortex was mapped. Similar mapping procedures were used to collect data from 14 experimentally naïve control animals. Because only NB-stimulated animals received connectors and acrylic headcaps used for NB-stimulation, it was impossible to be blind to experimental group during data collection. Briefly, a tracheotomy was performed to prevent breathing problems and head noise, and a cisternal drain was made to minimize cerebral edema. Then the right auditory cortex was exposed and the dura resected. The cortex was maintained under a thin film of silicone oil to prevent desiccation. Fluids with Ringer's solution and supplemental anesthetic (pentobarbital - 8 mg/ml) were periodically administered throughout the mapping procedure to maintain the animal's health and a state of areflexia.

All recordings were performed in a shielded, double-walled sound chamber. Frequency and intensity calibrations were performed with an ACO Pacific microphone (PS9200-7016) and Tucker-Davis SigCal software. The speaker (Motorola model #40-1221) was positioned directly opposite the contralateral ear at a distance of 10 cm. Tucker-Davis Technologies hardware and software (SigGen) were used for stimulus generation. Multiunit responses were recorded using parylene-coated tungsten electrodes (250 μm separation, 2 MOhm at 1 kHz, FHC Inc., Bowdoinham, ME) which were lowered ~550 μm below the cortical surface (layer IV/V). The neural signal was filtered (0.3-8 kHz) and amplified (10,000x). Action potential waveforms were captured using a software program (Brainware, Tucker-Davis Technology, Alachua, FL), and each recording site location was logged on a detailed digitized photo of the exposed auditory cortex using blood vessels as landmarks. At each site, a tuning curve consisting of 81

frequencies spanning from 1 to 32 kHz at 16 intensities spanning from 0 to 75 dB SPL was presented (1,296 tones, 25 ms duration, 5 ms rise and fall time, 1 repetition of each). The tones were randomly interleaved and presented every 500 ms. The total duration of each mapping experiment was similar in naïve and experimental groups (31 ± 1.2 and 26 ± 1.9 hours, $p > 0.1$).

Data Analysis

In total, 493 experimental sites (6 animals, 295 A1, 198 PAF sites) were compared to 588 sites from naïve controls (14 animals total, 390 A1, 198 PAF sites). For the naïve control groups, the A1 group consisted of 8 animals, while the PAF group contained 9 animals (3 naïve controls belonged to both the A1 and PAF control groups). The control group for each cortical field was selected so that every animal had at least 10 recording sites in the given auditory field. All experimental animals met this criterion for both A1 and PAF.

Tuning Curve Analysis

All sites from control and experimental rats were analyzed together in a blind, randomized batch to prevent experimenter bias. Several receptive field and latency characteristics were defined at each site. The lowest intensity that evoked a reliable neural response was defined as the threshold, and the frequency at which this response occurred defined the characteristic frequency (CF). Four bandwidths (BW10-BW40) were calculated as the range of frequencies (measured in octaves) which evoked reliable responses at 10, 20, 30 and 40 dB above threshold (Figure 1.1 *A* & *C*). A post-stimulus time histogram (PSTH) was constructed from all of the responses to tone-intensity combinations within the receptive field. The peak latency for each site was calculated as the time of the maximum number of spikes in the PSTH (Figure 1.1 *B* & *D*). The spontaneous firing rate at each site was estimated as the spike rate in the first 9 ms recorded after tone onset (before any neural response to sounds). For all of these measures,

experimental and control groups were compared using two-tailed unpaired Student's t-tests to determine if there were statistically significant differences in receptive field or response characteristics after NB-stimulation pairing.

Topography and map expansions

A1 was functionally defined on the basis of latency and tonotopy (Kilgard and Merzenich 1999). In general, sites with peak latencies less than 20 msec were classified as A1. Only 4% of A1 sites in this study exhibited longer latencies. The A1-PAF border was defined using the previously characterized change in topography and latency (Bao et al. 2001; Doron et al. 2002; Pandya et al. in review; Rutkowski et al. 2003). The dorsal and ventral borders of A1 were also defined by changes in topography and latency, while the anterior border of A1 was defined primarily by a reversal in topgraphy. Other boundaries were defined using sites non-responsive to auditory stimuli, or in some cases the limits of data collection.

Changes in cortical tone frequency representation were quantified by comparing the distribution of receptive field tuning (CF's) in experimental animals to naïve control animals. Since the percent of sites with CF's in any given frequency range could be biased if the spacing of recording sites was not even across the cortical surface (i.e. due to blood vessels), we also used the percent of cortical area measure used in earlier studies (Kilgard and Merzenich 1998a). Briefly, Voronoi tessellation was used to transform the discretely sampled surface into a continuous map using the assumption that each point on the map has the response characteristics of the nearest recording site. Since regions with above average sampling density have smaller tessellations, they do not bias estimates of the cortical response. The percent of the cortical area responding to each tone was estimated as the sum of the areas of all tessellations from sites (in A1 or PAF) with receptive fields that included the tone divided by the total area of the field.

Changes in PAF and A1 frequency representation were quantified by comparing the percentage of the cortical area of each field which responded to all presented tones in the tuning curve in naïve and experimental animals. Two-tailed unpaired Student's T-tests were used to determine the statistical significance of all comparisons.

RESULTS

Pairing NB-stimulation with a 19 kHz tone shifted the response of A1 and PAF neurons to favor high frequency tones over low frequency tones and increased frequency selectivity in PAF.

Example of Plasticity in Primary Auditory Cortex

The primary auditory cortex (A1) of each animal was defined by its location, tonotopic organization, narrow bandwidths and short latencies. NB-stimulation paired with a 19 kHz tone increased the area of A1 that responded to high-frequency tones. While 35% of A1 responded to a 19 kHz 60 dB SPL tone in the example control animal (Figure 3.2 *A*), 54% of A1 responded to the same tone in an NB-stimulated animal (Figure 3.2 *B*). The CF of sites increases systematically from posterior to anterior across the surface of A1. The coefficient of determination (r^2) between anterior-posterior location and CF for A1 sites in the example control animal is 0.68 ($p < 10^{-6}$, Figure 3.2 *A*). All A1 maps used in this study had large, significant coefficients of determination between anterior-posterior location and CF (r^2, mean ± s.e: 0.79 ± 0.02). NB-tone pairing did not eliminate the tonotopic organization of A1. The r^2 between anterior-posterior location in A1 and CF was 0.66 ($p < 10^{-19}$, Figure 3.2 *B*) for the experimental map shown. The mean r^2 value for A1 in all NB-stimulated rats was 0.79 ± 0.05.

Receptive fields in A1 were narrower than PAF sites (Figure 3.1 A and C), and did not change following NB-tone pairing. In both the representative control and experimental animals, the bandwidths 30 dB above threshold were approximately 2 octaves (Figure 3.2 C and D). The peak latencies for A1 sites were shorter than PAF, usually within 10-20 ms after tone onset (Figure 3.1 B). In the representative control animal, the average peak latency was 19.8 ± 2.6ms,

while in the representative experimental animal the average peak latency was 17.1 ± 0.7 ms (Figure 3.2 E and F). There was no significant change in A1 latencies in experimental animals compared to control animals (peak latency: control, 18.0 ± 0.4; experimental, 19.4 ± 0.7, p = 0.06).

Example of Plasticity in Posterior Auditory Field

The posterior auditory field (PAF) was defined based on its location posterior to A1, poor tonotopic organization, large receptive field sizes, and long response latencies. The anterior border of PAF was defined by the low frequency edge of A1, while dorsal and ventral borders of PAF were defined by sites which were non-responsive to tones. The far posterior border of PAF was often not as well demarcated because access was limited by the edge of the craniotomy. While the CF's of A1 sites in controls were fairly evenly distributed (in log space) between 1 and 32 kHz, a relatively large proportion of PAF sites were tuned to frequencies between 5 and 8 kHz (Figure 3.2 *A*). Receptive fields in PAF of the control animal usually spanned more than 3 octaves at BW30 (Figure 3.2 *C*), and many sites responded to all tones within the rat hearing range (Figure 3.1 *C*). The clustering of PAF CF's towards mid-frequencies may result from the large receptive field sizes and lower mid-frequency hearing thresholds of rats (Kelly and Masterton 1977).

In our example experimental animal, NB-stimulation paired with a 19 kHz tone decreased the area of the PAF cortex which responded to low frequency tones, but did not increase the total area of PAF that responded to high-frequency tones. While 82% of PAF responded to a 2 kHz 60 dB SPL tone in the example control animal (Figure 3.2 *A*), only 56% of PAF responded to the same tone in an NB-stimulated animal (Figure 3.2 *B*). In the control and experimental animals, 94% and 82% of PAF responded to a 19 kHz 60 dB SPL tone, indicating that the major effect of

NB-tone pairing may have been to decrease responses to unpaired tones which in turn lead to sharpened tuning curves. After NB stimulation, tuning curves in PAF became significantly narrower, and many more sites had CF's between 11 and 20 kHz (Figure 3.2 *B*). In the representative control animal, the average bandwidth of PAF sites was 3.9 ± .17 octaves, while the average bandwidth of PAF sites in the representative experimental animal was only 2.92 ± .16 octaves (Figure 3.2 *C* and *D*). The peak latencies in PAF for both the control and experimental animals were significantly longer than A1, between 30 and 80 ms (Figure 3.1 *B* and *D*, and Figure 3.2 *E* and *F*). The average peak latency of PAF sites for the representative control and experimental animals were 51.4 ± 3.2 ms and 60.7 ± 7.9 ms, respectively. NB-tone pairing did not significantly alter response latencies in PAF (peak latency, control: 53.1± 2.2 ms, experimental: 54.1± 2.3 ms, $p = 0.75$). These two example maps illustrate that both A1 and PAF exhibit shifts in their frequency tuning to favor high frequencies after NB-stimulation paired with a 19 kHz tone. However, only the secondary cortical area (PAF) showed increased frequency selectivity.

Posterior Auditory Field Topography

PAF showed evidence of weak tonotopic organization in control animals that was eliminated after NB-tone pairing. When all PAF sites from all nine control animals were examined together, we found a weak, but statistically significant, correlation between each site's anterior-posterior position (relative to the A1-PAF border) and CF ($r^2 = 0.09$, $p < 10^{-5}$, Figure 3.3 *A*). However, when each animal was considered separately, only 3 out of the 9 control rats showed significant correlations between position and CF. In the experimental group, there was no evidence of tonotopic organization when all PAF sites from all animals were grouped together ($r^2 = -0.09$, $p = 0.23$, Figure 3.3 *B*). Only one of the six experimental animals showed any evidence

of tonotopic organization in PAF when considered individually. The average coefficient of determination (r^2) between anterior-posterior axis location and CF in PAF for all individual experimental and control animals was 0.12 ± 0.03. The changes in frequency representation were not accompanied by a significant increase in the overall area of the cortical field in experimental animals compared to control animals for either A1 (1.94 ± 0.26 vs. 1.48 ± 0.17 mm^2, p=.13) or PAF (1.28 ± 0.22 vs. 0.89 ± 0.18 mm^2, p = .15).

NB Stimulation Reduced PAF Responses to Low Frequency Tones

NB-stimulation paired with a 19 kHz tone resulted in an increase in the proportion of sites in PAF which were tuned to high frequencies (Figure 3.4). In experimental animals, a larger percentage of sites had CF's which fell within half an octave of 16 kHz than control animals (mean percentage of total PAF sites in the 16 kHz bin for each animal, controls: 22% ±5, experimental: 37% ±10, p=0.11). In experimental animals, 28% ±4 of PAF sites had CF's within a half an octave of 8 kHz, compared to 41% ±6 in naïve controls (p = 0.11). There was no difference in the percentage of sites falling into the 2 or 4 kHz centered bins (Figure 3.4). These results are consistent with a shift in the CF of initially mid-frequency tuned sites (i.e. in the 8 kHz bin) towards the tone frequency paired with NB-stimulation. In the one animal that experienced a 9 kHz tone paired with NB-stimulation, the percentage of PAF sites in the 2 and 4 kHz bins (4% and 0%, respectively) were also decreased compared to naïve controls, providing further indication that the shift in tuning in PAF following NB-tone pairing was frequency-specific.

Pairing NB stimulation with a 19 kHz tone increased tone frequency selectivity in PAF. The average bandwidth 30 dB above threshold (BW30) decreased by 17%, from 3.6 ± 0.08 octaves in controls to 3.0 ± 0.08 octaves in experimental animals ($p<10^{-7}$). Sites tuned to higher

frequencies exhibited the largest reduction in BW. For sites with CF's within one half octave of 16 kHz, receptive fields were significantly narrower at each analyzed suprathreshold intensity (Figure 3.5). For example, BW30 of sites in the 16 kHz bin decreased by 30%, from 3.8 ± 0.14 octaves in control animals to only 2.7 ± 0.18 octaves in experimental animals ($p<10^{-6}$). Mid-frequency sites (8 kHz bin) showed a more modest bandwidth decrease and the low frequency bins did not show any change in bandwidths at any intensity above threshold (Figure 3.5).

The frequency-specific nature of the bandwidth decrease in PAF suggests the possibility that only sites that were activated by the 19 kHz tone paired with NB stimulation became more frequency selective, while sites that did not respond to the 19 kHz tone were unaffected. All PAF sites in both the control and experimental groups were subdivided into those whose receptive fields included 19 kHz played at 50 dB SPL (Tone In) and those that did not (Tone Out). As expected based on the selection criteria, the average bandwidth of the Tone In subset of sites was significantly larger than the average bandwidth of the Tone Out subsets in both control and experimental animals (Figure 3.6). The Tone In subset not only included sites which had CF's near the paired tone, but also included virtually all broadly tuned sites (i.e., with bandwidths greater than 4 octaves) regardless of their assigned best frequency.

NB-tone pairing decreased the bandwidth of the Tone In subset while the Tone Out subset of PAF sites remained unchanged (Figure 3.6). The average bandwidth 40 dB above threshold (BW40) of Tone In sites dropped by 0.79 octaves following NB-tone pairing, from 4.23 ± 0.12 octaves in control animals to 3.44 ± 0.12 octaves in experimental animals ($p < 10^{-5}$). Pairing did not alter the average bandwidth of Tone Out sites (BW40 in octaves of Tone Out sites, controls: 2.62 ± 0.16, experimentals: 2.58 ± 0.16, $p = 0.85$). These results imply that plasticity in PAF may have been restricted to those sites which were able to respond to the paired tone before NB-

tone pairing began (Figure 3.7). The increase in frequency selectivity in sites that responded to the 19 kHz tone paired with NB stimulation produced a decrease in the percentage of PAF which responded to low frequency tones. While 87% ± 3.2 of PAF responded to a 2 kHz tone played at 60dB SPL in the control rats, only 53%± 6.1 of PAF responded after NB-stimulation paired with a 19 kHz tone ($p = 0.0001$, Figure 3.8 *B*). Thus, 39% fewer sites responded to the 2 kHz, 60 dB SPL tone in experimental animals compared to naïve control animals. This decrease in responsiveness to low-frequency tones was observed for all tones with frequencies below 5 kHz played at intensities within the rat's hearing range (Figure 3.9 *C*). In contrast, there was only a modest increase in the percentage of PAF cortex which responded to high-frequency tones. For example, the percentage of PAF which responded to a 16 kHz 60 dB SPL tone increased from 65.7% ± 6.9 in control animals to 73.7% ± 6.3 of PAF in experimental animals ($p = .41$, Figure 3.8 *B*). Therefore, after NB-stimulation paired with a 19 kHz tone, the percentage of PAF cortex which responded to high-frequency tones increased only modestly, while the percentage of PAF cortex which responded to low-frequency tones decreased significantly (Figure 3.9).

NB Stimulation increased A1 response to high frequency tones

Primary auditory cortex (A1) showed an expanded representation of high-frequency sounds following NB stimulation paired with the 19 kHz tone, confirming previous studies of NB-tone pairing (Kilgard and Merzenich 1998a). The percentage of A1 sites which responded to a 16 kHz 60 dB SPL tone increased, from 40 % ± 3.62 in control animals to 54.08 % ± 5.29 in experimental animals ($p = 0.02$) (Figure 3.8 *A*). There were similar increases in the percentage of A1 sites which responded to tones between 10 and 17 kHz (at intensities between 50 and 70 dB SPL). In addition, the percentage of A1 sites which responded to low-frequency tones decreased slightly (percentage of A1 cortex responding to 2 kHz, controls: 41.7% ± 3.62,

experimentals: 31.54% ± 6.45, p = 0.13, Figure 3.8 *A*). Unlike the plasticity observed in PAF, A1 reorganization was not accompanied by significant alterations in receptive field size (BW 30, controls: 1.82 ± 0.05, experimentals: 1.78±0.05, p = 0.61). Therefore, NB-tone pairing in A1 appears to have created a shift of tuning curves towards the paired tone frequency, without changing receptive field sizes.

Frequency-Specific Changes in PAF Firing Rate

It is possible that NB-tone pairing increased the number of action potentials elicited by the paired (19 kHz) tone without significantly increasing the area of PAF cortex which responded to that tone. The percentage of cortex measure used in this and previous publications (Kilgard and Merzenich 1998a; Kilgard et al. 2001; Pandya et al. 2005) would not detect such an increase, because the measure only takes into account receptive field boundaries (i.e. tones are 'in' or 'out') and not variations in the evoked response to different tones within each site's receptive field. To determine whether NB-tone pairing increased responses to high-frequency tones, we quantified the mean response of all PAF sites in experimental and control animals to every presented frequency-intensity tone combination. At each site, the number of spikes evoked by each tone was averaged with the neighboring frequency and intensity steps (5 dB above and below and $1/16^{th}$ of an octave above and below each tone).

Pairing NB-stimulation with a 19 kHz tone did not increase the number of spikes elicited by high frequency tones, but did result in a large decrease in the number of spikes elicited by low frequency tones. In control animals, a 16 kHz 60 dB SPL tone elicited 0.69 ± 0.07 spikes per PAF site, while in the experimental group the same tone elicited 0.64 ± 0.06 spikes (p = 0.64). In contrast, a 2 kHz 60 dB SPL tone evoked 0.97 ± 0.09 spikes per site in controls compared to only 0.54 ± 0.06 spikes in experimental animals (p = 0.0002, Figure 3.10 *A*). Changes of similar

magnitude and statistical significance were observed in the responses to most tones played below 5 kHz (Figure 3.11). These results support our initial observation that NB-tone pairing caused a reduction in the bandwidth of PAF sites, but did not necessarily shift receptive fields towards the paired tone. If the reduction of bandwidth in PAF sites was due to shifts of individual neurons within the multi-unit clusters toward the paired frequency, we would have expected to see increased evoked responses to the paired tone. We saw no indication of increased responses to any high-frequency tone (Figure 3.11). Previous studies of A1 that systematically evaluated this issue observed that changes in selectivity of multi-unit responses closely mirrored the changes of well sorted single units (Kilgard and Merzenich 1998a).

When sites were subdivided by whether they responded to the paired stimulus (Tone In) or not (Tone Out), the decrease in response to low-frequency tones was found to originate primarily from the Tone In subset of sites (Figure 3.10 *B* and *C*). In control animals, Tone In sites responded to a 2 kHz 60 dB SPL tone with 0.99 ± 0.11 spikes per PAF site, but after NB-tone pairing, the same tone elicited only 0.52 ± 0.06 spikes ($p = 0.0002$). Although there was some evidence for decreased response strength to low-frequency tones in the Tone Out subpopulation (spikes per PAF site, controls: 0.89 ± 0.11, experimentals: 0.68 ± 0.09, $p = 0.16$, Figure 3.10 *C*), this decrease was small and not reliable. The response to high frequency tones was not increased in either the Tone In or Tone Out subgroups (Figure 3.10 *B* and *C*). This analysis, which required no judgments about tuning curve edges, supports our proposition that the primary effect of NB-stimulation pairing in PAF was to cause sites which responded to the 19 kHz tone to become more selective by decreasing their responses to low and mid-frequency tones (Figure 3.11).

Spontaneous firing increased in both PAF and A1 after NB-tone pairing. In PAF, the control group had a spontaneous firing rate of 2.75 ± 0.16 Hz, while the experimental group had a spontaneous rate of 4.68 ± 0.25 Hz ($p < 10^{-4}$). In A1, the control group's spontaneous firing rate was 3.74 ± 0 .16 Hz, while the experimental group had a spontaneous rate of 4.62 ± 0.21Hz ($p=0.0009$). The increase in spontaneous firing showed no evidence of frequency-specificity in either PAF or A1.

DISCUSSION

Summary of Results

NB-stimulation paired with a 19 kHz tone increased the cortical response to high frequency tones relative to other tone frequencies in both primary and non-primary auditory cortex. Consistent with earlier observations, the A1 representation of high frequencies increased without a significant decrease in the representation of low or mid frequency tones or change in average receptive field sizes (Kilgard and Merzenich 1998a; Kilgard et al. 2001). PAF showed a pattern of plasticity in response to NB-tone pairing which was distinctly different from A1. Rather than shifting tuning curves toward the paired tone, PAF sites became more selective. The principal effect observed in PAF was not an increase in the representation of the paired tone, but a decrease in the representation of low or mid-frequency tones. While we did not record from the same neurons before and after NB stimulation, it appears that sites which were responsive to the paired tone before NB stimulation increased their selectivity while low-frequency sites were left unaltered.

Changes in Spontaneous Firing and Cortical Plasticity

We observed increased spontaneous activity in both PAF and A1 after NB-stimulation pairing. Increased spontaneous activity has been observed in several studies of NB-stimulation pairing using diverse types of paired auditory stimuli (Metherate and Ashe 1993; Pandya et al. 2005), although not all studies of NB-stimulation pairing have observed increased spontaneous firing rate(Kilgard et al. 2001). Direct application of acetylcholine onto cortical neurons has also been shown to increase spontaneous firing in some cells(Metherate and Weinberger 1990),

implying that it may be a generalized effect of NB-stimulation. In contrast, receptive field properties such as frequency selectivity and response latency seem to change because of specific attributes of the auditory stimuli which were paired with NB-stimulation (Kilgard et al. 2001).

Previous Studies of Plasticity in Non-Primary Cortex

Receptive field narrowing may be a common feature of plasticity in broadly tuned non-primary cortical areas. Neurons in secondary cortical fields were more prone to bandwidth decreases than primary cortical neurons after tone-shock conditioning (Diamond and Weinberger 1986). In the ventroposterior area, which is similar to PAF in terms of response characteristics and location relative to A1, receptive fields were narrowed after stimulation of the ventral tegmental area(VTA) (Bao et al. 2001). The results are analogous to our observation that receptive field sizes in PAF were decreased after NB-tone pairing. However, secondary cortical areas do not always show receptive field narrowing when undergoing plasticity. Extensive training on an auditory frequency discrimination task led to frequency-specific reorganization of secondary field SRAF (suprarhinal auditory field) in trained rats, but did not affect receptive field sizes (Polley et al. 2006). This difference may have been because receptive fields in SRAF are relatively narrow even in naïve animals or because of the sensory statistics associated with the behavioral tasks.

Receptive field decreases have also been observed in secondary fields of the visual system after behavioral training. Training on an orientation discrimination task leads to smaller receptive fields in V4 (Yang and Maunsell 2004), Discriminating complex stimuli led to smaller receptive fields in the inferotemporal cortex of monkeys(DiCarlo and Maunsell 2003). In both of these cases, plasticity was limited to neurons which were active during performance of the

discrimination task, similar to our finding that PAF plasticity after NB-tone pairing may have been limited to sites which were activated by the paired stimulus.

Models of Plasticity

The PAF plasticity observed in this study could result from changes occurring in at least three different locations: A1, auditory thalamus, or PAF. Plasticity in PAF could be a direct reflection of map reorganization in A1. Anatomical studies have shown that A1 sends projections to PAF (Mascagni et al. 1993; Romanski and LeDoux 1993). In the cat, PAF requires active inputs from A1 in order to produce driven responses (Kitzes and Hollrigel 1996). The width of PAF receptive fields suggests that PAF neurons likely receive inputs from broad regions of A1 cortex rather than a tightly circumscribed region. High frequency tuned sites probably receive inputs from the more anterior regions of A1, while low frequency tuned PAF sites probably receive inputs from the more posterior regions of A1. Pairing a high frequency tone with NB stimulation leads neurons in anterior A1 to shift their tuning curves towards the paired frequency. PAF sites which receive inputs from the anterior region of A1 would now receive inputs from a smaller range of frequencies near the paired tone and therefore would decrease their receptive field sizes. On the other hand, low frequency-tuned PAF sites would still receive inputs from A1 neurons which responded to a broad range of frequencies and would therefore not change their receptive field sizes. This model could explain the pattern of plasticity which we observed in PAF, and could be used to make predictions about the results of future plasticity studies in PAF using more complex sounds based on known A1 results (Kilgard and Merzenich 2002, 1998b; Kilgard et al. 2001; Moucha et al. 2005; Pandya et al. 2005).

It is also possible that changes in the inputs PAF receives from the non-lemnisical thalamus could explain the observed plasticity in PAF. Like other secondary auditory fields, PAF receives

inputs from both the non-classical ascending pathway via the dorsal and medial divisions of the auditory thalamus and the classical ascending pathway via A1. Both the medial and dorsal divisions of the thalamus tend to have longer latency responses and broader tuning than the ventral MGN (Aitkin and Webster 1971; Bordi and LeDoux 1994; Calford 1983). Projections from these nuclei into PAF are not as strictly topographically organized as projections into A1 (Arnault and Roger 1990; Kimura et al. 2003; Malmierca 2003; Winer et al. 1999). This observation suggests that PAF response properties may be explained by its thalamic inputs, just as the organization of A1 is likely due to projections from the narrowly tuned and topographically-organized ventral MGN (Winer et al. 1999). Both the medial and dorsal divisions of the thalamus exhibit shifts in receptive field tuning and sometimes reductions in receptive field sizes after classical conditioning (Edeline and Weinberger 1992, 1991). Although plasticity in the medial and dorsal divisions of the thalamus are known to be sensitive to cholinergic drugs (Mooney et al. 2004), to our knowledge the direct effects of NB-stimulation pairing on receptive field properties of the thalamus have not been reported. There are no direct projections from NB to the auditory thalamus (Hallanger et al. 1987), so thalamic plasticity after NB-stimulation pairing would have to occur through some indirect route (Kolmac and Mitrofanis 1999). There are projections from A1 to the auditory thalamus (Hazama et al. 2004; Kimura et al. 2005; Winer and Larue 1987), which could cause changes in tuning in the dorsal and medial thalamus which reflects the plasticity in A1.

Since PAF receives projections directly from NB (Arnault and Roger 1990), it is also possible that the organization of PAF's intrinsic connections is directly altered by NB-tone pairing. In the visual system, training on some behavioral tasks has been shown to create cortical plasticity in secondary visual areas with no accompanying change in the primary visual cortex

(DiCarlo and Maunsell 2003; Ghose 2004; Ghose et al. 2002; Yang and Maunsell 2004). The plasticity which we observed in PAF after NB-tone pairing is likely due to some combination of changes in A1, MGB, and PAF.

Predictions for Future Studies

Our earlier studies have shown that the type of sounds paired with NB stimulation determines the form of plasticity generated in A1 (Kilgard and Merzenich 2002, 1998b; Kilgard et al. 2001; Moucha et al. 2005; Pandya et al. 2005). Pairing temporally modulated tones decreases frequency selectivity in A1, while pairing tones with many different frequencies sharpens frequency tuning. If the A1 feed forward model outlined above is correct, PAF receptive field narrowing would follow any A1 frequency map expansion, but would not necessarily follow plasticity that narrows frequency bandwidth in A1 without changing tonotopy. Sounds that broaden A1 tuning curves would have relatively little effect on PAF neurons which already receive convergent inputs.

Earlier findings indicate that secondary cortical areas may be more amenable to plasticity in response to complex stimuli than primary cortical fields (Diamond and Weinberger 1984; Weinberger et al. 1984). Behavioral training causes V4 and inferotemporal cortex neurons to increase their selectivity for trained stimuli (DiCarlo and Maunsell 2003; Ghose 2004; Yang and Maunsell 2004). It is currently unknown how PAF is altered by pairing NB-stimulation with complex spectrotemporal sequences. Our results and earlier studies of secondary auditory fields support the idea that non-primary auditory cortex is plastic and likely contributes to perceptual learning.

Technical Considerations

The methods of our current study did not allow us to record from the same neurons before and after NB-stimulation pairing. The finding that only the Tone In subsets of neurons were different between control and experimental animals implies that PAF plasticity was restricted to neurons which were responsive to the paired tone before NB-stimulation. However, it is also possible that the observed plasticity was caused by more generalized effects in which some PAF neurons shifted away from the paired tone (and thus joined the Tone Out group) while other PAF neurons shifted towards the paired tone (joining the Tone In group). While we favor the first explanation because it is less complicated (i.e., one type of plasticity limited to the activated population), the second explanation cannot be excluded without recording from the same neurons before and after NB-tone pairing.

All of the physiological recordings for this study were made under pentobarbital anesthesia. Several studies have indicated that anesthesia can change the response properties of auditory neurons (Gaese and Ostwald 2001; Zurita et al. 1994). In addition, some forms of plasticity are only expressed during the awake state, such as shifts in response properties during a difficult behavioral task (Fritz et al. 2005; Ito and Gilbert 1999; Li et al. 2004). Our observation that the changes in both A1 and PAF cortex caused by NB-tone pairing were present under deep anesthesia implies that the observed changes reflect plasticity in the circuitry of the auditory system, and that feedback activity from higher associational areas may not be necessary for some forms of experience-dependent plasticity to be expressed.

Conclusions

This study demonstrates for the first time that NB-tone pairing causes stimulus-specific plasticity in a non-primary cortical area. PAF sites showed a tendency to become selective for

the paired stimulus at the exclusion of other frequencies. This plasticity differs significantly from the concurrent plasticity in A1. Therefore, even simple stimuli can create complex patterns of plasticity across many stations of the auditory system. Recent behavioral and physiological studies have explored the role of primary auditory cortex in processing complex stimuli including speech sounds (Ohl and Scheich 1997; Reed et al. 2003; Sakai and Kudoh 2005; Steinschneider et al. 2003; Toro et al. 2005; Wang and Kadia 2001; Wetzel et al. 1998). It has been speculated that secondary fields may be specialized for extracting behaviorally relevant features from complex stimuli (Rauschecker 1998), and that learning-induced plasticity in these areas enhances this specialization (Ghose 2004). To fully understand perceptual learning, it will be critical to systematically explore plasticity in response to complex stimuli in both primary and non-primary sensory cortex.

APPENDIX 1

FIGURES

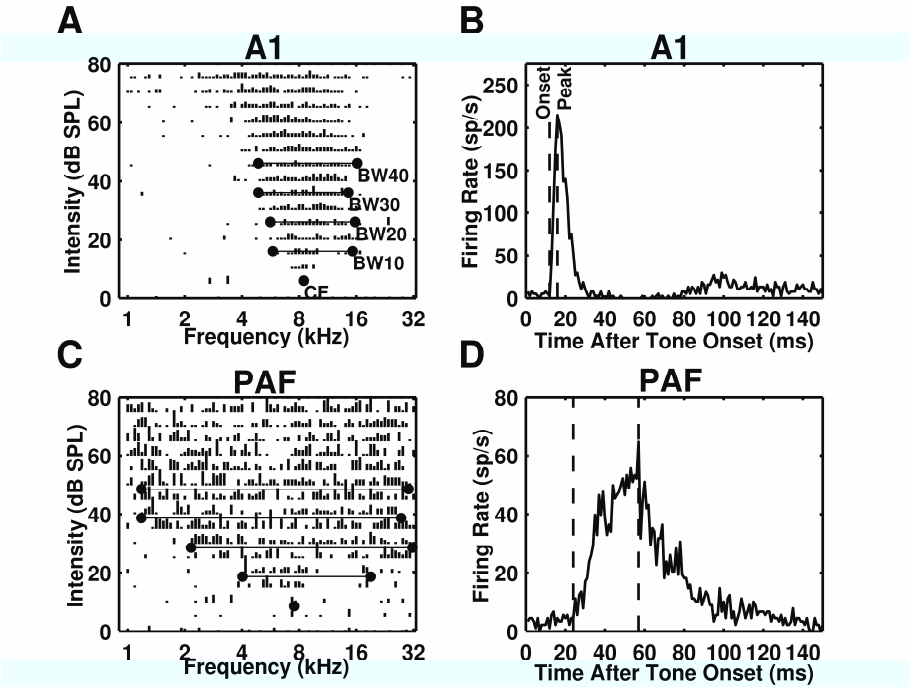

Figure 3. 1. Representative tuning curves and PSTHs demonstrating the larger receptive field sizes and longer latencies of posterior auditory field (PAF) sites compared to primary auditory cortex (A1) sites. (*A* & *C*) The following receptive field characteristics which were quantified for each site: characteristic frequency (CF), threshold, bandwidth 10 dB above threshold (BW10), BW20, BW30, and BW40. (*B* & *D*) A PSTH for each site was constructed from all of the responses within each site's receptive field. The peak, onset and end of peak latency for each site was then calculated from the PSTH (see Methods).

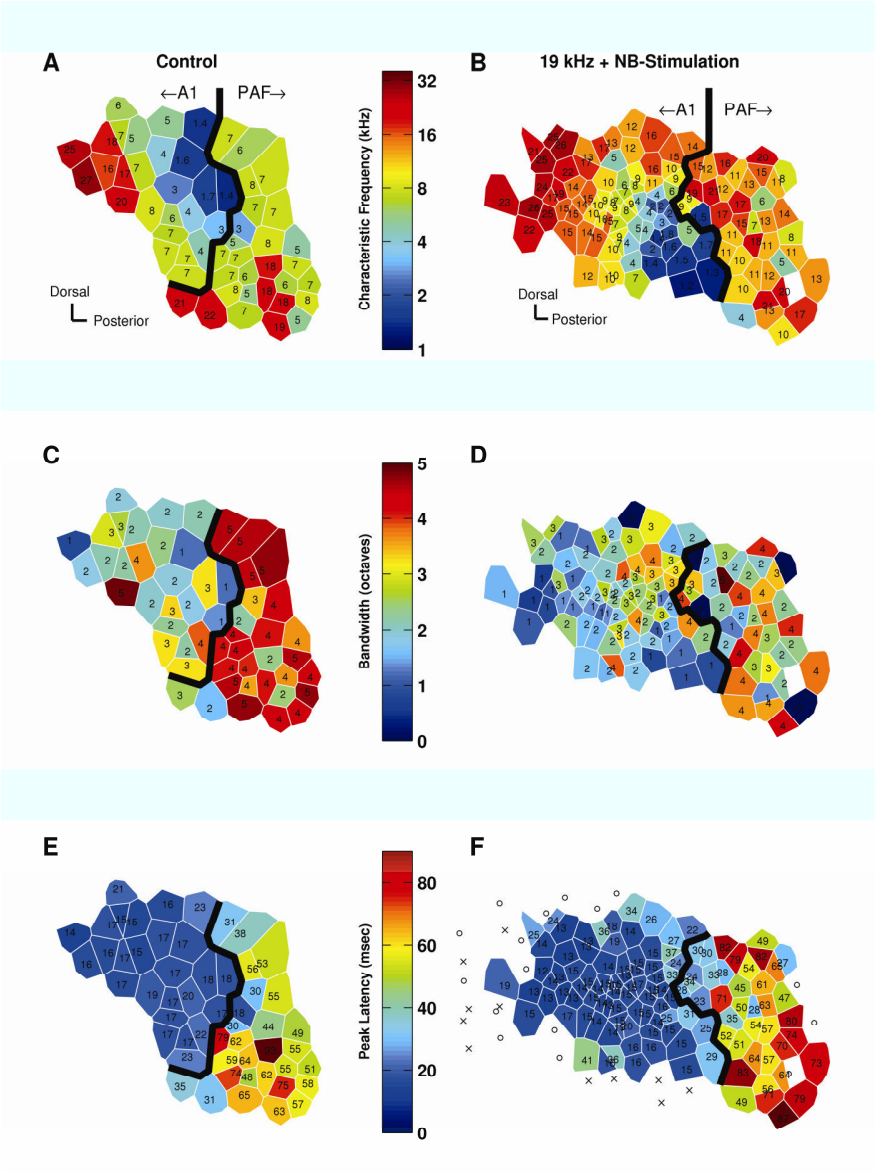

Figure 3. 2. Maps of A1 and PAF in an example control (left column) and experimental animal (right column) demonstrate the high frequency map expansion and decreased bandwidths after NB stimulation paired with a 19 kHz tone. Each polygon represents a single electrode penetration. The color of each polygon represents the value of the characteristic frequency (CF) (panels *A* & *B*), bandwidth 30 dB above threshold (panels *C* & *D*), or peak latencies (panels *E* & *F*) of the site. The thick black line indicates the border between A1 and PAF. Responses that did not respond to tones are indicated by an open circle (panel *F)*. Sites that responded to tones but did not meet our definition of A1 or PAF are indicated by an X (panel *F)*. The example control map did not have any sites which belonged to other auditory fields or any non-auditory sites. This map was selected for this illustration because of the large number of sites in both A1 and PAF. Most control maps focused on either A1 or PAF and were bounded by recording sites that were either non-responsive, non-A1, or non-PAF. The scale bars in panels *A* and *B* indicate a distance of 0.125 mm.

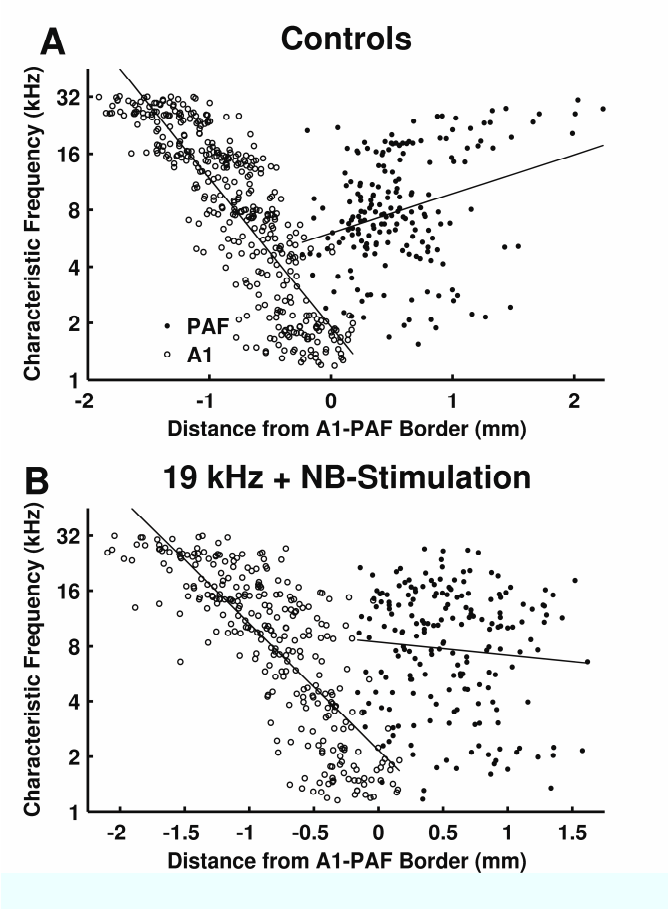

Figure 3. 3. Scatter plots of A1 and PAF with best-fit lines demonstrate that PAF is more loosely tonotopically organized than A1. In order to normalize for irregular borders and differing sizes of the A1 and PAF fields between animals, the A1 and PAF border was chosen as the mean position between the most posterior A1 site and most anterior PAF site. Because the border between A1 and PAF is frequently irregular, some A1 sites were located further posterior than the most anterior PAF site. A1 sites are denoted by open circles while PAF sites are denoted by points in the plot for controls (panel *A*) and for NB-stimulated animals (panel *B*).

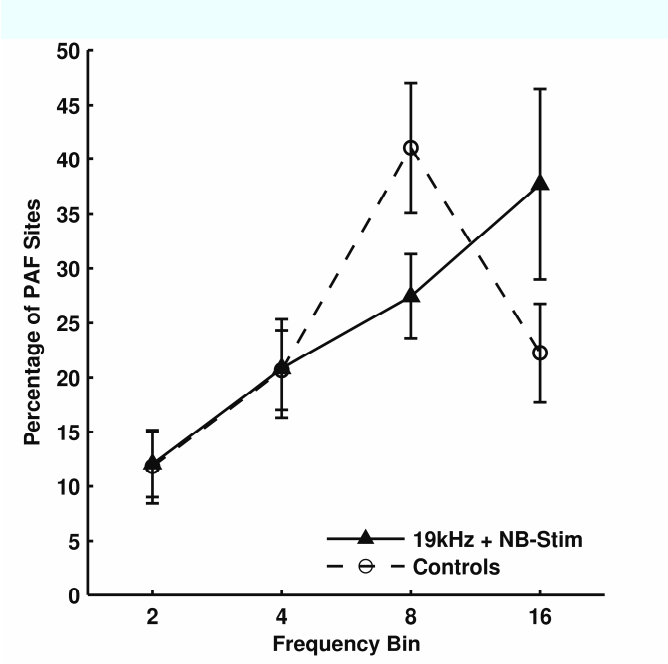

Figure 3. 4. Histogram of the proportion of PAF sites (±SEM) for each control or experimental group with characteristic frequency (CF) falling into each of 4 frequency bins, demonstrating the shift in tuning of PAF sites following NB-tone pairing. The one-octave wide bins are centered on 2, 4, 8, and 16 kHz.

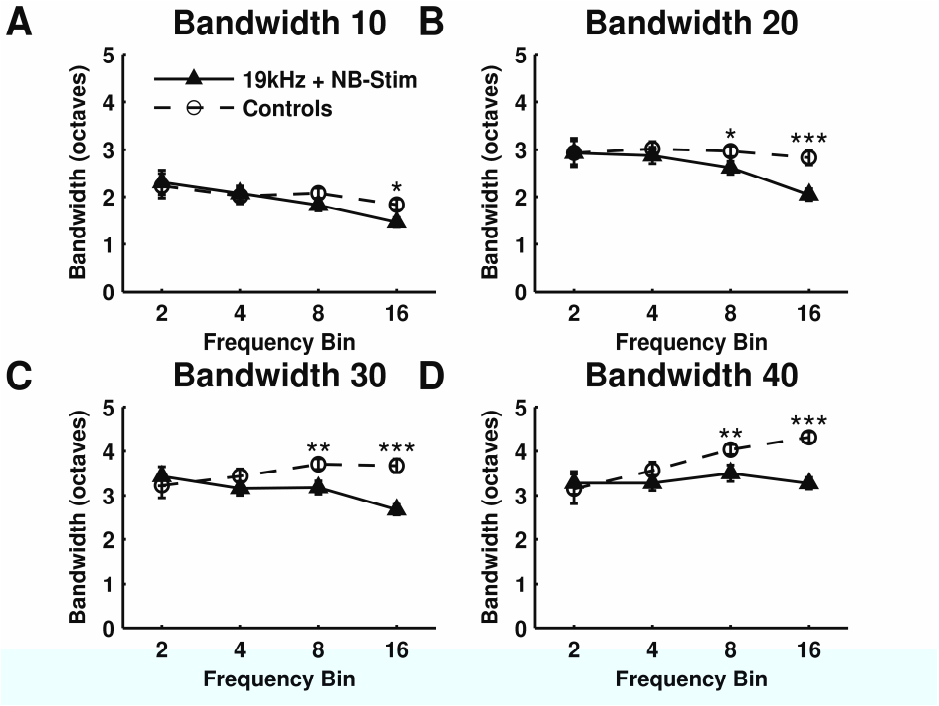

Figure 3. 5. Average bandwidth of PAF experimental sites decreased compared to controls in high frequency bins across all 4 bandwidth measures (BW 10-40). Each plot shows the average bandwidth (± SEM) in control and experimental sites with characteristics frequencies within each frequency bin for a different bandwidth measure. Asterisks indicate significance values of a t-test comparing control and experimental sites in each frequency bin (* - $p<0.05$, ** - $p<0.01$, * - $p<0.001$).**

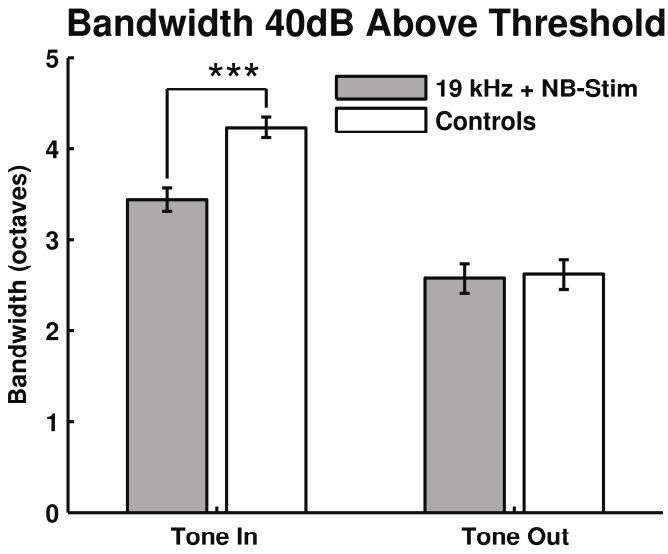

Figure 3. 6. The average bandwidth of PAF sites which contained the paired tone (19 kHz, 50 dB SPL) in their receptive field (Tone In) were decreased in control versus experimental animals. Tone Out sites did not change their receptive field sizes. BW40 was chosen for this measure because the paired tone fell closer to BW40 than other bandwidth measures in the receptive field of most PAF sites. However, the same pattern of results was observed for all bandwidth measures. Asterisks indicate level of significance (* - $p<0.05$, ** - $p<0.01$, * - $p<0.001$). The average bandwidths of Tone In sites were significantly larger than the average bandwidths of Tone Out sites in both control and experimental animals (see Results).**

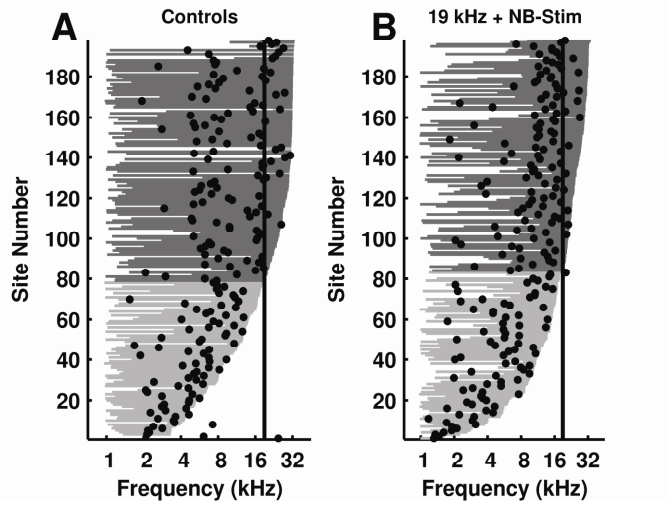

Figure 3. 7. NB-tone pairing narrows frequency tuning and shifts characteristic frequencies (CF) toward the paired tone frequency (19 kHz). Every PAF recording site for controls (*A*) and experimental animals (*B*) is shown sorted by the highest frequency contained within its receptive field for 50 dB SPL tones. Sites which included 19 kHz within their receptive field are plotted in dark gray, while sites which did not are depicted in light gray. The black circles denote the assigned CF of each site. The vertical black line on each panel is located at 19 kHz.

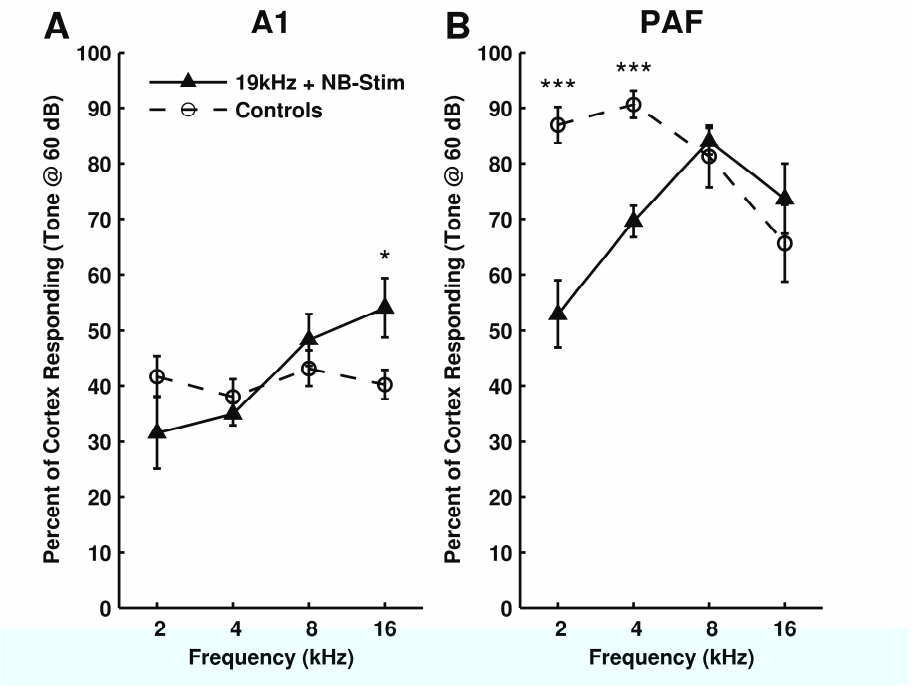

Figure 3. 8. Percentage of A1 and PAF that responds to several tones presented at 60 dB SPL. The average percentage of each field which responded to each tone frequency presented at 60 dB SPL is depicted. While A1 (panel *A*) showed an increase in the number of neurons which responded to high-frequency tones (such as 16 kHz), PAF (panel *B*) sites showed a decrease in the number of neurons which responded to low-frequency tones (2 and 4 kHz). Stars indicate level of significance in a comparison between controls and experimentals (* - p<0.05, ** - p<0.01, * - p<0.001).**

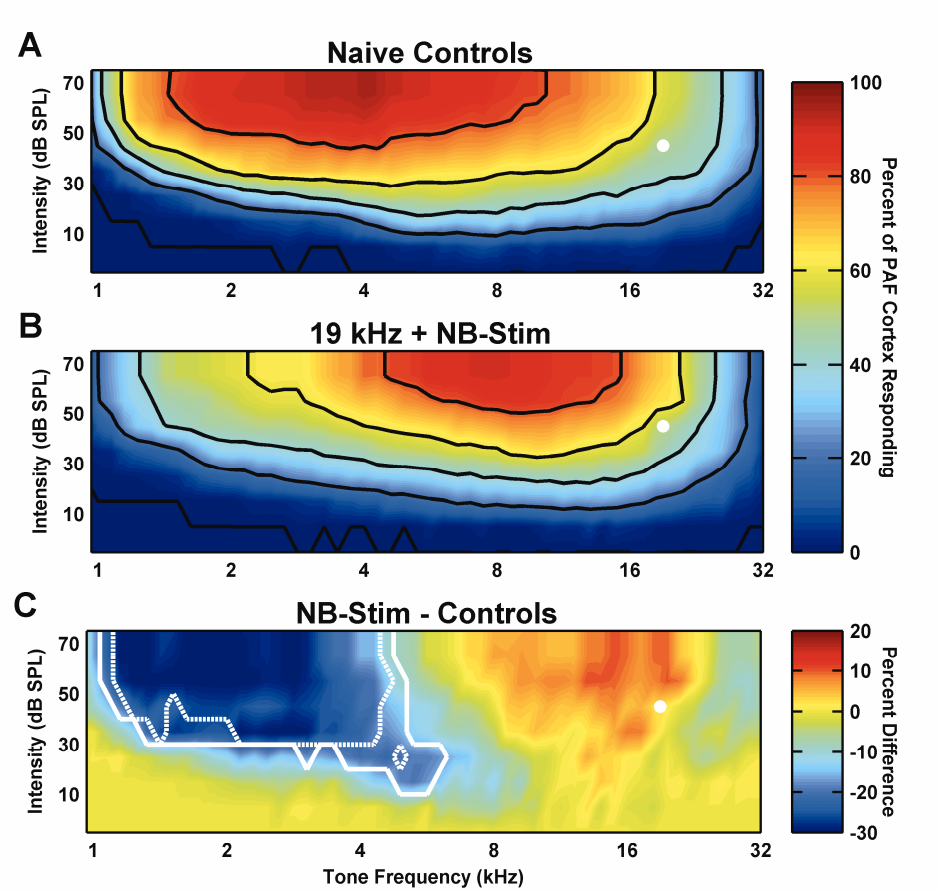

Figure 3. 9. The percentage of PAF that responded to all low-frequency tones decreased after NB-tone pairing. (*A*) The average percentage of PAF cortex in controls which responds to each tone frequency-intensity combination is shown. Contour lines on the plot indicate the tones which elicit responses from 0, 20, 40, 60 and 80% of PAF neurons. (*B*) The average percentage of PAF cortex in experimental animals responding to each tone frequency-intensity combination. The 60 and 80% contour lines were shifted towards high-frequencies in the experimental animals compared to controls. (*C*) The difference in the percentage of PAF responding to each tone-intensity combination (experimental – controls) is shown. White lines delineate the frequency-intensity combinations with a significant decrease in the percent of cortex responding after NB-stimulation pairing (solid – $p< 0.05$, dashed – $p<0.01$). No increases in the percentage of cortex responding were statistically significant. The white point in Panels A-C indicates the tone which was paired with NB-stimulation in experimental animals (19 kHz @ 50 dB SPL).

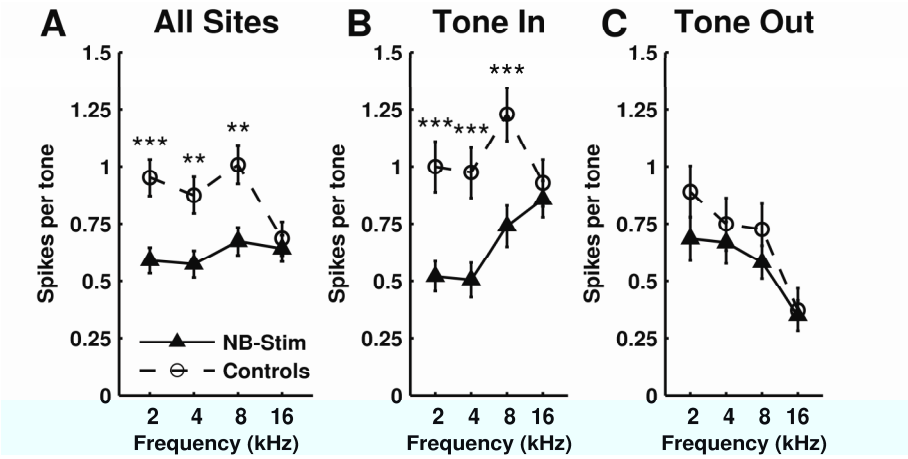

Figure 3. 10. Depiction of the number of spikes elicited by tones played at 60 dB SPL. Panel *A* shows the average evoked response for all PAF sites. Panels *B* and *C* show the responses of those subsets of sites which contained the paired tone within their receptive field (panel *B*) or did not (panel *C*). A decrease in the number of spikes elicited by low-frequency tones (panel A) was primarily caused by a decrease in responsiveness of the Tone In subset of sites (panel B). Asterisks indicate level of significance (* - p<0.05, ** - p<0.01, * - p<0.001).**

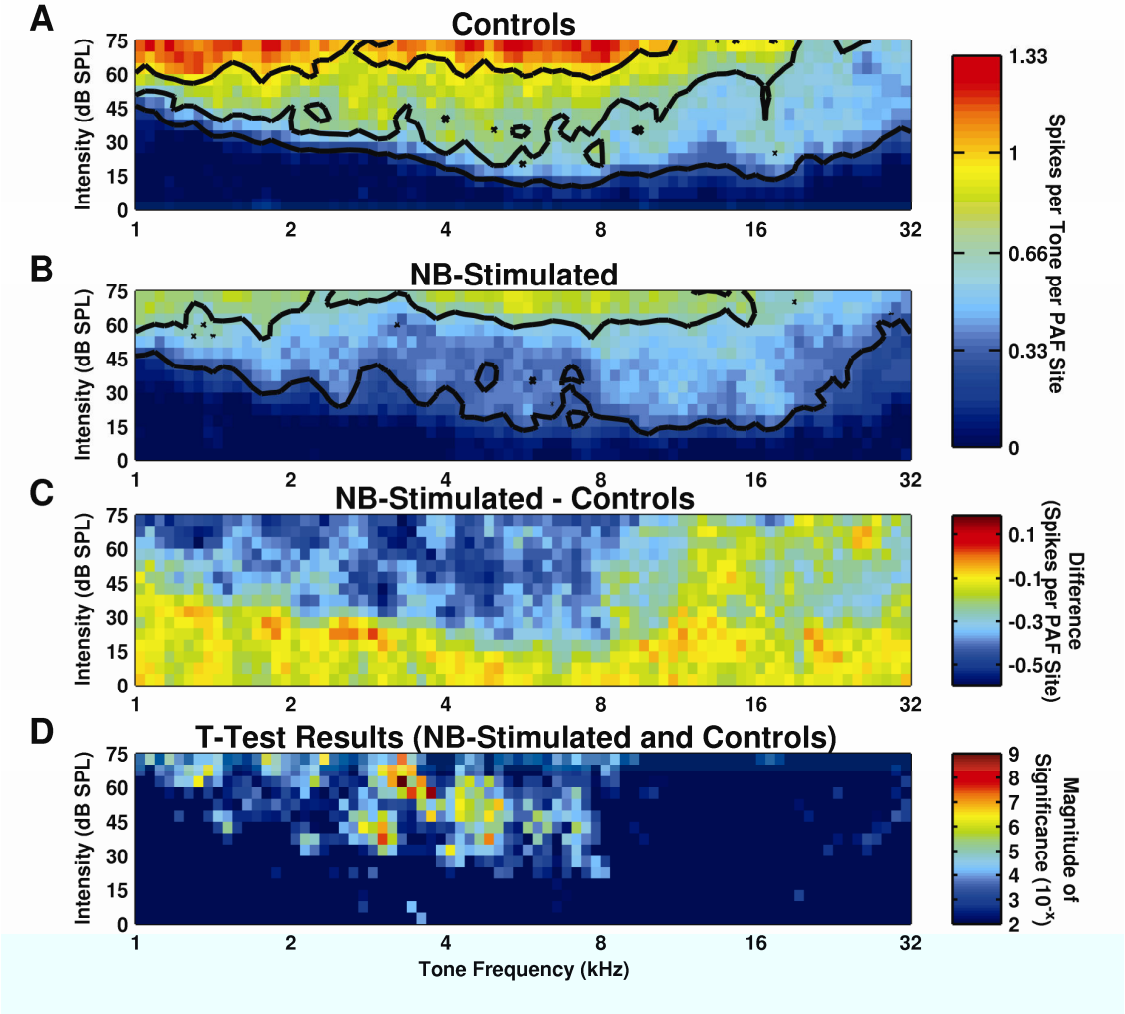

Figure 3. 11 Average number of spikes responding to each frequency-intensity combination per PAF site. For each site, the spontaneous rate (calculated as the activity to 0 dB SPL tones) was subtracted from the response to all the other frequency-tone combinations. Panel *A* shows the average number of spikes evoked from all control sites while panel *B* shows the average number of spikes from all experimental sites. The black lines in panels *A* & *B* delineate the 0.5, 0.75 and 1 spike per tone boundaries on each plot. Panel *C* shows the subtraction of the average of the control sites (panel *A*) from the average of the experimental sites (panel *B*). A zero (orange) indicates that there was no difference between the two groups in the number of spikes evoked by the frequency-intensity combination. Panel *D* shows the result of t-tests between the number of spikes evoked by each frequency-intensity combination in control and experimental sites. The color bar indicates degrees of significance (a value of 3 denotes $p<0.001$, while a value of 4 denotes $p<0.0001$).

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CHAPTER 4

CONCLUSION

The purpose of this dissertation was to examine the relationship between cortical plasticity and perceptual discrimination. Learning to perform a discrimination task is correlated with stimulus-specific plasticity in both human and animal species in all major sensory modalities and in the motor system (Buonomano and Merzenich 1998; Conner et al. 2005; Ghose 2004; Polley et al. 2006; Recanzone et al. 1993). The sensory inputs that are activated during a behavioral task guide the form that plasticity takes, while the release of neuromodulators acts as a gain control so that plasticity only occurs in behaviorally important contexts. However, learning to perform a discrimination task also involves a 'cognitive' component - specific knowledge of the requirements of the discrimination. This component of learning is thought to be mediated by higher cortical structures such as association cortex or pre-frontal cortex. Cortical association areas receive input from primary sensory cortices as well as from the nuclei that release neuromodulators. In turn, these association areas influence the neural activity of sensory cortices and neuromodulator-releasing nuclei through the activity of feedback connections (Gilbert and Sigman 2007).

One possible role of these feedback connections is to direct neural activity in sensory cortical areas so that plasticity takes on task-relevant characteristics. For example, Polley and colleagues showed that learning either a frequency or intensity discrimination using stimuli with very similar auditory characteristics can result in differential types of plasticity depending on the task demands (Polley et al. 2006). These results implied that plasticity was mediated not only by

activation of sensory and motivational circuitry, but also by top-down influences. These results also reveal an uncertainty as to the nature of the plasticity observed in primary cortical areas after discrimination training and the role of these areas during performance of a discrimination task. One possibility is that the auditory cortex functions primarily to process incoming sensory stimuli and then relays this information on to association areas with specific task-knowledge. The second possibility is that plasticity in primary cortical areas directly represents a specific memory of the discrimination task, and therefore plasticity must take place within a behavioral context in order to be functionally useful.

In Chapter 2, we used nucleus basalis stimulation pairing with an auditory stimulus to induce cortical plasticity in primary auditory cortex that was stimulus-specific without the influence of any specific task knowledge (ie, top-down information). It was not known whether this information could be 'useful' to animals when they went on to learn a discrimination task. We found that plasticity induced outside of a behavioral context improved learning and acquisition of the frequency discrimination task. This improvement in learning implies that at least part of the role of primary auditory cortex is to act as a sensory processor, and that the brain is able to take advantage of plasticity no matter what the context was when plasticity was induced. This finding is supported by studies in several other modalities. In some subjects, plasticity that has been induced because of peripheral injuries or in other learning situations can cause generalized improvement in discrimination. In the auditory system, subjects with steeply-sloping hearing loss functions tend to have better discrimination of tones that are close to the area of hearing loss (and presumably now have an expanded representation within the cortex) (McDermott et al. 1998). Subjects with specialized skills, such as professional musicians or Braille readers, tend to show enhanced responses to auditory or tactile stimuli and also show generalized improvements

in discrimination abilities using many different types of stimuli (Besson et al. 2007; Fahle 2005; Jednorog and Grabowska 2008). A study of tactile coactivation has shown that using passive stimulation of the finger to induce a temporary shift in the representation of a single digit in somatosensory cortex also leads to a temporary improvement in two-point discrimination performance(Godde et al. 2000). These results support our own findings and indicate that plasticity in primary cortical areas is able to confer some perceptual advantages when performing discrimination tasks no matter how this plasticity was induced. This further strengthens the hypothesis that plasticity in primary cortical areas represents an alteration in how basic stimulus properties are processed rather than storage of specific task knowledge.

The results of our study in Chapter 2 also indicate that receptive field plasticity in primary auditory cortex may be important for learning a discrimination task rather than performing a task that has already been mastered. For example, rats that had been well-trained to perform a discrimination task, exposed to NB-tone pairing with a behaviorally relevant tone, and then tested on the discrimination task after NB-tone pairing showed no stimulus-specific plasticity in the primary auditory cortex even though previous studies have indicated that all of these activities should promote plasticity (Kilgard and Merzenich 1998; Recanzone et al. 1993). Therefore, although plasticity in primary sensory cortex seems to be helpful early in the learning process, it may not be necessary for skilled task performance once the discrimination has been learned. This result has been mirrored in previous learning studies in both the visual and motor systems that have shown consolidation and reduction in plasticity as subjects become progressively more skilled. In the visual system, learning to perform a visual discrimination task resulted in plasticity in V1 that faded after further discrimination training with no decrement in performance(Yotsumoto et al. 2008). In the motor system, these shifts (from plasticity in both

the cortical-striatal and cortical-cerebellar pathways in a behavior to plasticity in only pathways) are associated with a transition from skilled to habitual performance of the motor task(Doyon and Benali 2005). This shift of plasticity in sensory and motor systems back to a normal state would allow subjects to learn and acquire new skills while still performing previously learned discrimination tasks. While simultaneous performance of many types of tasks is not often studied in laboratory environments, this skill would be essential for survival in real-world situations.

If discrimination performance has been improved on a permanent basis, then some aspect of the central nervous system must also be permanently altered. An important avenue for future study will be to identify where plasticity is persistent throughout the full course of task learning and performance. In Chapter 3 of this dissertation we presented the results of a pilot study indicating that nucleus basalis-tone pairing results in stimulus specific plasticity in both primary and secondary cortical areas(Puckett et al. 2007). Previous studies of plasticity after nucleus basalis -tone pairing and classical conditioning have shown that plasticity in primary auditory cortex is much more long-lasting that plasticity in either the thalamus or inferior colliculus, implying that plasticity becomes more permanent in higher sensory stations (Ji et al. 2001; Ma and Suga 2005; Zhang and Suga 2000). It would be important to examine plasticity in secondary and association areas of animals that have been well-trained to perform a discrimination task but no longer show overt signs of cortical plasticity in primary cortical areas.

Clinical relevance

The results of our studies provide support for the hypothesis that inducing cortical plasticity is important for improving functional recovery after stroke or other brain injury. Short-term plasticity techniques such as transcranial magnetic stimulation have already been used to

improve plasticity and functional performance after brain injury(Takeuchi et al. 2005). Our results indicate that creating longer-acting plasticity by activating neuromodulator systems could lead to more effective discrimination improvement. Many current studies already employ pharmacological agents such as amphetamines to improve the functional outcome of therapy after stroke(Walker-Batson et al. 2001; Walker-Batson et al. 1995). However, techniques such as nucleus basalis stimulation might be more effective than pharmacological agents because the timing of neuromodulator release can be specifically matched to target stimuli, leading to more rapid and specific plasticity.

Speeding the time course of recovery in patient populations could greatly improve functional recovery. For many patients, recovery is slowed because the behaviors that are practiced during therapy take a long time to become functionally useful. For example, if patients lose the use of an arm after stroke, they often learn to compensate and learn to perform tasks with only one hand. This means that plasticity created during physical therapy has to 'fight' with plasticity that is being developed because of this compensatory behavior (Kleim and Jones 2008). Combining physical therapy with a technique such as nucleus basalis -stimulation or a pharmacological intervention could speed functional recovery and might allow patients to recover normal function quickly rather than having to learn and unlearn compensatory behaviors.

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VITA

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