
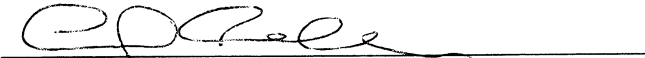


LONG-TERM AND SHORT-TERM PLASTICITY INDUCED BY EXPERIENCE
WITH SPECTROTEMPORALLY COMPLEX SOUNDS
IN RAT AUDITORY CORTEX

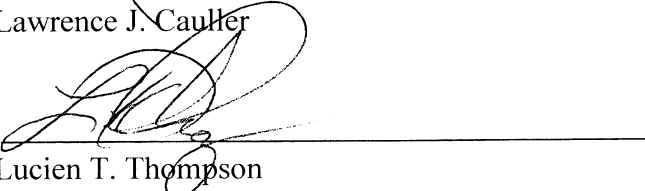
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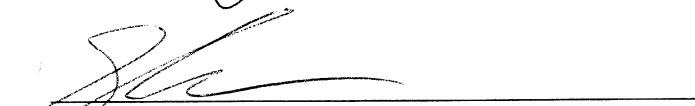
Michael P. Kilgard, Chair



Lawrence J. Cauller



Lucien T. Thompson



Stephen G. Lomber

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DEDICATION

"I can't believe impossible things." said Alice.

"I daresay you haven't had much practice," said the Queen.

"When I was your age, I always did it for half-an-hour a day.

Why, sometimes I've believed as many as six impossible things before breakfast."

- Lewis Carroll- Alice in Wonderland

This thesis is dedicated to hope that things can be better and to all those who believe in change and dedicate their lives redefining boundaries, whether set by politics or by nature. I do not know of other kinds of barriers. I will always admire your courage and selflessness and I hope there will never be a way to silence you.

LONG-TERM AND SHORT-TERM PLASTICITY INDUCED BY EXPERIENCE WITH
SPECTROTEMPORALLY COMPLEX SOUNDS
IN RAT AUDITORY CORTEX

by

RALUCA MOUCHA, M.S.

DISSERTATION

Presented to the Faculty of
The University of Texas at Dallas
in Partial Fulfillment
of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY IN COGNITION AND NEUROSCIENCE

THE UNIVERSITY OF TEXAS AT DALLAS

May, 2006

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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 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. It is acceptable for this dissertation to include as chapters authentic copies of papers already published, provided these meet the type size, margin, and legibility requirements. Where the student is not the sole author of a manuscript, the student is required to make an explicit statement in the introductory material to that manuscript describing the student’s contribution to the work and acknowledging the contribution of the other author(s). The signatures of the Supervising Committee, which precede all other material in the dissertation, attest to the accuracy of this statement.

April 2006

ACKNOWLEDGEMENTS

Many factors contributed to this accomplishment. Most importantly I think was a great deal of luck. I was lucky enough to possess the capacity to learn easily and to come to a world with a variety of choices and little barriers for those eager to learn. Added to this came hard work , good advice and help from all the people I came close too along this path. Thus the first name I want to acknowledge is that of the person I hold responsible for directing me onto this path: Dr Larry Cauller. As my advisor on my undergraduate honors thesis he guided me through my first neuroscience experiments in his laboratory. His enthusiasm and dedication for science were inspiring and contagious even while he was battling a life threatening condition. This first exposure made me realize that research was a good fit for my personality, my thirst of knowledge and that it would be a great means to challenge my mind as a way of life and for a good cause. The most important person responsible for my success after deciding to embark on the pursuit of a research degree was of course my advisor Dr Michael Kilgard. His most inspiring assets to me were his optimism and his real passion for his work. He is definitely someone who leads by example, always determined to convince his students that there are no real limits to what they can achieve.

I want to thank all my colleagues in the lab, especially Pritesh Pandya and Navzer Engineer with whom I worked on many projects together during the first years in the lab. We've developed a special bond as we went through the same hurdles together, shared successes and disappointments and learned from each other. The fact that we often share our spare time even after we've headed into different directions is testimony to the friendship that

we have. To Pritesh I will also always be in debt for taking the role of our pets guardian during my summer appointment in Prague (and this was no small task I'd say as we have nearly a Zoo). And while mentioning this I am thankful to Dean Bert Moore and Dr Aage Moller who enabled me to enrich my research experience by arranging this collaboration with the Institute of Experimental Medicine within the Academy of Sciences of the Czech Republic, and to Professor Josef Syka and all the members of his team for welcoming me in their laboratory.

I also want to mention Amanda Puckett, Cherie Percaccio, and Vikram Jakkamsetti for taking the time to read and help improve the manuscript drafts I have sent to them over time. Amanda was also my companion at several scientific meetings and we always had a good time together as well as stimulating discussions about science and everything else in the world. I thank Helen Chen for the extensive help she provided with the short-term plasticity experiments described in the last chapter and Andrew Sloan and Dr Rennaker for providing the valuable custom made microelectrode arrays used in these experiments.

My committee members Dr Steve Lomber, Dr Larry Cauller and Dr Tres Thompson guided me through the process of integrating all my ideas into a final coherent body of work, which this thesis resembles.

Finally, I could not have possibly endured this long and ambitious journey without the support of close friends and family located all over the world. Radu as the closest person to me, for the past decade now, certainly demonstrated enormous patience and humor along the way. He also forced me to have some balance during this process even though it felt crazy at times to catapult myself to an exotic location when so many assignments still lie

ahead of me. His passion for travel revealed to me that I am also part of a world full of natural and cultural wonders in addition to the ones of everyday science. I could not have asked for more enlightening experiences both professional and personal while pursuing my graduate degree and to all who contributed I am deeply and forever grateful.

LONG-TERM AND SHORT-TERM PLASTICITY INDUCED BY
EXPERIENCE WITH SPECTROTEMPORALLY COMPLEX SOUNDS IN
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The University of Texas at Dallas, 2006

Supervising Professor: Michael P. Kilgard

The goal of the described experiments was to document the effects of long-term and short-term experience with sounds that change in frequency on primary auditory cortex (A1). Previous studies by our laboratory have elaborated the relationship between acoustic experience and network plasticity demonstrating that 1) spectral variability and modulation rate of experienced sounds determine frequency selectivity of cortical neurons 2) the size of sensory area stimulated determine map reorganization and that 3) all these changes are influenced by background conditions. We have employed a well-established paradigm that uses stimulation of nucleus basalis (NB) paired with presentation of simple and modulated sounds to systematically reveal the effect of manipulating acoustic experience on cortical plasticity (Kilgard et al. 2001b). My experiments extend these results by pairing NB

stimulation with spectro-temporally complex sounds such as FM sweeps in different conditions varying the pattern of activation and the acoustic background. In addition I evaluated how changes in receptive field structure and temporal fidelity can arise from short-term experience with these sounds based on a spike-timing dependent plasticity model. In summary this thesis documents for the first time 1) how FM sweeps of one octave width are represented along three relay centers of the auditory pathway: Thalamus, A1, PAF, 2) how experience with FM sweeps changes the representation of these sounds and A1 properties (i) long-term (over ~20 days) and (ii) short-term (within minutes) and 3) how details of experience influence cortical plasticity with regard to (i) the extent of the sensory area activated by a stimulus and (ii) the background conditions in which a stimulus is presented. These results will be essential in understanding cortical processing of complex stimuli, complex activation patterns and processing of sound in complex acoustic environments, and will lay the ground for a new approach of experimental design in sensory systems.

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CHAPTER ONE
INTRODUCTION: CORTICAL PLASTICITY AND REHABILITATION

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ABSTRACT

The brain is constantly changing throughout every stage of life. It reorganizes continuously to adapt to environmental demands or endogenous changes (such as peripheral lesions). The plasticity of the nervous system has been refined over millions of years and expression of neural plasticity leads to outcomes that depend on many factors such as the nature of the experience, motivation, and contextual influences.

Guiding the expression of plastic changes in a direction that facilitates recovery of function is a primary goal of neurological rehabilitation. As the rules that govern neural plasticity become better understood, it will be possible to manipulate the sensory and motor experience of patients to induce specific forms of plasticity.

The first two sections of this review summarize our current knowledge regarding the factors that regulate cortical plasticity, and illustrate specific forms of reorganization induced by control of each one of these factors. In the last part we discuss evidence indicating that motivation, sensory input, and context are likely to be critical factors in directing expression of plasticity for therapeutic benefit. We discuss how the factors involved in experience-dependent induced plasticity can be manipulated to guide the expression of plasticity that is beneficial for recovery from brain injury.

1. Factors that regulate plasticity

Plasticity is the remarkable ability of developing, adult, and aging brains to adapt to a changing world. This potential is revealed whenever an organism must meet a new environmental demand or recover from nervous system damage. Sensory and motor systems exhibit plasticity following deprivation, skill learning, and injury. Experience dependent changes in brain function can be as subtle as a change in neuronal excitability (Engineer et al., 2004) or as dramatic as the rewiring of auditory cortex to process visual information (Sur et al., 1988). Cortical maps, receptive field size, neuronal firing rate, temporal precision, and combination sensitivity can all be modified in an experience dependent manner. The types of plasticity activated by specific situations depend on the nature of the inputs and the behavioral significance, apparently conveyed by a number of modulatory neurotransmitters (Fig 1).

1.1 Attentional modulation

Although neural plasticity is essential for adapting to changes in the environment, inappropriate plasticity could be destabilizing and even erase memories. As a result plasticity is regulated to prevent every random stimulus from modifying the brain. Attention is believed to be required for most forms of perceptual learning and regulates the plasticity associated with sensory experience. Many studies have shown that attention can directly affect firing rates of cortical neurons (Recanzone and Wurtz, 2000; Treue and Maunsell, 1999). Steinmetz et al., 2000 demonstrated the contribution of synchronized neuronal firing in directing attention to specific aspects of the environment. While recording from

populations of neurons in the secondary somatosensory cortex of monkeys, who were trained to shift attention between visual and tactile tasks, they observed an increase in firing rate of the neurons and in their degree of synchronization during the tactile task and not the visual task. Different forms of perceptual learning result, when subjects are required to attend to different stimulus features, even when the sensory inputs are identical (Ahissar and Hochstein, 1993). Exposure to moving dot patterns can improve motion direction discrimination ability even if the motion is undetectable (due to low coherence), so long as the subjects are actively attending a visual task (Watanabe et al., 2001; Seitz and Watanabe, 2003). These results suggest that the directed attention facilitates the learning of associated sensory features.

1.2 Neuromodulatory influences

How do individual cortical neurons 'know' what subjects are paying attention to? Considerable experimental evidence suggests that diffuse neuromodulatory systems instruct neurons which stimuli are attended. Several neuromodulators, including dopamine, norepinephrine, and acetylcholine, are known to regulate learning and memory in humans (Hasselmo, 1995). Synaptic plasticity is also enhanced by the presence of these neurotransmitters (Singer, 1986; Brocher et al., 1992). Direct injection of acetylcholine or norepinephrine into visual, somatosensory, or auditory cortex can even be used to stimulate plasticity in the intact brain (Greuel et al., 1988; McKenna et al., 1989; Delacour et al., 1990). Artificial electrical stimulation of the nuclei that naturally release them: nucleus basalis, locus coeruleus or ventral tegmental area (NB, LC or VTA), can also generate

stimulus specific neuronal plasticity (Kilgard and Merzenich, 1998; Bouret and Sara, 2002; Bao et al., 2003). Stimulants, like amphetamines, which increase the concerted release of several neurotransmitters can enhance cortical plasticity, while sedatives, like valium, can block it (Dinse et al., 2003). While such pharmacological substances that can globally up or down-regulate the various neuromodulatory systems are used clinically, surprisingly little is known about how the interaction of different modulatory neurotransmitters shapes plasticity in the intact brain.

1.3 Patterns of sensory activation

Prior studies have shown that sensory input determines the form of cortical reorganization. When animals or humans repeatedly practice a skill that engages a limited region of the sensory epithelium, the regions of the cortical map that respond to task-specific inputs are enlarged (Jenkins et al., 1990; Recanzone et al., 1992b; Recanzone et al., 1993; Elbert et al., 1995; Sterr et al., 1998). Depending on the spatial and temporal pattern of sensory activation encountered during training, cortical receptive fields (RF) can narrow or broaden, and response latency can increase or decrease. In owl monkeys trained on modulated tactile stimuli repeatedly delivered to one location resulted in increased RF's of cortical neurons and decreased response latencies (Recanzone et al., 1992a). In contrast, training on a task with tactile stimuli that moved across the skin cause receptive fields to shrink (Jenkins et al., 1990; Recanzone et al., 1992b). Tone frequency discrimination training decreased RF size and increased response latency of cortical neurons (Recanzone et al., 1993). Training on a visual orientation task increased the steepness of orientation tuning

in the trained region of the visual field (Schoups et al., 2001). These studies support the hypothesis that perceptual learning and cortical plasticity are specific to attended sensory features.

The rodent whisker system has proved to be an important model for examining how different spatial patterns of activity lead to different forms of cortical plasticity. Cutting a whisker substantially reduces input to the corresponding region of whisker cortex. This form of sensory reduction weakens the responsiveness of the deprived neurons and increases responsiveness to spared whiskers. However, the plasticity that results depends not only on the sensory restriction of the site's principal whisker, but also on the state of neighboring whiskers. The reduction in responsiveness to the deprived whisker is modest if all whiskers are trimmed and greatest if all the neighboring whiskers are spared (Glazewski et al., 1998). If all but one whisker is removed the responsiveness of spared whisker is increased. A checkerboard deprivation pattern causes responses to the deprived whiskers to decrease, but does not increase the response to the spared whiskers (Wallace and Fox, 1999). Finally, cutting all but two neighboring whiskers causes the receptive field of neurons in each region to shift toward the spared neighbor (Diamond et al., 1993). These results suggest that a mechanism for competition between sensory inputs is responsible for the different forms of reorganization.

1.4 Timing of sensory inputs

The temporal coincidence of sensory stimulation can be just as important as its spatial pattern in determining the direction and magnitude of cortical plasticity. Inputs that are correlated in time are more likely to drive a change than uncorrelated inputs. Coactivation of

tactile receptors by stimulation of a skin area with a solenoid disc induced broadening of receptive fields in somatosensory cortex, while stimulation of a single point on the skin did not (Pleger et al., 2003). In the developing auditory system co-activation of a large population of neurons by exposure to broadband noise caused RF broadening and degradation of the tonotopic map (Chang and Merzenich, 2003), while exposure to a single tone did not. Manipulations designed to increase correlated activation of fingers through surgical fusion or operant training lead to the development of multi-digit receptive fields in somatosensory cortex (Allard et al., 1991; Wang et al., 1995). The usual segregation of representations of distinct fingers in the cortex reflects the normal asynchronous use of each finger. After training with a bar that stimulated the distal versus proximal part of three fingers together, Wang et al reported segregation within a finger between proximal and distal segments rather than between fingers, which reflected the temporal synchrony of stimulation during training. In vitro and more recently in vivo studies have further demonstrated that the time window for correlated inputs to induce plasticity is on the order of tens of milliseconds (Tsodyks, 2002; Dan and Poo, 2004). These results indicate that the precise spatial and temporal pattern of inputs shape cortical networks due to operation of Hebbian synaptic plasticity.

1.5 Duration of experience

Many factors regulate the time course of learning and plasticity (Ebbinghaus, 1885; Dubnau et al., 2003). Fear conditioning can induce rapid and long lasting shifts of neuronal tuning towards the frequency of the conditioned tone (Bjordahl et al., 1998; Weinberger, 2003). However plasticity following skill learning or use dependent plasticity develops

gradually over time. The magnitude of effects often depends on duration of training and correlates with performance accuracy (Pleger et al., 2003). Motor map reorganization, which is accompanied by synaptogenesis and believed to underlie consolidation of motor skills, occurs during the late phase (after ten days) of motor skill learning (Kleim et al., 2004). The schedule of inputs can also determine the induction of stable versus reversible synaptic modifications (Mauelshagen et al., 1998). Repetitive delivery of LTP inducing stimuli with a spaced temporal pattern prevents the reversal of LTP due to subsequent spontaneous activity (Zhou et al., 2003). Stable synaptic modifications are also induced by visual experience when the exposure to unidirectional moving bars occurs in a spaced pattern (three sets of 60 flashes separated by five minutes) versus massed pattern (180 flashes continuously). If persistent synaptic changes are important for learning and memory, the effective use of training strategies that prevent their reversal is important. In behaviorally trained mice (Scharf et al., 2002) reported that temporally spaced training more effectively recruited protein synthesis and enhanced long term memory of contextual conditioning, while (Genoux et al., 2002) showed that massed training triggered more protein phosphatase 1 activity which suppresses memory formation. These results suggest that the schedule of training determines the duration of neural plasticity and learning.

1.6 Influence of background stimuli on plasticity

Psychologists and psychophysicists have known for decades that context (comprised of background unattended stimuli) influences perceptual learning. However only very recent data show that such influences are apparent even at the most simple level of the “learning

circuit”, directing plasticity of the responses of single neurons. Typically for better parametric control of stimuli, studies of sensory plasticity were conducted in environments stripped of such background, by using soundproof booths or gray backgrounds, and attention was directed only to the trained stimuli. Only recently more experiments have been designed to examine learning and plasticity in more naturalistic and complex settings.

In many cases, complex backgrounds actually improve learning. For example, contrast discrimination learning can be facilitated by stimuli surrounding the target stimulus. Practicing a contrast discrimination task in the presence of flankers with fixed contrast enabled subjects to improve their performance (Adini et al., 2002). A previous experiment by Kapadia et al, 1995 showed that a dim line object became easier to detect when flanked with a second collinear bar. This improvement in performance in humans paralleled an enhancement of neuronal responses in monkey V1 when analogous stimuli were presented (Kapadia et al., 1995). Auditory cortex neurons shift their frequency tuning toward a tone that is paired with foot-shock when presented in silence (Bakin and Weinberger, 1990; Dimyan and Weinberger, 1999) and away from the paired frequency when unpaired background stimuli are present (Ohl and Scheich, 1996).

While each of the above these studies support the idea that many factors regulate plasticity and learning, direct comparison of the interactions between these factors has proven difficult. Differences in the behavioral response, task difficulty, task goal, motivation, modality and species often confound the influence of the discussed factors on plasticity. Because these factors are so tightly interdependent it is difficult to tease apart their

importance in directing different forms of plasticity. Varying sensory patterns or adding a complex background, for example, would also affect task difficulty in most cases.

2. Sensory input paired with controlled release of neuromodulators.

Many studies that attempted to define the factors required to induce plasticity have done so by blocking plasticity. For example, studies using cholinergic blockade, either by pharmacological antagonists or targeted lesions, were significant in showing that the integrity of the cholinergic system is necessary for learning and plasticity (Fig. 2a,b,c). An important disadvantage of this approach is that role of additional factors that influence types of reorganization (such as nature of sensory inputs, timing and activation patterns) cannot be characterized while plasticity is blocked. Experimental paradigms that directly stimulate modulatory systems have proven valuable in documenting the influence of stimulus pattern, timing, and background conditions on cortical plasticity.

Pairing different sounds with electrical activation of the cholinergic nucleus basalis generates changes in cortical map and receptive field properties that closely parallel the different forms of plasticity resulting from operant training. For example, temporally modulated stimuli tend to increase receptive field size, while stimuli that activate different, but nearby, regions of the receptor surface tend to decrease receptive field size. The potential that the stimulus differences altered many aspects of learning during operant training made it impossible to be certain that the temporal and spatial properties of the stimuli were really the critical differences that determined receptive field size. The observation that the same

change in the sensory input leads to equivalent plasticity even in the absence of operant training supports the conclusion that stimuli shape the form of cortical plasticity.

Electrical stimulation allows precise control over the type, amount and timing of neuromodulator release associated with different sensory input. In natural learning, changing task contingencies are known to alter each of these important factors. (Richardson and DeLong, 1990, 1991) showed that novel sounds activate cholinergic NB neurons for a few trials, but habituate rapidly. The response is restored if the sound is associated with a reward or punishment. (Orsetti et al., 1996) showed that NB releases acetylcholine onto the cortex only during the learning phase of a simple lever press task, but not after the task is well learned. Electrical stimulation bypasses the natural triggers of NB activity and eliminates the natural brake on cortical plasticity. The consistency of electrical activation makes it possible to systematically compare how the type, amount and timing of neuromodulator release influence cortical plasticity when associated with sensory stimuli of differing spatial and temporal properties.

2.1 Attention can be substituted, but neuromodulators are necessary

Vast evidence exists that attention is required for most forms of perceptual learning. The selective release of neuromodulators during attentive states is critical for many forms of learning and plasticity (Hasselmo, 1995). Selective attention stimulates the release of several neurotransmitters (acetylcholine, dopamine, and norepinephrine) each of which are known to regulate synaptic plasticity. Thus plasticity dependence on behavioral state develops as a consequence of the neuromodulatory influences attention has on the cortex (Ahissar and

Hochstein, 1993). However attention is not obligatory if neuromodulator release can be experimentally induced.

2.2 Patterns of activation determine type of reorganization

Distinct types of cortical reorganization are generated when NB stimulation is associated with different sensory inputs. In a series of experiments we systematically varied the spatial and/or temporal structure of auditory stimuli and thus the pattern of activation of auditory neurons. Cortical topography, receptive fields, and response timing systematically varied with the temporal modulation and spatial distribution of activation. Repeated activation of a limited region by presentations of a single tone frequency resulted in expansion of the area responsive to the tone, and RF broadening. Distributing the activation over more frequency sectors by using seven tone frequencies resulted in a narrowing of RF, while an expansion of the sensory area corresponding to each frequency region was no longer possible (Kilgard et al., 2001). Modulated stimuli such as tone trains had similar effects to tones (map expansion and increased RF) when activation was focal (one carrier frequency), but opposite effects to tones (increased RF) if they activated several regions (seven different carrier frequencies). Such simple variations in patterns of auditory input revealed a relationship between activation patterns and cortical reorganization: (1) Sensory map expansion resulted from repeated focal activation and not from activation that was spatially distributed. (2) Unmodulated stimuli increased RF when they were applied focally and decreased RF when they activated several sectors. (3) RF's were increased by modulated stimuli regardless of spatial distribution (Kilgard et al., 2002). Table 1 summarizes the types of plasticity that

result from pairing NB stimulation with each pattern of sensory stimulation. The observation that RF size is increased by stimuli with high degree of temporal modulation and little spatial variability (tone trains) and decreased by stimuli with high spatial variability and no temporal modulation (unmodulated tones of varying frequency) is consistent with earlier observations of plasticity in operant trained monkeys. These results indicate that NB stimulation directs changes that are similar to operant induced plasticity even though the rats did not use the stimuli in any way.

Plasticity mechanisms are designed to increase the cortical processing capacity of behaviorally relevant inputs. Our finding that such changes seem to be a systematic function of spatial and temporal input statistics suggests that this may be attained by trade-offs between spatial and temporal precision of cortical neurons to better represent characteristics of the sensory stimuli.

2.3 Correlation of sensory inputs

Organization of primary auditory cortex can be differentially altered by sounds designed to decrease or increase input correlation across the frequency map (Pandya et al., 2005). Alternating activation of two non-overlapping auditory neuron populations by two tones of distant frequencies (2 kHz and 14 kHz) resulted in map segregation, decreased excitability and longer response latencies of the activated neurons. These changes did not occur when NB-stimulation was paired with a modulated noise burst that synchronously activated large populations of A1 neurons. An additional study found that pairing pulsed noises with NB stimulation in awake rats for 4 weeks disrupted tonotopic maps, and reduced

spontaneous discharge correlation in the primary auditory cortex (Bao et al., 2003). In the developing visual system alternating asynchronous electrical stimulation of the optic nerve prevents normal development of binocular visual responses (Stryker and Strickland, 1984), while monocular electrical stimulation results in stronger response to the activated eye, and the retention of most binocular responsiveness (Ohshima et al., 2002). These findings are in agreement with the Hebbian postulate that inputs with decreased correlation weaken cortical responses and supports other observations that primary sensory cortices segregate inputs that are asynchronous and integrate correlated inputs (Allard et al., 1991; Wang et al., 1995).

2.4 Duration of associative sensory pairing

The duration of NB-induced plasticity depends on the schedule of the pairing protocol. Repetitively pairing NB stimulation with a tone for several minutes causes a shift in frequency tuning that reverses within five hours (Zhang et al., 2005). Cortical map expansion builds with repeated pairings. One month of 300 NB-tone pairings per day increased the A1 representation of the paired frequency by twice as much as a week of pairing (Kilgard and Merzenich, 1998). After a month of pairing, NB induced map plasticity endures for at least 20 days (Carrasco et al., 2004). NB stimulation also increases the duration of cortical and subcortical plasticity induced by cortical microstimulation (Ma and Suga, 2003). These results support earlier observations that cholinergic modulation contributes to both short-term and long-term plasticity.

2.5 Background stimuli influence plasticity outcomes

Although background stimuli are known to influence task performance and plasticity (Kapadia et al., 1995; Adini et al., 2002), it has not been clear whether the differences are due to altered task difficulty or to some specific influence of the distracters. By directly pairing sensory stimuli with NB stimulation in different contexts we have examined the influence of background stimuli on plasticity while eliminating the task influences.

Pairing a single tone with NB stimulation results in a 20% increase in RF size. However, this RF expansion does not occur if the same tone-NB pairing is interleaved with flanking tones that are not associated with NB stimulation (Kilgard et al., 2001). Even expansion of cortical maps, typically induced with NB stimulation paired with auditory stimuli, can be blocked if during pairing other sounds are interleaved but not paired with NB. Repeated presentation of the word /SASH/ paired with NB stimulation caused expansion of the high frequency region of the cortical auditory map (Pandya et al., 2003). This frequency expansion probably reflects reorganization due to repeated stimulation of the high frequency sites by the initial letter /S/ which contains spectral energy at high frequencies. However when the phonemes of the word /S/, /A/ and /SH/ were separately presented, but not paired with NB stimulation, the tonotopic frequency map remained unchanged. This suggests that neural plasticity can be modulated by background stimuli previously thought to be irrelevant.

3. Clinical conclusions

It was proposed nearly two decades ago that cortical reorganization after injury may be the neural substrate for recovery of function after brain damage (Jenkins and Merzenich,

1987). More recent studies in primates have shown that rehabilitative training can influence direct reorganization to benefit recovery (Nudo et al., 1996). There is no longer a doubt that reorganization after brain lesions is shaped by the sensorimotor experiences in the weeks to months following injury. Hence it is important to effectively manipulate all factors that influence reorganization during this time. Not surprisingly factors that have been shown experimentally to enable plasticity can also be clinically manipulated to enhance therapeutic outcomes. For example cholinergic modulation which facilitates or blocks experience dependent plasticity also directly influences recovery (Conner et al., 2005). Thus, injury of the cholinergic system impairs plasticity and learning in healthy subjects as well as compensatory plasticity and recovery of function after brain lesion (Fig 2).

Attention is often impaired after brain injury and is likely important for training induced plasticity. Attention process therapy enhanced patient recovery after brain damage. The patients with the highest vigilance scores also received greatest benefit from the therapy (Sohlberg et al., 2000). If needed, attention can be modulated by medication. Intensive speech therapy combined with piracetam - a drug that enhances vigilance, facilitated rehabilitation of aphasic patients (Kessler et al., 2000). Other strategies that result in increased arousal and frustration such as constraint therapies are also proving to be more efficient than traditional occupational therapies (Taub and Uswatte, 2003). Neuromodulators that place the brain in a permissive state for experience dependent changes also augment the effects of training when administered systemically.

Drugs that act on noradrenergic, dopaminergic, serotonergic, and cholinergic systems have been shown in laboratory and clinical research to be pharmacological adjuvants in

neurorehabilitation (Phillips et al., 2003). Amphetamines lead to a diffuse increase of several modulators and can have a positive influence even when administered only as a single dose at the beginning of therapy (Feeney et al., 1982). Drug administration alone is not sufficient to aid recovery and must be paired with practice to have a beneficial effect. Administration of dextroamphetamine paired with sessions of speech language therapy increased the rate of improvement of aphasic patients during the early recovery period after stroke (Walker-Batson et al., 2004).

Targeted repeated sensorimotor stimulation is necessary in addition to neuromodulators to promote recovery. Motor maps are altered by skill acquisition not by repetitive use alone (Nudo, 1997). In somatosensory cortex postlesion changes were related to individual strategies and sensorimotor experience resulting from idiosyncratic behavior. The type of reorganization depended on the strategy used by individual monkeys to reacquire an object retrieval skill after an experimentally induced stroke (Xerri et al., 1998). These findings imply that cortical map plasticity can be influenced by the pattern of sensorimotor stimulation during behavioral treatment. In dysphagic stroke patients electrical stimulation of the pharynx results in motor cortex plasticity that is dependent on the pattern of stimulation (frequency, intensity and duration of stimulation) and correlates with improvement in swallowing function (Fraser et al., 2002). Various treatment strategies now effectively combine modulation of somatosensory input, administration of pharmacological adjuvants and cortical stimulation to improve outcomes of rehabilitation (review (Hummel and Cohen, 2005)).

The influence of background has not parametrically been studied in the context of neurorehabilitation. However studies have documented beneficial effects of general environmental enrichment in recovery after experimental brain infarcts (review (Johansson, 2004)). In addition enriched environments enhanced recovery when combined with training or drug therapy (Biernaskie and Corbett, 2001; Puurunen et al., 2001). Our results from plasticity experiments indicate that adding complex backgrounds during rehabilitative training (especially speech therapy) may aid in emphasizing and facilitating performance on specific tasks.

In conclusion therapies that optimize neural plasticity by integrating all the concepts described above are likely to lead to better outcomes (Table 2). Optimal modulator release can be accomplished by modulating attention and arousal either through task requirements or stimulating drugs. Stimuli used in training can be selected to address specific changes (rewiring) patients may need for recovery. The proper timing of sessions (spaced training rather than massed training) and duration should also be optimized for training effects to be stable and long term. Adding background stimuli may prove beneficial in many situations. Context stimuli can be used to emphasize aspects of a task or to incrementally increase task difficulty to maintain the patient's engagement and motivation. Ideally, the progress and efficacy of therapy should be monitored in each patient (and adjusted as needed) using brain imaging or evoked potentials.

We are now beginning to understand how many factors interplay in directing different forms of plasticity. Combining parameters such as activation pattern, timing and duration,

background, and the delivery of plasticity enhancing drugs in a synergistic way is essential to improving neuro-rehabilitation.

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APPENDIX CHAPTER ONE

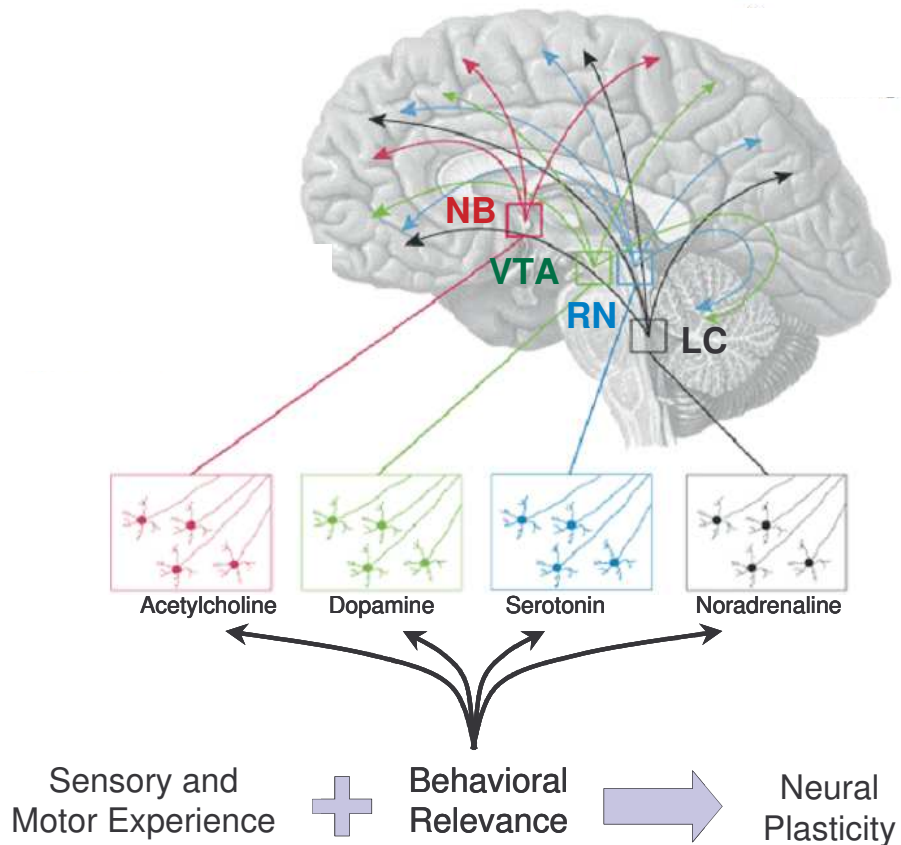


Figure 1.1. Several major neurotransmitter systems which project widely into the cortex are implicated in learning and experience dependent plasticity: Acetylcholine from the cholinergic Nucleus Basalis (NB), Dopamine from the Ventral Tegmentum (VTA), Noradrenaline from the Locus Coeruleus (LC), and Serotonin from the Raphe Nuclei (RN). In addition to these major neurotransmitters, GABA-ergic projections, histamine, and neuro-hormones also play a role in modulating plasticity. Their release can be triggered by behavioral state (attention), with drugs (amphetamines), and via direct electrical stimulation of their corresponding brainstem nuclei. If their release is associated with the occurrence of sensory stimuli, changes are induced at the level of the cortex that are stimulus specific and are known as experience dependent plasticity. Figure adapted from Karger Gazette No. 66.

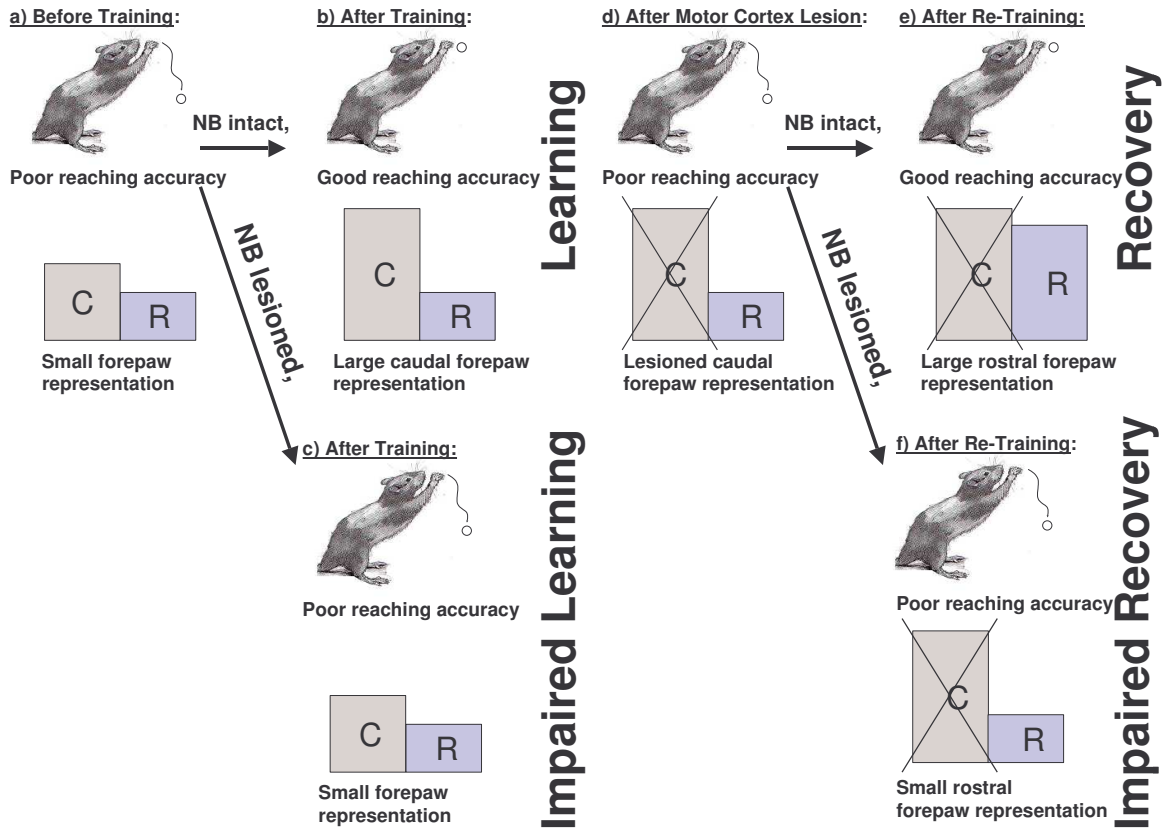


Figure 1.2. Rats can improve their reaching accuracy in a difficult task with behavioral training. This improvement correlates with an increase in somatosensory cortex of the caudal hand representation that is mainly engaged by the task (b). Without the normal input from the cholinergic nucleus basalis (i.e. after lesion of NB cholinergic neurons) rats cannot improve their accuracy with training and the somatosensory representation of the hand remains unchanged (c). Cortical lesions of the caudal hand representation after training (d) results in loss of accuracy that can be retrained, and expansion of the rostral hand representation (e). If nucleus basalis is lesioned during recovery, regaining of reaching accuracy is impaired and the rostral hand representation does not change (f). Results illustrated are from experiments by Conner et al, 2005.

Table 1. Plasticity induced by pairing NB with different sounds

NB stimulation paired with:	Plasticity observed:	References:
Single Tone	Map expansion + decrease latency	Kilgard and Merzenich, 1998
Tone Train	Map expansion + decrease latency + RF broadening	Kilgard and Merzenich, 2001
Distributed Tones	RF narrowing + increase latency	Kilgard and Merzenich, 1998
Distributed Tone Trains	RF broadening + temporal plasticity	Kilgard and Merzenich, 2001
Frequency Modulated Tones	RF broadening + decrease latency + decreased thresholds	Moucha et al., 2005
Complex Acoustic Sequence	Combination sensitivity + decrease latency + decreased thresholds	Kilgard and Merzenich, 2002
Background Sounds	Alters plasticity generated in silence	Moucha et al., 2005

Table 2. Factors regulating plasticity

Factors regulating plasticity	Effect	References:
Attention	Enhances stimulus driven plasticity via internal trigger of neuromodulator release	Hasselmo, 1995
Drugs	Achieve optimal levels of neuromodulators required for plasticity	Phillips et al., 2003
Pattern of stimuli	Determines form of plasticity (reorganization of sensory representations, temporal precision, spatial selectivity etc.)	Buonomano and Merzenich 1998
Temporal delivery (spaced vs. massed training)	Potential and stabilization of changes by stimulating protein synthesis mechanisms, and reducing phosphatases that prevent long term changes	Zhou et al., 2003; Scharf et al., 2002; Genoux et al., 2002
Duration of training	Consolidation of changes via synaptogenesis	Kleim et al., 2004

REFERENCES

- Adini Y, Sagi D, Tsodyks M (2002) Context-enabled learning in the human visual system. *Nature* 415:790-793.
- Ahissar M, Hochstein S (1993) Attentional control of early perceptual learning. *Proc Natl Acad Sci U S A* 90:5718-5722.
- Allard T, Clark SA, Jenkins WM, Merzenich MM (1991) Reorganization of somatosensory area 3b representations in adult owl monkeys after digital syndactyly. *J Neurophysiol* 66:1048-1058.
- Bakin JS, Weinberger NM (1990) Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Res* 536:271-286.
- Bao S, Chang EF, Davis JD, Gobeske KT, Merzenich MM (2003) Progressive degradation and subsequent refinement of acoustic representations in the adult auditory cortex. *J Neurosci* 23:10765-10775.
- Biernaskie J, Corbett D (2001) Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. *J Neurosci* 21:5272-5280.
- Bjordahl TS, Dimyan MA, Weinberger NM (1998) Induction of long-term receptive field plasticity in the auditory cortex of the waking guinea pig by stimulation of the nucleus basalis. *Behav Neurosci* 112:467-479.
- Bouret S, Sara SJ (2002) Locus coeruleus activation modulates firing rate and temporal organization of odour-induced single-cell responses in rat piriform cortex. *Eur J Neurosci* 16:2371-2382.

- Brocher S, Artola A, Singer W (1992) Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. *Brain Res* 573:27-36.
- Carrasco RA, Puckett A, Pandya PK, McMenemy A, Gibbons J, Heydrick C, Moucha R, Jain R, Kilgard MP (2004) Frequency map reorganization in rat primary auditory cortex enabled by basal forebrain activation endures for at least 20 days. In: Society for Neuroscience. San Diego, CA.
- Chang EF, Merzenich MM (2003) Environmental noise retards auditory cortical development. *Science* 300:498-502.
- Conner JM, Chiba AA, Tuszynski MH (2005) The basal forebrain cholinergic system is essential for cortical plasticity and functional recovery following brain injury. *Neuron* 46:173-179.
- Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. *Neuron* 44:23-30.
- Delacour J, Houcine O, Costa JC (1990) Evidence for a cholinergic mechanism of "learned" changes in the responses of barrel field neurons of the awake and undrugged rat. *Neuroscience* 34:1-8.
- Diamond ME, Armstrong-James M, Ebner FF (1993) Experience-dependent plasticity in adult rat barrel cortex. *Proc Natl Acad Sci U S A* 90:2082-2086.
- Dimyan MA, Weinberger NM (1999) Basal forebrain stimulation induces discriminative receptive field plasticity in the auditory cortex. *Behav Neurosci* 113:691-702.

- Dinse HR, Ragert P, Pleger B, Schwenkreis P, Tegenthoff M (2003) Pharmacological modulation of perceptual learning and associated cortical reorganization. *Science* 301:91-94.
- Dubnau J, Chiang AS, Tully T (2003) Neural substrates of memory: from synapse to system. *J Neurobiol* 54:238-253.
- Ebbinghaus H (1885) *Über das Gedächtnis. Untersuchungen zur Experimentellen Psychologie.* Leipzig: Duckner & Humbolt.
- Elbert T, Pantev C, Wienbruch C, Rockstroh B, Taub E (1995) Increased cortical representation of the fingers of the left hand in string players. *Science* 270:305-307.
- Engineer ND, Percaccio CR, Pandya PK, Moucha R, Rathbun DL, Kilgard MP (2004) Environmental enrichment improves response strength, threshold, selectivity, and latency of auditory cortex neurons. *J Neurophysiol* 92:73-82.
- Feeney DM, Gonzalez A, Law WA (1982) Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury. *Science* 217:855-857.
- Fraser C, Power M, Hamdy S, Rothwell J, Hobday D, Hollander I, Tyrell P, Hobson A, Williams S, Thompson D (2002) Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. *Neuron* 34:831-840.
- Genoux D, Haditsch U, Knobloch M, Michalon A, Storm D, Mansuy IM (2002) Protein phosphatase 1 is a molecular constraint on learning and memory. *Nature* 418:970-975.
- Glazewski S, McKenna M, Jacquin M, Fox K (1998) Experience-dependent depression of vibrissae responses in adolescent rat barrel cortex. *Eur J Neurosci* 10:2107-2116.

- Greuel JM, Luhmann HJ, Singer W (1988) Pharmacological induction of use-dependent receptive field modifications in the visual cortex. *Science* 242:74-77.
- Hasselmo ME (1995) Neuromodulation and cortical function: modeling the physiological basis of behavior. *Behav Brain Res* 67:1-27.
- Hummel FC, Cohen LG (2005) Drivers of brain plasticity. *Curr Opin Neurol* 18:667-674.
- Jenkins WM, Merzenich MM (1987) Reorganization of neocortical representations after brain injury: a neurophysiological model of the bases of recovery from stroke. *Prog Brain Res* 71:249-266.
- Jenkins WM, Merzenich MM, Ochs MT, Allard T, Guic-Robles E (1990) Functional reorganization of primary somatosensory cortex in adult owl monkeys after behaviorally controlled tactile stimulation. *J Neurophysiol* 63:82-104.
- Johansson BB (2004) Brain plasticity in health and disease. *Keio J Med* 53:231-246.
- Kapadia MK, Ito M, Gilbert CD, Westheimer G (1995) Improvement in visual sensitivity by changes in local context: parallel studies in human observers and in V1 of alert monkeys. *Neuron* 15:843-856.
- Kessler J, Thiel A, Karbe H, Heiss WD (2000) Piracetam improves activated blood flow and facilitates rehabilitation of poststroke aphasic patients. *Stroke* 31:2112-2116.
- Kilgard MP, Merzenich MM (1998) Cortical map reorganization enabled by nucleus basalis activity. *Science* 279:1714-1718.
- Kilgard MP, Pandya PK, Engineer ND, Moucha R (2002) Cortical network reorganization guided by sensory input features. *Biol Cybern* 87:333-343.

- Kilgard MP, Pandya PK, Vazquez J, Gehi A, Schreiner CE, Merzenich MM (2001) Sensory input directs spatial and temporal plasticity in primary auditory cortex. *J Neurophysiol* 86:326-338.
- Kleim JA, Hogg TM, VandenBerg PM, Cooper NR, Bruneau R, Remple M (2004) Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *J Neurosci* 24:628-633.
- Ma X, Suga N (2003) Augmentation of plasticity of the central auditory system by the basal forebrain and/or somatosensory cortex. *J Neurophysiol* 89:90-103.
- Mauelshagen J, Sherff CM, Carew TJ (1998) Differential induction of long-term synaptic facilitation by spaced and massed applications of serotonin at sensory neuron synapses of *Aplysia californica*. *Learn Mem* 5:246-256.
- McKenna TM, Ashe JH, Weinberger NM (1989) Cholinergic modulation of frequency receptive fields in auditory cortex: I. Frequency-specific effects of muscarinic agonists. *Synapse* 4:30-43.
- Nudo RJ (1997) Remodeling of cortical motor representations after stroke: implications for recovery from brain damage. *Mol Psychiatry* 2:188-191.
- Nudo RJ, Wise BM, SiFuentes F, Milliken GW (1996) Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science* 272:1791-1794.
- Ohl FW, Scheich H (1996) Differential frequency conditioning enhances spectral contrast sensitivity of units in auditory cortex (field A1) of the alert Mongolian gerbil. *Eur J Neurosci* 8:1001-1017.

- Ohshima M, Hata Y, Ichisaka S, Wakita M, Fukuda M, Kameyama K, Tsumoto T (2002)
Chronic electrical stimulation of afferents from one eye changes ocular dominance of
visual cortical neurons in kittens. *J Neurophysiol* 88:2147-2151.
- Orsetti M, Casamenti F, Pepeu G (1996) Enhanced acetylcholine release in the hippocampus
and cortex during acquisition of an operant behavior. *Brain Res* 724:89-96.
- Pandya PK, Engineer N, Moucha R, Rathbun DL, Vazquez J, Kilgard MP (2003)
Background Stimuli Control Cortical Map Expansion in Primary Auditory Cortex. In:
International Conference on Auditory Cortex. Magdeburg, Germany.
- Pandya PK, Moucha R, Engineer ND, Rathbun DL, Vazquez J, Kilgard MP (2005)
Asynchronous inputs alter excitability, spike timing, and topography in primary
auditory cortex. *Hear Res* 203:10-20.
- Phillips JP, Devier DJ, Feeney DM (2003) Rehabilitation pharmacology: bridging laboratory
work to clinical application. *J Head Trauma Rehabil* 18:342-356.
- Pleger B, Foerster AF, Ragert P, Dinse HR, Schwenkreis P, Malin JP, Nicolas V, Tegenthoff
M (2003) Functional imaging of perceptual learning in human primary and secondary
somatosensory cortex. *Neuron* 40:643-653.
- Puurunen K, Jolkkonen J, Sirvio J, Haapalinna A, Sivenius J (2001) Selegiline combined
with enriched-environment housing attenuates spatial learning deficits following focal
cerebral ischemia in rats. *Exp Neurol* 167:348-355.
- Recanzone GH, Wurtz RH (2000) Effects of attention on MT and MST neuronal activity
during pursuit initiation. *J Neurophysiol* 83:777-790.

- Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci* 13:87-103.
- Recanzone GH, Jenkins WM, Hradek GT, Merzenich MM (1992a) Progressive improvement in discriminative abilities in adult owl monkeys performing a tactile frequency discrimination task. *J Neurophysiol* 67:1015-1030.
- Recanzone GH, Merzenich MM, Jenkins WM, Grajski KA, Dinse HR (1992b) Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. *J Neurophysiol* 67:1031-1056.
- Richardson RT, DeLong MR (1990) Context-dependent responses of primate nucleus basalis neurons in a go/no-go task. *J Neurosci* 10:2528-2540.
- Richardson RT, DeLong MR (1991) Electrophysiological studies of the functions of the nucleus basalis in primates. *Adv Exp Med Biol* 295:233-252.
- Scharf MT, Woo NH, Lattal KM, Young JZ, Nguyen PV, Abel T (2002) Protein synthesis is required for the enhancement of long-term potentiation and long-term memory by spaced training. *J Neurophysiol* 87:2770-2777.
- Schoups A, Vogels R, Qian N, Orban G (2001) Practising orientation identification improves orientation coding in V1 neurons. *Nature* 412:549-553.
- Seitz AR, Watanabe T (2003) Psychophysics: Is subliminal learning really passive? *Nature* 422:36.
- Singer W (1986) The brain as a self-organizing system. *Eur Arch Psychiatry Neurol Sci* 236:4-9.

- Sohlberg MM, McLaughlin KA, Pavese A, Heidrich A, Posner MI (2000) Evaluation of attention process training and brain injury education in persons with acquired brain injury. *J Clin Exp Neuropsychol* 22:656-676.
- Steinmetz PN, Roy A, Fitzgerald PJ, Hsiao SS, Johnson KO, Niebur E (2000) Attention modulates synchronized neuronal firing in primate somatosensory cortex. *Nature* 404:187-190.
- Sterr A, Muller MM, Elbert T, Rockstroh B, Pantev C, Taub E (1998) Changed perceptions in Braille readers. *Nature* 391:134-135.
- Stryker M, Strickland S (1984) Physiological segregation of ocular dominance columns depends on the pattern of afferent electrical activity. *Invest Ophthalmol Vis Sci Suppl* 25:278.
- Sur M, Garraghty PE, Roe AW (1988) Experimentally induced visual projections into auditory thalamus and cortex. *Science* 242:1437-1441.
- Taub E, Uswatte G (2003) Constraint-induced movement therapy: bridging from the primate laboratory to the stroke rehabilitation laboratory. *J Rehabil Med*:34-40.
- Treue S, Maunsell JH (1999) Effects of attention on the processing of motion in macaque middle temporal and medial superior temporal visual cortical areas. *J Neurosci* 19:7591-7602.
- Tsodyks M (2002) Spike-timing-dependent synaptic plasticity - the long road towards understanding neuronal mechanisms of learning and memory. *Trends Neurosci* 25:599-600.

- Walker-Batson D, Smith P, Curtis S, Unwin DH (2004) Neuromodulation paired with learning dependent practice to enhance post stroke recovery? *Restor Neurol Neurosci* 22:387-392.
- Wallace H, Fox K (1999) The effect of vibrissa deprivation pattern on the form of plasticity induced in rat barrel cortex. *Somatosens Mot Res* 16:122-138.
- Wang X, Merzenich MM, Sameshima K, Jenkins WM (1995) Remodelling of hand representation in adult cortex determined by timing of tactile stimulation. *Nature* 378:71-75.
- Watanabe T, Nanez JE, Sasaki Y (2001) Perceptual learning without perception. *Nature* 413:844-848.
- Weinberger NM (2003) The nucleus basalis and memory codes: auditory cortical plasticity and the induction of specific, associative behavioral memory. *Neurobiol Learn Mem* 80:268-284.
- Xerri C, Merzenich MM, Peterson BE, Jenkins W (1998) Plasticity of primary somatosensory cortex paralleling sensorimotor skill recovery from stroke in adult monkeys. *J Neurophysiol* 79:2119-2148.
- Zhang Y, Hamilton SE, Nathanson NM, Yan J (2005) Decreased Input-Specific Plasticity of the Auditory Cortex in Mice Lacking M1 Muscarinic Acetylcholine Receptors. *Cereb Cortex*.
- Zhou Q, Tao HW, Poo MM (2003) Reversal and stabilization of synaptic modifications in a developing visual system. *Science* 300:1953-1957.

CHAPTER TWO

GENERAL SCOPE OF THESIS AND SIGNIFICANCE

The comprehensive aim of this work was to determine how short-term and long term experience with FM stimuli alters cortical responses to this important stimulus class. In summary these experiments were designed to characterize plasticity resulting from differential activation patterns (focal versus distributed), background conditions (silence versus presence of other sounds) and duration of experience (short-term versus long term). Parametric manipulation of sensory experience allows for exploration of plasticity rules that transform experience into specific neurophysiological changes such as: receptive fields narrowing or broadening, map reorganization or changes in temporal properties of sensory cortical neurons. These findings add to a growing body of evidence that input statistics play an important role in guiding representational plasticity that potentially contributes to perceptual learning.

Effective rehabilitation following peripheral or central nervous system damage appears to rely on plasticity mechanisms that are likely guided by sensory experience. A more complete understanding of the influence specific forms of experience have on the cerebral cortex will be useful in designing more effective strategies for improving functional recovery (Grimby et al., 2003). Our preliminary results indicate the sensory context within which rehabilitation is conducted may be as important as the tasks themselves in stimulating plasticity.

In addition a better understanding of the processing and representation of FM's has important implications for human and animal communication. Improved FM discrimination could potentially help rehabilitate specific language based learning impairments that are associated with deficient auditory processing of time varying sounds (Tallal et al., 1998).

Specific aim 1: Coding of frequency modulated sounds by auditory neurons in thalamus, A1 and PAF, Chapter 3

The aim of these experiments was to characterize auditory neuronal representation of one octave wide FM sweeps, and establish a baseline for plasticity experiments.

In the auditory system activation patterns are always intricate due to complex nature of sound and the simultaneous occurrence of all sounds. One essential step in understanding auditory sensory function is to probe with stimuli that are naturally present in the acoustic environment yet are still parametrically quantifiable. FM sweeps are complex time-varying sounds that are present in most species vocalizations, and have been effectively used to probe spectro-temporal integration of auditory neurons. High density microelectrode mapping techniques will be used to record responses of auditory neurons to this important class of sounds along three relay centers of the auditory pathway: thalamus, primary auditory cortex (A1) and posterior auditory field (PAF).

Specific aim 2: Cortical plasticity after long-term experience with frequency modulated sounds, Chapter 4

The aim of these experiments was to characterize changes in neuronal representation of one octave wide FM sweeps in primary auditory cortex as a consequence of repeated long-term experience with these sounds via NB stimulation associated with sound presentation. Previous studies have shown that pairing NB stimulation with sound presentation several hundred times a day for twenty days leads to cortical reorganization that is specific to the acoustic features of the paired sound (Kilgard et al., 2001; Kilgard and Merzenich, 2002). Using simple stimuli such as pure tones and tone trains they have shown that cortical plasticity is determined by activation pattern: restricted versus distributed over entire receptor surface, modulated versus unmodulated inputs and the presence of background sounds. To incorporate all these parameters we will characterize neuronal changes due to experience with FM sounds in three conditions. In experiment 1, a downward FM sweep covering a frequency range of one octave will be repeatedly paired with NB stimulation. In experiment 2, five different FM sweeps activating different regions of A1 will be paired with NB stimulation. Experiment 3 is identical to experiment 2 except that unpaired FM's of contrasting rates (faster or slower), or direction (upward sweeping) will be interleaved with the five FM sweeps paired with NB stimulation.

Specific aim 3: Cortical plasticity after short-term experience with sounds changing in frequency, Chapter 5

The aim of these experiments was to test whether changes in A1 properties can arise from short-term exposure to FM sounds using a paradigm based on a spike timing dependent plasticity model.

Spike timing-dependent modification of synaptic efficacy is a possible mechanism for experience-dependent plasticity of neuronal response properties. Modifying strength of specific synapses as predicted by the rules of spike timing-dependent plasticity (STDP) could cause the receptive field (RF) of a cell to shift toward strengthened inputs and away from weakened inputs. A daring study in cat visual cortex (Yao and Dan, 2001) provided evidence that shifts in direction tuning of V1 neurons were determined by the order and timing of pairs of visual stimuli. Their study opened new venues for investigation by showing that rules which operate at the synapse level could be reflected in the plasticity dynamics of networks of neurons *in vivo*.

In primary auditory cortex timing of auditory stimuli can directly affect timing of neuronal spiking and thus may play a role in activity dependent plasticity. Timing and order of neuronal activation can be manipulated using auditory stimuli such as tones and FM sweeps with different SOA's and sweep rates respectively. The RF would be predicted to shift closer to or further from the region stimulated during the induction protocol, depending on the timing and order of the consecutive inputs.

Table 2.1. Table of experiments

Experiment	Rats	Recordings
Aim 1 Coding		
FM coding	5	249
Aim 2 Long term plasticity		
1FM + NB	3	136
5FM + NB in silence	3	128
5FM + NB with background FM's	3	134
Naive	7	259
Aim3 Short term plasticity		
awake	9	95
anesthetized	2	20
Total	32	1021

REFERENCES

- Grimby G, Eriksson P, Nilsson M, Sjolund B (2003) [Neurobiology provides a scientific foundation for rehabilitation. Report from an international symposium].
Lakartidningen 100:2052-2055.
- Kilgard MP, Merzenich MM (2002) Order-sensitive plasticity in adult primary auditory cortex. *Proc Natl Acad Sci U S A* 99:3205-3209.
- Kilgard MP, Pandya PK, Vazquez J, Gehi A, Schreiner CE, Merzenich MM (2001) Sensory input directs spatial and temporal plasticity in primary auditory cortex. *J Neurophysiol* 86:326-338.
- Tallal P, Merzenich MM, Miller S, Jenkins W (1998) Language learning impairments: integrating basic science, technology, and remediation. *Exp Brain Res* 123:210-219.
- Yao H, Dan Y (2001) Stimulus timing-dependent plasticity in cortical processing of orientation. *Neuron* 32(2):315-323

CHAPTER THREE

RESPONSE CHARACTERISTICS TO FREQUENCY MODULATED SWEEPS IN
THALAMUS, PRIMARY AUDITORY CORTEX AND POSTERIOR AUDITORY FIELD
OF THE RAT

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ABSTRACT

Processing of short frequency modulated (FM) sweeps has not been extensively studied in the central auditory system. Here we report the neural representations of one-octave FM sounds along three centers of the auditory pathway: thalamus, primary auditory cortex (A1) and posterior auditory field (PAF). Dense microelectrode mapping techniques were used to collect multiunit responses from 249 sites in five barbiturate anesthetized rats. Nearly all sites had a strong onset response to these sounds and some had a 2nd excitatory peak (mostly in PAF) or oscillatory patterns (mostly in thalamus). Response strength in thalamus was more than two fold greater than the two cortical fields. Response latency increased along the ascending pathway, consistent with serial processing of auditory inputs. Responses to one-octave FM's were influenced by RF differences. PAF sites had wider bandwidths, while A1 and thalamus sites typically had asymmetric tuning skewed towards low frequencies. Direction selectivity systematically decreased with each station above thalamus. Onset responses were strongest for fast rates when FM sweeps moved toward the center of the receptive field, and non-rate selective for FM's that started near the center. Additional late action potentials were evoked in A1 in response to long duration (slow) FM's, but not in thalamus or PAF. These results indicate that receptive field properties strongly influence the central representation of short FM sounds.

INTRODUCTION

Frequency modulated (FM) sweeps are prevalent components of communication sounds in many vocalizations including human speech, suggesting that time-variant signals are effective carriers of information. The direction of FM sweeps in speech provides information about phonemic identity (Fitch et al., 1997; Rauschecker, 1999). Deficient FM processing accounts for impairments in speech discrimination described in the elderly (Wingfield et al., 1985), in language learning impaired children (Tallal et al., 1996) and in children with specific neurological disorders (Stefanatos, 1993).

Electrophysiological studies have shown that auditory neurons have preferences for specific parameters of these sounds. Tuning for FM modulation rate has been documented in the auditory nerve (Sinex and Geisler, 1981), cochlear nucleus (Moller, 1972, 1974; Britt and Starr, 1976), inferior colliculus (Clopton and Winfield, 1974; Rees and Moller, 1983; Felsheim and Ostwald, 1996), thalamus (Lui and Mendelson, 2003) and cortex (Gaese and Ostwald, 1995; Ricketts et al., 1998). Dynamic stimuli such as FM are more effective than tones in activating auditory cortical neurons (Whitfield and Evans, 1965) however cortical neurons process time-varying signals with less temporal fidelity (i.e. phase locking) compared to the auditory periphery. Yet at the perceptual level the integrity of auditory cortex is essential for discriminating direction of FM sounds, while frequency discrimination is spared by cortical lesions (Kelly and Whitfield, 1971; Wetzel et al., 1998; Ohl et al., 1999). Collectively these studies have shown, as might be expected from the prevalence of FM sweeps in behaviorally important sounds, that auditory neurons selective for FM rate and direction are found at all levels.

FM processing varies with field (PAF neurons prefer slower FM rates), species (cat neurons prefer downward sweeps while rat and ferret prefer upward), and type of FM (logarithmic versus linear) (Nelken, 2002). Despite the large number of studies on FM coding, little is known about responses to FM sounds that span narrow frequency ranges. Most studies of FM processing use broad FM sounds spanning multiple octaves that start and end outside of the neurons receptive field. As a result these sounds generally do not elicit an onset response and create activation patterns that are not evoked by FM transitions in natural sounds. The interaction between sound onset and FM may substantially alter neural selectivity for FM direction and rate.

FM components of animal and human vocalizations typically span less than one octave and begin soon after sound onset (Deglutte, 1997). For example, the onset and direction of the FM transition in the second formant distinguishes the English phonemes /ba/, /da/, and /ga/. The aim of our study was to document neuronal responses to one octave short FM sweeps and relate them to receptive field properties. These sounds are more similar to those present in natural environments than longer FM's yet remain parametrically quantifiable, and may provide an important next step in understanding auditory representation of natural sounds. Even though they span a narrow frequency range one octave sweeps can be easily learned in behavioral studies and discriminated according to their direction of modulation across various modulation rates and durations (Wetzel et al, 1998). The neural representation of such sounds isn't known, but could provide insights into how such categorical learning is achieved.

Recording and lesion studies have shown that the coding of stimulus features is often distributed across multiple locations in the sensory processing hierarchy. Despite this evidence relatively few studies have compared responses in multiple areas. FM's were studied in AAF and PAF of cat (Tian and Rauschecker, 1994, 1998) and gerbil (Schulze et al., 1997), AAF of ferret (Kowalski et al., 1995), lateral auditory belt of the rhesus monkey (Tian and Rauschecker, 2004) and MGN of rat (Lui and Mendelson, 2003). However, interpretation is complicated by differences in stimulus generation and species. Here we compare responses of neurons in the auditory thalamus primary and posterior fields of the rat.

METHODS

Electrophysiological recordings

Dense microelectrode mapping techniques were used to collect data from 249 microelectrode penetrations into the right auditory cortex of five adult female Sprague-Dawley rats (250-325g). Methods were similar to those described in previous publications from this lab (Kilgard et al., 2001; Engineer et al., 2004; Moucha et al., 2005). All protocols and recording procedures conformed to the Ethical Treatment of Animals (NIH) and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Dallas. Animals were anesthetized with sodium pentobarbital (50mg/kg). Supplemental pentobarbital (8mg/ml) was periodically administered intraperitoneally to maintain a state of areflexia during surgical procedures and recording sessions. The trachea was cannulated and humidified air was provided to ensure adequate ventilation and to minimize breathing related

noises. The skull was fixed in a palato-orbital restraint. The cisternae magna was drained of CSF to minimize cerebral edema. The temporalis muscle was reflected and the dura over the right auditory cortex was exposed through a craniotomy of approximately 6mm by 4mm. The dura was resected and the cortex was maintained under a layer of viscous silicone oil to prevent desiccation. A digitized image of the cortical surface was taken to aid in electrode placement and topographic reconstruction (Fig 1a). Electrocardiography and pulse oximetry were used to monitor circulatory function and to control the depth of anesthesia. Body temperature was monitored with a rectal probe and maintained at 37° with a heating pad (FHC). Proper hydration was maintained with lactated Ringers solution provided periodically during the course of the acute experiment.

Action potentials were recorded simultaneously from a pair of Parylene-coated tungsten microelectrodes (FHC, 2M Ω at 1 kHz) lowered orthogonally into the cortex using a micromanipulator. Cortical recordings were made at a depth of approximately 550 to 650 μ m, corresponding to layers IV/V in both A1 and PAF. In thalamus, recordings started at ~4000 μ m below the cortical surface and continued in increments of 100 μ m until non-responsive sites were encountered (Fig 1b). No histological verification was conducted for thalamic sites; however, most of the sites exhibited short latency responses and frequency tuning. It is possible that a portion of our sample includes non-lemniscal areas of the auditory thalamus.

Tucker-Davis Technologies neurophysiology hardware and software (Brainware) were used for signal filtering (0.3 to 8 kHz), amplification (10,000X), and data acquisition.

Recording thresholds were set to maximize the signal- to-noise ratio. Action potential waveforms crossing the threshold were recorded for more detailed quantitative analysis. As in our earlier experiments, potentials above approximately 0.18mV were considered action potentials. Neural responsiveness was quantified using multi-unit data. Penetration sites were chosen to avoid damaging blood vessels while generating a detailed and evenly spaced cortical map. For thalamic sites, up to 10 recordings could be obtained in one recording track, corresponding to 1mm depth.

Acoustic Stimulation and Recording

During acute mapping, recordings were made in a shielded, doubled-walled sound chamber (Acoustic Systems) and sounds were presented via free-field using a calibrated speaker. The speaker was positioned 10 cm from the ear at 0° elevation and 90° azimuth for all acute experiments. Tucker-Davis Technologies hardware and software (SigGen) were used for stimulus generation.

Auditory frequency response tuning curves (spectral receptive fields) were determined by presenting 81 frequencies from 1 to 32 kHz at each recording site for cortical locations. For thalamic sites, responses to 41 frequencies from 1 to 32 kHz were recorded. Each frequency was presented at 16 intensities ranging between 0 and 75 dB SPL (1296 or 656 total stimuli). Tuning curve tones were randomly interleaved and separated by at least 475 msec between presentations to minimize adaptation effects. All tones had 5 msec rise-fall times (ramps) and were 25 msec in total duration.

Frequency modulated sweeps. Responses to FM sweeps of different direction, rates, and starting frequency were also recorded. Every FM sweep used in this study spanned a

single octave. Three different sweep rates were presented: 40, 160, and 640 ms duration (corresponding to 25, 6.25, and 1.56 oct/s). FM sweeps always started at 1, 2, 4, 8, 16, or 32 kHz. At each site sweeps were presented that cover one octave above and one octave below the best frequency. Because the frequency range of each FM sweep is kept constant (1 octave), the speed (rate of change in frequency) covaries with duration such that: fast FM's are also short duration (40ms) and slow FM's are long duration (640ms). At fast rates it takes less time to sweep through one octave than at slower rates.

The onset and offset of all sounds was linearly ramped over 3 ms. Responses to a total of 12 randomly interleaved FM stimuli (3 rates x 2 octaves x 2 directions) were recorded at each site (Fig 3). Each sweep was repeated twenty times at 800 ms intervals to minimize adaptation.

Data analysis

Tuning curve analysis MATLAB (Mathworks) was used for all analysis. To prevent the possibility of experimenter bias, an experienced blind observer determined tuning curve parameters. The parameters were defined using custom software that displayed raw spike data but not the penetration location or the identity of the animal. For each site, the characteristic frequency, threshold, bandwidth (10, 20, 30 & 40 dB above threshold), and latency data were recorded. The characteristic frequency was defined as the frequency where a response is obtained to the lowest stimulus intensity (i.e. threshold). Bandwidth was defined as the frequency range (in octaves) that activated the neurons. Asymmetry of RF was calculated using an index as in Godey et al, 2005:

$$ASI = \log_2(F_{hi}/CF) - \log_2(CF/F_{lo}) / \log_2(F_{hi}/CF) + \log_2(CF/F_{lo})$$

F_{hi} and F_{lo} are the high and low frequency borders of the excitatory RF at 20 dB above threshold. When the asymmetry index (ASI) is zero the RF is symmetric relative to the CF. Negative or positive values indicate a skewing towards low or high frequencies respectively. Neural onset latency was defined as the time from stimulus onset where the neural response was above background activity (determined from the 16 intensities at the best frequency and its two neighboring frequencies (best frequency \pm 1/16th of an octave). Peak latency was the time needed to reach peak response in the PSTH. The end-of-peak latency or the termination of response was the time after stimulus onset at which activity returned to spontaneous levels.

FM direction selectivity The response to each FM was quantified as the number of action potentials from onset to 60 ms after the end of the sound (average of 20 repetitions). Spikes within a time interval that matches the duration of each FM and 60ms after were analyzed. This corresponds to a time window of 100ms for the fast, 220ms for the medium, and 700ms for the slow FM respectively. Only sweeps that evoked at least 1 spike in either direction were compared

The responses of A1 neurons to pairs of sweeps that begin at a frequency within the excitatory receptive field and sweep one octave up or down (i.e. 4-8 kHz and 4-2 kHz) are typically indistinguishable because the response is largely determined by the onset frequency which is identical for the two sweeps. Thus we compared FM sounds that sweep in opposite direction but cover the same frequency range (octave) such as 4-2 and 2-4 kHz, and 8-4 and 4-8 kHz for units where 4 kHz was closest to CF.

A standard metric known as the direction selectivity index (DS) was used to quantify the difference between the two directions of these sounds. The DS of individual recording was computed at every site using the following formula:

$$DS = (R_{\text{forward}} - R_{\text{reverse}}) / (R_{\text{forward}} + R_{\text{reverse}})$$

R is the response (in number of spikes averaged from 20 repetitions) elicited by the forward or reverse sweep. A value of 0 indicates no preference either for forward or reverse while a value of 1 or -1 indicates a perfect selectivity for the forward or reverse sounds respectively. The presence of statistically significant direction selectivity was also evaluated at each site using a t-test to compare the response to upward and downward FM's.

Operationally defining Thalamus, A1, and PAF.

A1 and PAF were functionally defined on the basis of latency, receptive field size, and tonotopy (Kilgard and Merzenich, 1999), and Pandya et al, submitted). A1 was defined on the basis of its short latency (8-20 ms) responses and continuous tonotopy. Boundaries will be determined using non-responsive and non-A1 sites. For thalamic sites, we included all sites within a penetration or recording track in which an objectively defined CF could be obtained. If a CF was not obtained, it was excluded from all analyses. We could not identify subregions of the thalamus since we did not do lesions or reconstructed electrode tracks histologically. We expect that the majority of recordings were from the ventral division because we densely sampled the thalamus and ventral is the biggest. Consistent with this expectation most tracks showed tonotopy as documented in earlier studies characterizing the lemniscal thalamus.

Statistical analysis was done using MATLAB. Statistical significance between response measures was evaluated using a t-test. Error bars on all figures reflect standard error of the mean.

RESULTS

Responses to tones

In thalamus, we recorded tuning curves from 104 sites with best frequencies ranging from 1.41 to 32 kHz. These cells typically had short-latency responses and monotonic v-shaped tuning curves. As recording depth along the thalamic tracts increased CF's also significantly increased with depth, illustrating tonotopic organization ($p < 0.0001$, $r = 0.4321$). (Fig2b). The average bandwidths for the thalamic population were 1.0 ± 0.01 and 2.4 ± 0.01 (mean \pm SEM) octaves at 10 and 40 dB above threshold respectively. The average first-spike and peak latencies for the thalamus responses were 10.8 ± 0.4 and 24.4 ± 1.2 msec respectively. The strength of the evoked response was on average 2.7 ± 0.12 spikes from each thalamic site.

We recorded from 72 A1 sites with best frequencies ranging from 1.03 kHz to 32.5 kHz. These sites also typically had short-latency phasic responses but did not respond as strongly as thalamic sites to tones. A1 sites showed significant tonotopic organization with CF's systematically decreasing along the antero-posterior axis ($p < 0.0001$, $r = 0.83$). (Fig2c). The average bandwidths for this population at 10 and 40 dB above threshold were 1.1 ± 0.01 and 1.8 ± 0.01 . The first spike latency for A1 sites was longer than thalamic sites with a

mean of 15.9 ± 1.7 msec while the average peak latency was 20.5 ± 2.2 msec. Response strengths was on average 1.7 ± 0.1 spikes per tone at each A1 penetration.

PAF recordings were derived from 73 recording sites with best frequencies ranging from 1.2 to 30 kHz. No significant tonotopy could be seen in these recordings ($p > 0.1$, $r = 0.18$).

(Fig2c). The average bandwidths of these sites were 1.9 ± 0.1 and 3.8 ± 0.1 . Sites in PAF had substantially longer first-spike and peak latencies than both A1 and thalamic sites. The average first spike latency and peak latency were 31.5 ± 1.8 and 49.9 ± 3.9 msec respectively. The average response strength to tones was 1.8 ± 0.1 spikes per tone.

In summary, the frequency bandwidth measures at both 10 and 40 dB above threshold indicated that the most broadly tuned neurons were consistently found in PAF while the most narrowly tuned neurons were found in A1. Neurons in the auditory thalamus had broader tuning than A1, but narrower tuning than PAF. The response strength to tones in PAF was similar to the average strength in A1, but was weaker than the average strength in the thalamus. In addition, latency comparisons revealed that auditory thalamus had the shortest onset latencies and PAF neurons had the longest latencies of the three areas measured. This data is summarized in Table 1.

Responses to FM sweeps

At each site we quantified responses to one octave FM sweeps that moved toward or away from the center of the receptive field. Each FM traversed either the low or high side of the tuning curve. We will refer to the FM sweeps that cover the low frequency range as Lup and Ldown, and to the FM's covering the higher octave as Hup and Hdown (Fig 1). To evaluate rate selectivity each sweep was presented with 40, 160, or 640 ms durations. As a

result, a total of 12 sweeps (2 directions x 3 rates x 2 octave ranges) were presented. Since PAF neurons have wide frequency bandwidths, the FM sweeps generally began and ended within the excitatory receptive field regardless of which octave was covered. For A1 and thalamus, some FM's began outside of the excitatory receptive field. Receptive fields in A1 and thalamus tend to be skewed towards frequencies lower than CF, such that the higher FM sweeps were more likely to cross the edge of the tuning curve than were the lower FM sweeps (see table 1 for BW and RF asymmetry quantifications). Thus each FM evoked different responses depending not only on direction and/or rate but also on which area of the RF they traverse.

Response Strength

Responses to FM sweeps had similar characteristics as responses to tones in the three fields. As for tones, latency in response to FM's was shortest in thalamus, and longest in PAF. Thalamus also had the strongest instantaneous firing compared to the cortical fields (Fig 3a,b). The most common response profile for all three fields was a burst of action potentials soon after sound onset after which activity returned to baseline regardless of FM duration (46% Thal, 70% A1 and 67% of PAF sites). Many sites responded with a second peak of firing more than 100ms after the onset response: 26% Thal, 16% A1, and 33% PAF sites. Some sites had an oscillatory pattern in their response, either to the longer duration FM's in 17% Thal and 14% A1 sites, or to all FM's - only in 10% of thalamic neurons. Such oscillations have been previously described in thalamus under pentobarbital anesthesia (Cotillon-Williams and Edeline, 2003).

Even though thalamus had more synchronous discharges compared to the cortical fields (Fig 3b) the difference in average number of driven spikes over 100 ms was small because A1 and PAF respond over a longer interval (Fig 3a,c). Response strength varied with FM start frequency. In thalamus response strength was significantly greater for sweeps with start frequencies close to CF (Ldown and Hup) as compared to FM sweeps spanning the same frequency range with start frequencies an octave above or below CF. Neurons in A1 responded equally to sweeps that traversed the low frequency side of their receptive fields, Lup (mean 1.64 ± 0.14 spikes) and Ldown (mean 1.72 ± 0.15 spikes). Sweeps that traversed the high side of the RF and start frequency close to CF evoked stronger responses (mean 1.7 ± 0.16 spikes) than their opposite sweeping FM pair with start frequency near the border of the RF (mean 1.26 ± 0.14 spikes). In PAF response strength was not significantly different for any of the two pairs of FM sounds. These results suggest that direction selectivity for one octave FM's is dependent on the frequency range and recording site within the auditory system.

FM direction selectivity

FM responses varied depending on the direction of the sweeps relative to the center of the RF. Direction selectivity was quantified using the standard DS index (see methods) and found to be highest in thalamus: 42% of sites had a significant preference for downward FM's covering the lower octave (DS = -0.31 ± 0.06) and 47% for upward FM's covering the higher octave (DS = $+0.39 \pm 0.06$) at the medium rate. As discussed above the preference was predominantly for the direction of the FM with start frequency closest to the CF (Fig 4a). In A1 34% of neurons had a significant preference for upward FM's covering the higher

octave ($DS = + 0.20 \pm 0.09$) at the medium rate, but no direction preference for FM sweeps spanning the lower octave (Lup vs Ldown). PAF exhibited no direction preference for either octave. Fig 4b shows mean DS values of sites with a statistically significant direction preference. This selection increased the average DS, as expected, but did not alter the conclusion that auditory thalamus is more direction selective than auditory cortex.

Population PSTH's derived from FM responses indicate that the number of evoked action potentials may miss some aspects of FM coding (Fig5). In addition to greater response strength, preferred sweeps also had better response timing quantified as the time to reach the peak response or peak latency. In thalamus, on the lower frequency side of the RF, responses to the downward FM had shorter peak latency (mean 23.73 ms) compared to the upward sweeping FM's (mean 26.55 ms), Fig 5a. Downward FM's covering this frequency range (lower octave relative to CF) start at a frequency close to the CF sweeping outward while their upward pairs start near the low frequency border of the RF sweeping towards CF. On the high frequency side of the RF responses to upward FM's had shorter peak latencies, mean 15.20 ms compared to mean 17.59 ms for downward FM's. In this condition upward FM's had start frequencies in the vicinity of CF and elicited stronger responses than the downward pairs with start frequency near the high frequency edge of the RF (Fig 5d).

In A1 responses were asymmetric. For sweeps traversing the low frequency RF side responses differed only in latency while on the high frequency side the difference was also in response strength (Fig 5b,e). Responses to sounds starting near the center of the RF (Ldown) had shorter peak latency mean 28.19 ms compared to responses to sounds starting near the low frequency border of the RF (Lup) mean 32.93 ms, while response strength was similar

for these sounds (Fig 4c). In contrast responses to sounds starting near the center of the RF (Hup) had increased response strength and shorter peak latency (mean 29.10 ms) compared to responses to sounds starting near the high frequency border of the RF (Hdown), mean 33.74 ms. Thus sounds sweeping towards CF from lower frequencies elicit a later excitatory response while sounds sweeping towards CF from higher frequencies also elicit a weaker response in A1 compared to sounds that start near CF. Judging from response strength alone it appeared that in A1 there is a preference for sounds that start near CF only if they traverse the high frequency RF side (Fig 4c). However this analysis suggest that A1 responds better to both FM sounds that start near CF (Ldown and Hup) but this preference is displayed differently on the low vs. the high side of the RF.

In PAF response strength was similar on both sides of the CF, however PAF sites had slower response latency (mean 35.68 ms) to sounds sweeping in from the high frequency side of the RF (Fig 5c,f) compared to mean 31.28 ms for FM's that start close to CF. On average responses to FM sounds with start frequencies near CF had shorter peak latencies even when response strength was not different. In all three fields these differences in responses were observed at all three FM rates.

FM rate selectivity

To assess rate selectivity we compared responses to fast and slow FM sweeps. Because the FM sweeps always covered one octave, rate and duration covary such that fast sweeps were also short duration (40 ms) while slow sweeps were long duration (640 ms). Differences in responses elicited by the onset of the sounds in the first 100 ms are most likely a result of selectivity for the rate of the sounds. However medium and slow FM sweeps are

still ongoing until 160 ms and 640 ms respectively thus differences in spike rate after the onset response are more likely due to duration of sounds. The response time course in each field is illustrated by the population PSTH's of responses to fast, medium and slow FM's, in Fig 6. We compared responses to fast versus slow FM sweeps over two time windows (onset 0-100ms and late 100-700ms) to differentiate between rate and duration selectivity. As with direction selectivity the preferred rate differed with the sweep frequency range.

In thalamus 26 % of neurons fired significantly more spikes (1.1 ± 0.2) to the fast rate compared to slow during the onset response (1-100 ms from sound onset), for FM's sweeping towards CF from the low frequency side (Lup) Fig 7a. Nine percent of neurons fired more spikes (on average 0.4 ± 0.1) to the fast rate for FM's sweeping towards the CF from the high frequency side (Hdown). There was no significant difference for FM's sweeping out of the RF (Ldown, Hup). Thus thalamus had a general preference for sounds sweeping faster towards the CF from both the low and high frequency side of its RF. During the late response (100-700ms) thalamus did not fire significantly more spikes for the longer duration sounds/slow FM's (Fig 7b).

In A1 8 % of neurons fired more spikes (0.3 ± 0.1) during the onset, to the fast FM rate sweeping towards CF from the low frequency side. And 10% of neurons fired more spikes (on average 0.6 ± 0.1) to the fast rate for FM's sweeping towards the CF from the high frequency side (Fig 7a). No significant difference in responses to different FM rates when sweeping outward. In addition, during the late response, A1 fired more spikes for the longer duration sound both for FM's sweeping into the RF (39% sites, 1.4 ± 0.4 spikes) and out of the RF (46% sites, 1.6 ± 0.6 spikes) on the low frequency side (Fig 7b).

In PAF 7% of neurons fired significantly more spikes (on average 0.4 ± 0.1) during the onset, to the fast rate for FM's sweeping towards the CF from higher frequencies (Hdown). During the late response (100-700 ms) PAF neurons did not fire significantly more spikes to the long duration FM's.

In summary thalamus, A1 and PAF preferred stimuli that sweep fast towards their RF. A1 had more sensitivity for sound duration. Between fields, neurons likely to exhibit rate preference were found in thalamus, A1 and PAF. Neurons more likely to exhibit duration preference over a long time window (600ms) were A1 neurons. Duration sensitivity as direction selectivity was also asymmetrical, being expressed only for stimuli traversing the low frequency side of the RF in either direction. Assuming that this reflects an asymmetry of RF such as a skewing towards low frequencies, it could be hypothesized that the duration sensitivity would reflect a preference for stimuli that spend more time within a neurons RF. Thus A1 preferred stimuli that sweep into the RF fast, and sweep out slow.

DISCUSSION

In the present study we have compared responses to tones and one octave wide FM sweeps in three areas of the auditory pathway: thalamus, A1, and PAF. Responses to tones in these three areas are consistent with earlier reports. Thalamus had stronger responses and shorter latencies compared to the cortical fields. PAF had the same response strength as A1 but broader frequency tuning and longer response latency (as in (Doron et al., 2002)). These differences in responses to tonal inputs are likely responsible for differences the response to FM sounds.

Responses to FM sounds have been characterized by past studies as a short bursts of spikes when the frequency trajectory of the sweep approaches the BF and reaches a particular value named the effective instantaneous frequency (F_i). However this is a typical characteristic of responses to broad sweeps that start and end well outside the frequency tuning range of the cells. In our study responses to FM sweeps that span only one octave look quite similar to responses to tonal stimuli. The differences in responses to each FM depend mostly on the onset frequency and sweep direction relative to the excitatory RF. Field differences can be generally explained by differences in RF shape. Thalamus responds stronger to FM's with start frequencies near their CF compared to A1 and PAF. In PAF the lack of response difference can be attributed to its wide excitatory bandwidths such that sounds sweeping through one octave start well inside the RF (Fig 1d). Thalamus and A1 have narrower bandwidths compared to PAF. The reason only thalamus shows a bias towards sounds that start inside their RF could be due to stronger inhibitory sidebands such that sounds with onsets near RF borders always elicit a weaker response. Asymmetries in A1 responses when sweeps traverse the low versus the high frequency side of the RF can be explained by the skewing of A1 excitatory response areas towards low frequencies (table 1). It is possible that such asymmetry results from upward spread of masking in the cochlea (Egan & Hake 1950).

Comparison to other studies

FM's have been studied in different species, under different anesthesia, methods or recording techniques (single units vs. multi-units, vs. pattern of activity), stimulation

paradigms (free field vs. earphones), and very importantly different FM sounds. All of which alone or in combination could account for differences in findings.

FM sounds can vary along multiple dimensions: 1. form of modulation (linear, exponential, sinusoidal), 2. modulation range (frequency band covered by sweep –ranging from narrow excursion 1-2 octaves wide, to broad frequency range up to 5 octaves wide), 3. modulation rate (rate of change of frequency), 4. direction of modulation (upward from low to high frequencies or downward) and 5. intensity. It is difficult to characterize responses along all these dimensions in a single study. Various experimenters have chosen various combinations of FM parameters to employ in their studies, from sinusoidal linear or logarithmic five octave wide sweeps, to transient FM of a continuous tone. A variety of adaptive, inhibitory or other mechanisms could be involved by each class of FM sounds.

As a result DS maps are different for linear vs. logarithmic FM's (Nelken and Versnel, 2000), and for continuous (Shamma et al., 1993) vs. separate sweeps (Heil et al., 1992; Mendelson et al., 1993). In ferret DS is either not present (Kowalski et al., 1995), or present for upward sweeps (Nelken and Versnel, 2000), depending on the paradigm.

A single study using narrow 2kHz FM sweeps reported that cat cortical neurons prefer FM's that cross the RF edge and sweep towards the BF (Phillips, 1985). At first glance this seems to be in discordance with our findings reported here. However what they and all of the above studies had in common was that all the FM sounds used were designed to exclude any masking caused by FM onsets either by using long FM's starting very far from the RF, or using sinusoidal modulation, or embedding a sweep into a continuous tone. We purposefully chose to use short excursion sweeps due to their resemblance to sweeps naturally

encountered, and also chose not to mask their onset because such masking could contribute to the coding mechanisms for natural sounds. Our findings reported here substantiate that responses to such sounds cannot be predicted from responses to long excursion or onset masked FM's. We also show that coding strategies are different along three different processing stations of the auditory system, thalamus, primary auditory cortex and posterior auditory field.

Technical considerations

Our study did not determine whether individual neurons were direction or rate selective to one octave FM sweeps because we recorded multi-unit responses. We can only report the "behavior" of clusters of neurons that contributed to our recordings at each penetration site. Multi fields and comparisons to earlier studies showed columnar organization within the auditory cortex, therefore it is possible that units recorded within the same cluster had similar response characteristics. Our recordings were performed under barbiturate anesthesia. Anesthesia could alter FM tuning because inhibition has been shown to be highly susceptible to anesthesia. However in a study using tone sequences, Brosch et al, 2005, found forward inhibition and forward facilitation in the auditory cortex of awake monkeys that were similar to those observed in the auditory cortex of anesthetized monkeys with the same sequences. (Brosch et al., J. Neurophysiol, 1999). This suggests that at least some anesthetics, ketamine as was used in the Brosch study, do not result in qualitatively different response properties to temporally modulated stimuli such as tone sequences. Gaese and Ostwald 2001, have shown that some neuronal response properties are influenced by barbiturate anesthesia but it is not known how and if this affects responses to stimuli such

FM sweeps since different response characteristics may be influenced differentially by anesthesia.

CONCLUSIONS

We have documented that one octave FM sweeps evoked different responses depending on which area of the RF they activated. Such response differences could not be captured by previous studies because commonly used long FM sweeps only differed in direction and rate, but not onset frequency. In contrast short FM's differ from each other in onset frequency and sweep direction similar to the way formant transitions in phonemes such as /ba/, /da/, /ga/ differ. Our findings suggest that forward masking seem to play an important role in the coding of these sounds. Clusters of neurons which were strongest activated by FM's were masked by the onset, and did not differentiate in their response between direction or rate of these sounds. Thus neurons which could categorize short FM sweeps are likely the ones with CF's away from the start frequency of the sweeps because they displayed rate selectivity for FM sounds sweeping towards their RF.

Ineffective forward masking could be among the underlying mechanisms of disorders that manifest deficiency in discrimination of FM sounds.

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APPENDIX CHAPTER THREE

RESPONSE PARAMETERS	Thalamus n=104	A1 n=72	PAF n=73	Diff
Threshold (dB)	19.7 ± 0.1	18.9 ± 1.3	14.8 ± 1.1	§ †
BW20 (oct)	1.7 ± 0.1	1.5 ± 0.1	3.0 ± 0.1	§ †
Min. Latency (ms)	10.8 ± 0.4	15.9 ± 1.7	31.5 ± 1.8	‡ § †
Time to peak (ms)	24.4 ± 1.2	20.5 ± 2.2	49.9 ± 3.9	§ †
Response Strength (spikes/tone)	2.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	‡ †
Spontaneous	7.6 ± 0.7	5.2 ± 0.4	4.0 ± 0.3	§ †
RF asymmetry	-0.12 ± 0.04	-0.18 ± 0.05	-0.04 ± 0.04	§

Table 1. Basic Response Properties of recorded sites in the thalamus, A1, and PAF to Tones in the Receptive Field. Values indicate mean and standard error of the mean.

‡ = Thal vs A1; § = A1 vs PAF; † = Thal vs PAF; two-tailed ttest, $p < .05$

PAF was significantly different from Thal for all response parameters, and from A1 for all except response strength. A1 and Thal were similar except for latency and response strength (Thal is faster, and fires more spikes).

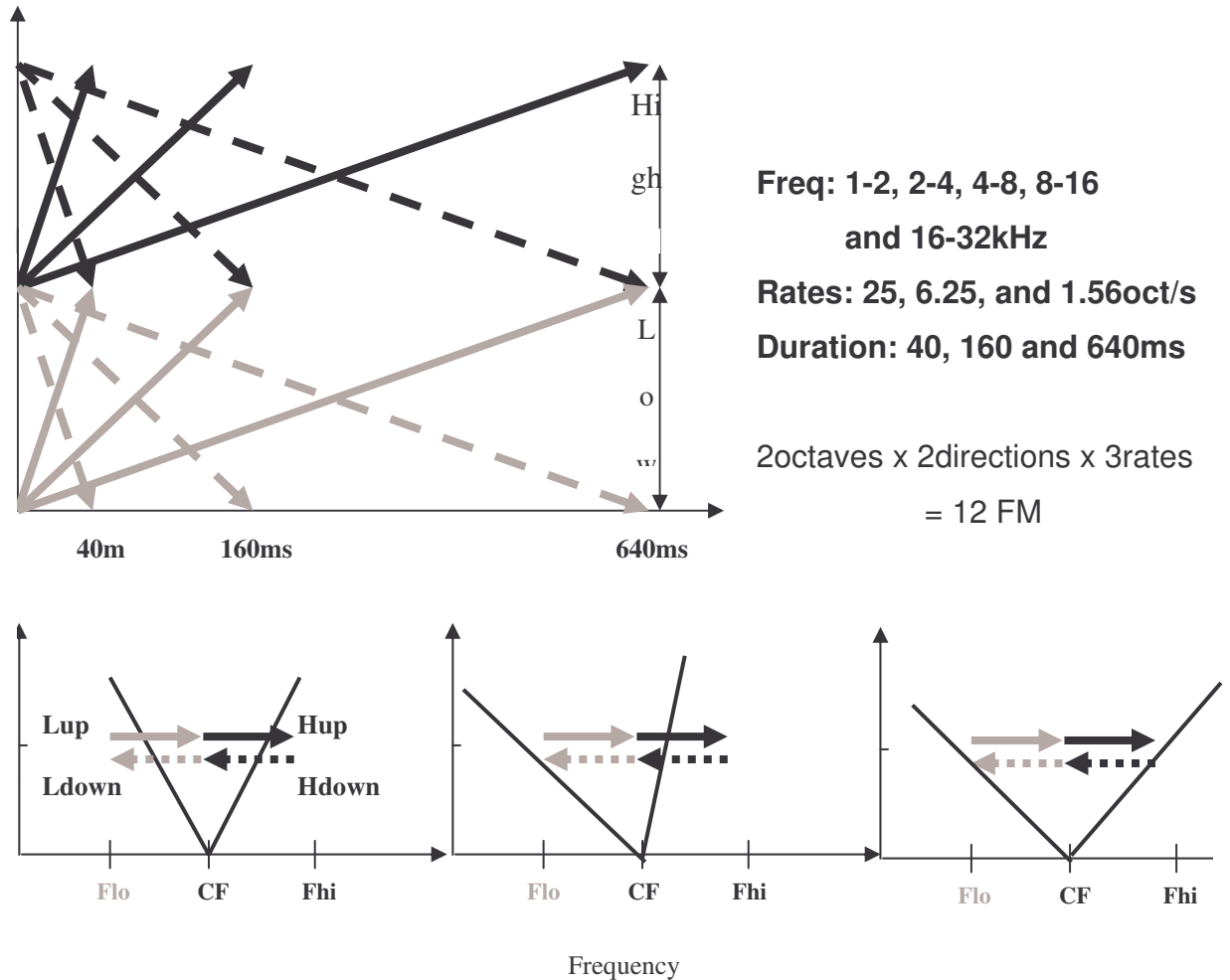


Figure 3.1. Schematic illustration of FM stimuli presented at each recording site. The frequency range of any FM was one octave: 1-2, 2-4, 4-8, 8-16 or 16-32 for upward sweeps and 2-1, 4-2, 8-4, 16-8 or 32-16 for downward FM sweeps. Only octave ranges matching the units frequency response areas (tuning curves) were played. At each site two successive octaves were chosen such that the lower octave upward sweep (Lup) started at a frequency lower than the CF of the site (Flo) while the higher octave downward sweep (Hdown) started at a frequency above CF (Fhi). Each octave pair (up&down) traversed a different area of the RF. The sweep intensity was 25 dB over CF threshold. Average auditory tuning curves have a bandwidth of less than 2 octaves at 20dB above threshold thus our stimuli likely traversed the tuning curve edges on both sides as illustrated in panel b. If tuning bandwidths were wider both start and end frequencies of any octave pair could be within the tuning curve (panel d). If tuning curves are skewed only one octave range likely crosses tuning curve boundaries (c). FM sweeps were presented at three different rates for a total of 12 FM stimuli at every site (2 octaves x 2directions x 3 rates).

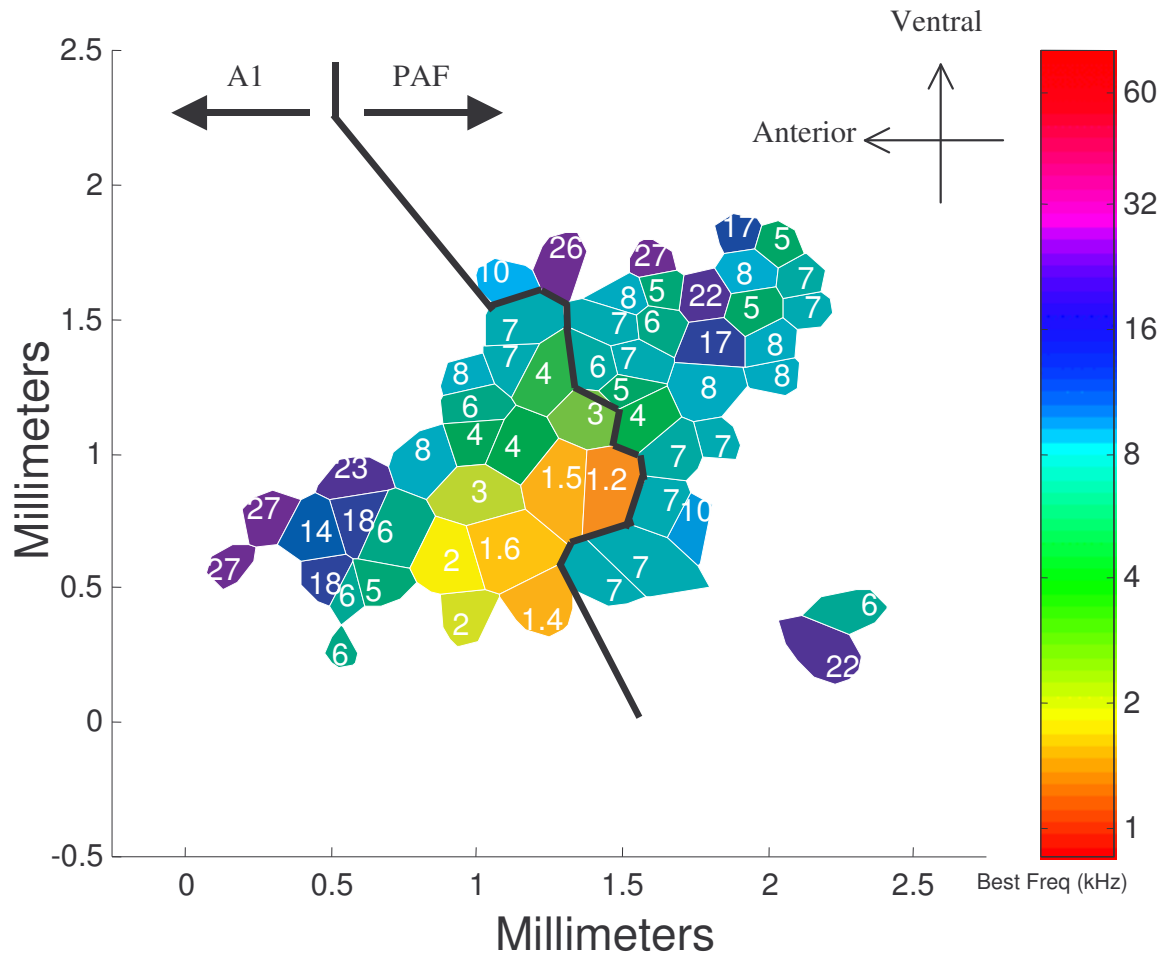


Figure 3.2. a) Example of a cortical map of best frequencies derived from recordings in one rat. Each polygon represents a cortical site and the numbers indicate the CF of each site. A1 was defined based on short latency narrow bandwidth and orderly progression of frequency preference from low to high (tonotopy). PAF sites have long latencies large bandwidths and no tonotopic gradient. The border line between A1 and PAF separates sites with response profiles belonging to the different fields.

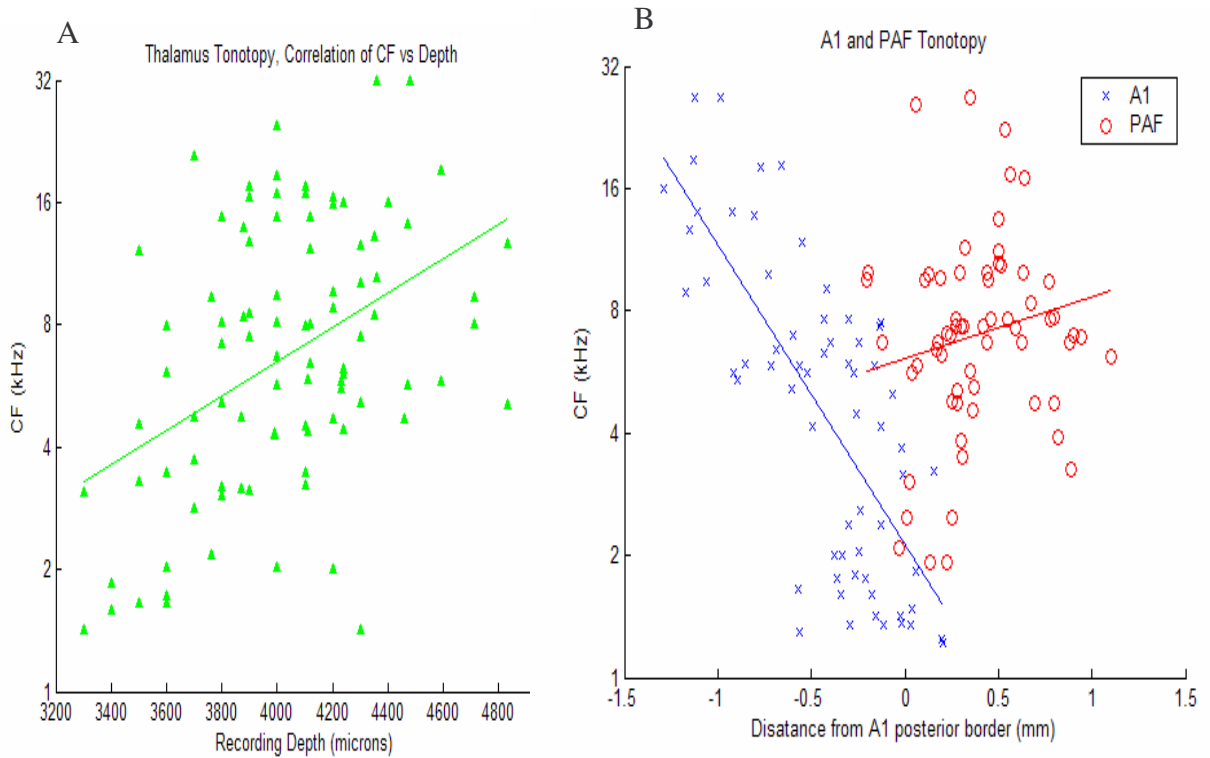


Figure 3.2. b) Scatterplot of thalamic CF's plotted versus recording depths illustrating tonotopic low to high organization of MGN responses along the latero-medial axis. c) Comparison of tonotopic gradients of the two cortical fields. A1 shows strong correlation of CF versus distance with low to high progression along the postero-anterior axis. No tonotopy is evident in PAF.

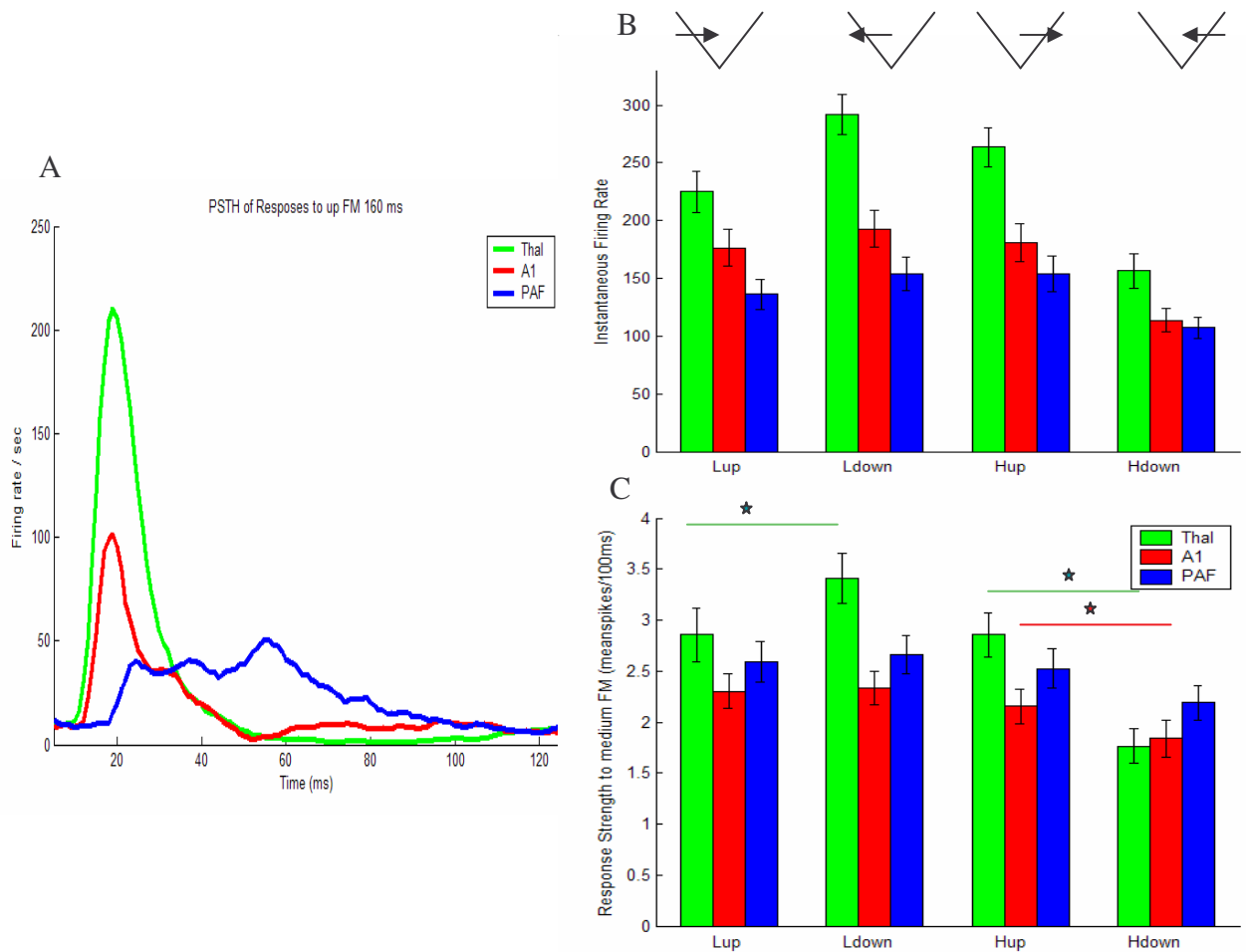


Figure 3.3. a) Population PSTH of responses to an upward sweep of 160 ms. Response strength is bigger in thalamus compared to the cortical fields, and latency is shorter. Thalamus has faster and more synchronous responses to FM's.

b) Peak firing rate in each field. Thalamus has a higher firing rate for FM sounds that start near its CF (Ldown and Hup) and a higher firing rate than A1 and PAF. A1 firing rate is higher than PAF for sounds traversing its lower RF side (Lup/down).

c) Field comparison of the mean response to a medium FM sweep averaged over 100ms from sound onset. Even though there are differences in timing of responses the overall number of spikes elicited by FM stimuli during a 100 ms time window is similar in the three areas. As seen in panel a) A1 fires most of its spikes in the first 50ms while PAF continues to fire almost twice as long.

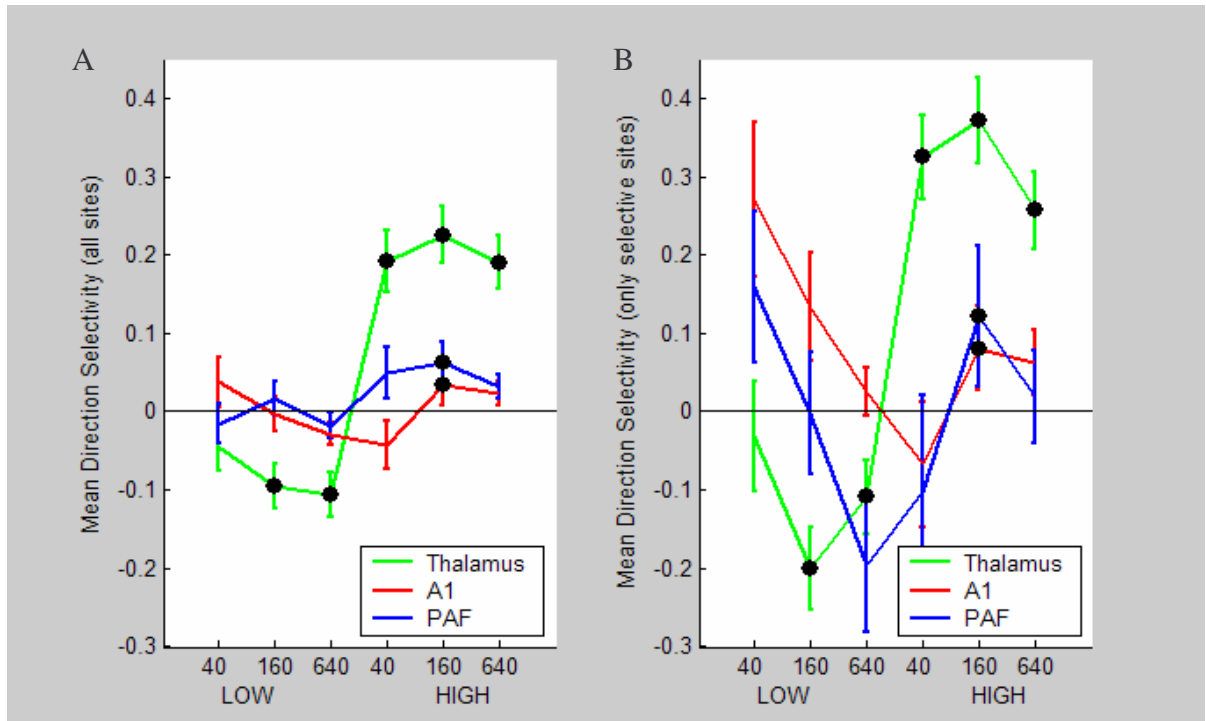


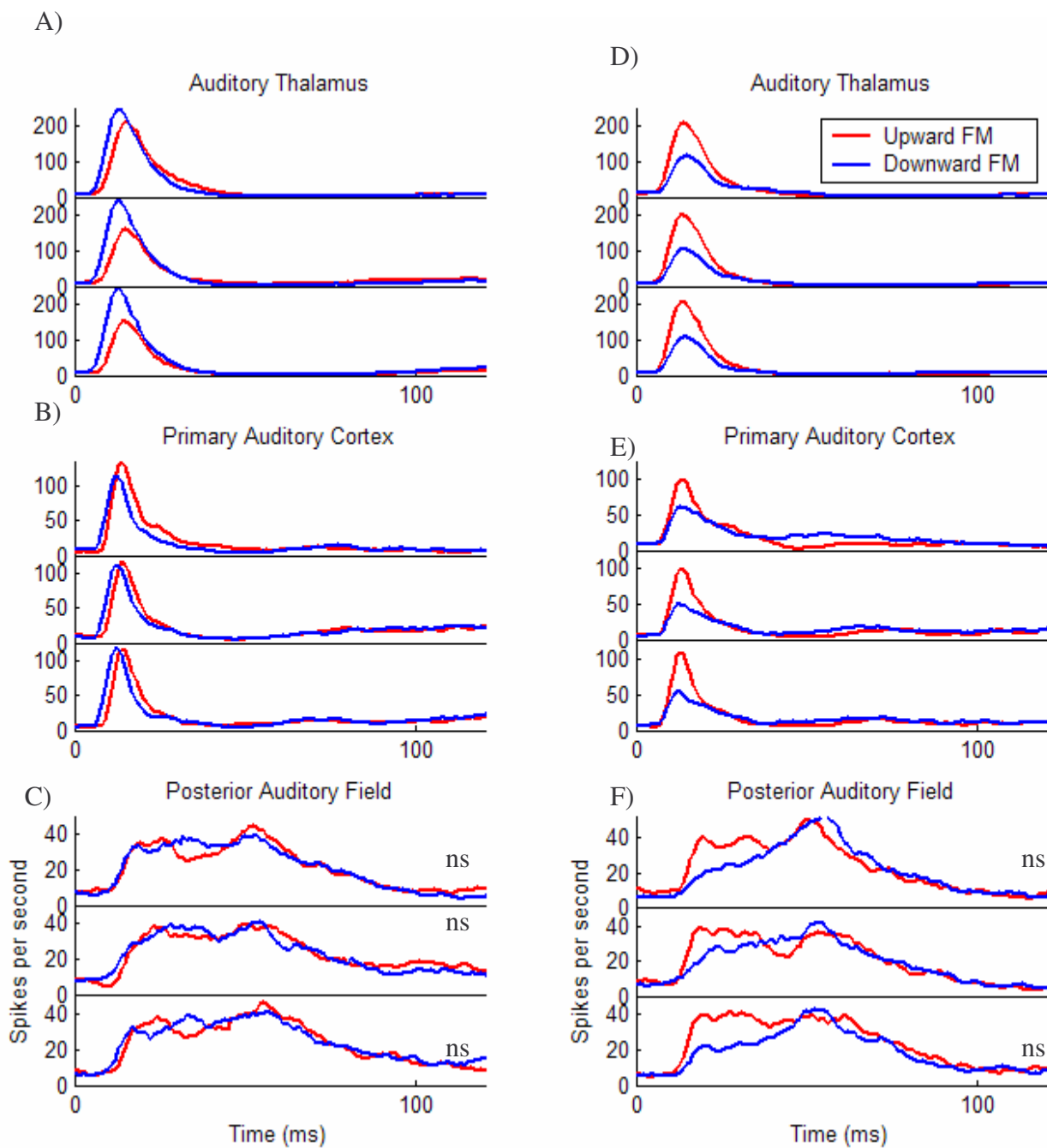
Figure 3.4. a) Mean DS index of all sites for all of the six up & down FM pairs: Low octave at 40, 160, 640ms and High octave same three rates. Full circles indicate significance ($p < 0.01$). Negative values indicate a preference for downward while positive values for upward FM's. Thalamus exhibits strong direction selectivity and preferred direction depends on FM octave range. Preference is for downward FM 's spanning the lower octave and upward FM's spanning the higher octave. This bias reflects a real preference for the sounds starting near its CF (Ldown and Hup).

b) Mean DS index of selective sites (sites with DS significantly different from zero) The same trend as in panel (a) is evident with DS numbers more indicative of the degree of preference. A DS index of $|0.33|$ reflects a response magnitude twice as large for the preferred direction.

Figure 3.5. Population PSTH's of responses to up (red) and down (blue) FM sweeps are overlaid. Each column shows responses to one of the octave ranges (right column – lower octave, left column – higher octave). For each field responses to the three FM rates (fast, medium, slow) are shown in subplots. Not significant differences are noted with “ns”. Responses to stimuli crossing the low frequency side of RF are different from the ones to FM sounds traversing the high side of RF. Most responses favor the direction of the FM with start frequency near the center of the RF (Ldown and Hup). For the high octave FM's all fields have increased response strengths (fire more spikes) to the “near center” upward FM (red line in panels d,e,f). For the low octave FM's fields differ in response strength and/or latency in this case favoring the “near center” downward FM (blue). Thalamus responds faster and stronger (**a**), A1 responds only with shorter latency (**b**) while PAF responses are not different to the two FM directions (**c**).

Low Frequency RF 

High Frequency RF 



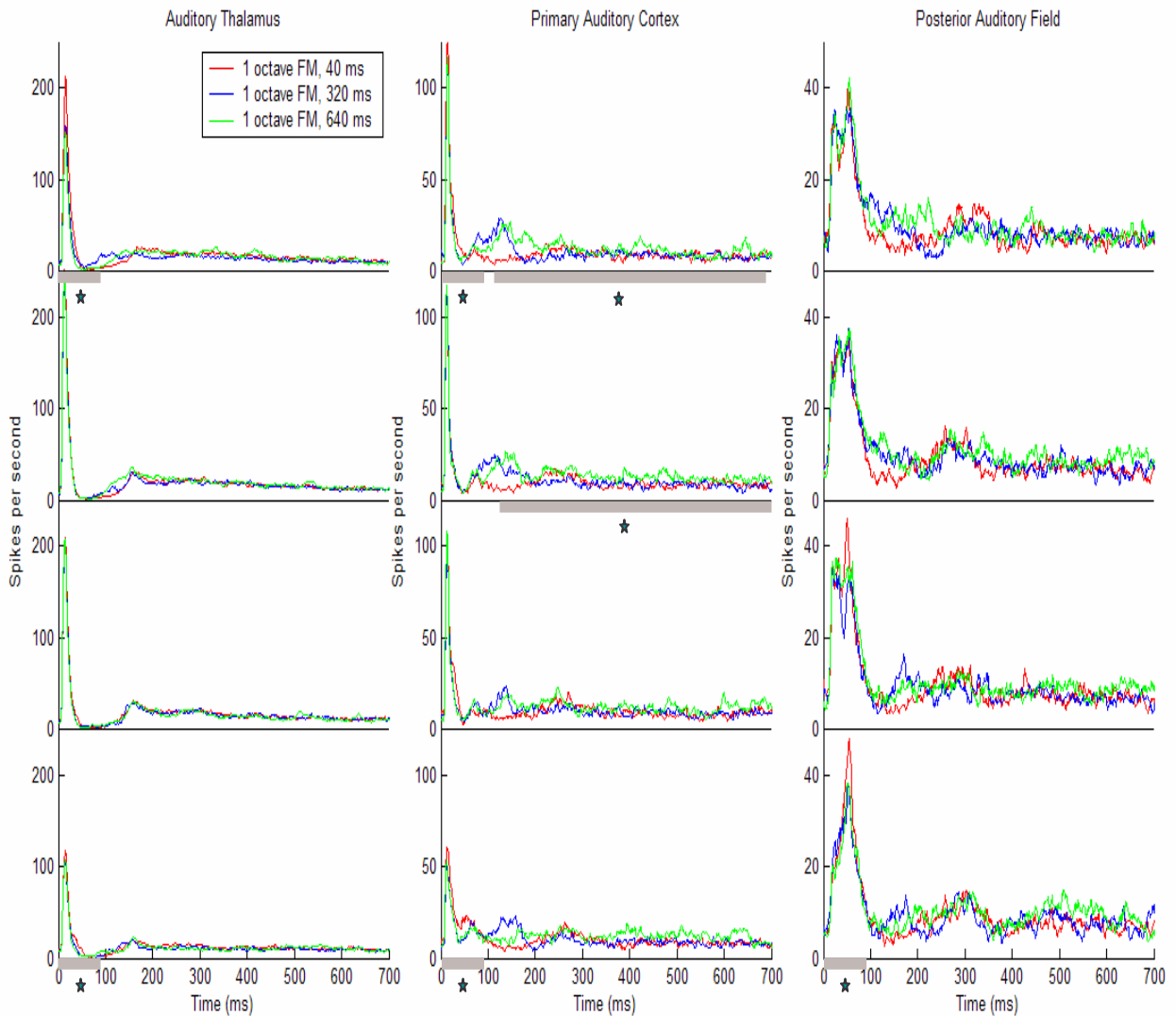


Figure 3.6. Responses to each rate are plotted in a color: red=fast, blue=medium, green=slow. Each column is a different field. For each field the response to the four FM stimuli are shown in each row (Lup, Ldown, Hup, Hdown). Lines and stars indicate time windows with significant differences in responses between the fast and slow rate. During the onset response 0-100 ms Thalamus and A1 fire more spikes to the fast FM rate (Lup and Hdown). Over the late response window (100-700ms) A1 fires more spikes for sounds that are still playing (slow rate) traversing the lower frequency RF.

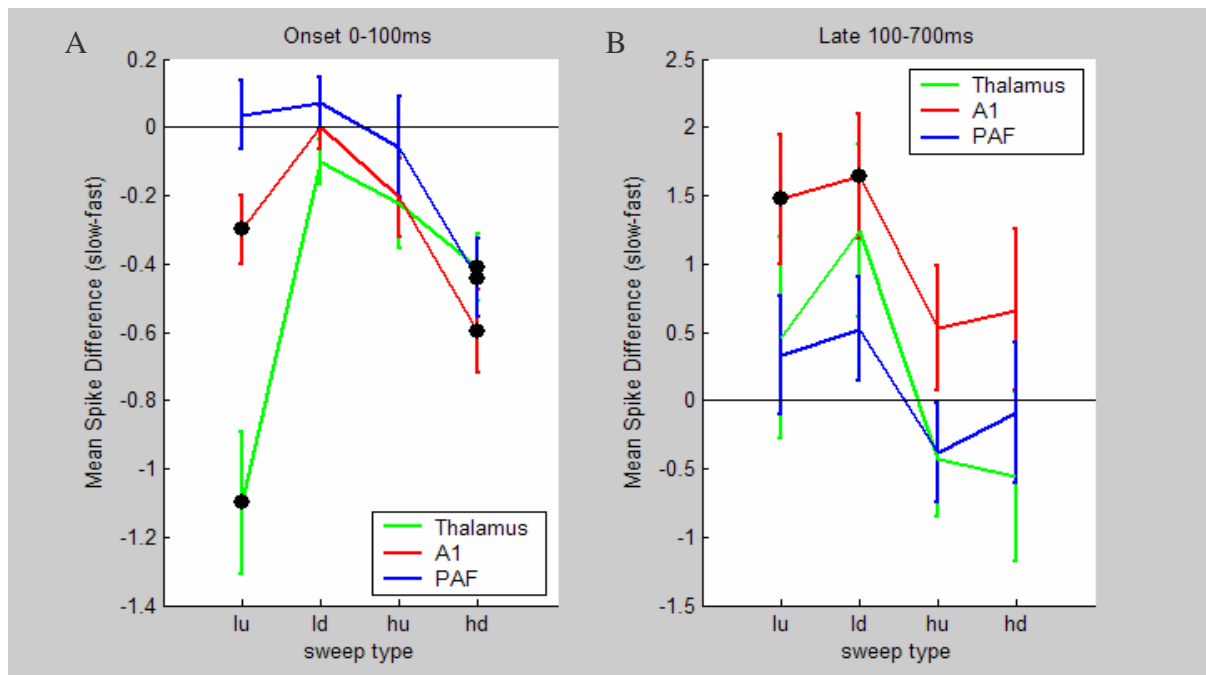
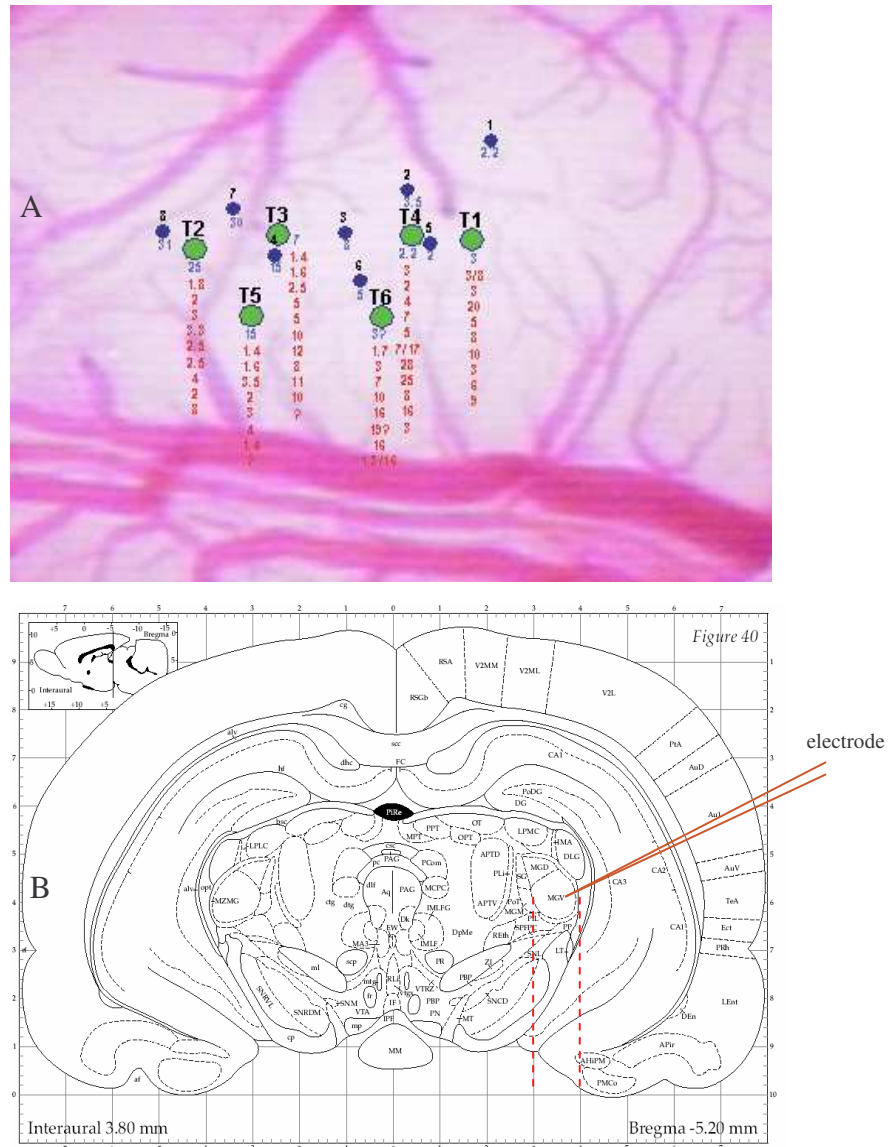


Figure 3.7. Mean difference in responses to a short FM sweep (40 ms) compared to a slow FM sweep (640ms) over the time matching the duration of the FM sounds. Responses to each of the four FM sounds played are compared (Lup, Ldown, Hup, Hdown). Filled circles indicate significant differences ($p < 0.01$).

a) During the onset response Thalamus and A1 fire more spikes to all fast FM's. PAF fires more spikes to the fast higher octave FM.

b) During the late response 100-700ms the fast FM is off while slow FM's are still ongoing. A1 fires more spikes for the slow lower octave FM's of either direction.



List of Abbreviations

A1	primary auditory cortex
AAF	anterior auditory field
BF	best frequency
BMF	best modulation frequency
BW	bandwidth
BMR	best modulation rate
CF	characteristic frequency
DS	direction selectivity index
Fi	instantaneous frequency
FMR	frequency modulation rate
FM	frequency modulation
FRA	frequency response area
FRF	frequency response function
LFP	local field potential
MU	multiunit
P	posterior auditory field
PFR	peak firing rate
PAF	posterior auditory field
RU	response elicited by an upward FM
RD	response elicited by a downward FM
RCF	rate of change of frequency
SFM	sinusoidal frequency modulation
SAM	sinusoidal amplitude modulation
Thal	thalamus

REFERENCES

- Britt R, Starr A (1976) Synaptic events and discharge patterns of cochlear nucleus cells. II. Frequency-modulated tones. *J Neurophysiol* 39:179-194.
- Clopton BM, Winfield JA (1974) Unit responses in the inferior colliculus of rat to temporal auditory patterns of tone sweeps and noise bursts. *Exp Neurol* 42:532-540.
- Cotillon-Williams N, Edeline JM (2003) Evoked oscillations in the thalamo-cortical auditory system are present in anesthetized but not in unanesthetized rats. *J Neurophysiol* 89:1968-1984.
- Deglutte B (1997) Auditory Neural Processing of Speech. In: *The Handbook of Phonetic Sciences* (Laver WHaJ, ed), pp 507-538. Oxford: Blackwell.
- Doron NN, Ledoux JE, Semple MN (2002) Redefining the tonotopic core of rat auditory cortex: physiological evidence for a posterior field. *J Comp Neurol* 453:345-360.
- Engineer ND, Percaccio CR, Pandya PK, Moucha R, Rathbun DL, Kilgard MP (2004) Environmental enrichment improves response strength, threshold, selectivity, and latency of auditory cortex neurons. *J Neurophysiol* 92:73-82.
- Felsheim C, Ostwald J (1996) Responses to exponential frequency modulations in the rat inferior colliculus. *Hear Res* 98:137-151.
- Fitch RH, Miller S, Tallal P (1997) Neurobiology of speech perception. *Annu Rev Neurosci* 20:331-353.
- Gaese BH, Ostwald J (1995) Temporal coding of amplitude and frequency modulation in the rat auditory cortex. *Eur J Neurosci* 7:438-450.

- Heil P, Rajan R, Irvine DR (1992) Sensitivity of neurons in cat primary auditory cortex to tones and frequency-modulated stimuli. II: Organization of response properties along the 'isofrequency' dimension. *Hear Res* 63:135-156.
- Kelly JB, Whitfield IC (1971) Effects of auditory cortical lesions on discriminations of rising and falling frequency-modulated tones. *J Neurophysiol* 34:802-816.
- Kilgard MP, Merzenich MM (1999) Distributed representation of spectral and temporal information in rat primary auditory cortex. *Hear Res* 134:16-28.
- Kilgard MP, Pandya PK, Vazquez J, Gehl A, Schreiner CE, Merzenich MM (2001) Sensory input directs spatial and temporal plasticity in primary auditory cortex. *J Neurophysiol* 86:326-338.
- Kowalski N, Versnel H, Shamma SA (1995) Comparison of responses in the anterior and primary auditory fields of the ferret cortex. *J Neurophysiol* 73:1513-1523.
- Lui B, Mendelson JR (2003) Frequency modulated sweep responses in the medial geniculate nucleus. *Exp Brain Res* 153:550-553.
- Mendelson JR, Schreiner CE, Sutter ML, Grasse KL (1993) Functional topography of cat primary auditory cortex: responses to frequency-modulated sweeps. *Exp Brain Res* 94:65-87.
- Moller AR (1972) Coding of amplitude and frequency modulated sounds in the cochlear nucleus of the rat. *Acta Physiol Scand* 86:223-238.
- Moller AR (1974) Coding of sounds with rapidly varying spectrum in the cochlear nucleus. *J Acoust Soc Am* 55:631-640.

- Moucha R, Pandya PK, Engineer ND, Rathbun DL, Kilgard MP (in press) Background sounds contribute to spectrotemporal plasticity in primary auditory cortex. *Exp Brain Res.*
- Nelken I, Versnel H (2000) Responses to linear and logarithmic frequency-modulated sweeps in ferret primary auditory cortex. *Eur J Neurosci* 12:549-562.
- Ohl FW, Wetzel W, Wagner T, Rech A, Scheich H (1999) Bilateral ablation of auditory cortex in Mongolian gerbil affects discrimination of frequency modulated tones but not of pure tones. *Learn Mem* 6:347-362.
- Rauschecker JP (1999) Auditory cortical plasticity: a comparison with other sensory systems. *Trends Neurosci* 22:74-80.
- Rees A, Moller AR (1983) Responses of neurons in the inferior colliculus of the rat to AM and FM tones. *Hear Res* 10:301-330.
- Ricketts C, Mendelson JR, Anand B, English R (1998) Responses to time-varying stimuli in rat auditory cortex. *Hear Res* 123:27-30.
- Schulze H, Ohl FW, Heil P, Scheich H (1997) Field-specific responses in the auditory cortex of the unanaesthetized Mongolian gerbil to tones and slow frequency modulations. *J Comp Physiol [A]* 181:573-589.
- Shamma SA, Fleshman JW, Wiser PR, Versnel H (1993) Organization of response areas in ferret primary auditory cortex. *J Neurophysiol* 69:367-383.
- Sinex DG, Geisler CD (1981) Auditory-nerve fiber responses to frequency-modulated tones. *Hear Res* 4:127-148.

- Stefanatos GA (1993) Frequency modulation analysis in children with Landau-Kleffner syndrome. *Ann N Y Acad Sci* 682:412-414.
- Tallal P, Miller SL, Bedi G, Byma G, Wang X, Nagarajan SS, Schreiner C, Jenkins WM, Merzenich MM (1996) Language comprehension in language-learning impaired children improved with acoustically modified speech. *Science* 271:81-84.
- Tian B, Rauschecker JP (1994) Processing of frequency-modulated sounds in the cat's anterior auditory field. *J Neurophysiol* 71:1959-1975.
- Tian B, Rauschecker JP (1998) Processing of frequency-modulated sounds in the cat's posterior auditory field. *J Neurophysiol* 79:2629-2642.
- Tian B, Rauschecker JP (2004) Processing of frequency-modulated sounds in the lateral auditory belt cortex of the rhesus monkey. *J Neurophysiol* 92:2993-3013.
- Wetzel W, Ohl FW, Wagner T, Scheich H (1998) Right auditory cortex lesion in Mongolian gerbils impairs discrimination of rising and falling frequency-modulated tones. *Neurosci Lett* 252:115-118.
- Whitfield I, Evans E (1965) Responses of Auditory Cortical Neurons to Stimuli of Changing Frequency. *J Neurophys* 28:655-672.
- Wingfield A, Poon LW, Lombardi L, Lowe D (1985) Speed of processing in normal aging: effects of speech rate, linguistic structure, and processing time. *J Gerontol* 40:579-585.

CHAPTER FOUR
BACKGROUND SOUNDS CONTRIBUTE TO SPECTROTEMPORAL PLASTICITY IN
AUDITORY CORTEX

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ABSTRACT

The mammalian auditory system evolved to extract meaningful information from complex acoustic environments. Spectrotemporal selectivity of auditory neurons provides a potential mechanism to represent natural sounds. Experience-dependent plasticity mechanisms can remodel the spectrotemporal selectivity of neurons in primary auditory cortex (A1). Electrical stimulation of the cholinergic nucleus basalis (NB) enables plasticity in A1 that parallels natural learning and is specific to acoustic features associated with NB activity. In this study, we used NB stimulation to explore how cortical networks reorganize after experience with frequency-modulated (FM) sweeps and how background stimuli contribute to spectrotemporal plasticity in rat auditory cortex.

Pairing an 8-4 kHz FM sweep with NB stimulation 300 times per day for 20 days decreased tone thresholds, frequency selectivity, and response latency of A1 neurons in the region of the tonotopic map activated by the sound. In an attempt to modify neuronal response properties across all of A1, the same NB activation was paired in a second group of rats with five downward FM sweeps, each spanning a different octave. No changes in FM selectivity or receptive field (RF) structure were observed when the neural activation was distributed across the cortical surface. However, the addition of unpaired background sweeps of different rates or direction was sufficient to alter RF characteristics across the tonotopic map in a third group of rats.

These results extend earlier observations that cortical neurons can develop stimulus specific plasticity, and indicate that background conditions can strongly influence cortical plasticity.

INTRODUCTION

Sensory experience can alter the organization of adult somatosensory (Merzenich et al. 1990; Recanzone et al. 1992b), visual (Gilbert 1996), and auditory (Recanzone et al. 1993; Weinberger and Bakin 1998; Dimyan and Weinberger 1999; Kilgard et al. 2001b) cortex. The correlation between changes in neuronal responsiveness and behavioral performance (Recanzone et al. 1992c; Ohl et al. 2001; Super et al. 2001) suggests that these changes might constitute the neural basis for perceptual learning (for a review see Das 1997). This reasoning is complemented by evidence that precise details of sensory experience differentially shape cortical plasticity. Tuning properties of cortical neurons change depending upon stimulus parameters, neuromodulatory influences and contextual circumstances. Decreases as well as increases in neuronal responses to acoustic conditioned stimuli (CS+) have been documented, depending on various task parameters including task difficulty, type of training (i.e. fear conditioning or NB stimulation), or the presence of non-reinforced stimuli (CS-) (Bakin and Weinberger 1990; Edeline and Weinberger 1993; Ohl and Scheich 1996; Dimyan and Weinberger 1999).

Repeated pairing of a tone with foot shock results in a specific increase in response to the CS+ frequency, while presentation of the same tone and shock on an unpaired schedule leads to a general increase in response to all tones, called sensitization (Bakin and Weinberger 1990). When a tone is presented repeatedly without the shock, neurons in primary auditory cortex (A1) habituate and respond with fewer action potentials specifically to the repeated tone frequency (Condon and Weinberger 1991). The demonstration that RF plasticity is gated by arousal and is specific to input features indicates that sensory

experience alters the neural representation of behaviorally relevant and irrelevant events (reviewed in Weinberger and Bakin 1998).

Neurons in the cholinergic nucleus basalis (NB) respond to stimuli that have been associated with either rewards or aversive stimuli (Sarter et al. 1999; Sarter et al. 2001). Activation of the NB has been used as a substitute for behavioral arousal in gating plasticity and has been shown to create changes specific to features of sensory stimuli associated with increased NB activity. Cortical maps (Kilgard and Merzenich 1998a), RF size and structure (Metherate and Weinberger 1989; Weinberger and Bakin 1998; Dimyan and Weinberger 1999; Kilgard et al. 2001a), temporal response properties (Kilgard and Merzenich 1998b; Mercado et al. 2001), and combination sensitivity (Kilgard and Merzenich 2002) of A1 neurons can be altered by pairing different sounds with NB stimulation. In many cases the resulting plasticity parallels CS+ specific plasticity generated by behavioral training. RF size can be increased or decreased depending on the spatial variability and modulation rate of sensory inputs associated with a behavioral task or NB stimulation. Modulated stimuli repeatedly delivered to one site on the receptor surface increase RF size and decrease response latency, while unmodulated stimuli delivered to different locations decrease RF size and increase response latency (Recanzone et al. 1992a; Recanzone et al. 1993; Kilgard et al. 2001a).

Non-reinforced stimuli (CS-) also influence the expression of neural plasticity. Pairing foot-shock with a single tone frequency in the context of many others generates neural plasticity that is in the opposite direction compared to the plasticity induced by tone-shock pairing presented in a silent background (Bakin and Weinberger 1990; Ohl and

Scheich 1996). The background sounds caused the best frequency of A1 neurons to move away from the tone associated with shock rather than toward it. Background tones also influence the expression of RF size. Pairing a single tone with NB stimulation results in a 20% increase in RF size. However, this RF expansion does not occur if the same tone-NB pairing is interleaved with flanking tones that are not associated with NB stimulation (Kilgard et al. 2001a). While these experiments and many others indicate that cortical plasticity is guided by sensory input patterns, the complex relationship between input patterns and plasticity remains poorly understood.

Frequency modulation is nearly ubiquitous in natural communication sounds. FM sweeps have been used to probe spectrotemporal coding by auditory neurons in several species (reviewed in (Eggermont 2001). A1 neurons have been shown to be selective for FM parameters such as rate and direction (reviewed in Nelken 2002). Lesion studies indicate that FM direction discrimination depends on the integrity of primary auditory cortex (Kelly and Whitfield 1971; Wetzal et al. 1998). Here we report that experience with frequency-modulated (FM) sounds influences plasticity in A1.

The aim of the current study was to determine how experience with FM stimuli in silence or in a background of contrasting sounds alters cortical responses to this important stimulus class. FM sweeps were paired with electrical activation of nucleus basalis to generate spectrotemporal plasticity in rat A1. In experiment 1, a downward FM sweep covering a frequency range of one octave was repeatedly paired with NB stimulation. In experiment 2, five different FM sweeps activating different regions of A1 were paired with NB stimulation. Experiment 3 was identical to experiment 2 except that unpaired FM's of contrasting rates

(faster or slower), or direction (upward sweeping) were interleaved with the five FM sweeps paired with NB stimulation. The results of this study were presented earlier in abstract form (Moucha et al. 2001a; Moucha et al. 2001b).

METHODS

Sixteen adult female Sprague-Dawley rats were used for this study. Nine rats were implanted with NB stimulation electrodes. The experimental data used in this study was derived from 398 A1 recording sites from these rats. Recordings from 259 A1 sites from seven rats served as control data (Table 1). All procedures were carried out in accordance with the guidelines laid down by the US National Institute of Health and the UT Dallas Institutional Animal Care and Use Committee.

Chronic Implantation and Electrical Stimulation

The NB stimulation used in this study was identical to our previous reports that included more detailed technical descriptions (Kilgard and Merzenich 1998a; Kilgard and Merzenich 1998b; Kilgard and Merzenich 1999; Kilgard et al. 2001a; Kilgard et al. 2001b; Kilgard and Merzenich 2002). Briefly, experimental rats were pentobarbital anesthetized and implanted with platinum bipolar stimulating electrodes into NB using sterile stereotaxic techniques (7.0 mm below cortical surface; 3.3mm lateral and 2.3mm posterior from bregma). Leads were attached to screws over cerebellum and cortex to allow for recording of the global electroencephalogram (EEG) during the subsequent sound-NB pairing phase of the study. After two weeks of recovery each animal was placed in a sound shielded, calibrated test chamber and received ~300 pairings of an acoustic stimulus with NB stimulation per day

for ~20 days under one of the three experimental conditions. Custom software was used to control the auditory stimuli (generated with a Tucker-Davis D/A converter, Alachua, Florida USA) and trigger NB stimulation (train of 20 biphasic pulses, 100Hz, 0.1ms pulse width, current level 70-150 μ A, beginning at the termination of each paired sweep).

Acoustic Stimulation

In experiment 1, the sound repeatedly paired with NB stimulation was a downward FM sweep (160ms duration, 6.25oct/s) spanning a single octave (8-4 kHz). In experiments 2 and 3, five downward FM sweeps each spanning a different octave (2-1, 4-2, 8-4, 16-8, 32-16 kHz) were randomly interleaved and paired with NB stimulation (Figure 1). Each sweep was presented at ~25 dB above rat hearing threshold based on behavioral and neural responses (Kelly and Masterton 1977; Kilgard and Merzenich 1999). Each of the five octaves was presented at 60, 55, 45, 40, and 50 dB SPL respectively. Acoustic and electrical stimuli did not evoke any observable behavioral responses, but did generate reliable EEG desynchronization for 1-2 seconds if stimulation occurred during slow wave sleep.

Neurophysiological recording

Twenty-four hours after the final NB stimulation session, responses of auditory cortex neurons to tones and FM sweeps were quantified with high-density microelectrode recordings. Animals were pentobarbital anesthetized (50 mg/kg body weight), and maintained in a state of areflexia throughout the surgical procedure and during recordings. To ensure a proper level of anesthesia and stable physical condition, the animal's electrocardiogram and blood oxygen concentration were also monitored. The skull was

supported in a head holder leaving the ears unobstructed. The trachea was cannulated to ensure adequate ventilation. The cisternae magnum was drained of CSF to minimize cerebral edema. The auditory cortex was exposed via a wide craniotomy, the dura mater was resected and a thin layer of viscous silicon oil was applied to prevent desiccation. Recording of action potentials were made in a double-walled sound chamber from two Parylene-coated tungsten microelectrodes (FHC, Bowdoinham, Maine USA) lowered orthogonally into right auditory cortex to a depth of 550 μ m (layers IV/V). Multiunit data was collected from 30-65 sites in each animal. Neural signal was filtered (.3 to 8 kHz), amplified (10,000x), and resulting spike-waveforms crossing a fixed threshold were sampled. Tucker-Davis neurophysiology hardware and Brainware software were used for stimulus production, online spike sorting and data acquisition.

Frequency-intensity tuning curves were derived at each site, by presenting 81 frequencies spanning 5 octaves, at 16 intensities ranging between 0 and 75dB (1296 total stimuli). Responses to FM sweeps of different direction, rates, and starting frequency were also recorded. Every FM sweep used in this study spanned a single octave. Six different sweep rates were presented: 20, 40, 80, 160, 320, and 640 ms (corresponding to 50, 25, 12.5, 6.25, 3.12, and 1.56 oct/s). FM sweeps always started at 1, 2, 4, 8, 16, or 32 kHz. At each site sweeps were presented that covered one octave above and one octave below the best frequency. The onset and offset of all sounds was linearly ramped over 3ms. Responses to a total of 24 randomly interleaved FM stimuli (6 rates x 2 octaves x 2 directions) were recorded at each site. The sweeps had the same intensities as those used during NB

stimulation (see above). Each sweep was repeated thirty times at 1s intervals to minimize adaptation.

Data Analysis

All analysis was conducted using custom Matlab (MathWorks, Natick, Massachusetts USA) programs. Tuning curve parameters were defined blind to experimental condition and recording location, using an interface specifically designed for this purpose. Thresholds, characteristic frequency (CF), response strength, minimum latency, time to end of response, and excitatory frequency tuning range or bandwidth (BW at 10,20,30,40dB over threshold) were determined for each site (Fig.2a). Tone response strength was estimated as the average number of spikes in response to tones with intensities greater than threshold and frequencies within the maximum excitatory bandwidth for each site (as determined from the frequency-intensity tuning curves, Fig.2a). Latency was derived from post stimulus time histogram (PSTH) created by summing responses to all the tones within each site's tuning curve. The minimum latency was defined as the time from stimulus onset to the earliest consistent response. Peak latency was the time to the maximum instantaneous firing rate. The time to end of driven response was the time to return to spontaneous activity levels Fig.2c. A1 was defined on the basis of its short latency (8-20 ms) responses and continuous tonotopy.

Boundaries were determined using non-responsive and non-A1 sites.

Unpaired two tailed t-tests were used to evaluate the effects of NB-FM pairing on frequency selectivity, response strength, latency, and direction selectivity.

FM direction selectivity (DS) was quantified using the following index:

$$DS = (R_{up} - R_{down}) / (R_{up} + R_{down})$$

where R is the response (in number of spikes averaged from 30 repetitions) elicited by the upward or downward FM sweep. Spikes occurring from 8ms after sweep onset until 40ms after the end of each sweep were analyzed. The average spontaneous firing rate was estimated from the 8ms before the onset of a driven response and subtracted. The responses of A1 neurons to pairs of sweeps that begin at a frequency within the excitatory receptive field and sweep one octave (up or down) are typically indistinguishable because the response is largely determined by the onset frequency which is identical for the two sweeps. Thus we compared sweeps that swept through the same octave in opposite directions (i.e. had reversed start and end frequencies, such as 4-8 vs 8-4 kHz).

RESULTS

In this study, we compared responses to tones and FM sweeps from naïve animals (n=259 sites from 7 rats) with responses from animals who heard FM sweeps paired with NB stimulation (n=398 sites from 9 rats). The three experimental groups differed in the number of FM sweeps paired with NB activation (1 or 5), and the presence or absence of background sweeps (0 or 15) interleaved between the paired sweeps (Table 1 and Figure 1).

Experiment 1

Animals in the first group heard a downward FM sweep (8-4 kHz, 160ms duration) paired with NB activation 300 times per day over a period of three weeks. While repeated NB stimulation paired with an unmodulated tone nearly doubled the percent of A1 neurons responding to the paired tone frequency (Kilgard and Merzenich 1998a), pairing the 8-4 kHz sweep with NB stimulation did not alter the A1 map of tone frequency. The percent of

cortex responding to 45 dB tones at 2, 4, 8, and 16 kHz in the experimental group were not significantly different from naïve rats (36 ± 7 vs. $38\pm4\%$, 37 ± 6 vs. 44 ± 3 , 43 ± 3 vs. 45 ± 6 , 41 ± 4 vs. 39 ± 3 , respectively).

Responses to upward and downward FM sweeps were recorded at each site to document changes in FM direction selectivity. Although A1 neurons in rats rarely exhibit a high degree of direction preference for one octave wide sweeps, some neurons do exhibit some preference. To measure direction selectivity we compared responses to FM pairs that swept through the same octave but in opposite directions (i.e. 8-4kHz and 4-8kHz). After pairing an 8-4 kHz sweep with NB stimulation no downward direction selectivity developed (DS= 0.14 ± 0.05 and 0.13 ± 0.05 for control and experimental rats, respectively).

Although pairing a single FM sweep with NB stimulation did not alter direction preference or frequency topography, temporal and receptive field properties were altered in the region of auditory cortex activated by the paired sweep. NB activation paired with the 8-4 kHz sweep altered frequency selectivity, response threshold, latency, and strength of A1 neurons with best frequencies from 4 to 16 kHz (Table 2). In this experiment the FM sweep paired with NB activation was 8-4kHz presented at ~25 dB above rat hearing and neuronal threshold (see methods). Based on previous data from our lab at 20 dB over threshold the excitatory frequency bandwidth (BW₂₀) of neurons in naïve rats is ~ 2.0 octaves (see also Table 2). Thus an 8kHz tone corresponding to the start frequency of the paired sweep would likely activate neurons with CF's one octave below and above eight (4-16kHz). The mean tone threshold in these neurons was 2 dB quieter than neurons in naïve animals. Receptive field size (bandwidth at 20 dB above threshold) was increased by one-fifth of an octave. The

minimum response latency was decreased by more than 2 milliseconds (Fig.2). Response threshold, frequency selectivity, and latency were not altered in the flanking regions of the A1 frequency map (Table 2). CF's 1-4kHz and 16-32kHz are both towards the edges of the rat hearing range. It has been documented that neuronal thresholds are higher and receptive fields (BW) are narrower for these neurons (Kelly and Masterton 1977; Kilgard and Merzenich 1999). The same trend is apparent in Table 2, thresholds were 26.45 dB as compared to 15.0 dB for CF's 4-16, and BW20 1.46 octaves as compared to 1.9 octaves. Because both CF groups (1-4kHz and 16-32kHz) showed no significant RF changes after FM – NB pairing their results were pooled. These results indicate that this paradigm generates plasticity that is specific to the region of the tonotopic map most strongly activated by the FM sweep. However the average number of spikes evoked by tones within each site's receptive field was increased across A1.

Experiment 2

Earlier studies with spatially restricted stimuli also reported response plasticity in restricted regions of sensory maps (Bao et al. 2001; Xerri et al. 1996; Irvine et al. 2001). It is possible to generate plasticity that is not restricted by using inputs that are distributed across the receptor surface. For example, pairing NB stimulation with unmodulated tones of different frequencies (1.3, 2, 3, 4, 5, 7, 9, 11.2, and 14 kHz) narrowed receptive fields and increased the minimum latency of A1 neurons across the tonotopic map (Kilgard et al. 2001a). The aim of experiment 2 was to determine whether distributing FM sweeps evenly across the cochlea could generate plasticity (in this case, broader RF's and shorter latencies as documented in experiment 1) that generalized across the A1 frequency map. NB

stimulation was paired with downward FM sweeps that spanned five different frequency ranges (2-1 kHz, 4-2 kHz, 8-4 kHz, 16-8 kHz, 32-16 kHz). In contrast to the earlier studies, no plasticity of any sort resulted from the repeated pairing (Table 3). A1 topography, receptive field properties, latency, and FM direction selectivity were all indistinguishable from naïve rats. Response strength to downward FM's did not increase after the repeated pairing of down FM with NB stimulation, however response strength to upward FM sweeps was somewhat decreased (Table3).

Experiment 3

Previous evidence indicates that non-reinforced inputs can influence the expression of cortical plasticity in auditory, visual and somatosensory modalities. (Bakin and Weinberger 1990; Edeline and Weinberger 1993; Ohl and Scheich 1996; Dimyan and Weinberger 1999; Moore et al. 1999; Kilgard et al. 2001a). To test whether receptive field and FM response plasticity could be altered by background sounds, we repeated experiment 2 with additional unpaired FM sweeps randomly interleaved between the sweeps paired with NB activation (Figure 1). The fifteen background sweeps each spanned one octave, but differed from the paired sweeps in that they were of opposite direction (i.e. upward sweeping FM's) or different frequency modulation rate (i.e. faster or slower), as described in methods (Table 1). The additional sweeps were designed to provide greater contrast between paired and unpaired sounds that might influence the representation of the paired FM sweep.

Frequency selectivity, response latency and threshold all significantly decreased as a result of adding background unpaired FM's. The changes in receptive field size and minimum latency were of the same magnitude as in experiment 1, but generalized across the

A1 frequency map (Table 3). The addition of contrasting FM sweeps decreased the minimum response threshold by nearly 5 dB compared to naïve animals. The threshold decrease was significantly greater than the decrease observed in experiment 1 ($p < 0.05$, for neurons in the 4-16 kHz CF range). This experimental paradigm resulted in a decrease in average number of spikes elicited by all FM sweeps. However the temporal characteristics of the response to FM sweeps was changed (Figure 3). No preference for downward sweep direction or rate developed (data not shown).

In summary, NB stimulation paired with FM sweeps alters A1 responses as a function of both the number of paired sounds and the background in which the sounds were presented. Temporal sharpening and broader frequency tuning resulted from NB stimulation paired with FM sweeps that activated either a restricted region of the map or were presented in the context of contrasting FM sweeps. Since identical NB activation (strength, repetitions, time course) was associated with sound presentation in each experimental group, we conclude that the differential plasticity documented here was a result of the differential auditory experience of each group.

DISCUSSION

The aim of this study was to explore aspects of experience-dependent plasticity in primary auditory cortex. We investigated how plasticity mechanisms activated by NB stimulation alter cortical response properties following experience with FM sounds. Our results show that experience with a single octave wide FM sweep alters receptive field properties (threshold and bandwidth), response strength, and minimum latency of auditory

cortical neurons in a frequency specific manner. In contrast, experience with multiple FM sweeps spanning the rat hearing range does not alter receptive field properties or the temporal fidelity of A1 neurons. However, if the same auditory experience occurs in the context of background sounds, receptive fields and temporal response properties are changed across A1.

Receptive Fields

Earlier studies documented that RF size can be altered by sensory input characteristics. Frequency discrimination training resulted in smaller RF's in auditory cortex (Recanzone et al. 1993). In contrast, a task requiring detection of amplitude modulation rate of a stimulus applied to a single digit resulted in RF broadening in somatosensory cortex (Recanzone et al. 1992b). Due to the large number of differences between these studies it was not possible to be sure whether task parameters or the pattern of sensory input resulted in the opposite effects on RF size. Such differential plasticity was also observed after pairing identical NB stimulation with different patterns of sensory inputs. Pairing NB stimulation with a single modulated tone broadened RF's, while pairing with two unmodulated tones more than an octave apart narrowed RF's. Sounds that were both modulated and distributed across the receptor surface resulted in intermediate RF plasticity. These results suggest that modulation rate and number of locations activated by the sensory input influence RF size. The present findings may shed new light on a classic RF plasticity experiment conducted in monkeys. Several weeks of exposure to a spinning disk that brushed across several digits decreased RF's in somatosensory cortex (Jenkins et al. 1990). Because the original explanation of the spinning disk result was that moving stimuli generate narrow receptive

fields (Merzenich et al. 1990), we had expected FM stimuli to decrease RF size in the present experiment. Our results (using the auditory analog of light brushes to the skin) indicate that the initial interpretation of the monkey experiment may be incomplete. It should be noted however that behavioral training likely engages other neuromodulatory systems than the NB stimulation used in the present study. Our observation that background stimuli can shape the expression of RF plasticity suggests many more studies will be needed before we can reliably predict how untested sensory experiences will influence cortical plasticity.

Response Latency

Response latency of cortical neurons has also been shown to change as a function of sensory experience. Frequency discrimination training increased latencies of AI neurons in owl monkeys (Recanzone et al. 1993), while temporal tasks decreased response latencies (Recanzone et al. 1992c). Results from NB stimulation experiments which attempted to mimic the patterns of sensory input in the primate studies closely parallel these findings (Kilgard et al. 2001a). Although some theoretical work has been done to understand the plasticity mechanisms that influence cortical response latency (Song et al. 2000) little is known about the effects of complex input patterns. At present the most parsimonious explanation of the latency results following behavioral training or NB stimulation is that modulated inputs tend to decrease response latency while nonmodulated stimuli tend to increase latency. A complex sound sequence (tone-tone-noise) paired with NB activation caused a 30% decrease in cortical processing time (Kilgard and Merzenich 2002). In the present study, experience with an 8 to 4 kHz FM sweep caused minimum response latency to

decrease within the region of the map activated by the input. When multiple FM sweeps were paired with NB activation such that all regions of the map were equally activated neuronal latencies were not affected. However, when unpaired background sounds were interleaved with the paired sounds, latencies decreased across the A1 frequency map. These results suggest that the spectral and temporal features of both behaviorally relevant and irrelevant sounds have the potential to influence the response latencies of cortical neurons.

Response strength

The number of spikes evoked by a tone can also be modified by experience (Engineer et al. 2001). Pairing modulated tones (i.e. 15 Hz train of 9 kHz tones) with NB stimulation increased spikes per tone by 40% (Kilgard et al. 2001a), while pairing unmodulated tones of the same frequency had no effect on neural excitability. Our observation of increased response strength after pairing NB stimulation with FM stimuli supports an earlier report by Mercado and colleagues (Mercado et al. 2001). It is not yet clear why response strength was not increased after pairing five different FM sweeps with NB stimulation. Distributing the inputs across five octaves resulted in fewer paired inputs to each cortical sector. Although this could explain the lack of response strength plasticity, it would not explain why RF and latency plasticity were equally strong in experiments 1 and 3. In experiment 3 the addition of background sounds resulted in more sounds activating each region, with the distinction that not all were paired with NB stimulation.

Background stimuli

Studies of associative learning have shown that neuronal tuning can change both towards the conditioned frequency, when presented in silence (Bakin and Weinberger 1990; Dimyan and Weinberger 1999), or away from the paired frequency, when unpaired stimuli are presented (Ohl and Scheich 1996). Our current results indicate that background unpaired FM sweeps have a significant influence on RF and latency plasticity in A1. Stimuli or silent intervals were delivered every 10 seconds (Fig.1) therefore this influence cannot be attributed to the effect of acetylcholine at the time the background sounds are presented. Introducing only 1-s separation between the sensory input and NB activation is sufficient to block NB-induced plasticity (Metherate and Ashe 1991, 1993). Another consideration in interpreting the effects attributed to the background sounds is the difference in stimulation rate in our experimental conditions. Because we kept NB stimulation identical in each experimental group, pairing of five different FM sweeps (~300 times/day for ~20 days) resulted in fewer pairings of each sweep (i.e. frequency interval), compared to the group where a single FM sweep was paired (~300 times/day for ~20 days). However the interpretation that this might lead to less plasticity is partly incorrect when we take into consideration the different effects observed in our third group which received identical pairing in the presence of unpaired background sounds. We favor the interpretation that the differential plasticity in experiments 2&3 is due to the difference in background conditions between these two experiments, since stimulation rate was the same. Such dependence of plastic effects on contextual circumstances may improve learning in noisy environments.

Responses to FM sweeps

Neurons in several species have been shown to exhibit precise spectrotemporal selectivity to behaviorally relevant complex stimuli (Wang et al. 1995), for a review see (Suga 1989)). Our experiments were designed to explore the neural mechanisms that may give rise to this selectivity. Plasticity induced by cholinergic modulation creates changes in cortical responses specific to the experienced stimulus, thus by pairing NB activation with FM sweeps we investigated if neurons can become selective for this class of sounds. Earlier studies of FM exposure during development have suggested that direction selectivity of auditory neurons can be altered by sensory experience (Clopton and Winfield 1976; Poon et al. 1990). We observed no change in direction selectivity toward the direction of the paired sweep. This seems to be in accord with a recent study by Mercado and colleagues using FM-NB stimulation pairing, however we did not see a general increase in response strength to FM sounds as they reported. We show that neurons had a slight preference for upward direction which confirms findings by other investigators studying this species (Gaese and Ostwald 1995) and (Ricketts et al. 1998). Direct comparison however with previous studies is made difficult due to differences in FM parameters used (both frequency range and rate). Some discrepancies could be explained by the difference in the way FM sweeps are generated. For example direction selectivity maps are different for linear vs. logarithmic FM's (Nelken and Versnel 2000), and for continuous (Shamma et al. 1993) vs. separate sweeps (Heil et al. 1992; Mendelson et al. 1993). Thus it appears that FM direction selectivity depends strongly on the paradigm used to measure it.

Comparison of plasticity generated by rapidly modulated and unmodulated tones

The present results are part of a series of experiments that use identical NB stimulation paired with different sounds to determine how sensory features influence cortical response plasticity. However, most of the experiments completed to date have focused on relatively simple tonal stimuli. The long-term goal is to understand how complex input patterns direct neural plasticity. Despite the limited set of sounds tested so far, a number of important generalizations are apparent. The decreased threshold observed in the current study appears to be specific to FM stimuli as it was not observed when NB stimulation was paired with amplitude modulated or unmodulated pure tones (Table 4). Increased response strength was observed only after amplitude-or frequency-modulated tones that activated a restricted region of A1 were paired with NB stimulation. Shorter minimum latency and broader receptive fields resulted from pairing NB stimulation with narrow band FM sounds or unmodulated tones that activate a restricted receptor region. Unmodulated inputs distributed across the cochlea, was the only combination that lengthened latency and narrowed receptive fields. Although FM sweeps and pure tones generate very similar patterns of local activation, pairing a single FM sweep with NB stimulation does not generate the map plasticity that results from pairing a simple tone.

The observation that increases in bandwidth often co-occur with decreases in latency suggests the two effects could be related. For example stronger afferent inputs could explain these effects. However the processes giving rise to the observed changes are likely to be complex. Receptive field expansion or contraction and latency shortening or lengthening

could result from (i) lowering or raising of spike thresholds, (ii) increased or decreased synaptic strength, (iii) added or reduced number of connections, (iv) reduced or increased inhibition, and/or (v) shifts in the balance of inputs toward thalamo-cortical or cortico-cortical synapses respectively.

CONCLUSIONS

In summary, we show that (i) differential changes in receptive field properties of cortical neurons can be induced in adult animals by altering sensory experience, and that (ii) background sounds play an important role in shaping cortical plasticity. Temporal sharpening and broader tuning curves resulted from pairing one octave FM sweeps with NB stimulation, only when restricted to one region of the map or presented in the context of background FM sweeps. These findings add to a growing body of evidence that input statistics play an important role in guiding representational plasticity that contributes to perceptual learning. Effective rehabilitation following peripheral or central nervous system damage appears to rely on plasticity mechanisms that are likely to be guided by sensory experience. A more complete understanding of the influence specific forms of experience have on the cerebral cortex will be useful in designing more effective strategies for improving functional recovery (Grimby et al. 2003). The current results indicate the sensory context within which rehabilitation is conducted may be as important as the tasks themselves in stimulating plasticity.

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APPENDIX CHAPTER FOUR

GROUPS	Sounds Paired with NB Stimulation	Background Sounds	No. of Rats	No. of A1 sites
EXPERIMENT 1 Single FM sweep + NB	8-4 kHz (160ms)	None	3	136
EXPERIMENT 2 Five FM sweeps + NB	2-1, 4-2, 8-4, 16-8 & 32-16 kHz (160ms)	None	3	128
EXPERIMENT 3 Five FM sweeps + NB with background FM's interleaved	2-1, 4-2, 8-4, 16-8 & 32-16 kHz (160ms)	1-2, 2-4, 4-8, 8-16 & 16-32 kHz (160ms) 2-1, 4-2, 8-4, 16-8 & 32-16 kHz (40 or 640ms)	3	134
CONTROLS	None	None	7	259
Totals:			16	657

Table 4.1. Experimental design

RESPONSE PARAMETERS	SITES WITH CF's 4 to 16 kHz			SITES WITH CF's < 4 and > 16kHz		
	8→4 kHz sweep paired w/NB (n = 71)	Naïve Controls (n=130)	Change	8→4 kHz sweep paired w/ NB (n=65)	Naïve Controls (n=129)	Change
Threshold	13.01±0.87	15.0 ±0.58	-2.0 dB*	24.71 ±1.01	26.45 ±0.82	-1.73 dB
Bandwidth 20 dB above Threshold	2.10 ±0.05	1.90 ±0.06	0.19 octaves*	1.40 ± 0.08	1.46 ±0.05	-0.05 octaves
Minimum Latency	16.09±0.45	18.31±0.41	-2.22 ms**	16.83 ±1.10	17.50 ±0.63	-0.66 ms
End of Peak Latency	37.27 ± 0.62	35.87 ±0.45	1.40 ms	35.45 ±1.26	35.81 ±1.01	-0.36 ms
Mean Response to Tones	1.59 ±0.12	1.09 ±0.06	0.49 spikes**	1.35 ± 0.09	1.05 ±0.05	0.30 spikes**
Response to FM 8-4 kHz 160ms	1.41±0.15	1.12±0.09	0.29 spikes	N/A	N/A	N/A
Response to FM 4-8 kHz 160ms	1.79±0.20	1.46±0.14	0.33 spikes	N/A	N/A	N/A

Table 4.2. Pairing an 8-4 FM sweep with NB stimulation generates plasticity in the region of the cortical map activated by the sweep. Values given are the mean and standard error of the mean. P-values were derived from two-tailed t-tests. * p<0.05, ** p<0.01 compared with naïve controls. N/A = non applicable (FM 8-4kHz or 4-8kHz were not presented). The distribution of CF values in control and experimental groups was not different. For each experimental group CF range (minimum and maximum value in kHz), mean (distance away of any CF from 1kHz in octaves) and standard deviation (in octaves) were as follow: sites with CF's 4-16 kHz controls (4.23, 15.91, 3.14, 1.91), experimental (4.04, 15.85, 3.13, 1.97) and sites with CF's <4 and >16 controls (1.14, 31.92, 2.63, 4.80), experimental (1.31, 31.89, 3.07, 4.60).

FIVE DOWNWARD FM SWEEPS PAIRED WITH NB STIMULATION					
RESPONSE PARAMETERS	Naïve Controls n=259	Experiment2 in silence n=128	Change	Experiment 3 with background n=134	Change
Threshold	20.85 ± 0.62	19.20 ± 0.72	-1.65 dB	15.96 ± 0.84	-4.89 dB **
BW20	1.67 ± 0.04	1.72 ± 0.06	0.05 octave	1.85 ± 0.09	0.18 octave *
Min. Latency	17.75 ± 0.30	18.39 ± 0.52	0.64 ms	15.89 ± 0.38	-1.86 ms **
End of peak	35.57 ± 0.39	36.68 ± 0.71	1.11 ms	33.05 ± 0.46	-2.51 ms**
Response to tones	1.07 ± 0.04	0.98 ± 0.05	-0.08 spikes	1.07 ± 0.06	0.00 spikes
Response to Down 160ms FM	0.78 ± 0.02	0.60 ± 0.01	-0.18 spikes	0.42 ± 0.02	-0.36 spikes**
Response to Up 160ms FM	0.98 ± 0.01	0.77 ± 0.01	-0.21 spikes	0.69 ± 0.00	-0.29 spikes**

Table 4.3. Pairing Multiple FM sweeps with NB stimulation does not lead to significant plasticity unless background (unpaired) sweeps are also presented. Values given are the mean and standard error of the mean P-values are derived from two-tailed t-tests. * $p < 0.05$, ** $p < 0.01$ compared with naïve controls. The distribution of CF values in control and experimental groups was not different. For each experimental group CF range (minimum and maximum value in kHz), mean (distance away of any CF from 1kHz in octaves) and standard deviation (in octaves) were as follow: controls (1.14, 31.91, 2.87, 1.29), experiment 2 (1.20, 32.0, 3.05, 1.33), experiment 3 (1.18, 32.20, 2.88, 1.28).

Sounds Paired with NB Stimulation

Response Parameters	Unmodulated Single Tone	Unmodulated Single Tone + Background	Single Tone AM	Single FM	Multiple Tones	Multiple Tones AM	Multiple FM's	Multiple FM's + Background
Threshold	-	-	-	<i>Decrease</i>	-	-	-	<i>Decrease</i>
Response Strength	-	-	Increase	Increase	-	-	-	-
Bandwidth 20dB above Threshold	Increase	-	Increase	Increase	<i>Decrease</i>	Increase	-	Increase
Minimum Latency	<i>Decrease</i>	<i>Decrease</i>	<i>Decrease</i>	<i>Decrease</i>	Increase	-	-	<i>Decrease</i>
Map Plasticity	Increase	Increase	Increase	-	-	-	-	-

Table 4.4. Different forms of plasticity result from pairing single or multiple tones that are unmodulated, amplitude-modulated (15Hz), or frequency-modulated (1 octave decrease in 160 ms) paired with identical NB stimulation. Left: plasticity resulting from pairing a single FM sweep or a tone with NB stimulation were similar. Right: Plasticity resulting from pairing multiple FM sweeps or tones with NB stimulation were dissimilar

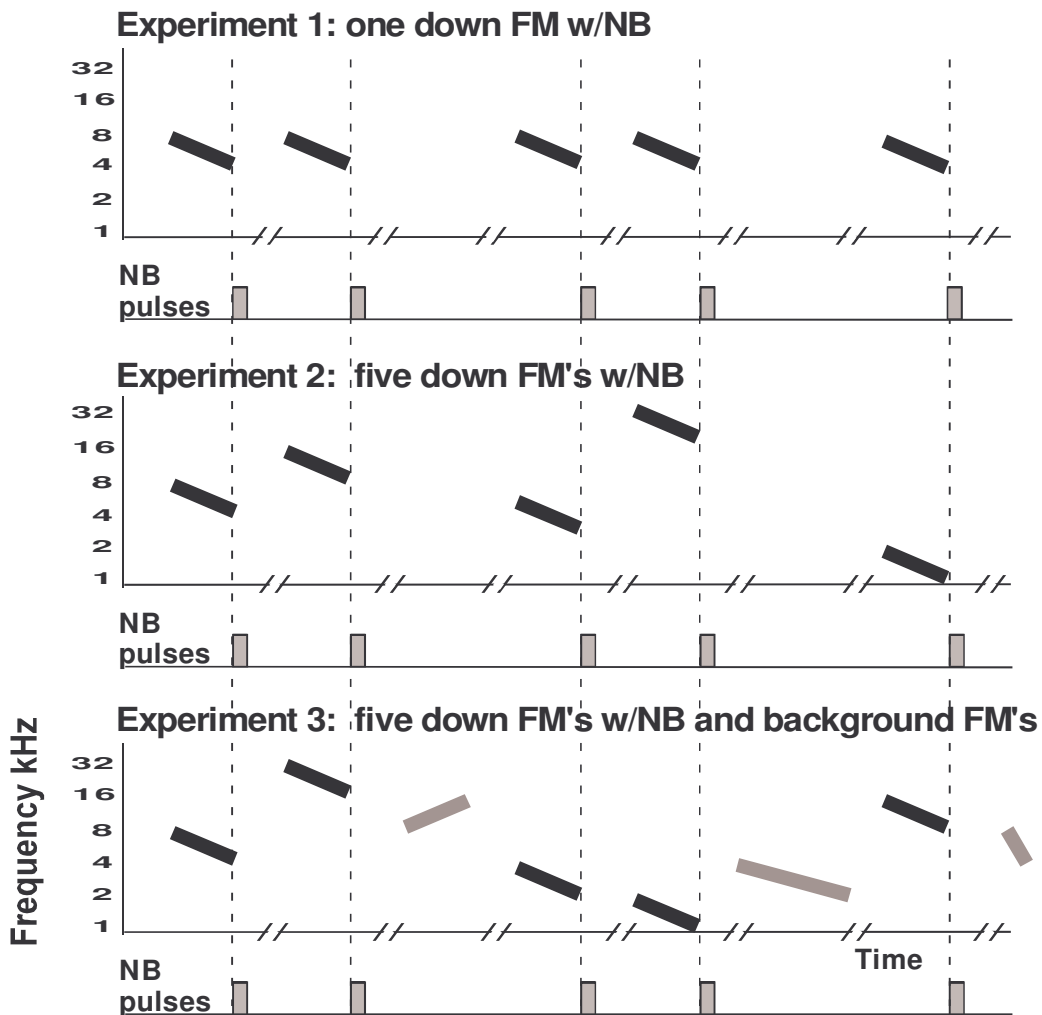


Figure 4.1. Nucleus Basalis (NB) stimulation was paired with downward one octave FM sweeps (160 ms duration) 300 times per day for 20 days. **A)** In experiment 1, an 8 to 4 kHz sweep was paired with NB stimulation (rectangles denote a train of 20 pulses). **B)** In experiment 2, five different one octave downward sweeps were paired with NB stimulation. **C)** In experiment 3, additional unpaired FM sweeps were randomly interleaved between the five sweeps paired NB stimulation. These background FM sweeps also spanned one octave, but were shorter (40 ms), longer (640 ms), or swept in the opposite direction as the paired sounds. Stimuli or silent intervals were delivered every 10 seconds (// indicate 10s gap). Total duration of daily exposure was 2-3 hrs.

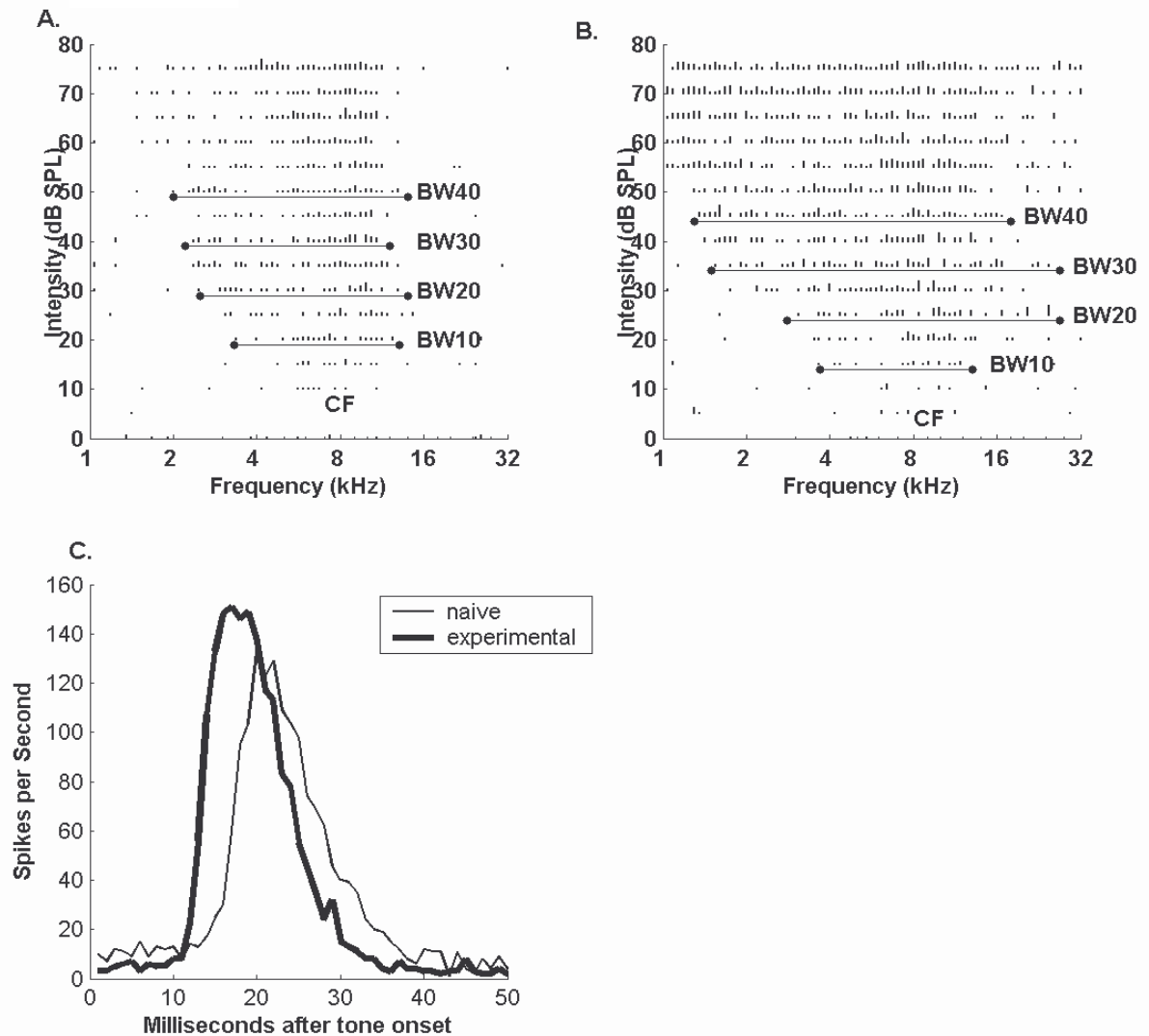


Figure 4.2. Receptive field and latency plasticity after pairing NB stimulation with FM sweeps A&B. Representative tuning curves from naïve (A) and experimental (B) animals. Frequency intensity tuning curves were derived from responses to 81 frequencies spanning 5 octaves, at 16 intensities ranging between 0 and 75 dB. The length of each line is proportional to the number of spikes. C) Peri-stimulus time histograms (PSTH), for the recording sites in A&B. Minimum response latency is defined as the time from stimulus onset to the earliest consistent response. The time to the maximum response and end of response were also quantified. Decreased latencies and neuronal thresholds, and broadened bandwidths (BW at 10, 20, 30, and 40 dB above threshold) resulted from pairing NB stimulation with FM sweeps (Tables 1 and 2).

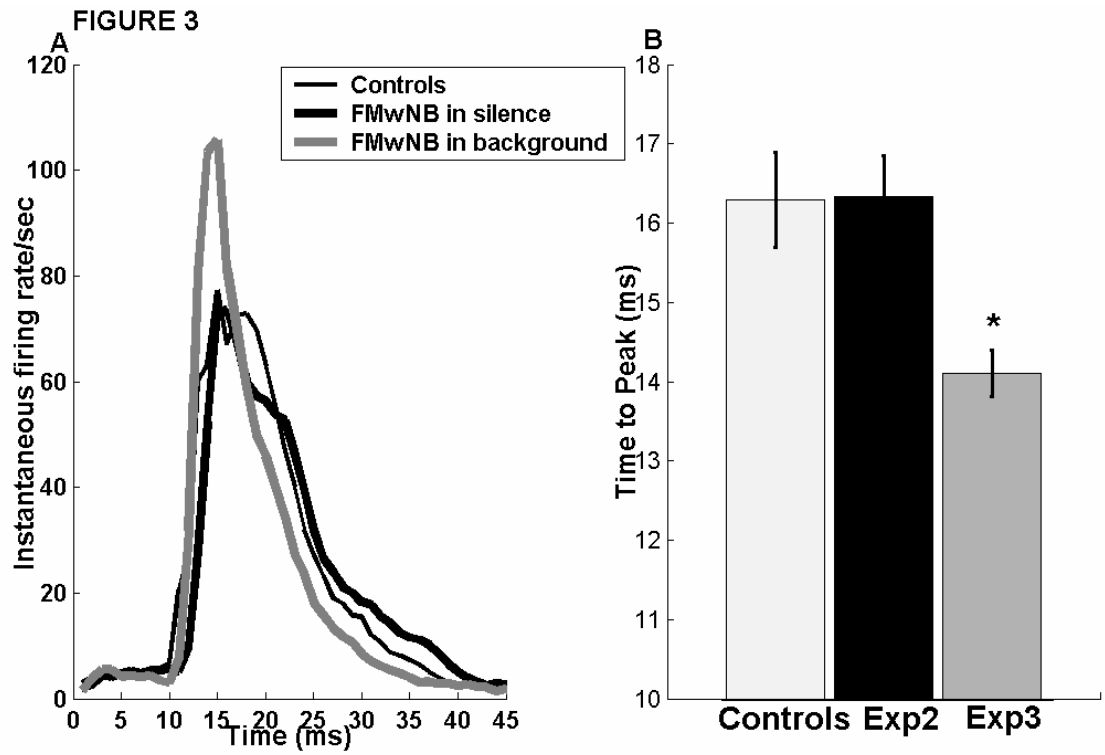


Figure 4.3. a) Population PSTH's of responses to the paired FM sweeps in control and experimental groups. The addition of background FM sweeps interleaved with the sweeps paired with NB stimulation increased the response coherence of responses to the paired FM. **b)** The addition of background sounds also decreased peak latency.

List of Abbreviations

A1	Primary Auditory Cortex
AM	Amplitude Modulated
BW	Bandwidth
CF	Characteristic Frequency
DS	Direction Selectivity
FM	Frequency Modulated
NB	Nucleus Basalis
PSTH	Post Stimulus Time Histogram
RF	Receptive Field

REFERENCES

- Bakin JS, Weinberger NM (1990) Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Res* 536: 271-286.
- Bao S, Chan VT, Merzenich MM (2001) Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature* 412: 79-83
- Clopton BM, Winfield JA (1976) Effect of early exposure to patterned sound on unit activity in rat inferior colliculus. *J Neurophysiol* 39: 1081-1089.
- Condon CD, Weinberger NM (1991) Habituation produces frequency-specific plasticity of receptive fields in the auditory cortex. *Behav Neurosci* 105: 416-430
- Das A (1997) Plasticity in adult sensory cortex: a review. *Network:Comput Neural Syst* 8: R33-R76
- Dimyan MA, Weinberger NM (1999) Basal forebrain stimulation induces discriminative receptive field plasticity in the auditory cortex. *Behav Neurosci* 113: 691-702
- Edeline JM, Weinberger NM (1993) Receptive field plasticity in the auditory cortex during frequency discrimination training: selective retuning independent of task difficulty. *Behav Neurosci* 107: 82-103
- Eggermont JJ (2001) Between sound and perception: reviewing the search for a neural code. *Hear Res* 157: 1-42.

- Engineer ND, Pandya PK, Vazquez JL, Rathbun DL, Moucha R, Kilgard MP (2001)
Auditory experience improves response characteristics in rat primary auditory cortex
neurons. In: Society for Neuroscience, vol 27, San Diego, CA
- Gaese BH, Ostwald J (1995) Temporal coding of amplitude and frequency modulation in the
rat auditory cortex. *Eur J Neurosci* 7: 438-450.
- Gilbert CD (1996) Learning and receptive field plasticity. *Proc Natl Acad Sci U S A* 93:
10546-10547.
- Grimby G, Eriksson P, Nilsson M, Sjolund B (2003) [Neurobiology provides a scientific
foundation for rehabilitation. Report from an international symposium].
Lakartidningen 100: 2052-2055
- Heil P, Rajan R, Irvine DR (1992) Sensitivity of neurons in cat primary auditory cortex to
tones and frequency-modulated stimuli. II: Organization of response properties along
the 'isofrequency' dimension. *Hear Res* 63: 135-156
- Irvine DR, Rajan R, Brown M (2001) Injury- and use-related plasticity in adult auditory
cortex. *Audiol Neurootol* 6: 192-195
- Jenkins WM, Merzenich MM, Ochs MT, Allard T, Guic-Robles E (1990) Functional
reorganization of primary somatosensory cortex in adult owl monkeys after
behaviorally controlled tactile stimulation. *J Neurophysiol* 63: 82-104
- Kelly JB, Masterton B (1977) Auditory sensitivity of the albino rat. *J Comp Physiol Psychol*
91: 930-936.

- Kelly JB, Whitfield IC (1971) Effects of auditory cortical lesions on discriminations of rising and falling frequency-modulated tones. *J Neurophysiol* 34: 802-816.
- Kilgard MP, Merzenich MM (1998a) Cortical map reorganization enabled by nucleus basalis activity. *Science* 279: 1714-1718.
- Kilgard MP, Merzenich MM (1998b) Plasticity of temporal information processing in the primary auditory cortex. *Nat Neurosci* 1: 727-731.
- Kilgard MP, Merzenich MM (1999) Distributed representation of spectral and temporal information in rat primary auditory cortex. *Hear Res* 134: 16-28.
- Kilgard MP, Merzenich MM (2002) Order-sensitive plasticity in adult primary auditory cortex. *Proc Natl Acad Sci U S A* 99: 3205-3209.
- Kilgard MP, Pandya PK, Vazquez J, Gehi A, Schreiner CE, Merzenich MM (2001a) Sensory input directs spatial and temporal plasticity in primary auditory cortex. *J Neurophysiol* 86: 326-338.
- Kilgard MP, Pandya PK, Vazquez JL, Rathbun DL, Engineer ND, Moucha R (2001b) Spectral features control temporal plasticity in auditory cortex. *Audiol Neurootol* 6: 196-202.
- Mendelson JR, Schreiner CE, Sutter ML, Grasse KL (1993) Functional topography of cat primary auditory cortex: responses to frequency-modulated sweeps. *Exp Brain Res* 94: 65-87

- Mercado E, Bao S, Orduna I, Gluck MA, Merzenich MM (2001) Basal forebrain stimulation changes cortical sensitivities to complex sound. *Neuroreport* 12: 2283-2287.
- Merzenich MM, Recanzone GH, Jenkins WM, Grajski KA (1990) Adaptive mechanisms in cortical networks underlying cortical contributions to learning and nondeclarative memory. *Cold Spring Harb Symp Quant Biol* 55: 873-887
- Metherate R, Weinberger NM (1989) Acetylcholine produces stimulus-specific receptive field alterations in cat auditory cortex. *Brain Res* 480: 372-377.
- Moore CI, Nelson SB, Sur M (1999) Dynamics of neuronal processing in rat somatosensory cortex. *Trends Neurosci* 22: 513-520
- Moucha R, Pandya PK, Vazquez J, Engineer N, Rathbun D, Kilgard MP (2001a) Plasticity of spectrotemporal coding in primary auditory cortex enabled by cholinergic modulation. In: Association for Research in Otolaryngology, St. Petersburg Beach, Florida
- Moucha R, Pandya PK, Vazquez JL, Engineer ND, Rathbun DL, Kilgard MP (2001b) Background stimuli contribute to cortical plasticity in rat primary auditory cortex. In: Society for Neuroscience, vol 27, San Diego, CA
- Nelken I (2002) Feature Detection by the Auditory Cortex. In: Popper RRFaAN (ed) *Integrative Functions in the Mammalian Auditory Pathway*, vol 1. Springer Verlag, New York, pp 358-416

- Nelken I, Versnel H (2000) Responses to linear and logarithmic frequency-modulated sweeps in ferret primary auditory cortex. *Eur J Neurosci* 12: 549-562.
- Ohl FW, Scheich H (1996) Differential frequency conditioning enhances spectral contrast sensitivity of units in auditory cortex (field A1) of the alert Mongolian gerbil. *Eur J Neurosci* 8: 1001-1017.
- Ohl FW, Scheich H, Freeman WJ (2001) Change in pattern of ongoing cortical activity with auditory category learning. *Nature* 412: 733-736.
- Poon PW, Chen XY, Hwang JC (1990) Altered sensitivities of auditory neurons in the rat midbrain following early postnatal exposure to patterned sounds. *Brain Res* 524: 327-330.
- Recanzone GH, Merzenich MM, Jenkins WM (1992a) Frequency discrimination training engaging a restricted skin surface results in an emergence of a cutaneous response zone in cortical area 3a. *J Neurophysiol* 67: 1057-1070.
- Recanzone GH, Merzenich MM, Jenkins WM, Grajski KA, Dinse HR (1992b) Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. *J Neurophysiol* 67: 1031-1056.
- Recanzone GH, Merzenich MM, Schreiner CE (1992c) Changes in the distributed temporal response properties of SI cortical neurons reflect improvements in performance on a temporally based tactile discrimination task. *J Neurophysiol* 67: 1071-1091.

- Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci* 13: 87-103
- Ricketts C, Mendelson JR, Anand B, English R (1998) Responses to time-varying stimuli in rat auditory cortex. *Hear Res* 123: 27-30.
- Sarter M, Bruno JP, Turchi J (1999) Basal forebrain afferent projections modulating cortical acetylcholine, attention, and implications for neuropsychiatric disorders. *Ann N Y Acad Sci* 877: 368-382
- Sarter M, Givens B, Bruno JP (2001) The cognitive neuroscience of sustained attention: where top-down meets bottom-up. *Brain Res Brain Res Rev* 35: 146-160
- Shamma SA, Fleshman JW, Wiser PR, Versnel H (1993) Organization of response areas in ferret primary auditory cortex. *J Neurophysiol* 69: 367-383
- Song S, Miller KD, Abbott LF (2000) Competitive Hebbian learning through spike-timing-dependent synaptic plasticity. *Nat Neurosci* 3: 919-926
- Suga N (1989) Principles of auditory information-processing derived from neuroethology. *J Exp Biol* 146: 277-286.
- Super H, Spekreijse H, Lamme VA (2001) Two distinct modes of sensory processing observed in monkey primary visual cortex (V1). *Nat Neurosci* 4: 304-310.

- Wang X, Merzenich MM, Beitel R, Schreiner CE (1995) Representation of a species-specific vocalization in the primary auditory cortex of the common marmoset: temporal and spectral characteristics. *J Neurophysiol* 74: 2685-2706
- Weinberger NM, Bakin JS (1998) Learning-induced physiological memory in adult primary auditory cortex: receptive fields plasticity, model, and mechanisms. *Audiol Neurootol* 3: 145-167.
- Wetzel W, Ohl FW, Wagner T, Scheich H (1998) Right auditory cortex lesion in Mongolian gerbils impairs discrimination of rising and falling frequency-modulated tones. *Neurosci Lett* 252: 115-118.
- Xerri C, Coq JO, Merzenich MM, Jenkins WM (1996) Experience-induced plasticity of cutaneous maps in the primary somatosensory cortex of adult monkeys and rats. *J Physiol Paris* 90: 277-287

CHAPTER FIVE
ORDER AND TIMING OF AUDITORY SIMULI DO NOT DETERMINE SHIFTS IN
TUNING OF AUDITORY CORTEX NEURONS IN VIVO

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ABSTRACT

Learning and experience change neuronal response properties in numerous ways. It is presumed that these changes result from continuous modifications in neuronal connectivity dependent on recurrence of certain activity patterns. At the cellular level spike times and order determine changes in synaptic strength - a phenomenon described as spike-timing dependent plasticity (STDP). It is not known if such synaptic mechanisms underlie plasticity observed in vivo following sensory experience. In this study we tested whether order and timing of auditory stimuli can change the response properties of auditory neurons, in the direction predicted by the STDP model.

Sprague-Dawley adult rats were implanted with tungsten multi-channel electrode arrays and recordings were made from auditory cortex in awake rats. Shifts in receptive fields (RF) were measured from isointensity tuning curves derived at each site before and after repetitive conditioning with a two-tone sequence. The tone frequencies were low (L) and high (H) with respect to the CF of the site examined, and their order and time interval within the sequences was varied.

Conditioning with a low-high sequence caused some RF to shift towards the low frequencies at tone intervals less than 35ms. Similarly a high-low sequence induced shifts towards the high frequencies. However, across the population of neurons examined there was no significant shift in tuning demonstrating stability of auditory receptive fields to this type of conditioning.

Cellular mechanisms related to spike timing between pre and postsynaptic neurons could provide a potential explanation for the short-term receptive field shifts observed, since timing

of tonal activation directly influences spike timing in auditory neurons. Nevertheless our method did not verify this hypothesis because overall we could not measure reliable shifts induced by our protocol. This is consistent with other observations in vivo (Schulz et al, 2004). Potential impediments for seeing this type of plasticity in vivo preparations are discussed.

INTRODUCTION

Experiments in mammalian sensory cortex have shown that large populations of neurons are substantially reorganized when required to learn novel stimuli and adapt to changing situations. The expression and implementation of this plasticity depends on the statistics of specific input patterns and the context in which the information is experienced. The exact mechanisms of such dynamic shifts in network properties are not known but believed to be the result of correlation-based Hebbian plasticity. Thus sensory input patterns that induce correlated firing of neurons are more likely to induce changes than input patterns that decorrelate neuronal spikes. In somatosensory cortex manipulations designed to increase or decrease input correlation using behavioral training support this hypothesis. Following tactile training in owl monkeys, stimuli applied synchronously to three fingers resulted in these fingers being integrated in their representation, whereas fingers to which stimuli were applied asynchronously became segregated in their representation (Wang et al., 1995). In the visual system alternating asynchronous electrical stimulation of the optic nerve prevents normal development of binocular visual responses (Stryker and Strickland, 1984), while monocular electrical stimulation results in stronger response to the activated eye, and the

retention of most binocular responsiveness (Ohshima et al., 2002). In vitro and more recently in vivo studies have further demonstrated that the time window for correlated inputs to induce plasticity is on the order of tens of milliseconds (Tsodyks, 2002; Dan and Poo, 2004). This is known as spike timing dependent plasticity (STDP) and is based on the finding that at the cellular level the order of pre and postsynaptic spikes can produce either depression or potentiation when they occur within 10 ms (Markram and Tsodyks, 1996; Bi and Poo, 1998). Spike timing-dependent modification of synaptic efficacy is a possible underlying mechanism leading to experience-dependent changes of neuronal response properties. Modifying strength of specific synapses as predicted by the rules of STDP would cause the receptive field (RF) of a cell to shift toward strengthened inputs and away from weakened inputs. In cat visual cortex (Yao and Dan, 2001), provided evidence that shifts in direction tuning of V1 neurons are determined by the order and timing of pairs of visual stimuli. Direction tuning shifted towards the direction of a repeatedly presented moving grating within a pair of gratings of different directions (+15 and - 15 degrees from the preferred direction of the neuron) when it was presented 8ms ahead from the second grating and not when it was 8ms after it. Their study showed for the first time that rules which operate at the synapse level could be reflected in the plasticity dynamics of networks of neurons in vivo. STDP was also demonstrated in somatosensory barrel system in vivo. Using whisker deprivation patterns the spike timing that normally occurs within the barrel cortex in response to whisker stimulation can be manipulated causing neurons in layer IV to fire before layer II or vice-versa. Such manipulations showed that in the barrel system there is a good

correlation between the effect of STDP at the cellular level and in vivo (reviewed in (Fox and Wong, 2005)).

In primary auditory cortex timing of auditory stimuli can directly affect timing of neuronal spiking and thus may play a role in activity dependent plasticity. Based on STDP predictions receptive fields of auditory neurons would shift closer to or away from the regions activated during repetitive stimulation, depending on the timing and order of consecutive auditory inputs (Fig 2). Such short-term shifts in tuning are potential underlying mechanisms for the plasticity observed after long-term experience with spectro-temporally complex sounds. Here we tested whether parametric manipulations in order and timing of consecutive auditory inputs (such as tone pairs or frequency modulated sweeps) result in RF shifts of auditory cortical neurons in the direction predicted by STDP. After repetitive sequential activation with a low followed by a high frequency tone, separated by 10 or 20 ms we predicted a shift in neuronal tuning towards the lower side of the frequency spectrum. Similarly repetitive presentation of upward FM sweeps at a rate of 20ms/octave would induce a shift towards lower frequencies due to always activating the low frequency neurons first.

METHODS

A total of 39 Sprague Dawley adult rats were used for this experiment. Thirty six rats were implanted with multichannel electrode arrays for awake recordings. In three rats recordings were done under barbiturate anesthesia to test the influence of state on auditory cortical responses. Recordings from nine of the implanted rats were used for analysis. The

remaining rats were eliminated for various reasons: complications during acute surgery, nonfunctioning electrodes, not enough days of stable recordings. Only rats from which stable recordings could be obtained for a minimum of three consecutive days were included in analyses. Stable recordings could be obtained for up to two months in some rats.

Chronic Implantation for Awake Multichannel Recordings

Animals were pentobarbital anesthetized (50 mg/kg body weight), and maintained in a state of areflexia throughout the surgical procedure and during recordings. To ensure a proper level of anesthesia and stable physical condition, the animal's electrocardiogram and blood oxygen concentration were also monitored. The skull was supported in a head holder leaving the ears unobstructed. Rats received prophylactic treatment with ceftizox antibiotic (20 mg/kg), dexamethazone (4 mg/kg), and atropine (1 mg/kg). Six bone screws were used to anchor the electrode assembly. The temporalis muscle was reflected and partially removed to expose the temporal bone. The auditory cortex was exposed via a wide craniotomy, and the dura mater resected. Recording of action potentials was made in a double-walled sound chamber from two Parylene-coated tungsten microelectrodes to a depth of 550 μm (layers IV/V) to determine the location of A1. Once the auditory responsive area was delineated a multichannel (3x5) array of polyimide-insulated electrodes (Neural Engineering Lab, Dr. Rennaker, OU) was then lowered orthogonally into auditory cortex (Fig 1) to a depth of 550 μm (layers IV/V). The array was made of sixteen 50 μm diameter tungsten wires spaced 250 μm center-to-center. The wires were insulated with polyimide and had an average impedance of 60 k Ω at 1 kHz in saline. Fourteen wires were used for recording and the remaining two were used as reference and ground wires. The reference

wires (50 μm diameter tungsten , Teflon insulation) were embedded in the array and stripped of their insulation prior to implantation. Teflon was used, because it can be easily removed from the wires chosen to act as a low impedance reference. The brain was then sealed with a thin layer of kwik-cast (World Precision Instruments, FL, USA) and the array was cemented to the skull using nail acrylic. The animals were monitored during recovery and received post-surgical antibiotic treatment for five days after implantation.

Acoustic Stimulation

For awake recordings animals were placed in sound attenuated boxes. Frequency and intensity calibrations were made with an ACO Pacific microphone (PS9200-7016) and Tucker Davis SigCal software. The speaker was positioned 20 cm from the ear at 0° elevation and 90° azimuth and rats were allowed to move freely within a small cage during sound presentation and recordings. A custom-made 18-channel commutator (Plastics One, Roanoke, VA) allowed for movement while maintaining the connection from the implant and the high impedance headstage to a digital biological amplifier. Tucker Davis hardware (RP2 Real Time Processor, RX5-2DSP Pentusa Base Station) and software (RPvds, SigGenRP, Brainware) was used for stimulus generation and extracellular multiunit recordings.

Isointensity tuning curves were derived for each channel from presentation of 25 frequencies ranging from 1-32 kHz in 1/5 octave steps, at ~25 dB above neuronal threshold, randomly interleaved at 1s separation, 60 repetitions each. After this baseline was established one of two acoustic stimulation patterns were presented: either a tone pair or an frequency modulated (FM) sweep. The two-tone sequence consisted of a low frequency (L) and a high frequency (H) tone. The tone frequencies were selected as a function of each sites tuning

such that they were within the excitatory frequency range of the site, and were separated by 7 log-steps. For example at a site with tuning bandwidth spanning 1-3.5 kHz we played a 1.15 kHz (L) and a 3.16 kHz (H) tone. The order of the tones within the sequence was either L-H, H-L, L-L or H-H. Each tone was 20 ms duration and the intervals between the onsets of the two tones (stimulus onset asynchrony, SOA) was either 25, 30, 35, 45, 65 or 85 ms. The tone sequences were separated by 500 ms and repeated 200 times. The FM sweeps were logarithmic upward 1-32 kHz, at one of three durations/rates 50 ms (100 oct/s), 100 ms (50 oct/s) or 200 ms (25 oct/s). The sweeps were continuously played for 400 repetitions. Because of the high number of manipulations not all stimulation protocols (i.e. orders and intervals) could be tested during a typical eight-hour recording session and thus were divided out over several days.

Data Analysis

For the short-term plasticity experiments shifts in tuning were quantified after short term experience with either FM sweeps of three different rates or two-tone sequences at two different orders and six different intervals as described above. We calculated the difference between the average number of spikes evoked by each tone frequency of the 25 presented to determine the isointensity tuning curves before and after the conditioning protocol (pre response minus post response). Negative values for frequencies lower than the CF indicated a leftward shift in tuning while positive values for frequencies higher than CF indicated a rightward shift towards high frequencies. All calculations had spontaneous spiking activity subtracted. Spontaneous activity was calculated and averaged from 10 ms recordings prior to

sound presentation. All analysis was conducted using custom Matlab (MathWorks, Natick, MA) programs.

RESULTS

We hypothesized that short-term plasticity in auditory cortex may result as a function of timing and order between subsequent auditory stimuli *in vivo*. This hypothesis was based on evidence that at the cellular level timing and order between pre and postsynaptic spikes determine the direction and magnitude of synaptic plasticity known as STDP.

We analyzed extracellular neuronal recordings from chronic multichannel electrode arrays over auditory cortex in nine adult Sprague Dawley rats. To determine the effect of cortical state on neuronal responses, in three non-implanted rats recordings were obtained under pentobarbital anesthesia. We evaluated the effect of repetitive conditioning with pairs of auditory stimuli on auditory frequency tuning. The order and timing between stimuli was varied to assess the effects of different activation conditions.

Conditioning induced changes in frequency tuning in auditory cortex

To quantify shifts in frequency tuning we recorded isointensity tuning curves of auditory neurons before and after conditioning with one of two sets of auditory stimuli. One set was a tone sequence of a High (H) and Low (L) frequency tone presented in four different combinations (orders): H-L, L-H, H-H, L-L at 25ms SOA. Each order was presented 200 times at a repetition rate of 2 Hz. The second stimulus set was a frequency modulated sweep, changing continuously in frequency from either L-H (upward sweep) or H-L (downward

sweep), presented 400 times constantly (what it non-stop or just 2Hz). The frequency tuning of each site was compared pre and immediately post conditioning by subtracting post-pre tuning curves. Some sites exhibited shifts in tuning resembling STDP predictions. Figure 2 shows shifts in tuning dependent on order of stimulation, after conditioning with a L-H vs. H-L tone sequence. Repeated presentation of a L followed by a H tone at 35 ms SOA caused a significant increase in responses to the first stimulus in the sequence (L) and a shift in tuning towards low frequencies (Fig2,b). By comparison, repeated presentation of a H followed by a L tone at the same site caused a significant decrease in responses to the second stimulus in the sequence (L) causing a shift in tuning towards high frequencies (Fig2, a). Both changes were frequency specific, increases and decreases occurred at the frequency of the low tone 1.15 kHz and not at neighboring frequencies 1 or 1.3 kHz (Fig 2,c). However no significant changes were measured in responses to the frequency of the H tone in the sequence regardless weather it was first or second presented within the sequence. Dependence of tuning shifts on order of activation was not consistent across all recordings. Mean responses from all channels before and after conditioning were not significantly different regardless of the stimulus order in both tone sequences or FM sweeps (data not shown). As might be evident from Fig 2, the lack of a change across all the sites examined was not always due to lack of plasticity at individual sites. Rather there was no consistent pattern or direction of changes in frequency preference. Tuning curves could be narrowed, broadened, shifted high or low, or left unchanged with little correlation to the stimulus configuration used in conditioning. Figure 3 shows examples of some other forms of plasticity observed.

Effect of timing of auditory activation

If STDP like mechanisms are underlying frequency plasticity conditioned changes should depend on the temporal proximity between auditory stimuli. We evaluated the influence of spacing between stimuli by presenting the same tone sequences previously described at six different SOA's (25, 30, 35, 45, 65 or 85 ms), and the FM stimuli at three different sweep rates (25, 50, 100oct/s). Frequency tuning was compared before and after conditioning with each interval. We observed no effect of interval between consecutive auditory stimuli on magnitude of RF shifts (Fig 5). Frequency tuning did not significantly change at any of the tested SOA's or FM rates.

DISCUSSION

We evaluated the effects of varying tone sequence order and inter-tone intervals on direction of plasticity in auditory cortex neurons. Based on our conditioning protocol we could not verify the hypothesis that shifts in frequency tuning follow STDP-like rules in the rat auditory cortex in vivo. On average frequency tuning of cortical sites remained stable after hundreds of consecutive presentations of two-tone sequences and FM sweeps designed to induce RF shifts based on STDP. Our results are in conflict with in vitro and in vivo reports from other modalities. Plasticity dependent on order and timing of sensory activation has been documented in the visual and somatosensory systems (reviewed in (Fox and Wong, 2005). Differences in techniques and possible modality specific mechanisms could account for the disparate findings and are discussed here.

1) Only few studies of STDP have been conducted in vivo, in visual and somatosensory cortices, using either whole cell recordings (Shulz et al., 2004; Meliza and Dan, 2006) or extracellular recordings from single neurons (Yao and Dan, 2001; Fu et al., 2002; Celikel et al., 2004). If the effects of spike timing dependent plasticity are only measurable at single synapses our method of multiunit extracellular recording cannot sort out such effects as we are recording responses from groups of cells located in the proximity of our electrode tip.

2) No studies yet have been conducted in awake preparations. In slice preparations of *Xenopus* visual tectum, activity induced synaptic plasticity is reversed either by subsequent spontaneous activity or by exposure to random visual inputs (Zhou et al., 2003). This finding suggests that the lack of plasticity in our preparation could be attributed to cancellation of the effect due to higher spontaneous levels of neuronal activity during awake states. Even though our results were not different under barbiturate anesthesia, due to our limited sample of recordings under anesthesia, we cannot rule out the influence of increased spontaneous activity in the awake state.

3) Our electrode arrays were chronically cemented in place over auditory cortex at 500 μ depths. Possible variations in brain swelling post surgery result in variations in recording depth as the array cannot move with the brain tissue. In visual system different plasticity effects have been reported in superficial layers II/III (cortico-cortical synapses) versus layer IV (thalamo-cortical synapses). STDP effects were only measurable in layers II/III (Schuett et al., 2001). Transfer of the effects to the contralateral hemisphere also

suggests that such plasticity is predominantly cortico-cortical in origin in the visual system since V1 is the first station integrating information from both eyes (Yao et al., 2004).

Therefore if our recording depth varied depending on the condition of the brain over time it may explain why plasticity effects were inconsistent as we may have recorded from different layers at different times. The only way to verify this would have been to perform histology after each recording session, which would have been impractical in our design.

4) To maximize our yield of recording sites we used a dense 16 channel microwire array. Due to spacing requirements between wires the breadth of the array was slightly larger than the surface area of A1, which meant that some electrodes were possibly placed in secondary auditory fields. Neurons in secondary auditory fields can undergo plasticity that is in the opposite direction from A1 (Puckett et al., 2003). Even though only a minority of our channels could have been placed outside A1 (due to mapping of A1 prior to insertion) this was an additional source of noise in our data.

5) The optimal stimulus parameters to induce short term plasticity may differ in the auditory modality compared to visual or somatosensory. Stimulus specific adaptation in A1 has several time scales concurrently, spanning many orders of magnitude, from hundreds of milliseconds to tens of seconds (Ulanovsky et al., 2004). In addition forward masking of responses to a second stimulus in a pair depends not only on time interval but also on frequency distance between two consecutive tones (Brosch and Schreiner, 1997). Using the best frequency of a site in a two-tone pair to match the visual protocol where the best orientation of a V1 neuron was chosen (Yao and Dan, 2001) would always mask the response to any second stimulus.

6) The STDP rule in the auditory system may have slightly different characteristics. Experiments performed in slice at ascending synapses from layer IV to layer II/III cells in A1 reveal a temporal asymmetry that resembles STDP at positive EPSP-spike pairing intervals of 5-20ms, but not when pairing order is reversed (Manis et al., 2003).

7) Finally, it is possible that the auditory system is more resilient to short-term changes. Adaptation mechanisms underlying visual illusions such as after image effects or motion after effects are not known in the auditory system.

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APPENDIX CHAPTER FIVE

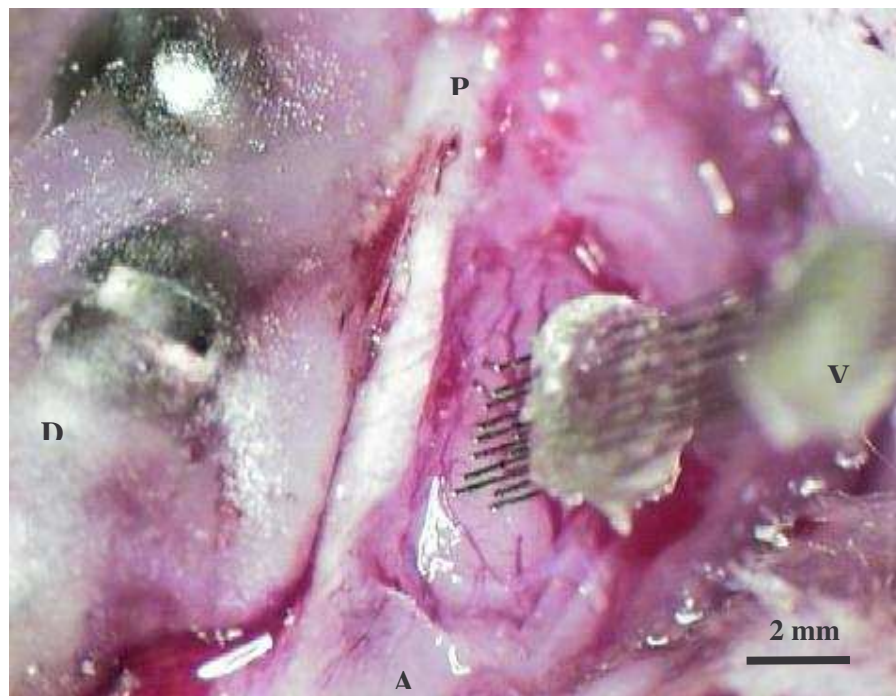


Figure 5.1. Positioning of the electrode array over auditory cortex before insertion

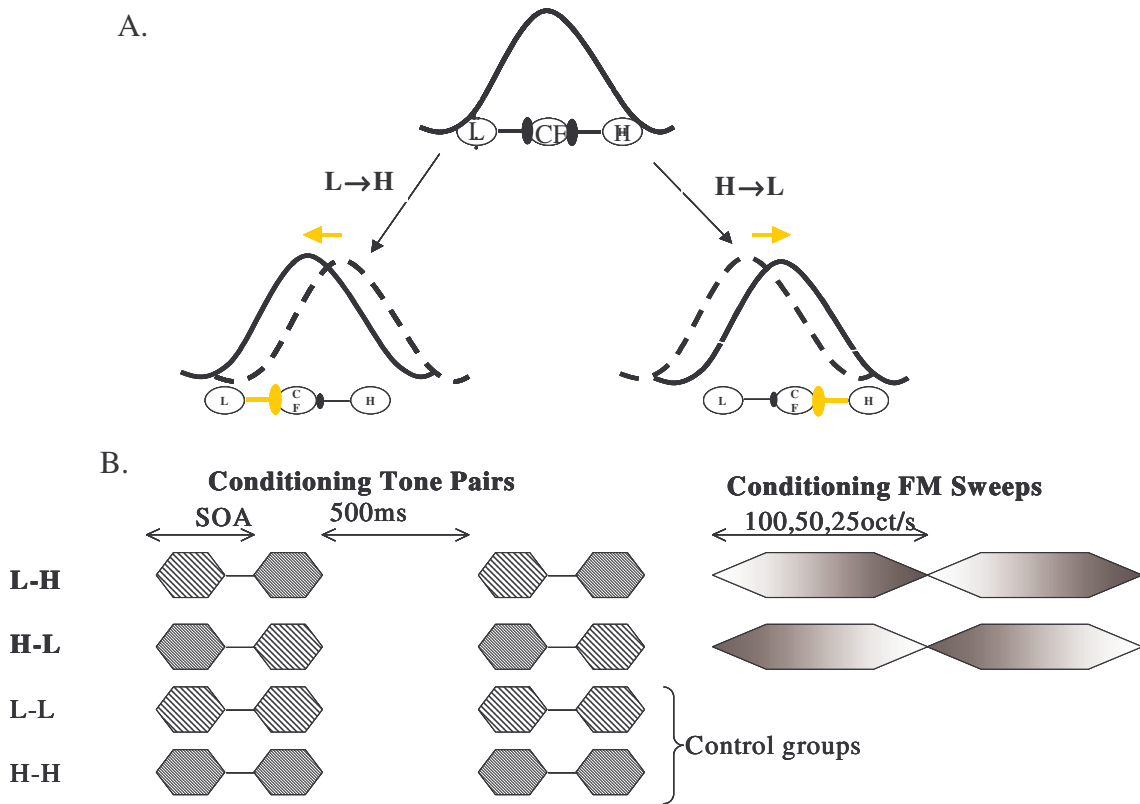


Figure 5.2. a) A simplified schematic in which spike timing dependent modification of intracortical connections can result in shifts in frequency tuning of auditory neurons. Circles represent cortical units and their preferred frequency is indicated by the text inside (L=low frequency tuned site, H=high frequency tuned site). CF is the central frequency of the unit recorded and its bell-shaped tuning curve is shown above. L→H represents conditioning with tones activating low frequency tuned sites before the high ones, while H→L indicates conditioning in the opposite direction. The thickness of connecting lines between circles symbolizes synaptic strengths. Tuning curves below show expected shifts in frequency tuning based on spike timing dependent strengthening and weakening of intra-cortical connections. b) Experimental Design for the Induction of Shifts in Frequency Tuning with Paired Conditioned Stimuli. Two types of conditioning stimuli were used in our study: conditioning tone pairs and conditioning FM sweeps. Each stimulus set was designed to generate two main activation patterns (orders) L→H and H→L. Thus tone pairs consisted of two tones of frequencies lower and higher than CF: L-H, H-L and FM's were sweeping upward (low to high) or downward (high to low) covering the whole frequency range from 1-32kHz. Two additional tone pairs were presented to serve as controls H-H and L-L.

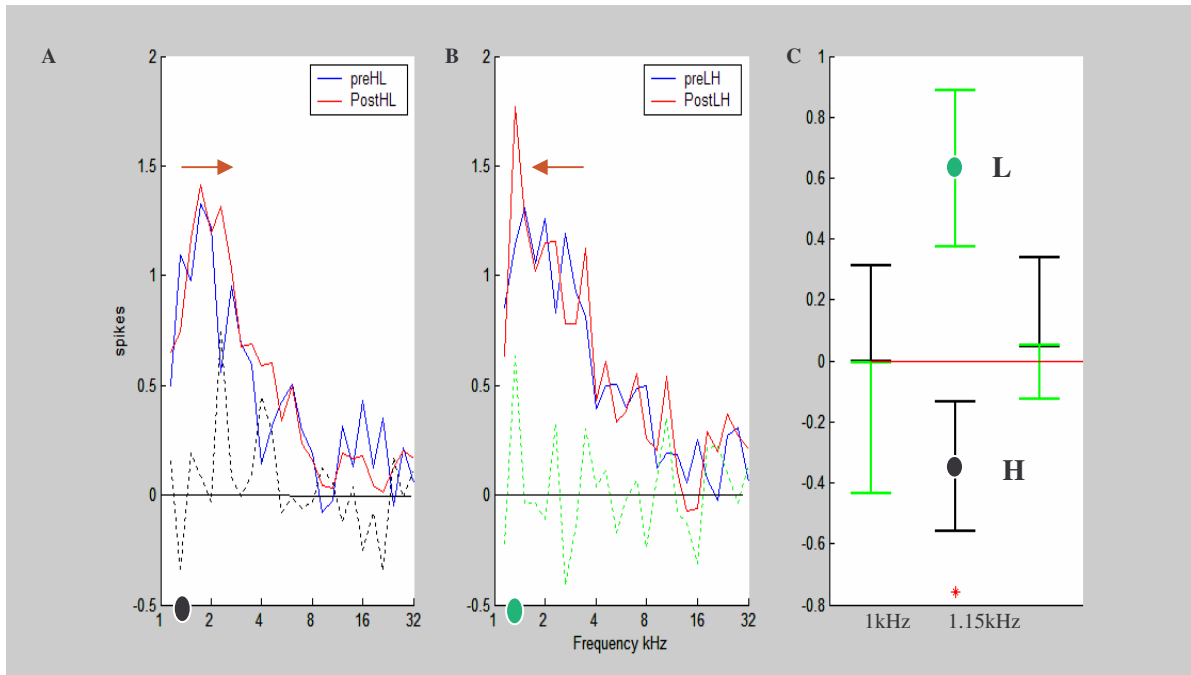


Figure 5.3. Shifts in frequency tuning dependent on order of conditioning tones, in one rat. Isointensity tuning before (blue), and after (red) conditioning with a H-L sequence (a), and a L-H sequence (b). Dotted lines (black or green) are the pre-post difference in tuning after HL and LH conditioning respectively. A positive value indicates an increase in response to a specific frequency after conditioning, while negative values indicate a decrease. Increases in responses to high frequencies and decreases to low indicate a receptive field shift towards high frequencies as shown in (a). A shift in the opposite direction is shown in (b). Panel (c) shows the mean differences before and after between the responses to the conditioned L frequency (1.15 kHz) compared to two adjacent frequencies (1 and 1.3kHz). Responses to L increased after L-H conditioning and decreased after H-L conditioning. Responses to adjacent frequencies did not change. Data is pooled from different days from the same channel, in one rat.

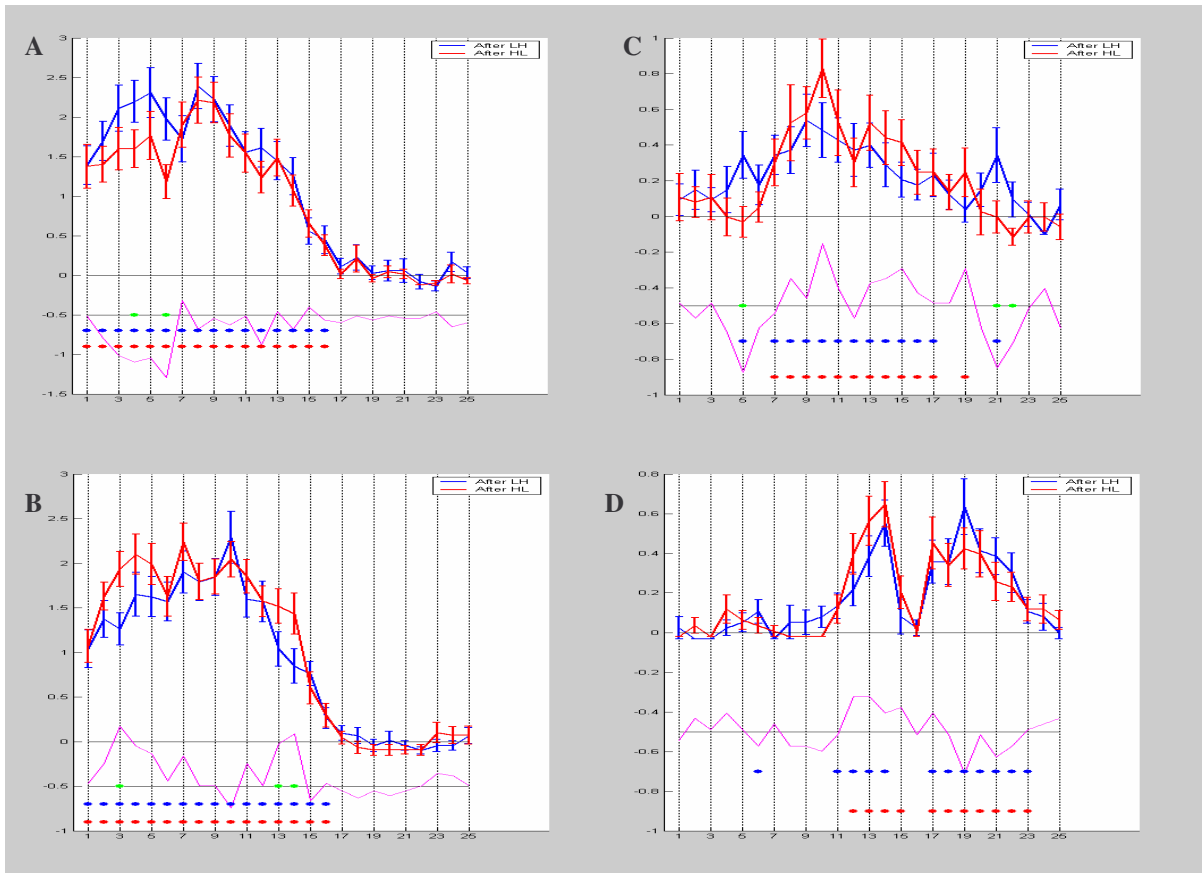


Figure 5.4. Examples of frequency plasticity at individual sites, following two-tone conditioning. Each subplot shows frequency tuning curves following conditioning with L-H (blue) and H-L (red) tone pairs. The purple line below shows the difference function between the two. Green dots point out significant differences between L-H and H-L induced frequency shifts, blue and red dots indicate the frequency response range (bandwidth) of each tuning curve. Repeated H-L activation resulted in a significant decrease of responses to low frequencies as compared to L-H activation (**a**). At a similarly tuned site the same conditioning caused the opposite change (**b**) and an additional increase of responses to high frequencies (red vs blue) with no change in bandwidth of tuning. Panel (**c**) shows an example of narrowing in tuning after H-L activation, compared to L-H. The site in panel (**d**) shows no significant difference in frequency tuning following conditioning with either stimulus.

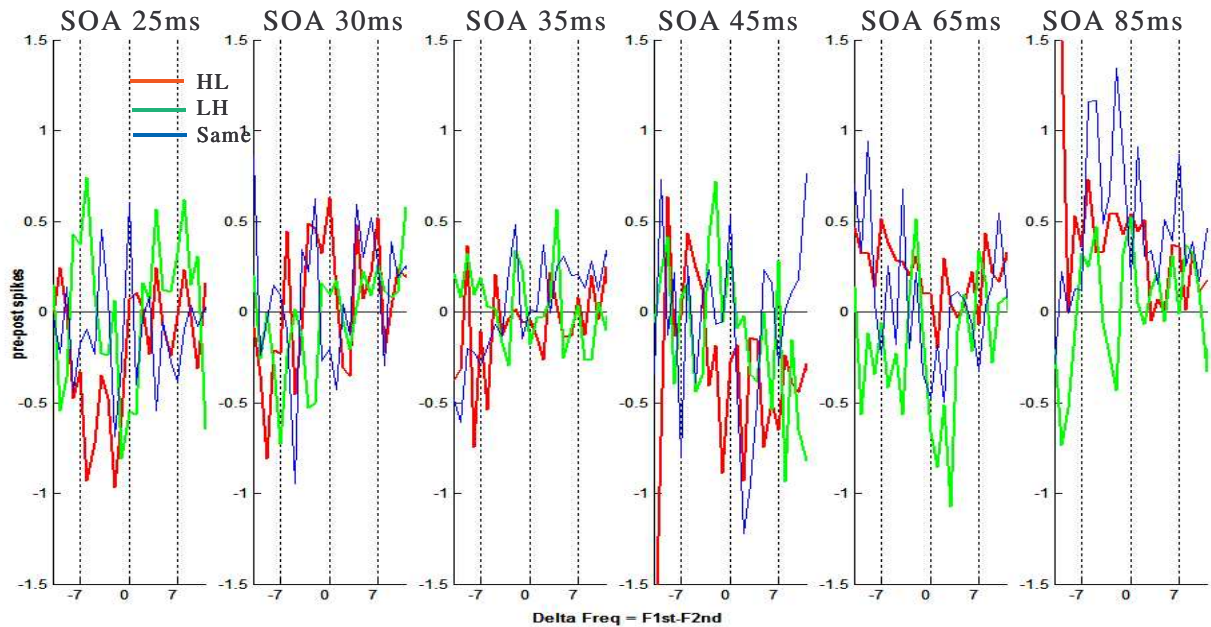


Figure 5.5. Group data showing shifts in tuning for the different orders and intervals between tone stimuli. Each subplot is data after conditioning with a different interval. Plots show pre minus post difference in responses to the 25 frequencies presented for the tuning curve. Green lines show pre-post responses after L-H, red lines after H-L and blue lines after H-H and L-L conditioning. On the abscissa zero represents the frequency of the first tone (F1) in the sequence. Negative and positive values indicate that the frequency of the second tone (F2) was higher (L-H) or lower (H-L) than F1, specifically F2 was always seven frequency steps away from F1. Thus if response changes were specific to the frequencies of the tone pairs, differences would be apparent at points zero (F1) or ± 7 (F2). An increase in responses to low frequencies after L-H conditioning would be indicated by a positive difference between pre and post (green line) in the left quadrant. An increase in responses to high frequencies after H-L conditioning (red line) would be indicated by a positive difference between pre and post in the right quadrant. This data shows no significant differences between pre and post responses after conditioning at any of the tested intervals. Shifts in any direction and at any frequency can be observed for any time intervals of the sound pairs.

REFERENCES

- Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J Neurosci* 18:10464-10472.
- Brosch M, Schreiner CE (1997) Time course of forward masking tuning curves in cat primary auditory cortex. *J Neurophysiol* 77:923-943.
- Celikel T, Szostak VA, Feldman DE (2004) Modulation of spike timing by sensory deprivation during induction of cortical map plasticity. *Nat Neurosci* 7:534-541.
- Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. *Neuron* 44:23-30.
- Fox K, Wong RO (2005) A comparison of experience-dependent plasticity in the visual and somatosensory systems. *Neuron* 48:465-477.
- Fu YX, Djupsund K, Gao H, Hayden B, Shen K, Dan Y (2002) Temporal specificity in the cortical plasticity of visual space representation. *Science* 296:1999-2003.
- Manis P, Wu H, Wang Y, Fleming R, Bortone D (2003) Synaptic plasticity at ascending synapses on Layer II/III neurons in rat primary auditory cortex. In: *Society for Neuroscience*. Washington DC.
- Markram H, Tsodyks M (1996) Redistribution of synaptic efficacy between neocortical pyramidal neurons. *Nature* 382:807-810.
- Meliza CD, Dan Y (2006) Receptive-field modification in rat visual cortex induced by paired visual stimulation and single-cell spiking. *Neuron* 49:183-189.

- Ohshima M, Hata Y, Ichisaka S, Wakita M, Fukuda M, Kameyama K, Tsumoto T (2002) Chronic electrical stimulation of afferents from one eye changes ocular dominance of visual cortical neurons in kittens. *J Neurophysiol* 88:2147-2151.
- Puckett A, Pandya P, Dai W, Engineer N, Moucha R, Rathbun D, Kilgard M (2003) Nucleus Basalis Induced Plasticity in Primary and Posterior Auditory Fields. In: International Conference on Auditory Cortex. Magdeburg, Germany.
- Schuett S, Bonhoeffer T, Hubener M (2001) Pairing-induced changes of orientation maps in cat visual cortex. *Neuron* 32:325-337.
- Shulz D, Brasier D, Feldman D (2004) Spike-timing dependent plasticity investigated using whole-cell recording in rat somatosensory cortex in vivo. In: Society for Neuroscience Meeting. San Diego.
- Stryker M, Strickland S (1984) Physiological segregation of ocular dominance columns depends on the pattern of afferent electrical activity. *Invest Ophthalmol Vis Sci Suppl* 25:278.
- Tsodyks M (2002) Spike-timing-dependent synaptic plasticity - the long road towards understanding neuronal mechanisms of learning and memory. *Trends Neurosci* 25:599-600.
- Ulanovsky N, Las L, Farkas D, Nelken I (2004) Multiple time scales of adaptation in auditory cortex neurons. *J Neurosci* 24:10440-10453.
- Wang X, Merzenich MM, Sameshima K, Jenkins WM (1995) Remodelling of hand representation in adult cortex determined by timing of tactile stimulation. *Nature* 378:71-75.

- Yao H, Dan Y (2001) Stimulus timing-dependent plasticity in cortical processing of orientation. *Neuron* 32:315-323.
- Yao H, Shen Y, Dan Y (2004) Intracortical mechanism of stimulus-timing-dependent plasticity in visual cortical orientation tuning. *Proc Natl Acad Sci U S A* 101:5081-5086.
- Zhou Q, Tao HW, Poo MM (2003) Reversal and stabilization of synaptic modifications in a developing visual system. *Science* 300:1953-1957.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

A task of principal importance in neuroscience is to understand how the nervous system is modeled by the sensory experiences we have as we interact with our environment. What pattern of activity in the brain gives us the percept of a sensory stimulus, for example a sound, and how does this pattern change as certain stimuli become important? The goal of my dissertation research was to characterize how auditory neurons represent and adapt to experience with spectro-temporally complex sounds. I have selected an important class of stimuli frequency modulated (FM) sweeps. FM's are sounds that change in frequency over time resulting in a moving pattern of activation across the cochlea (analogous to brushes applied to the skin or moving bars of light activating the retina). Due to their resemblance to stimuli that are more likely encountered naturally, moving stimuli have been extensively used to characterize neuronal responses and plasticity in other modalities such as the somatosensory and visual systems. FM sweeps are ubiquitously encountered in nature as components of communication sounds in many species including birds, rodents, and primates. As formant transitions they are important perceptual cues in speech sounds.

Despite the fact that natural FM sweeps are narrow in frequency range, primarily broad FM sweeps covering up to five octaves have been previously studied. In chapter three I have characterized responses of auditory neurons to one octave FM sweeps at different processing levels in the auditory system: thalamus, A1 and PAF. My findings reveal very important aspects related to coding of narrow FM sweeps, the influence of receptive field

(RF) differences between auditory fields on the responses to these sounds in each field, and the importance of forward masking. Previous studies have used FM sounds designed to exclude any masking caused by FM onsets by i) using broad FM's starting very far from the RF, ii) using sinusoidal modulation or iii) embedding a sweep into a continuous tone. By choosing narrow excursion sweeps and viewing the onset response as part of the natural coding mechanism I have shown that auditory neurons do not exhibit coding strategies as predicted by studies of broad excursion or onset masked FM's. Direction and rate selectivity do not follow the same rules for a narrow FM versus a broad one. For narrow FM's direction preference is determined by the start frequency of the sweeps in relation to the RF of each unit. Thus RF characteristics have more importance than previously thought, to the extent that response variations in distinct auditory areas can be explained by their RF differences such as size and asymmetry. Rate selectivity also depends on start frequency of short FM's. Only neurons that were not masked by the onset of the sweep (i.e. not tuned to the onset frequency) exhibited different firing patterns to different speeds of the sound. Thus depending on the frequency range traversed by FM sweeps, specific groups of neurons code different FM parameters. These findings provide a strong argument that results derived from studies of simple or even complex stimuli that are unnatural are not always likely to explain or predict how more natural stimuli are coded by sensory neurons.

Similarly, experience with spectro-temporally complex sounds such as FM sweeps resulted in plasticity that was not predictable from plasticity described after experience with simple tones. The topic of chapters four and five was to characterize changes in responses of

primary auditory cortex neurons after short and long-term experience with one octave FM's. When modulated or unmodulated tonal stimuli were repeatedly paired with nucleus basalis (NB) activation the resulting changes at the level of A1 were an increase of the frequency area responding to the paired tone frequency and a RF broadening. Pairing FM sweeps with NB decreased response latency, broadened receptive fields, and increased sensitivity to quiet tones. Interestingly, these changes were restricted only to A1 regions activated by the sound frequency range, but did not cause an expansion of this cortical region as previously seen with tones. Even though the responses to tones and one octave FM's in A1 are similar in that they evoke a burst of spikes over a time window of 10 – 20 ms from the sound onset, the changes in the response following repeated pairing with tones versus FM's were not similar. Experience with modulated tones of different carrier frequencies that resulted in distributed activation of several frequency regions changed the rate of neuronal responses. Maximum following rate increased or decreased to match the rate of the tone trains paired with NB. When the starting frequencies of the FM sweeps were varied to distribute the activation across the map of frequency no plasticity occurred. Thus far my findings suggest that the brain exhibits different "learning" strategies for stationary versus moving stimuli. Pairing NB stimulation with downward one octave FM's that covered five different octaves only resulted in threshold and latency plasticity if other FM's of different rates and direction were also presented but not paired. This suggests that neural plasticity can be modulated by background stimuli previously thought to be irrelevant. In summary all these findings provide additional evidence that forms of experience dependent plasticity are reliably related to the nature of sensory stimulation. Changes induced at the cortical level directly depend on whether

experienced stimuli are stationary or moving across the receptor area, activate a focal region or are distributed, and whether background stimuli are present or not. A better understanding of the influence of each of these parameters on possible outcomes of plasticity provides an important insight that could be used to direct specific forms of cortical plasticity for therapeutic benefits.

VITA

Raluca Moucha was born in the picturesque town of Brasov, Romania, on July 10th, 1967. She attended the historical Johannes Hontherus Gymnasium in German language, followed by Andrei Saguna High School where she graduated in 1985. She subsequently studied Dentistry at the University of Medicine of Cluj Napoca and Bucharest in Romania. Her dentistry studies were abruptly interrupted when she left Romania following the uprising in 1989 against the communist dictatorship. She lived in Frankfurt, Germany as a refugee until 1995 when she was granted immigration to the USA. Here, in Dallas, she began to build back her academic background by attending the University of Texas at Dallas for premed requirements and decided to change her career path from Dentistry to Neuroscience. She received her BS in Neuroscience, *Summa cum Laude* in 1998. She was also the recipient of the Dean's Award for Excellence in Neuroscience. She continued with her doctoral studies at UTD in the laboratory of Dr Michael Kilgard. During her graduate degree she was the recipient of an exchange scholarship for a research collaboration with the Institute of Experimental Medicine within the Academy of Sciences of the Czech Republic. Her future goals are to continue her investigation of the factors contributing to changes in the neural processing of information with the final hope of revealing how precise manipulation of these factors can aid neural rehabilitation.