Title
Adult auditory cortical plasticity modulated by locus coeruleus activity

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Publication Date
2006

Peer reviewed|Thesis/dissertation

Adult Auditory Cortical Plasticity Modulated by Locus Coeruleus Activity

by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Neuroscience

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Dedicated to my mom and dad.

Acknowledgments

My years at UCSF and in San Francisco have been an experience that has greatly influenced my development and knowledge of myself and my future desires. This experience has allowed me to question, to answer, and then to reevaluate the answers and also the questions themselves. This might not have been possible without the influence and character of Michael Merzenich, someone I consider a friend for life.

As I scanned the spectrum of potential graduate programs, it became obvious to me who I wanted to follow. Before I was even accepted to graduate school, I knew I wanted to explore and discuss the ideas Mike has brought to the table and the brain over the years. I knew I was going to join his lab before I left UCSD, where I spent my undergraduate years. And as it has worked out, I wouldn't change a thing.

I was very excited when I realized that my request to have Mike as one of my interviewers during the interview process was granted. I prepared for the meeting by reviewing his and related literature. After a few minutes of hospitality, I found myself in a discussion that was aggressive, challenging, engaging, and also confrontational. This only further affirmed my decision that he was going to be my mentor.

The course of my graduate schooling could not have been scripted. There have been ups and downs and in-betweens, and Mike has always supported my

exploration of new perspectives and my attempt to seek out my mission. Without him, I might not have made it this far.

While working in Mike's lab, there have be several scientists that have been instrumental in my development. Shaowen Bao dedicated an extraordinary amount of his time to the development of my surgery, technical, and analysis skills.

Etienne De villers-sidani, Daniel Polley, and David Blake all put up with my sometimes stupid and simple technical and computer questions and were engaging in various scientific and other discussions over coffee. Fabrizio Strata (a.k.a. Fab), the coffee master, I consider a great friend and added much vigor and light-heartedness to the laboratory environment. Cathy Garabedian and Edward Chang I consider myself fortunate to have as my peers; we spent many late nights and early mornings in simultaneous experiments.

The entering class of 1999 was a great group of characters. As one of the last groups single-digit classes entering UCSF, we became a tight-knit bunch at times; I thank the entire squad. Specifically I would like to thank Sam Sober, Steven Shabel, and Shannon Shields.

Over the course of my rotations I gained a great amount of perspective on the variety that is neuroscience. I thank Lily Jan, Ken Miller, Nicholas Preibe, Tim Roberts, Rob Edwards, Cori Bargmann, Steve Finkbeiner, Patricia Janak, Antonello Bonci, Howard Fields, Allison Doupe, Louis Reichardt, Stanley Pruisner, and Roger Nicoll for their input and guidance during this and other portions of my graduate experience.

Science and life are not done in a vacuum and I would like to thank Cheryl Bates, Tess Kornfield, Luke Paquin, and the members of Pink Clouds for all their support.

Cait Garvey is an extraordinary person. I am fortunate to know her and to have her in my life.

I would also like to thank Lee Bollinger and Frederick Seitz for their inspiration.

Ultimately, I owe everything to my parents who have shown me the way without holding the reins. They prepared me to accept any challenge I deem worthy.

Finally, I would like to thank the rats and mice who, for the most part were very gracious in their participation in these and other experiments.

"If we now look a little more closely at these forces, and at our own present economic system, then we can see that our own theoretical criticism is borne out by experience."

-Karl R. Popper

Adult Auditory Cortical Plasticity Modulated by

Stimulus-Paired Locus Coeruleus Activity

Jacob J. Bollinger

Abstract

Sensory stimuli associated with novelty or reinforcement activate the brain's neuromodulatory nuclei. The release of neuromodulators throughout the neuraxis allows the adult brain to refine its functional connectivity and adapt to its environment. One of these nuclei is the locus coeruleus (LC), which supplies the neuromodulator norepinephrine (NE) throughout the forebrain including the primary auditory cortex (A1). The current study examines the long-term effects of stimulus-paired NE signals on functional representations in A1.

Previous studies have demonstrated that A1 plasticity can be induced through stimulus-paired nucleus basalis (Weinberger et al. 1998, 2003, 2006; Kilgard et al. 1998a, 1998b, 2002; Bao et al. 2003) or ventral tegmental area (Bao et al. 2001) activity. The following experiments were designed to extend our understanding of neuromodulation-induced A1 plasticity to the LC-NE system.

Pairing tone-pip trains with LC stimulation in awake, unrestrained, adult rats for 20 days induced general and frequency-specific expansions in A1, as well as alterations in post-stimulus responses, without any corresponding changes in response bandwidths or thresholds, examined under anesthesia.

Cortical characteristics were examined in a group of animals in which the LC was stimulated alone, without paired auditory stimuli. After a stimulation regimen similar to that of the paired groups, the size of the area representing A1 was modestly and generally increased, while a large general increase in post-stimulus responses was recorded. These effects were observed without any alterations in bandwidths or thresholds, implying that the very strong increases in responsivity were not due to a general decrease in sensitivity or to a decrease in response selectivity. I argue that this increase may be due to an increase in excitability attributable to a weakening of stimulus-driven inhibition.

To examine the LC-specificity of these effects, it was ablated in control rats prior to experimental manipulation. Pretreatment with N-(2-chloroethy)-N-ethyl-2-bromobenzylamine (DSP-4), an LC-specific neurotoxin, eliminated the aforementioned effects after the same pairing regimen, and induced a slight reduction of A1.

These data demonstrate that the adult auditory cortex retains the capacity for receptive field, tonotopic-map, and response profile plasticity and that stimulus-paired LC activity plays an important role in remodeling the adult brain.

Christopher Schreiner

1-05-2007

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Chapter 1: Introduction

Introduction

It is now well established that mammalian primary cortices in the auditory, visual, and somatosensory modalities are not disordered representations of their respective sensory epithelia, but are rather ordered topographic tablas in which neighboring microcircuits within the defined area respond electrically to analogous stimulation of the related sensory surface (Buonomano and Merzenich 1998). More recently, many studies have shown that detailed features of these cortical representations manifest an animal's experience, peripheral input sources, and input schedules, and that this topography can be substantially modified by altering these variables.

Three decades of experiments from many laboratories have contributed to a wider understanding of the specifics of topographical reorganization and plasticity (Kaas et al. 1983; Merzenich et al. 1983; Merzenich et al. 1984; Merzenich et al. 1990; Merzenich and Jenkins, 1993). For example, the somatotopic cortical representations of the hand in area 3b were examined before and after the amputation of digit D3 (Merzenich et al. 1984). Within two to eight months post-amputation, much of the area that had previously represented digit D3 now responded to inputs from adjacent fingers or the subjacent palm. These results indicated that the cortex responded plastically to input competition. Topographic reorganization of A1 has also been demonstrated to correspond to changes in perceptual acuity in a simple training task (Recanzone et al. 1993). The tonotopy of the primary auditory cortex has also been shown to be modified via cochlear lesions (Robertson and Irvine 1989).

Recanzone et al. 1993 showed an increase in the cortical representation of a restricted frequency range in primary auditory cortex of adult owl monkeys that was correlated with the animal's performance in a frequency discrimination task. It has become clear from numerous studies that not only are primary cortices plastic via invasive or traumatic means which also occur in nature, but also via manipulations that are more modest in scale and possibly more applicable to daily behavior, changes in behavioral contingencies, and the corresponding behavioral modifications.

Though it clear that many primary sensory cortices are plastic, the primary auditory cortex has certain experimental advantages. One of the most important advantages in the following experiments is the ability to reliably deliver an approximately identical stimulus (in terms of spectra and intensity) in many successive trials. There are numerous experimental limitations to the study of other modalities in a similar manner. Retinal position can be very difficult to accurately fix in an awake behaving animal, and the instruments used to deliver tactile stimuli are not as reliably precise. There are also variabilities and artifacts caused by the movement of a behaving animal. In the auditory domain, the main source of stimulus variability is the animal's body and pinna position, but this causes much less of an impact in the rat (in terms of stimulus reliability) than those in other modalities. Another advantage of the auditory cortex as a model of plasticity is that its response properties and topography are fairly well characterized in the rat.

Recanzone's and Ahissar's work on attention-driven plasticity that was specific to the attended modality despite identical presented auditory and tactile stimuli, inspired the experimental design used by Kilgard, according to his thesis.

Kilgard concluded that attention gates the correlation-based rules that modify connection strengths and network dynamics in the adult cortex. From this hypothesis he employed an auditory-stimulus-paired-with-nucleus-basalis-activity experimental design (Kilgard et al. 1998, 2002). This design has led to an enriched understanding of nucleus basalis-driven auditory cortical plasticity and subsequently, that of the VTA (Bao et al. 2001, 2003). Here we turn our focus to the neuromodulatory impacts of a third important contributor, the locus coeruleus (LC), a distinct cluster of approximately 3000 noradrenergic neurons (in the rat) located in the dorsal pons and designated as the A6 and caudal A4 cell groups. As I will discuss in some detail, the LC has been implicated in the early organization of behavioral modification perhaps via the neuromodulation of certain brain areas.

The LC is driven by many facets of everyday experience. Previous studies have begun to unveil the specifics of LC-driving stimuli and their relevance to behavioral situations. The connectivity, synaptic, intracellular, and empirical evidence of the mechanisms that drive and/or are affected by LC activity all work in concert and orchestrate stimulus storage and behavioral modification. Below, I attempt to summarize the literature that is most relevant to the following experiments included in this thesis, the implications of the experimental design, and the reach and scope of the results.

LC neuronal firing occurs during orienting responses, stressors, adaptive transitions in behavior, and the initiation of novel response strategies (Pennartz 1996). Single LC units have been shown to respond to innocuous (tones, lights, food, etc.)

and stressful or aversive stimuli, opiate withdrawal, visceral stimuli, bladder or colon distension, and sciatic or vagus nerve stimulation.

Activity in the LC is driven and modified by its anatomical connectivity and by diverse neurochemical mechanisms. Afferents to the LC core (cell bodies) are dominated by metenkephalin, CRF, and GABAergic inputs. The nucleus prepositus hypoglossi (PrH) is the main source of GABA and the paragigantocellularis (PGi) provides topographic CRF and glutamatergic inputs. The PGi receives input from parts of the hypothalamus, PAG, nucleus of the solitary tract, the caudal medullary reticular formation, and the parabrachial nucleus. Stimulation of the PGi has been substituted for LC stimulation in some paradigms; PGi activation by glutamate enhances memory for emotional and spatial events in rats (Clayton and Williams 2000). It is likely that this anatomical connectivity dictates the specificity of LC responsiveness to the above behaviorally relevant stimuli and the effects of this responsiveness in target areas.

Activity in the LC can induce various sensory alterations. Electrical stimulation of the LC produces antinociception, decreases dorsal horn responses to noxious stimuli, and significantly increases the spinal content of NA metabolites. These effects are mediated by postsynaptic alpha 2-Rs in the lumbar spinal cord (Jones 1991). Perhaps the LC is preparing the central nervous systems for efficiency in the face of behavioral challenge.

LC activation enhances the coding of salient sensory cues in projection areas, while performing a permissive function damping the responses to behaviorally irrelevant or distracting stimuli thereby facilitating transitions between behavioral

states. Increases in levels of NA mediated by the LC activity appear to enhance the influence of extrinsic input on cortical representations via a decrease in horizontally induced EPSPs and a smaller decrease in afferent-evoked EPSPs. NA has been proposed to modulate the signal-to-noise ratio in a range of sensory modalities including auditory, somatosensory and visual, by altering the relative influence of recurrent excitation mediating the internal representation of sensory input (Hasselmo et al. 1997). NA induces a 10-40% decrease in EPSP amplitude while increasing intrinsic reactivity (without membrane potential changes) in layer II/III pyramidal cells activated by intracellular current pulses or by extracellular stimulation of layer V pyramidal cells in sensorimotor and non-sensory cortex. Dodt el al. 1991 suggests that this decreased synaptic input would only transmit temporally coherent signals to target cells whose excitability was increased by NA.

NA has been shown to depress excitatory synaptic transmission in CA3 cultures, pyriform cortex, and neocortex via activation of alpha-Rs. The possible selective suppression of intrinsic but not afferent synaptic transmission by NA might be enhanced by the apparent specificity of NA innervation for layers other than IV in the neocortex. NA is proposed to switch the dynamics of cortical function from a state in which activity is primarily determined by intrinsic synapses mediating recall based on previous learning, to a state in which extrinsic stimulation predominates (Hasselmo 1995).

The postsynaptic, presynaptic and autoreceptor-mediated effects of NA and the resulting physiological modifications likely orchestrate the hypothesized roles of

the LC in behavioral modification. In the following paragraphs, I will discuss some of the literature that has attempted to address these issues.

In urethane-anesthetized or awake rats, NA or alpha 1-R agonists that predominately activate postsynaptic receptors, or systemic alpha 2-R antagonists that inactivate both inhibitory autoreceptors in the LC (net increase in LC activity) as well as postsynaptic receptors, have been demonstrated to decrease spontaneous and evoked activity while increasing the threshold and frequency selectivity of most single units, effects that can be mimicked with GABA. However, when NA was applied in the presence of bicuculline at doses able to block GABAergic inhibition, the inhibitory effects on spontanous and evoked activity were still present, possibly indicating a non-GABAergic mechanism. These effects were a function of cortical depth where supragranular layers display only inhibition and infragranular layers were excited at low concentrations. NA might be attenuating all sensory signals in a way that behaviorally relevant information will be represented and encoded with high fidelity and precision. Consistent with this possibility, selectively increased responses arising from a background of decreased activity have been observed (Manunta and Edeline 2000).

Electrical stimulation of the LC results in early and late IPSPs and an EPSP recorded in the mPFC. The early and late IPSPs are mediated by Cl- and K+ currents, respectively. Antidromic activation of LC-projecting mPFC neurons by LC stimulation likely contributes to the complex synaptic events in pyramidal cells of the mPFC. The IPSPs elicited by antidromic activation of mPFC GABAergic neurons by LC stimulation, may improve the signal-to-noise ratio and favor a better

responsiveness of neighboring neurons to NA released in the mPFC (Branchereau 1996).

In general, the weak inhibitory cortical responses due to LC stimulation are localized throughout all layers and are mediated by beta-Rs while slow excitatory cortical responses are more prominent in the middle and deeper layers and are likely mediated by alpha 1-Rs and beta-Rs. (One might note the difference between the aforementioned response modifications due to alpha1-R agonists and alpha2-R antagonists, and the effects due to electrical stimulation of the LC mentioned here.) Activation of beta-Rs results in an enhanced excitability and responsiveness to a depolarizing input via a decrease in Ca++-dependent K+ conductance (IAHP) and decreased spike frequency adaptation. This effect appears to be a general mechanism underlying NA influence on cortical pyramidal cells because it can be found in nearly every cortical pyramidal cell exhibiting spike frequency adaptation in a wide variety of cortical regions including cingulate, sensorimotor, visual, and auditory areas, and within CA1, CA3, and the DG. If there is no synaptic input, this influence may have no effect at all. However, if there is afferent input while beta-Rs are stimulated, the train of APs will occur at a higher rate, particularly after the first 50ms (McCormick and Williamson 1991). I will later propose these mechanisms as integral to the effects recorded in the core experiments described in this thesis.

The thalamus is widely known as a main source of sensory input to the cortex. The LC is known to innervate and induce physiological changes in the thalamus; the thalamus is a prominent suspect in the mechanisms of behavioral modification hypothetically attributed to the LC. In the thalamus, NAergic neurons of the LC

innervate both the reticular nucleus and primary sensory nuclei, but the densities vary between species. In vitro application of NA to neurons of the LGNd, MGNd, nucleus reticularis (nRT), AV nucleus, and the parataenial (PT) nucleus resulted in a slow depolarization and increased responsiveness, a decrease in input conductance, and an increase in slow membrane time constant, via a decrease in a leak potassium conductance. Therefore, NA facilitates neuronal responsiveness to both excitatory and inhibitory inputs. These effects are likely due to activation of alpha 1-Rs, which are densely present throughout the entire dorsal thalamus (where alpha 2-Rs and beta 1-Rs are also present in moderate densities). This depolarization effectively suppresses burst firing and promotes the occurrence of single-spike activity. The NA-induced inhibition of burst firing is likely the result of inactivation (via the NA-induced slow depolarization) of the low-threshold T-type Ca++ current responsible for the Ca++ bursting. The neurons exhibiting these responses to NA have the electrophysiological properties of thalamocortical relay cells or of the GABAergic neurons of the reticular nucleus. Via these mechanisms, NA can effectively inhibit the generation of thalamocortical rhythms and greatly facilitate the faithful transfer of information from the thalamus to the cortex (McCormick and Prince 1988). In contrast, the more the thalamocortical system is isolated from the ascending brainstem cholinergic and NAergic activating systems, the higher the probability of the emergence of oscillation in the thalamocortical system (Buzsaki, Kennedy, Solt and Zeigler 1991). Any condition that interferes with these nRT-relay cell interactions can be regarded as anti-oscillatory, anti-epileptic and anti-tremor.

In vivo and in vitro NA and ACh have been demonstrated to alter the filtering character of corticothalamic synapses in rats. ACh (or muscarinic agonists) and NA (or alpha2-R agonists) depress the efficacy of corticothalamic synapses while enhancing their frequency-dependent facilitation, resulting in a stronger depression of lower-frequency than of higher frequency input. This results in a high-pass filtering of thalamocortical information, such that input >20Hz preferentially elicits spiking in postsynaptic neurons during arousal and increased pontine reticular formation activity (which includes the LC, the laterodorsal tegmentum, the pedunculopontine tegmentum, and the raphe nuclei) (Castro-Alamancos and Calcagnotto 2001). The various mechanisms discuss above are perhaps intricately involved in behavioral modification and also the cortical results discussed in this thesis.

The hippocampus has long been known to be essential for certain types of conditioning. As I will later outline, the experimental design in the following experiments of this thesis is very similar to a classical conditioning paradigm. In turn, I feel it vital to review some of the literature pertaining to the roles of LC and noradrenaline in the hippocampus. In the hippocampus and prior to behavioral contingency, an auditory stimulus evokes a small response. After it became a predictor of food, the sensory stimulus elicited a more reliable and larger response. Combined with LC stimulation, a relevant auditory stimulus presentation further enhanced this response (Segal and Bloom 1976).

The effect of NA on the oscillatory properties of individual hippocampal microcircuits might also depend upon the localization of NA-Rs to specific IN subtypes. It has been demonstrated in CA1 that an interaction between two groups of

INs, GABA-A slow and GABA-A fast cells, may contribute to the theta and gamma rhythms respectively. GABA-A slow cells inhibit both pyramidal and GABA-A fast cells; this interaction contributes to nested gamma/theta rhythms in the hippocampus (Banks et al. 2000). NA-modulation of subgroups of these INs might instill specific oscillatory properties during different states of vigilance and across task demands, in order to align the proper internally- and extrinsically-driven representations of reward contingencies.

NA can increase the probability of eliciting LTP in the hippocampus (Bliss et al. 1983) and neocortex (Brocher et al.1992). Inhibitors of CAMKII or PKC, both potentially modulated by NA, block LTP induction by tetanic stimulation. Potential mechanisms of this LC-induced increase in LTP probability include modulation of EAA-Rs, GABA-Rs, voltage-gated K+ and Ca++ channels, and Ca++-activated K+ channels. In neocortical pyramidal cells, there is clear evidence for T-, N-, L-, and P-type Ca++ currents all of which are potentially modulated by NA. Potential effects of modulation of these currents include reductions in transmitter release, alterations in dendritic integration, and modulation of amplitude and frequency of back-propagating action potentials. In these cells N-, P-, and Q-types are coupled to the sAHP and the mAHP which are involved in spike rate adaptation, while L currents contribute to inward currents (not AHPs). Perhaps some or all of the mechanisms discussed in the preceding paragraphs contribute to the effects I will discuss in this thesis.

The LC is one part of a neuromodulatory system that has multiple hubs. Another important nucleus in this realm is the nucleus basalis which has been demonstrated to be involved in cortical plasticity and also reciprocally interacts with

the LC. NAergic-LC neurons have been shown to synapse directly onto AChergic NB neurons and to activate both alpha-1 and beta-Rs on these neurons (Detari et al. 1999). NA depolarizes and excites ACheric NB cells while producing a dose-dependent increase in gamma-ECoG activity (desynchronization), a decrease in delta activity, and an increase in waking and arousal (Cape and Jones 1998).

Electrical stimulation of the LC excites the majority of F-cells (AChergic cells active during fast wave gamma cortical EEG which likely project to cortical pyramidal cells and, maybe also cortical interneuron-projecting GABAergic cells) and inhibits the majority of S-cells (cells that have a higher rate during slow wave cortical EEG activity) in the NB (Dringenberg and Vanderwolf 1997). Thus the LC likely modulates cortical EEG independently as well as via activation of the NB. In addition, the cortical EEG is tightly coupled to the firing mode of thalamocortical relay cells; bursting and single-spike activity of these neurons are associated with synchronized and desynchronized cortical EEG, respectively (McCormick 1989).

LC neurons normally increase their firing rates in response to a footshock (FS); Rats with AChergic lesions via the neurotoxin AF64A show impaired acquisition and retention in a passive avoidance task. The FS-evoked increase in LC discharge is significantly reduced in AF64A-treated animals in comparison with controls. These findings suggest that the AChergic system is involved in the early component of LC activation to FS and therefore might direct LC-mediated plasticity to stressors or noxious stimuli (Nishiike 2001).

The electrocorticogram (ECoG) is a measure of cortical electrical activity and components of this measure are labeled specific event-related potentials (ERPs) based

on their approximate latency. One of the most intensively studied ERPs is the P300 or P3 which is elicited in response to novel and/or task-related stimuli and therefore might be related to LC activity. Monkeys with damage to the LC cell bodies showed decreased P239 and P372 responses relative to their pre-lesion measures, decreased acoustic startle reflexes, enhanced behavioral responsiveness to novelty, and increased distractibility and exploratory behavior toward novel objects (Pineda, Foote, and Neville 1989).

In a five-choice serial retention paradigm used to evaluate visuospatial attention in rats, lesions of the LC impair performance only when additional attentional demands are placed upon the subject, e.g. distracters or temporally unpredictable targets. In addition, cortical ACh efflux shows a robust and task-related increase during established contingent performance; NA efflux increased only transiently in contingent subjects after task onset but show sustained increases in non-contingent subjects on the first day when the contingencies changed. These data implicate cortical ACh in aspects of attentional functioning, but highlight a specific involvement of the cortical NA system in detecting shifts in the predictive relationship between instrumental action and reinforcement (Dalley et al. 2001).

The LC also modulates the activity of neurons in the ventral tegmental area (VTA), another important neuromodulatory nucleus. When the LC is stimulated with a single pulse or a train of pulses (to simulate bursting activity), the activity of VTA neurons is increased and decreased respectively. Thus, the LC regulates VTA activity in a manner that is dependent upon the rate of LC depolarization. Galanin, which inhibits VTA neurons, is released from LC terminals in a similar frequency-specific

manner. In the cholinergic system, it has been shown that only high frequency stimulation of the diagonal band induces galanin co-release in the ventral hippocampus. Galanin release from the LC in the VTA might occur in a similar frequency-specific manner; the LC might inhibit VTA activity when the LC is in burst mode. As I will discuss later, this is a potential mechanism to explain certain behaviors of rats after a regimen of LC stimulation (see Chapter 6 on behavior).

Galanin receptors are also located in the amygdala and galanin microinjections into the lateral ventrical or amygdala induce anxiolytic and anxiogenic effects, respectively; aberrant galanin release can induce symptoms of anxiety (Wrenn and Crawley 2001). Some of the observed behavioral effects of our experiments might be the result of similar galanin influences (see chapter 6 on behavior).

The LC has the capacity to discriminate the relative importance of behaviorally important stimuli. The effects of mechanical and thermal cutaneous sensory stimulation on the activity of the LC and the peripheral sympathetic nerve (PSN) were investigated in rats. Noxious mechanical stimulation caused a parallel, virtually identical increase in LC and PSN activity, and both systems showed a biphasic excitation-inhibition response accompanied by increases in arterial blood pressure and heart rate. However, the two systems differ in their ability to adapt during prolonged noxious thermal stimulation. LC neurons adapt completely and returned to baseline firing rate within 5 min, while PSN activity is unchanged throughout the stimulation period. In contrast to the adaptation of LC responses to prolonged noxious thermal stimulation, the LC did not adapt during prolonged hemorrhage. This indicates a discriminatory capacity of the LC system in monitoring

sensory and autonomic stimuli. Only those stimuli of imperative importance to the animal are associated with continuing robust LC discharge (Elam et al. 1986). In the following experiments in this thesis we will attempt to mimic this imperative importance, but link it to an otherwise innocuous stimulus.

Behaviorally, LC activity has been linked to shifts in behavioral contingencies, activity that actually precedes the animal's shifts in performance to complete the new task reliably. The LC was investigated in a visual discrimination task in which monkeys were required to release a lever within 665ms only to the CS+ and not the CS- stimulus to receive a juice reward. Monkeys were over-trained for several months on one stimulus contingency. In this task, LC neurons were selectively and phasically activated by CS+ stimuli (onset latency ~140ms) for the over-trained contingency, but not for other task events. The contingency was then reversed. Very soon after contingency reversal, there was a rapid and transient increase in the tonic activity of LC neurons, and soon all LC neurons became selectively responsive to the new target and non-responsive to the old target (new non-target). Neural excitation of the LC was detected within the first 100 new target stimulus presentations, and extinguished within 100 presentations of the new non-target. This is a fairly difficult task, and monkeys require over 1000 trials to establish a new behavioral contingency to a criterion of fewer than 5% task errors. LC neurons however, exhibit very good discrimination within the first 500 trials of a new contingency. In other words, LC neurons acquire the new response profile before accurate behavioral performance on the new task is established. These results imply that the LC is likely involved in the early organization of the proper behavioral

responses to novel reward associations (Kubiak et al. 1998). In the experiments outlined in this thesis, I attempt to unveil the potential cortical manifestations of behavioral modification.

Aberrant LC activity might be involved in the symptoms of schizophrenia. Schizophrenics are unable to ignore distracting or irrelevant sensory stimuli and therefore cannot attend to relevant aspects of the environment. This and other disturbances can be viewed as a defect in gating of afferent sensory information, a disturbance that has been proposed to result in sensory overload of the brain as an information processor with limited capacity. Many schizophrenics are in a continuous state of over-arousal and have elevated plasma NA levels. NA levels are also 300% of control values in the ventral septum of schizophrenics. Such symptoms may be the result of an aberrant LC-NAergic system (Hornykiewicz 1986).

In the following experiments, we wish to induce a very large, temporally precise activation of the LC which will subsequently induce a large efflux of NA from LC terminals in the cortex. NA levels as measured in the mPFC were significantly higher with phasic activation of the LC compared with tonic activation; bursts of LC activity may be more effective in increasing terminal NA release in target areas (Florin-Lechner et al. 1996). These data and others provide the foundation by which the stimulation portion of the experiment was designed.

There are many established means of lesioning the LC. In the following experiments we used a intraperitoneal injection regimen of (n-)2-chloroethyl)-n-ethyl-2-bromobenzylamine hydrochloride (DSP-4) outlined in previous publications where the concentrations of NA in the hippocampus and neocortex were markedly reduced

by treatment with DSP-4 while the concentrations of dopamine and serotonin were unaffected (Al-Zahrani et al. 1998). DSP-4 was the neurotoxin we chose to lesion the LC to determine if the effects we have seen require an intact LC.

NA-depleted rats extinguish much more slowly than controls after the behavioral contingency is removed. Lesions of the dorsal NA-bundle produce a similar extinction deficit known as "the dorsal bundle extinction effect" or DBEE. Results of such studies implicate the NA system in three limbic forebrain functions, each of which may be regarded as a component of attention: limiting, stimulus identification, and mismatch detection. Mason (1980) argued that the most satisfactory explanation from the literature is that the DBEE results from impairment of sensory attention, or in the ability to notice changes in salient cues.

In summary, the LC has been demonstrated to be involved in the early organization of the proper behavioral responses to novel reward associations via limiting, stimulus identification, and mismatch detection which likely result from the LC's modulation of cortical dynamics from a state in which activity is primarily determined by intrinsic synapses mediating recall based on previous learning, to a state in which extrinsic stimulation predominates. Various molecular mechanisms in multiple cortices and nuclei (e.g. neocortex, hippocampus, thalamus, VTA, nucleus basalis) likely work in concert to perform these tasks for the animal. In the following experiments we examine the long-term effects of auditory stimulus-paired LC activity on functional representations in A1.

Chapter 2: Examination of A1 Properties in Adult Naïve Animals

Chapter 2: Examination of A1 Properties in Adult Naïve Animals

Abstract

The auditory cortex has been studied in some detail in many species and via many experimental means. In all mammalian species studied to date, the topography of the cochlea is maintained in the primary auditory cortex (Merzenich and Brugge, 1973; Merzenich et al. 1975; Merzenich et al. 1976; McMullen and Glaser, 1982; Aitkin et al. 1982; Lelly et al. 1986; Sally and Kelly 1988; Dear et al. 1993; Thomas et al, 1993; Stiebler et al. 1997; Batzri-Izraeli et al. 1990; Suga and Jen, 1976; Tunturi 1950, Imig et al. 1977; Reale and Imig, 1980; Hellweg et al., 1977; Romani et al., 1982; Jen et al., 1989). The primary auditory cortex of the rat has been characterized in terms of both frequency and amplitude in earlier studies. In the following experiments, we measure A1 properties which we will attempt to modulate in later experiments.

Introduction

Rat A1 neurons have been characterized as responding to short tone pips with short latency, phasic responses. A1 has a repeatable tonotopy with an approximately rostral-caudal orientation, with lower frequency representation occurring more rostrally. Perpendicular to tonotopy is an "iso-frequency" representation in which neurons within each "iso-frequency band" respond preferentially to tone pips of similar frequency. In this study, auditory cortical neurons were probed for their spectral response profiles. Responses were then reconstructed to reveal a map of the tonotopy.

In light of previous studies, neuronal response samples were identified as falling within A1 if they met specific criteria. Response profiles should be consistent and have onset latencies of 7-12 milliseconds (as determined from the peri-stimulus time histograms (PSTHs)). Characteristic frequencies (CFs) should fall into a rostral-caudal tonotopic gradient. Once the recordings that meet these criteria are collected, they are subsequently reconstructed (based on the penetration matrix, and CF analysis) to produce a "map" of the multiunit recordings or a map of A1. In addition, response profiles were analyzed in terms of PSTH, bandwidth, threshold, and response amplitudes (fraction and percentage) across frequency and amplitude.

Methods

Surgical anesthesia was induced with pentobarbital sodium (50 mg/kg). Throughout the surgical procedures and during the recording session, a state of areflexia was maintained with supplemental doses of dilute pentobarbital (8 mg/ml, i.p.). The trachea was cannulated to ensure adequate ventilation. The cisternae magnum was drained of cerebrospinal fluid to minimize cerebral edema. The skull was secured in a head holder leaving the ears unobstructed. After the right temporalis muscle was reflected, auditory cortex was exposed and the dura was resected. The cortex was maintained under a thin layer of viscous silicone oil to prevent desiccation. Recording sites were marked on an amplified digital image of the cortical surface vasculature. Cortical responses were recorded with parylene-coated tungsten microelectrodes (1–2 MegaOhms at 1 kHz; FHC, Bowdoinham, ME). Recording sites were chosen to sample evenly from the auditory cortex while avoiding blood vessels.

At every recording site the microelectrode was lowered orthogonally into the cortex to a depth of 470–550 micrometers (layers 4/5), where vigorous stimulus-driven responses were obtained. The neural signal was amplified (10,000X), filtered (0.3–10 kHz), and monitored on-line. Acoustic stimuli were generated using TDT System II (Tucker-Davis Technology, Alachna, FL) and delivered to the left ear through a calibrated earphone (STAX 54) positioned inside the pinnae. A software package (SigCal, SigGen, and Brainware; Tucker-Davis Technology) was used to calibrate the earphone, generate acoustic stimuli, monitor cortical response properties on-line, and store data for off-line analysis. The evoked spikes of a neuron or small cluster of neurons were collected at each site. Single units were isolated either on-line using spike amplitudes, or off-line using principal components of the spike waveform. Frequency-intensity receptive fields (RF) were reconstructed in detail by presenting pure tones of 50 frequencies (1–30 kHz; 0.1 octave increments; 25 msec duration; 5 msec ramps) at eight sound intensities (0–70 dB SPL in 10 dB increments), for a total of 400 stimuli, to the contralateral ear at a rate of two stimuli per second. The tones were presented in a random, interleaved sequence. In off-line RF analysis, activity after a tone was considered spontaneous and was removed from further analysis if all of the eight neighboring tones in the frequency-intensity stimulus grid (i.e., tones within +/- 0.1 octave of the frequency and within +/- 10 dB of the intensity) failed to activate the neurons. A computer program was used to automatically characterize the tuning curve of each site as the cortical response threshold as a function of frequency. The characteristic frequency (CF) of a cortical site was defined as the frequency at the tip of the tuning curve. When a tuning curve had a broad tip or multiple peaks, the

median frequency at the threshold intensity was chosen as the CF. Response bandwidth 30 dB above threshold (BW30) was defined as the bandwidth of the tuning curve 30 dB above the tip. For multi-peaked tuning curves, the response bandwidth was defined as the range from the lowest to the highest frequency at 30 dB about the most sensitive tips that activated the cortical site, possibly encompassing the frequencies in a trough of the tuning curve that did not activate the cortical neurons. The CF and BW30 were automatically determined using computer programs. They were well correlated with those "blindly" characterized by an experienced observer [$r>0.95$; $p<0.0001$ for both CF and BW30]. The response latency was defined as the time from stimulus onset to the earliest response (4 X SD above baseline activity) for five frequencies that were nearest the CF at 70 dB SPL. To generate cortical maps, Voronoi tessellation ("Voronoi" is a Matlab function; The Mathworks, Inc.) was performed to create tessellated polygons, with the electrode penetration sites at their centers. Each polygon was assigned the characteristics (e.g., CF) of the corresponding penetration site. In this way, every point on the surface of the auditory cortex could be linked to the characteristics experimentally derived from a sampled cortical site that was closest to this point. The boundaries of, and the multiunits to be included in the primary auditory cortex were functionally determined using the following criteria: (1) primary auditory neurons generally have a continuous, single-peaked, V-shaped receptive field, (2) CFs of the AI neurons are tonotopically organized with high frequencies represented rostrally and low frequencies represented caudally and 3) onset latencies occur 7-12 ms after stimulus onset. All statistical analyses were done

using Matlab. Unless specified otherwise, statistical significance was assessed using unpaired two-tailed t tests. Data are presented as mean +/- SEM.

Results

Size and location of A1.

The auditory cortex in the rat can be reliably located using the lateral suture and blood vessels as references (Sally and Kelly, 1988). Primary auditory cortex (A1) is located approximately 1 mm dorsal to the horizontal portion of the suture and approximately 1.5 mm posterior to the ventral portion of the suture. The average A1 area in naïve (control) rats was 1.248 +/- 0.1732 mm^2 (see figure 2.6).

Tuning Curves.

Most tuning curves collected from rat cortex that met our criteria for A1 inclusion, had the characteristic "slanted triangle" shape similar to that described in earlier publications (Sally and Kelly, 1988). Figure 2.2 is a representative. BW30 is calculated 30 dB below the threshold. Characteristic frequency (CF) is calculated as the frequency at which the multiunit is most consistently activated at the lowest stimulus intensity.

Map and Response Properties of the Primary Auditory Cortex in Adult Naïve Animals.

In female adult naïve Sprague Dawley rats, A1 was mapped in a dense series of multiunit recordings (1-8 neurons) in which neurons were tested in their response

properties to 400 stimuli; 8 intensities for 50 different frequencies (25 ms tone pips) (Figure 2.1 displays an abbreviated diagram of the recording setup). These responses were plotted to yield a tuning curve (Fig. 2.2). Dot size is a linear function of stimulus-evoked discharge amplitude. From this tuning curve, a characteristic frequency (CF) was determined. The CF is defined as the frequency at which the multiunit responds in a consistent manner at the lowest stimulus intensity (dB). The CF of a multiunit in relation to the CFs of surrounding mulitunits (isofrequency and tonotopic domains) is one of the major criteria used to determine if the response has been recorded in A1.

All responses were accumulated into a post-stimulus time histogram (PSTH Fig. 2.3) which included all responses of a multiunit to the 400 stimuli. The onset of a multiunit's response in this PSTH is the other major criteria in determining if a multiunit is to be included in A1; multiunits with response onsets ranging from 7-12 msec that also meet CF criteria were included in the pool of A1 recordings.

Once these multiunits were scrutinized against these criteria, their CFs were mapped into a penetration matrix which was referred to a picture of the cortex with documented penetration locations (Fig. 2.4). From the CFs and penetration matrix, Voronoi tessellation was used to generate CF maps of A1 (Fig 2.5). Cortical sizes for the control group are presented in Figure 2.6.

In these animals, response areas were also calculated across frequencies for a given intensity. Figure 2.7 shows an example of this analysis from a single naïve animal calculated in both fraction of A1 responding as well as absolute area of A1

responding (mm^2). Average response area plots for individual intensities for the group of naïve animals will later be compared to those of other experimental groups.

PSTH analysis was performed in which PSTHs were averaged within frequency bins according to the CF of the penetration. 0.5 octave bins surrounding 4kHz, 9kHz, and 20kHz were used. Examples of this analysis for an individual animal and for the naïve group are shown in Figure 2.8.

The results discussed here define the control data for the following experimental manipulations. All of the above data is similar to what has been previously collected in A1 of the rat.

Figure 2.1; Acute A1 Mapping Procedure

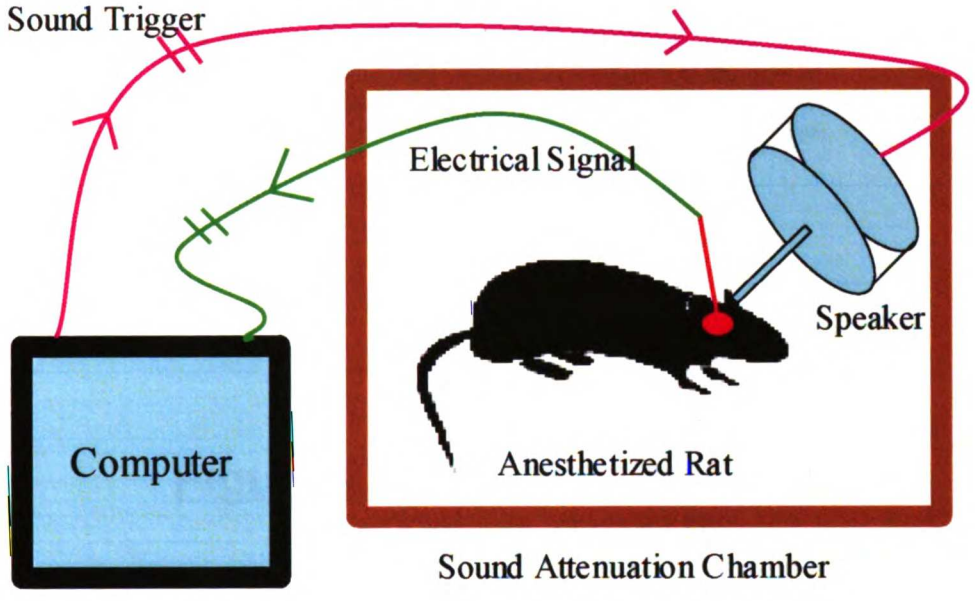


Figure 2.2; Spectrotemporal Receptive Field (STRF)

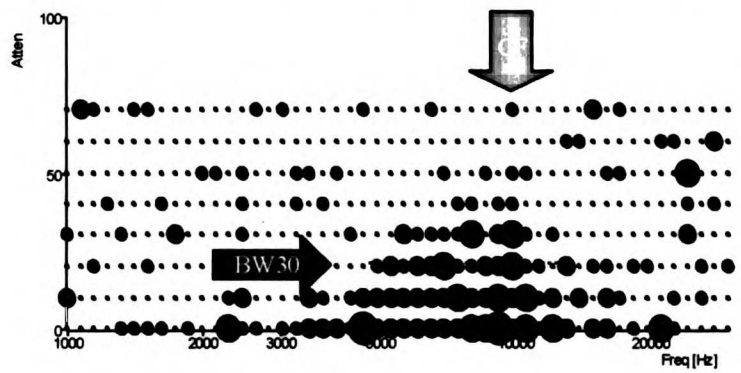


Figure 2.3; Peristimulus Time Histogram (PSTH)

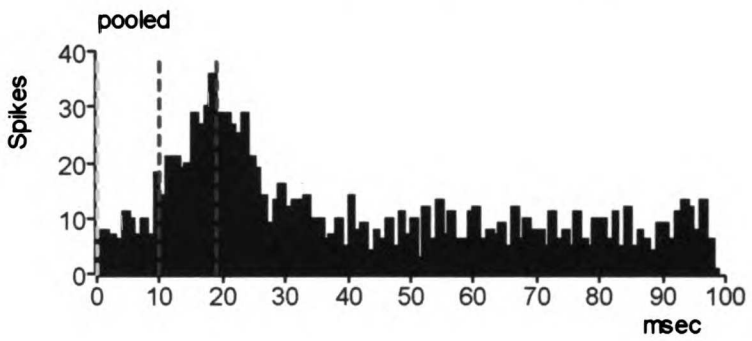


Figure 2.4; A1 Anatomy/ Penetration Matrix

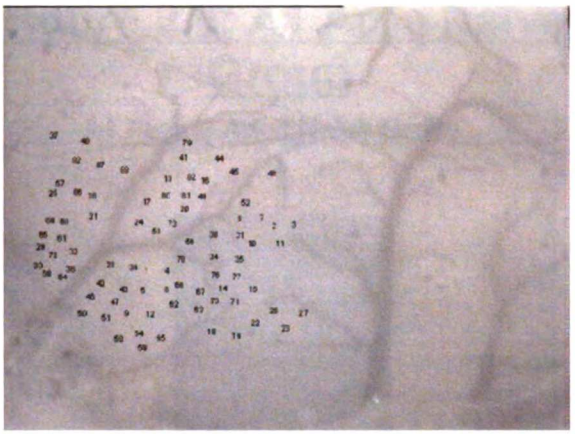


Figure 2.4; Adult Naïve A1 Map

Figure 2.6; A1 Area Naïve Group

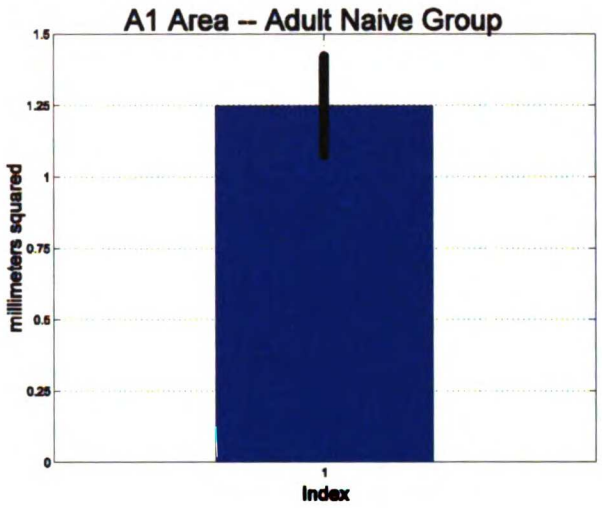
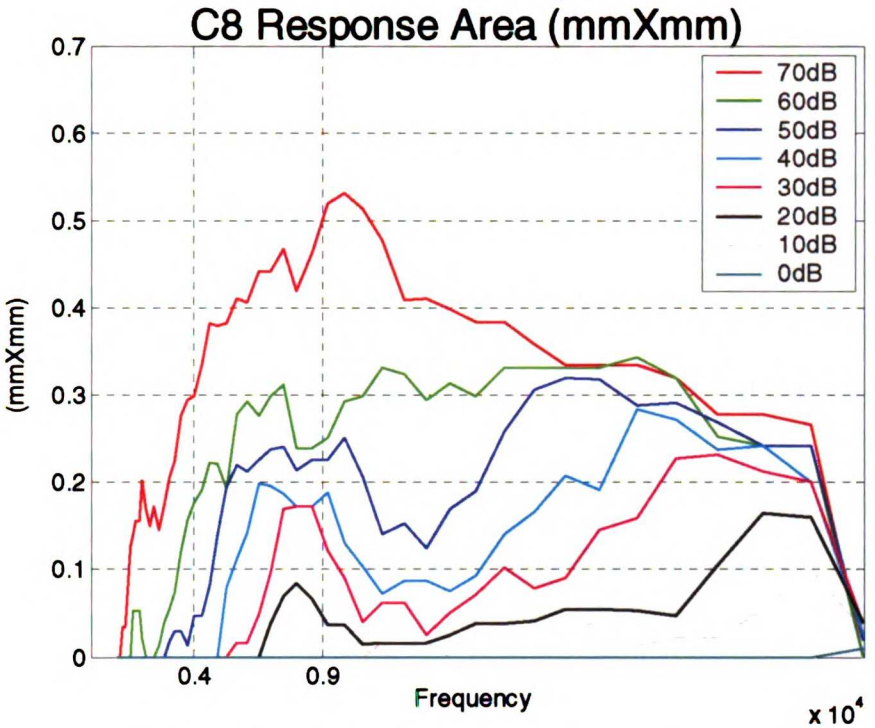


Figure 2.7a-b; Response Area Naïve Animal



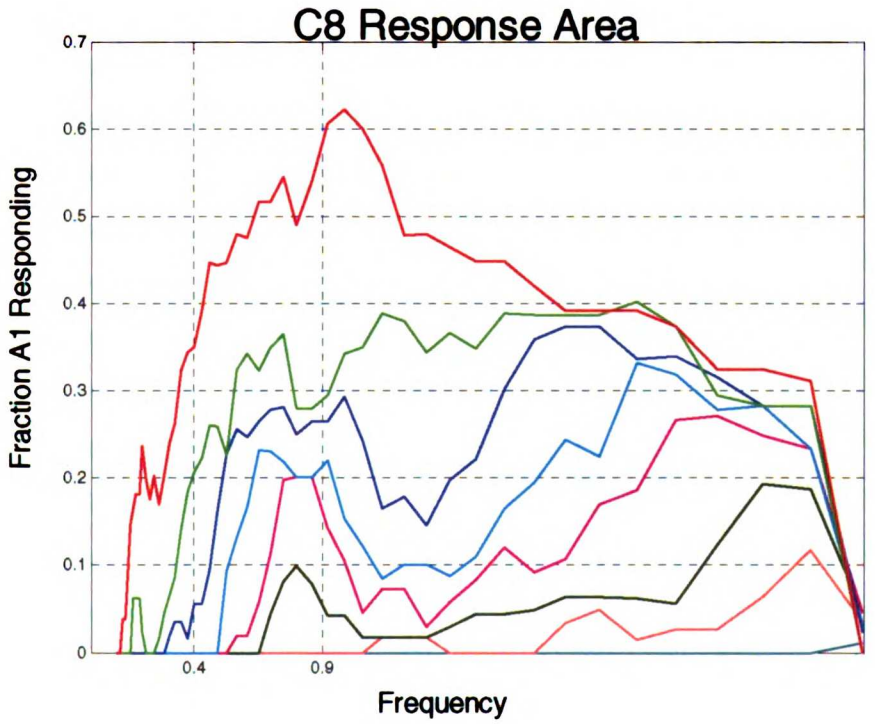
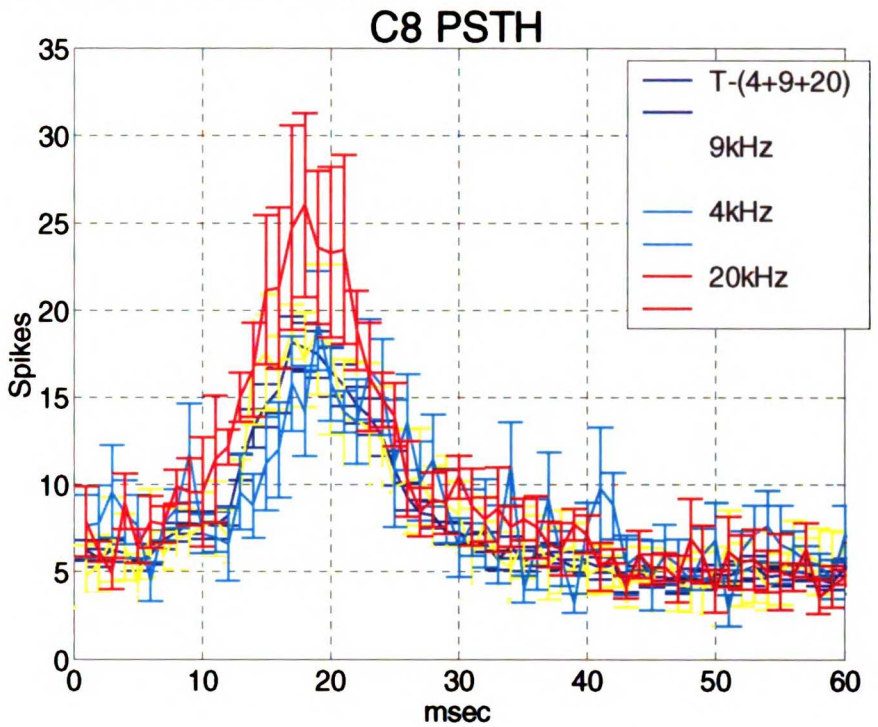


Figure 2.8a-b; Averaged PSTHs Naïve Animal and Naïve Group



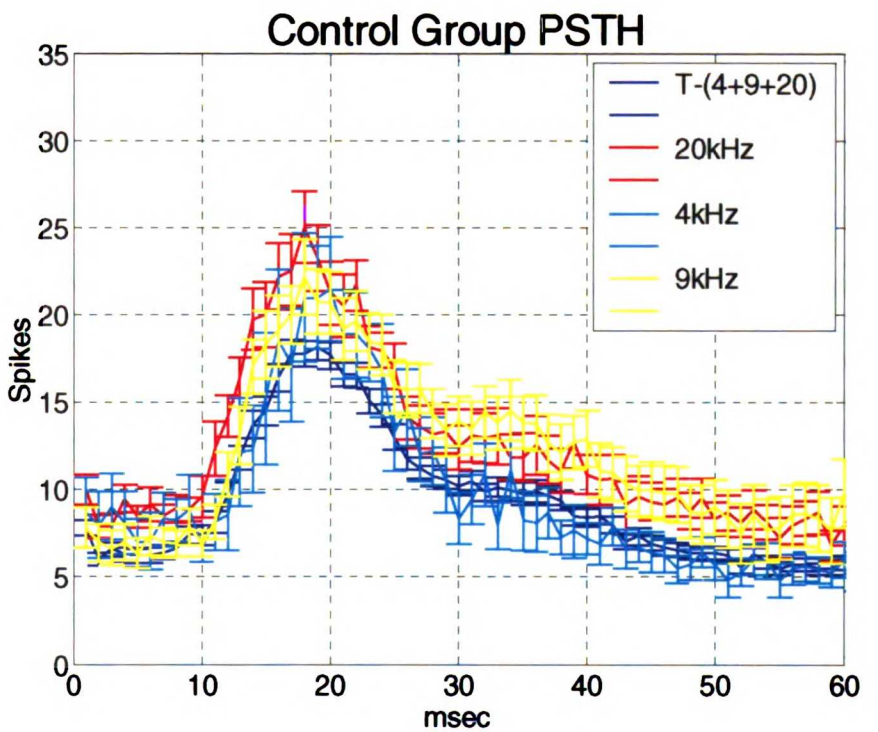


Figure 2.1 Schematic of the A1 Mapping procedure. Stimuli are triggered by the computer with Tucker-Davis technologies SigGen software and delivered through a speaker with a tube inserted in the animal's left ear. Recordings are made from the contralateral hemisphere amplified and collected in Brainware software.

Figure 2.2 Tuned responses for a neuron recorded within a representative A1 penetration. CF (orange arrow) is the frequency that elicits a consistent neural response at the lowest intensity, or the threshold. BW30 is the range of frequencies the neurons are responsive to at 30 dB above threshold (red arrow and dotted line).

Figure 2.3 Peristimulus time histogram (PSTH) from this tuning curve. Note that the onset latency is <10 msec. See text for further details.

Figure 2.4 Photograph of the left hemisphere of the rat from a typical A1 mapping experiment in a naïve rat. The microelectrode penetration matrix is overlaid on an image of the cortical microvasculature. To generate cortical maps, Voronoi tessellation ("Voronoi" is a Matlab function; The Mathworks, Inc.) was performed to create tessellated polygons, with the electrode penetration sites at their centers. Each polygon was assigned the characteristics (e.g., CF) of the corresponding penetration site.

Figure 2.5 Representative CF map of a right primary auditory cortex of a naïve rat corresponding to the tuning curve, PSTH, and penetration matrix shown above. Each polygon represents one penetration. Color represents each site's characteristic frequency. Non-responsive and auditory responding non-A1 sites are marked with O's and X's respectively.

Figure 2.6 Bar graph of the primary auditory cortical areas (mm^2) from the naïve group. Graph is an average of four representative naïve maps. Error bar is the standard error (SD/(sq. rt of n)).

Figure 2.7 a) Response area of a representative naïve animal. Colors (from red to grey) represent individual intensities, with red being the highest (70dB). X-axis is frequency and y-axis represents the fraction of the total A1 area responding at an individual intensity across frequencies. b) Response areas reconstructed in the same animal. All is the same as in a), except that the y-axis represents actual (not proportional) areas (in mm^2).

Figure 2.8 a) Averaged (compound) PSTHs for a representative naïve animal. Color represents 4kHz (pale blue), 9kHz (orange), and 20kHz (red) centered bins for which the PSTHs were averaged. The average PSTH for all penetrations within this animal are shown in dark blue. Bars are standard errors. b) Averaged PSTHs across the same bins for all animals within the naïve group. Note that individual animals were very similar to the averages across animals within the group, indicating a high consistency

of this response measure across animals. Note that every spike in this 0-60 msec PSTH epoch was counted and averaged.

Chapter 3: Examination of A1 Properties After LC-Stimulation-Tone-Pip Pairing (4kHz and 9kHz)

Chapter 3: Primary auditory cortical plasticity via LC-stimulation: tone-pip pairing

Abstract

Auditory cortical areas represent sensory inputs that have passed through several interacting filters. Stimuli enter our brains most waking milliseconds, but those stimuli that might require immediate attention or that have been previously learned to be as such, activate systems that are designed to enable retention of the stimulus itself and the response to that stimulus that has gained the animal the most reward without excessive risk. The perception of risk or relevance, and their plastic influences on stimulus-driven activity are indistinguishable because the very systems that induce fight or flight hormonal responses or sensations (for example) are also enabling the retention of the stimuli that might have induced such a state. The same argument works for reward and hedonic impact. I will however work towards an almost mutually exclusive relationship between types of stress and types of hedonic impact.

One nucleus that has received much attention in this realm is the locus coeruleus (LC). Once a stimulus activates the LC, the incoming stimulus will intersect with internally generated noradrenaline (NA) signals. Though the incoming stimulus-generated activity is an approximation of the stimulus, the NE signal that accompanies this activity is an internally generated relevance flag that plausibly allows this stimulus-driven activity to have certain privileges that raise the probability of sending suprathreshold signals across synapses and in turn enabling Hebbian changes in connections and synaptic strengths to occur. In the following experiments, episodic electrical stimulation of the LC paired with an auditory stimulus resulted in

substantial alterations in A1 tonotopy and response profiles that were differential, depending on the auditory stimulus. This suggests that input characteristics drive specific receptive field alterations independent of behavioral response or knowledge of task rules.

Introduction

The LC has long been implicated in arousal. However, arousal reflects a fundamental property of behavior that has proven difficult to define or link to specific neurobiological mechanisms and/or systems. According to Aston-Jones and Cohen 2005, phasic LC activation is driven by the outcome of task-related decision processes and is proposed to facilitate ensuing behaviors and to help optimize task performance.

In Aston-Jones, Rajkowski, and Kubiak (1997), the LC was investigated in a visual discrimination task in which monkeys were required to release a lever within 665ms only to the CS+ and not the CS- stimulus to receive a juice reward. Monkeys were over-trained for several months on one stimulus contingency. In this task, LC neurons were selectively and phasically activated by CS+ stimuli (onset latency ~140ms) for the over-trained contingency, but not for other task events. The contingency was then reversed. Very soon after contingency reversal, there was a rapid and transient increase in the tonic activity of LC neurons, and soon all LC neurons became selectively responsive to the new target and non-responsive to the old target (new non-target). Neural excitation of the LC was detected within the first

100 new target stimulus presentations, and extinguished within 100 presentations of the new non-target.

This is a fairly difficult task, and monkeys required over 1000 trials to establish a new behavioral contingency to a criterion of fewer than 5% task errors. LC neurons however, exhibited very good discrimination within the first 500 trials of a new contingency; they acquired the new response profile before accurate behavioral performance on the new task was established. The LC is likely involved in the early organization of the proper behavioral responses to novel reward associations (Kubiak et al. 1998).

In rats, the effect of NE in the auditory cortex has been investigated via iontophoresis experiments. Manunta and Edeline (2004) evaluated how repeated presentation of brief pulses of NE concomitant with presentation of a particular tone frequency changes the frequency tuning curves of auditory cortical neurons. Neurons previously tuned within ¼ octave of the paired frequency exhibited selective tuning modifications towards the paired frequency after 100 pairing trials. This suggests that NE can modify cortical responses in an acute preparation.

Taken together, these studies indicate that there are likely acute cortical modifications occurring in tasks that engage the LC including simple discrimination, and that the LC likely directs behavioral modification and the sculpting of the most successful response as well as the retention of this sculpting and modification. The following study attempted to extend the investigation to include the examination of chronic plasticity effects due to repeated LC activation linked with a stimulus similar to those previously used. This pairing does not require a learned behavioral response,

as opposed to the Aston-Jones experiment in which a response is required, and employs a more natural activation of the LC-NE system and all its downstream effects than attempted in previous studies of cortical plasticity.

Methods

Implantation and Pairing: Female Sprague Dawley rats (280–350 gm; 3–6 months old) were anesthetized with sodium pentobarbital. Platinum bipolar stimulating electrodes (SNE-200, Rhodes Medical Instruments, Woodland Hills, CA; 0.1 mm tip diameter and 0.5 mm separation) were stereotaxically implanted into the right locus coeruleus (1.2 mm lateral and 4.0 mm caudal to lambda crossing, 6.0 mm below the cortical surface; bregma 2 mm below lambda). Three bone screws were threaded into a burr hole on the skull to anchor the electrode assembly. Leads were attached to the screws over the cortex to monitor cortical EEG during LC stimulation. All procedures were approved under University of California San Francisco Animal Care Facility protocols. After a 2 week recovery period, rats were tested for the threshold of current microstimulation (10 biphasic pulses of 0.1 msec duration at 100 Hz) necessary to desynchronize cortical EEG. A small four-pin connector was used to record the EEG and deliver electric current pulses to the stimulating electrode. Rats with a desynchronization threshold at or below 200 microamperes were included for further study. A group of 8 awake and unrestrained rats received pairings of pip trains (six 50 msec pulses; 5 msec on/off ramps; delivered at a rate of 20 pulses per second; 70 dB total sound pressure level, 4kHz or 9kHz) with LC microstimulation (10 biphasic pulses of 0.1 msec duration at 100 Hz, initiated 100 msec after sound onset, typically

100–200 microamperes). The tone-pip stimulus was generated using the LabView (National Instruments, Austin, TX). Approximately four hundred pairing trials were delivered in daily sessions, with a random inter-trial interval in the range of 12–28 sec. Pairing took place in a 25x25x25 cm wire cage located in a 50x50x50 cm sound-attenuation chamber lined with 3 inch acoustic foam. Use of this sound chamber accomplished approximately 40 dB attenuation of sounds occurring outside the chamber. The chamber was illuminated and well ventilated. After 20 d (5 d/week, 4 weeks) of pip train pairing, the auditory cortex was mapped 24 hr after the last pairing session.

Histology: Subsequent to A1 mapping, animals were perfused and fixed in 4% paraformaldehyde. An electrolytic lesion was done in order to identify electrode placement. 10 microamperes of current was delivered for 15 seconds with positive polarity. A Simpson Electric Company stimulator was used for this step. The brains were then removed and allowed to equilibrate in a 30% sucrose solution. Sections 50 micrometers in thickness were cut on a cryostat and collected in 0.1 M phosphate buffer, pH7.2-7.4. Sections were mounted onto 0.5% gelatin-subbed slides using a small paintbrush and Petri dish of 0.2% gelatin solution. Slides were dried at room temperature for a minimum of 2-3 days.

Neutral Red: Tissue sections were re-hydrated in a descending ethanol series, beginning with 95%, then 70%, 50%, and finally in distilled H2O. Sections remained in each solution for approximately 5 minutes. Slides were placed in 1% Neutral Red solution (made in ddH2O, with 1 drop of glacial acetic acid per 100mL) for 5 minutes. The exact staining time varied, depending on section thickness and tissue

condition. Slides were washed extensively in dH2O until the wash water was colorless. Slides were placed through serial ascending ethanols, starting at 50% (2x), 70% (2x), 95% (2x), and 100% (2x). Slides were kept in each wash for 2-3 minutes and were agitated occasionally to promote destaining. They were then dehydrated in xylene (2x) for 5 minutes. Slides were coverslipped with 24x60 mm coverslip with DPX (Sigma, Cat# 317616).

Cresyl Violet: Tissue sections were dehydrated in ascending ethanols, starting at 95% (2x), and 100% (2x). Slides were kept in each wash for 15-20 minutes. They were dehydrated in xylene (2x) for 5 minutes and then re-hydrated in descending ethanol series, beginning with 100%, 95%, then 70%, 50%, and finally in distilled H2O. They were left in each solution for approximately 5 minutes. Slides were placed in 0.5% Cresyl Violet solution (made in ddH2O, with 0.75ml of glacial acetic acid per 250mL) for 5 minutes. The exact staining time varied, depending on the section thickness and tissue condition. Slides were washed extensively in dH2O until the wash water was colorless. They were placed through serial ascending ethanols, starting at 50% (2x), 70% (2x), 95% (2x), and 100% (2x). Slides were kept in each wash for 2-3 minutes and were agitated occasionally to promote destaining. They were then dehydrated in xylene, 2x for 5 minutes. Slides were coverslipped with 24x60 mm coverslip with DPX (Sigma, Cat# 317616).

Results

The physiologically defined area of the primary auditory cortex (A1) was larger in paired animals compared to adult naïve female Sprague Dawley rats. This expansion was not limited to the paired frequency, but included all frequencies in A1.

In the first set of experiments, animals were given LC stimulation of approximately 100 microamperes paired with a series of 6, 50 ms tone pips of either 4kHz (n = 4) or 9kHz (n = 4). LC stimulation was delayed from tone-pip onset by 100ms (Fig 3.1). Animals received approximately 400 paired trials per day over 20 days. An abbreviated diagram of the pairing procedure is shown in figure 3.2. Note that the animal was actually in a sound-transparent, wire cage, and not in a plastic sound-reflective one as pictured. Animals' auditory cortices were mapped 24 hours after the last pairing session. Figures 3.3a and b show histological confirmation of electrode placement in the LC. Circle indicates contralateral LC.

The auditory cortices of tone-pip-paired animals were mapped and examined for response properties for this first experiment. Figure 3.4 shows a comparison of maps between a 9kHz-paired and naïve rat; Figure 3.5 shows a comparison between a 4kHz-paired and naïve animal. The bar represents one millimeter. The differences in overall sizes of the maps are quantified in Figure 3.6, which includes the overall A1 areas of all animals from these different groups. The size of A1 is larger in paired animals versus naïve animals; the average size of A1 in the of 4kHz-paired group was 1.8803 +/- 0.1306 mm^2 ($p < 0.05$ relative to naïve group) and the average size of A1 in the 9kHz-paired group was 2.581 +/- 0.1658 mm^2 ($p < 0.01$ relative to naïve group).

LC-stimulation-tone-pip-pairing induces frequency-specific alterations

In addition to the general expansion of A1 size, LC-stimulation-tone-pip-pairing also induced a frequency-specific expansion within A1. There is an increase in the area representing the paired frequency (Figs. 3.8a-h) in addition to and perhaps related to the overall increase in A1 size. This increase occurs at all intensities in the 9kHz-paired group because of the very large increase in overall size, but only occurs down to 40dB in the 4kHz group arguably because of the more modest overall increase in A1 size in this group (data not shown).

There is also an increase in the fraction of A1 representing the paired frequency. In the series of plots below, the fractions of A1 responding across frequencies is represented (40dB-70dB, Figs. 3.7a-h)). At the highest intensity, there appears to be a saturation of response and therefore there is little or no difference in the fraction of A1 responding to the paired frequency compared with naïve animals. However, at intensities between 40dB and 60dB there is a clear increase in this fraction. This is a fairly narrow window and implicates intensity specificity in the fractional relationship between paired and non-paired frequencies in the paired groups. These effects appear to be at the expense of frequencies immediately adjacent to the paired frequency where there are dips in the fractions representing those immediately adjacent frequencies.

LC-stimulation-tone-pip pairing induces changes in neuronal spiking properties across A1

In addition to the alterations in tuning and A1 maps, LC-stimulation-tone-pip pairing induces many changes in the spiking properties of multiunits across A1. Summed poststimulus time histograms (PSTH) were calculated for all multiunits. These summed PSTHs include all responses of a given multiunit to the 400 stimuli delivered in the A1 mapping procedure to calculate each tuning curve. Summed and then averaged PSTHs were grouped into 0.5 octave bins. The responses for multiunits within the 0.5 octave bin closest to the paired frequency were prolonged in comparison to other bins, as well as an average of all other bins within a paired animal and also in comparison to those for naïve animals. In addition, within a paired animal, responses in all bins except that of the paired frequency were smaller in amplitude compared to the bin of the paired frequency (ANOVAs: 4kHz-paired group: and also in comparison to that of naïve animals (fig. 3.9a-c). This decrease may be due to an alteration in the balance of inhibition and excitation.

These PSTH effects appear, in part, to be a reflection of alterations in the bandwidths of binned multiunits. Here, the threshold of the multiunit is defined as the minimum intensity to which the neurons reliably respond to the delivered stimuli; this value (dB attenuation) often corresponds to the response at or near the CF of a given multiunit as neurons are likely to respond most reliably at the lowest intensity at or near their respective CFs. The bandwidths at 30 dB below threshold of all frequency bins sampled from all groups are shown in Figure 7.3; thresholds are in 7.4. At best there is a slight effect on BW30s of the paired frequency bins. There

appears to be no consistent effect on threshold. These data will be discussed further in chapter 6.

Discussion

These results raise several questions including: 1) Why does the pairing protocol result in an enlarged A1? 2) What purpose might a generally enlarged A1 serve for the animal? The neurons that are part of A1 in these animals are not necessarily new auditory-responsive neurons as they may have been part of other auditory fields prior to the pairing protocol. Instead, I propose that these neurons develop lower thresholds over the course of the pairing and in turn require less voltage input to spike and therefore respond at shorter latencies causing them to meet our criteria for A1 inclusion. There is much precedent for this in the literature. As mentioned in the review, NE has been demonstrated to inhibit leak K+ channels as well as Ca++-activated K+ Channels in cortical pyramidal cells. Either of these effects or both in combination might cause the resting potential of these cells to be raised and therefore lower their spiking thresholds and shorten their latencies. In addition, spontaneous activity that would otherwise be subthreshold, might become suprathreshold and induce a spread of activity whose source is not external but internal. Over-excitation of the LC might induce a state of exaggerated excitability.

The importance of this enlargement is a more speculative subject. I propose that the pairing protocol alerts the animal that the auditory modality is persistently the most consistent predictor of LC activity and therefore the most consistent predictor of stress or novelty. I propose that the LC is involved in producing the stress response

(perhaps through its activation of the HPA axis), and is not merely activated by the stress itself. Although other modalities are stimulated during the epochs of LC stimulation, the auditory modality is phasically activated in a manner that is most temporally correlated with LC activity. Perhaps it is the cortical horizontal connections that spread activity within the auditory cortex while NE is present, the combination of which induces a permissive excitability that is related by modality and not necessarily by isofrequency proximity even though isofrequency proximity may be a more dominant factor.

This horizontal spread and it's unsynchronized character might also explain the resulting spread of activity in the temporal domain that will be discussed in later chapters. This plasticity can therefore still be Hebbian and not necessarily synchronized with the initial input of activity to the cortex from the periphery. Rather, the increased excitability of regions adjacent to the isofrequency region activated by the periphery, combined with horizontal spread of activity might cause the neurons on the postsynaptic side of layer three to fire soon enough to create Hebbian plasticity within layer three; this could include both excitatory and inhibitory neurons. Given this, the cortical population activity resulting from a particular pip in the tuning curve stimulus set, would not have to be synchronized to be a product of Hebbian plasticity.

In addition to the large general expansion of A1, it is also demonstrated that this plasticity is not proportional across frequencies represented in A1. Instead the representation of the paired frequency is preferentially expanded. Past publications have discussed the likelihood that this type of plasticity is related to the learned

behavioral salience of this specific stimulus, and its recognition and identification in the environment. This might be the case here as over time the animal repeatedly associates the frequency of the paired stimulus with a stressful or novel event. This plasticity is a plausible neural substrate of these memories.

The mechanism of this plasticity might be related to the mechanism of the general expansion discussed in Chapter 1. As the auditory stimulus traverses the neuraxis and reaches the auditory cortex, this activity is then propagated via horizontal connections. The progressive lowering of the thresholds of cortical pyramidal cells across the pairing protocol, along with this auditory cortical activity within and across cortical columns, could lead to Hebbian strengthening between synapses and the progressive spread of excitation to more distant neurons. Because the activity within and near the isofrequency region representing the paired stimulus occurs in a smaller temporal window, spread of Hebbian plasticity is more likely to promote the spread of representation of the paired stimulus into neighboring regions and thus promote the frequency specificity of the plasticity. This is further supported by the data in figures 3.7 and 3.8 in which the level of plasticity is shown to be less at further distances from the area representing the paired stimulus.

These adaptations in cortical responsiveness hint at the roles of the LC and NE in plasticity and learning. It appears that the pairing protocol increases the excitability of longer latency neurons in a manner that decreases their latencies. In addition, the neurons less proximal to the paired frequency respond with fewer spikes within the animal and in comparison to naïve animals, which implies increased inhibition as well. Therefore these portions of the cortex are responsive faster but with less

intensity to auditory stimuli other than the paired stimulus. In contrast, neurons more proximal to the isofrequency region representing the paired stimulus respond for a longer duration and likely in a less synchronized manner across the population. The reason for this is unclear, but might allow for a propagation of plasticity to downstream synapses that spans a broader time window.

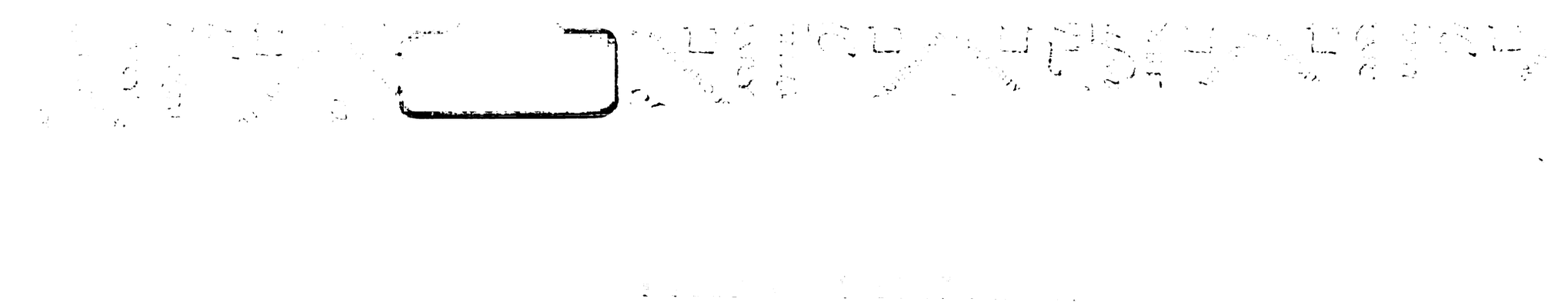
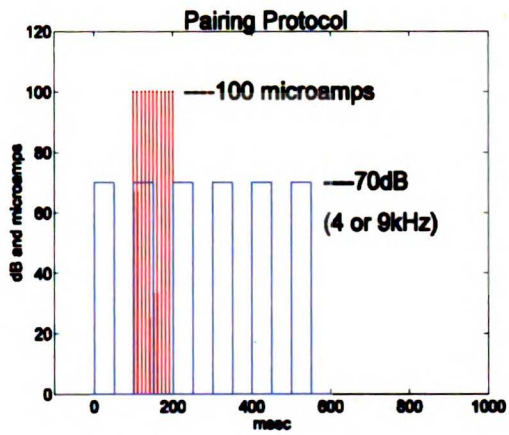


Figure 3.1; Pairing Protocol



*300-400 Trials/Day

*20 Days of Pairing

*Interstimulus Interval = 12-28 secs

Figure 3.2; Pairing

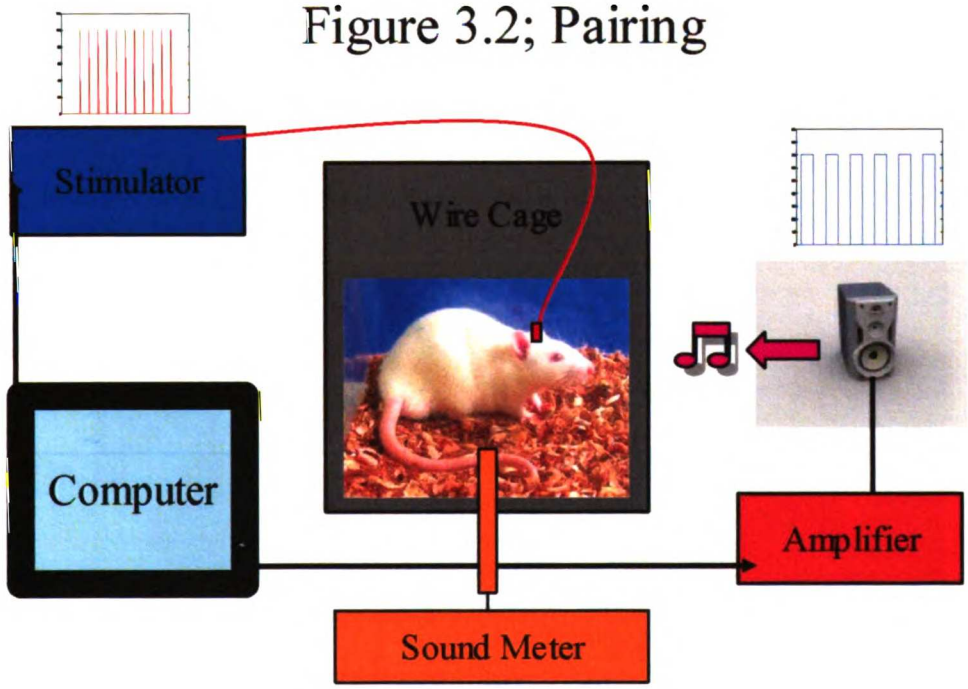


Fig3.3a; Histological Confirmation

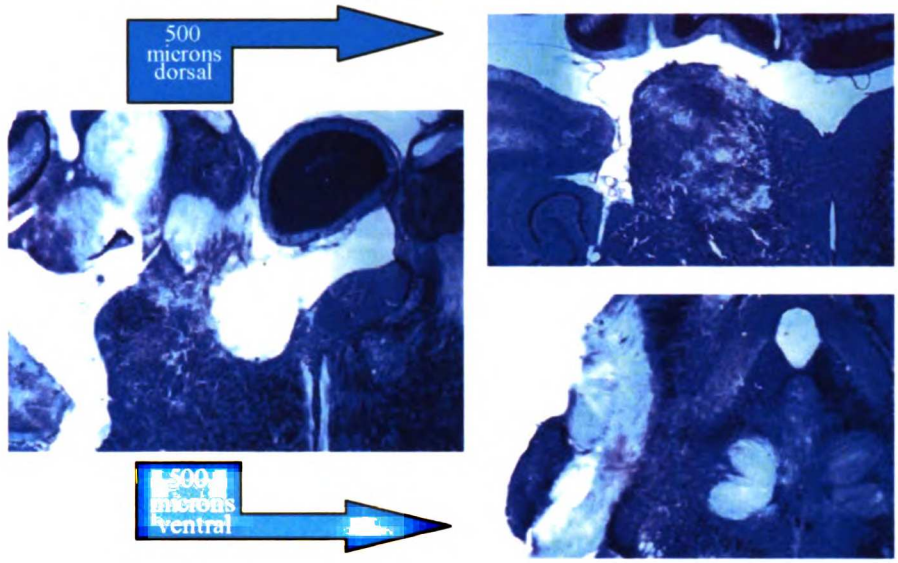


Fig3.3b Histological Confirmation

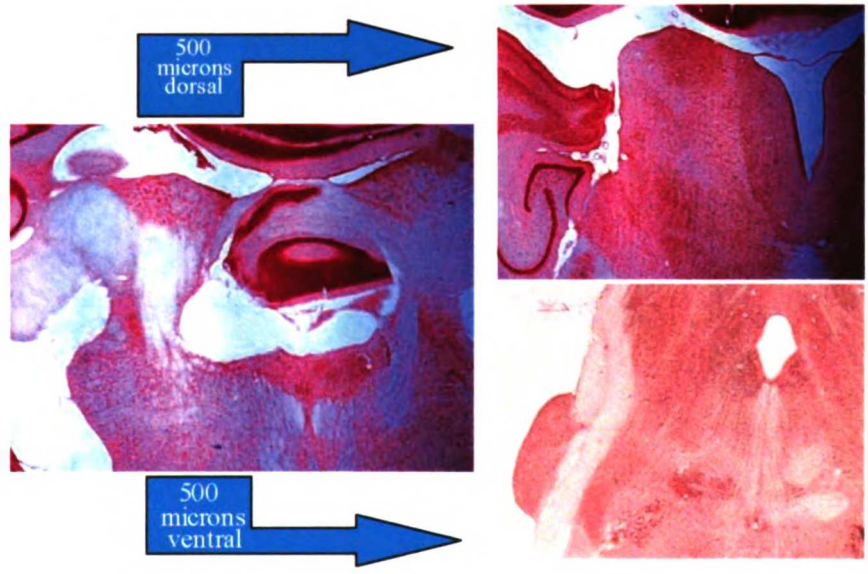


Figure 3.4; Comparison of Naïve and 9kHz-Paired Maps

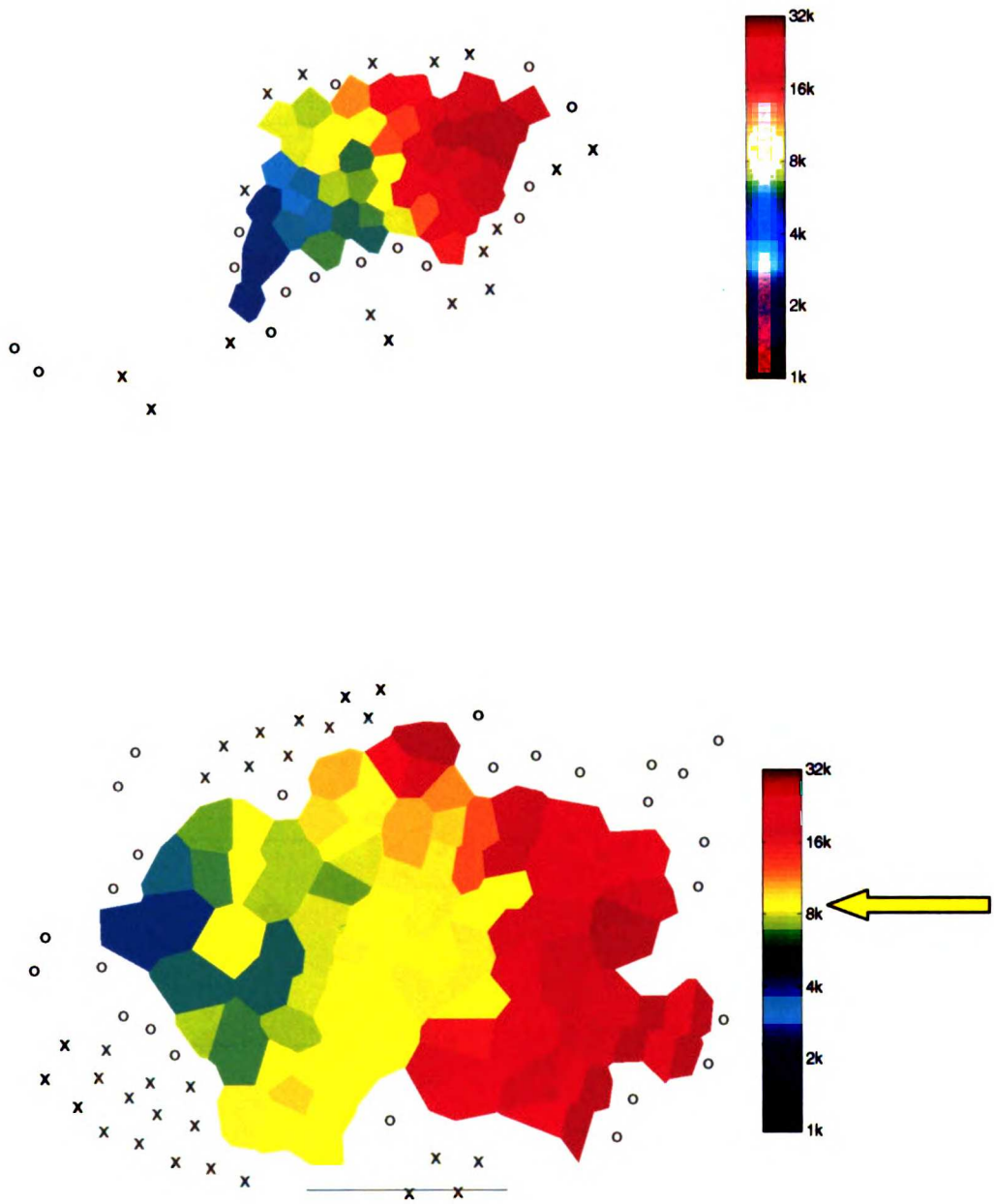


Figure 3.5; Comparison of Naïve and 4kHz-Paired Maps

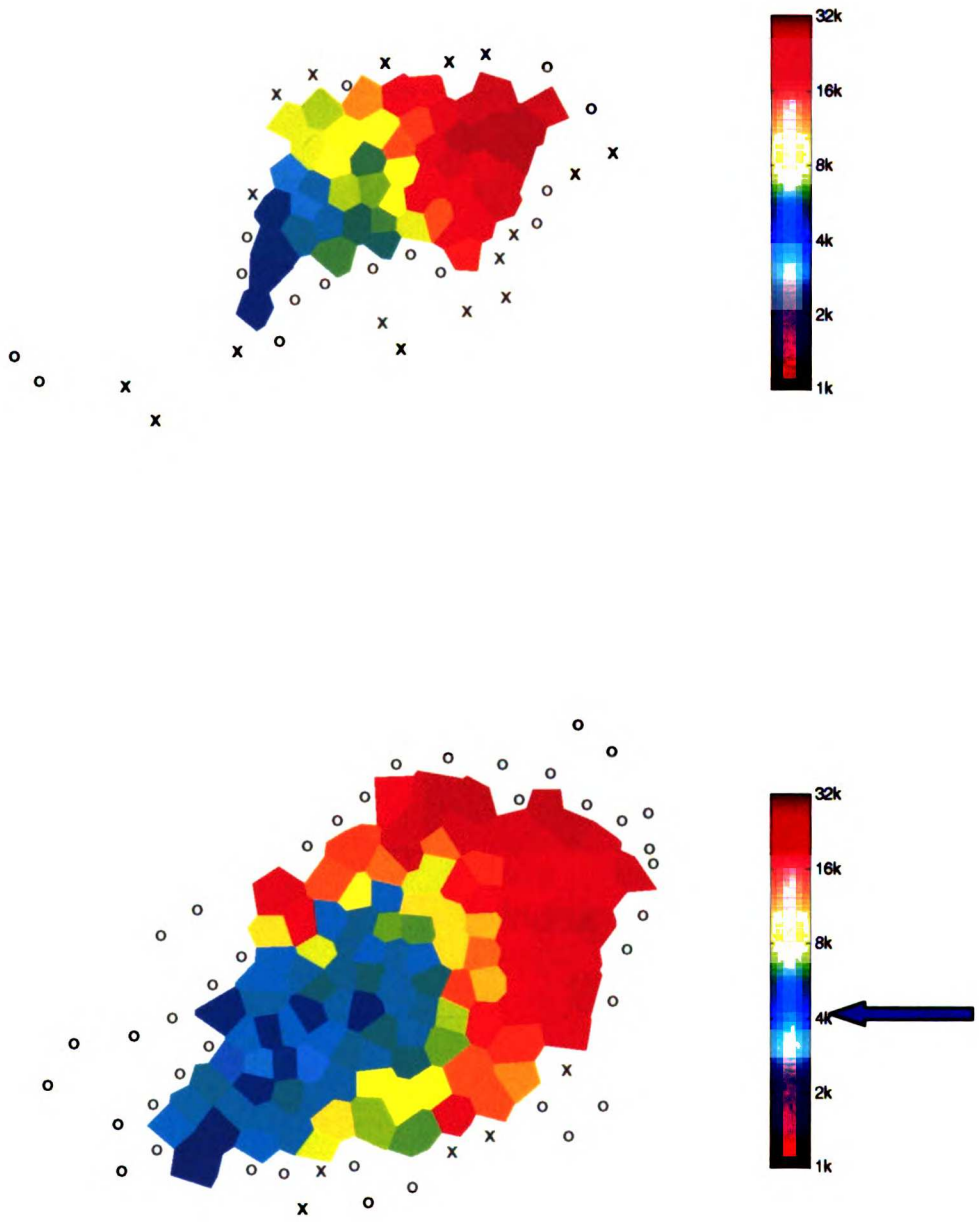


Figure 3.6; A1 Areas Naïve vs. Paired Groups

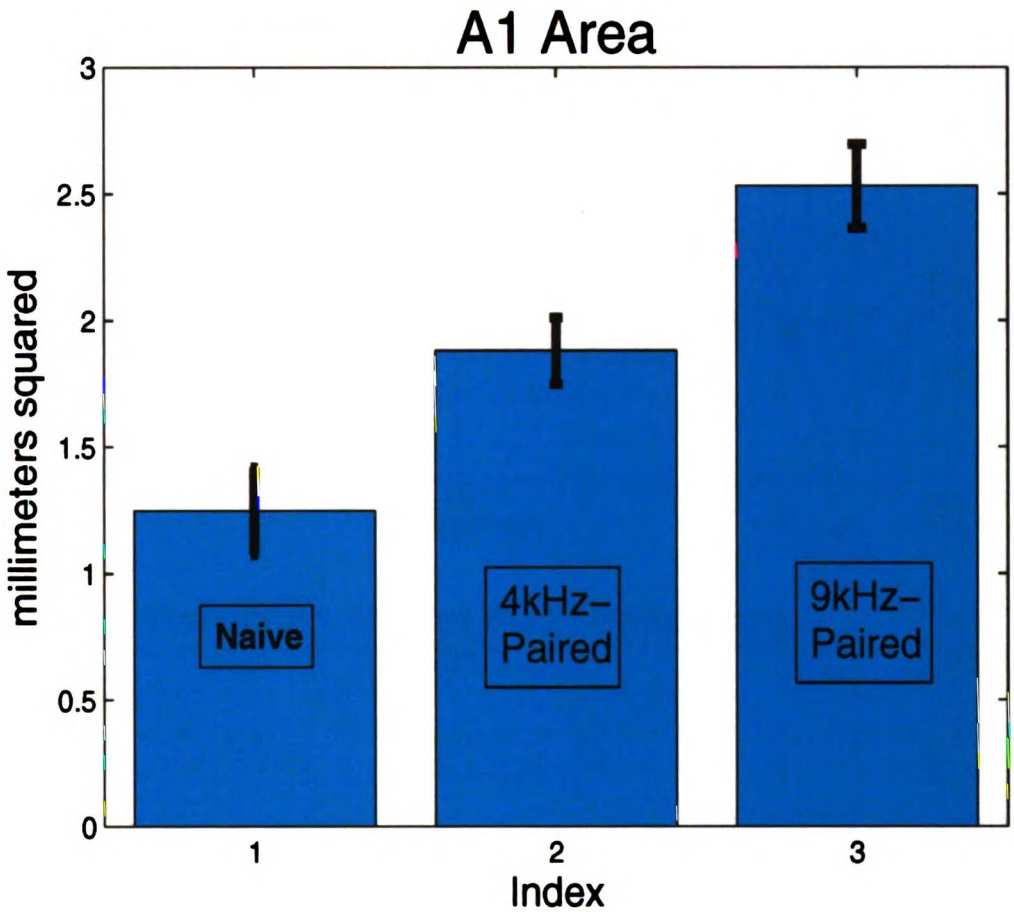
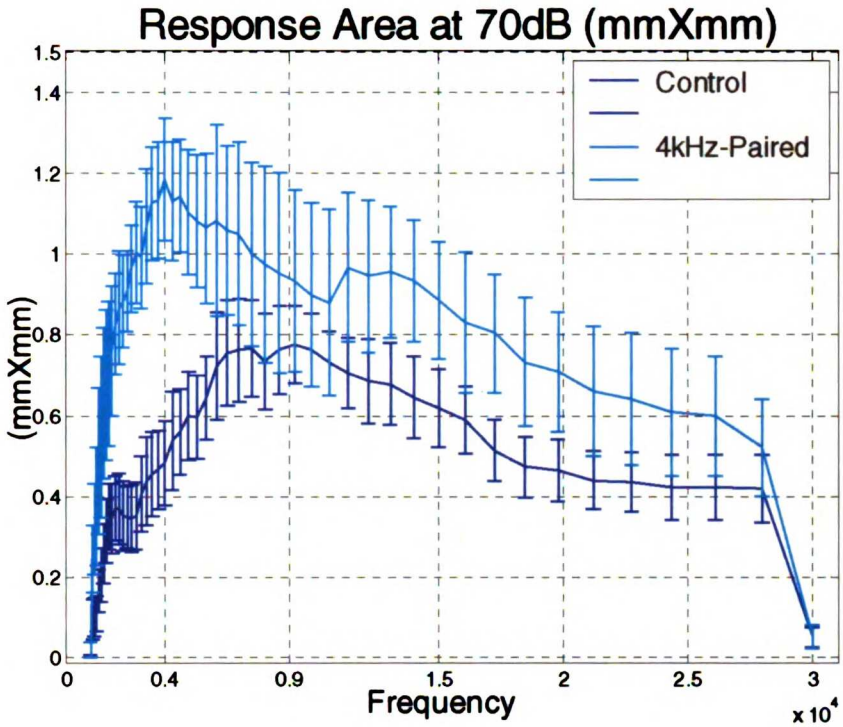


Figure 3.7a-d; Response Areas Naïve vs. 4kHz-Paired Groups (mm^2)



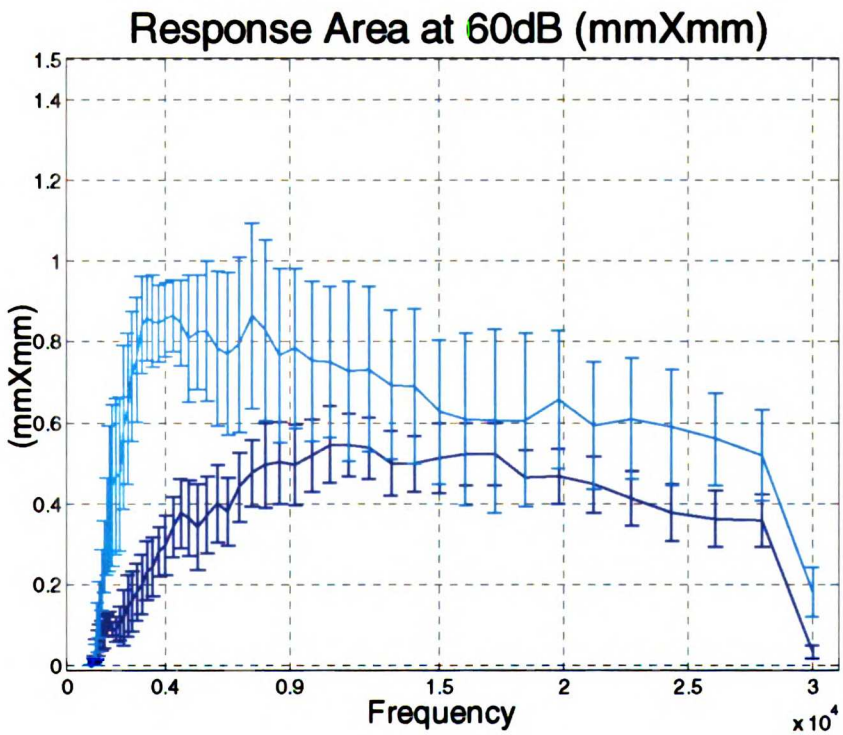
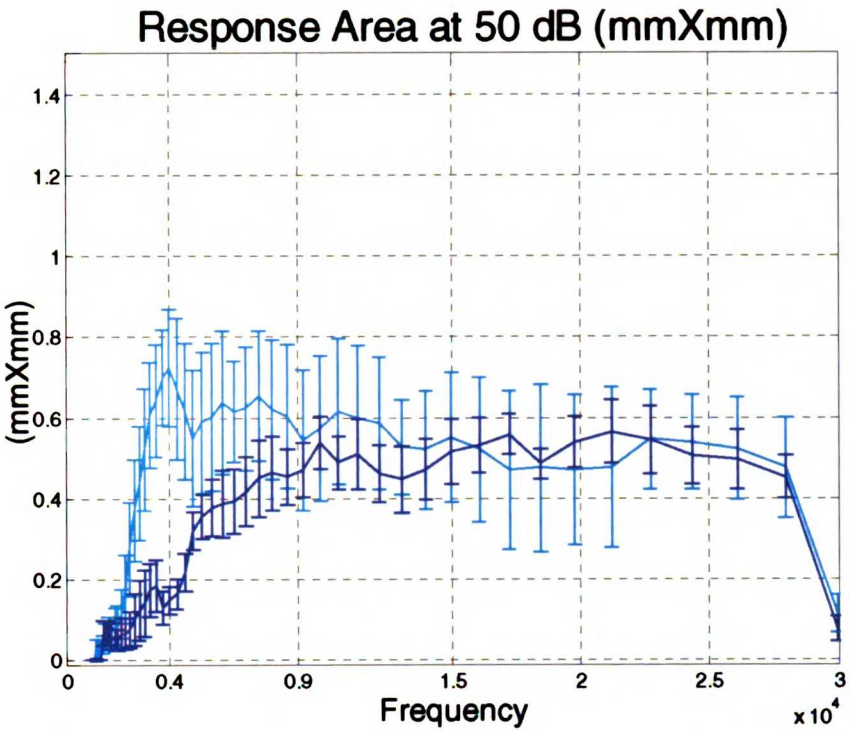


Figure 3.7a-d (cont.); Response Areas Naïve vs. 4kHz-Paired Groups (mm^2)



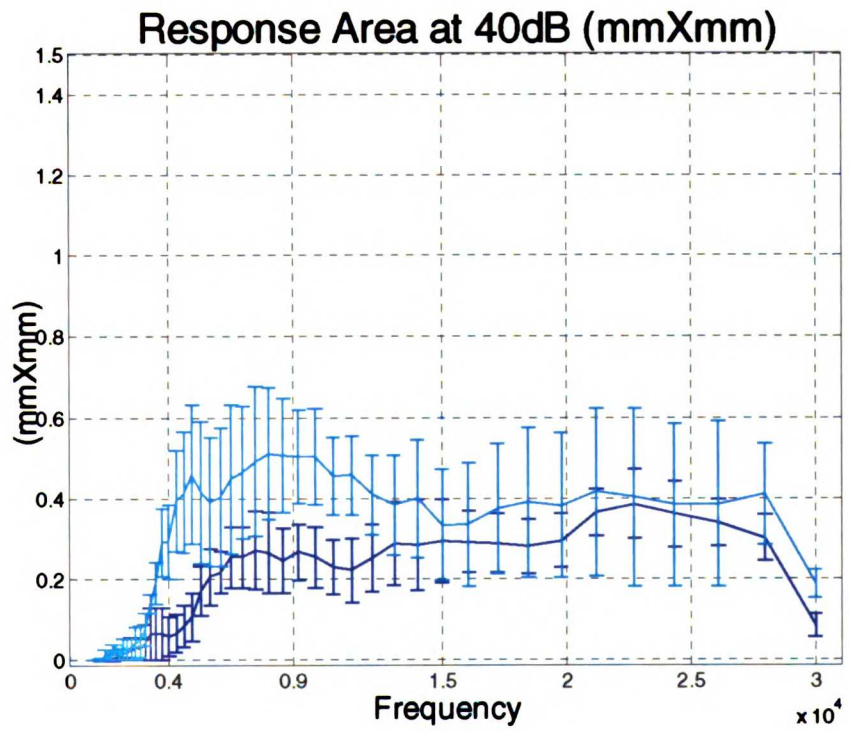
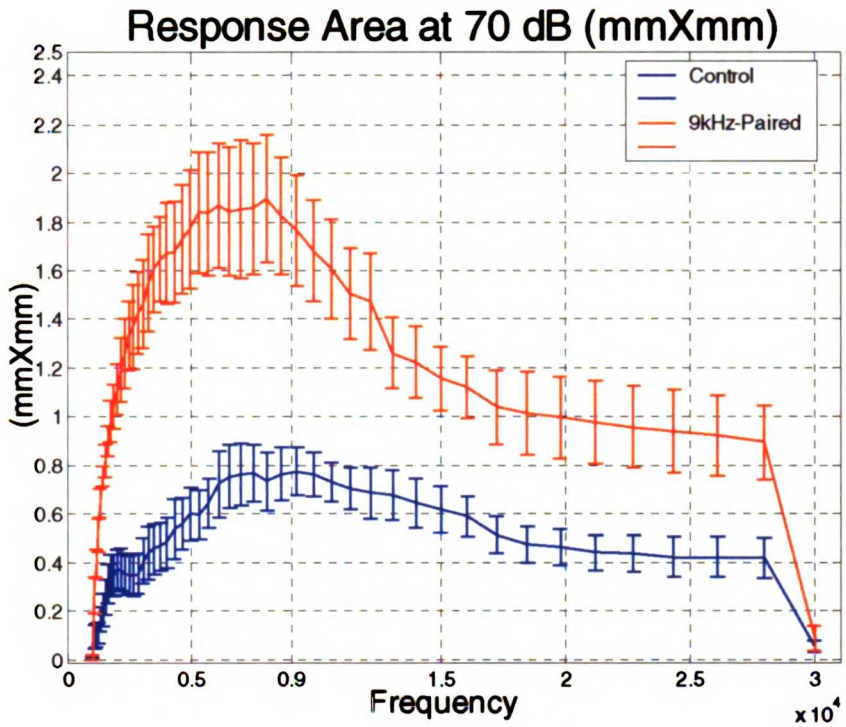


Figure 3.8e-h; Response Areas Naïve vs. 9kHz-Paired Groups (mm^2)



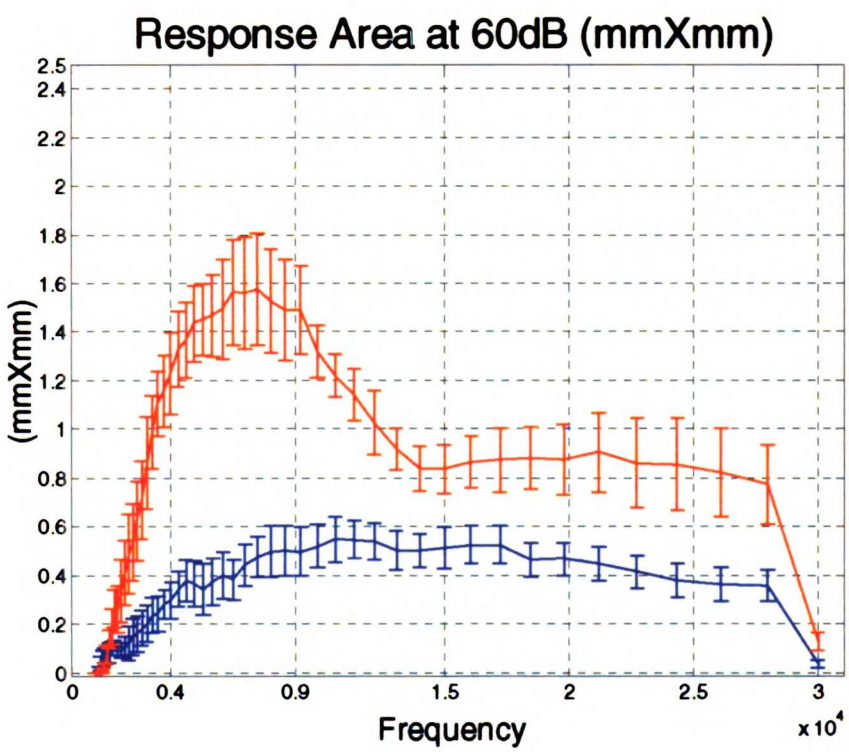
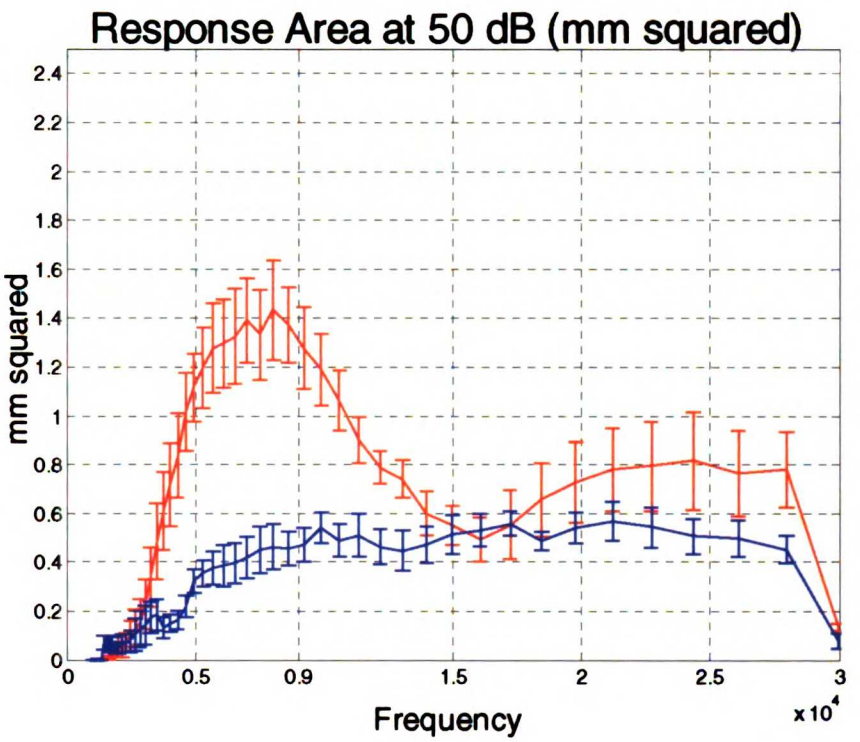


Figure 3.8e-h (cont).; Response Areas Naïve vs. 9kHz-Paired Groups (mm^2)



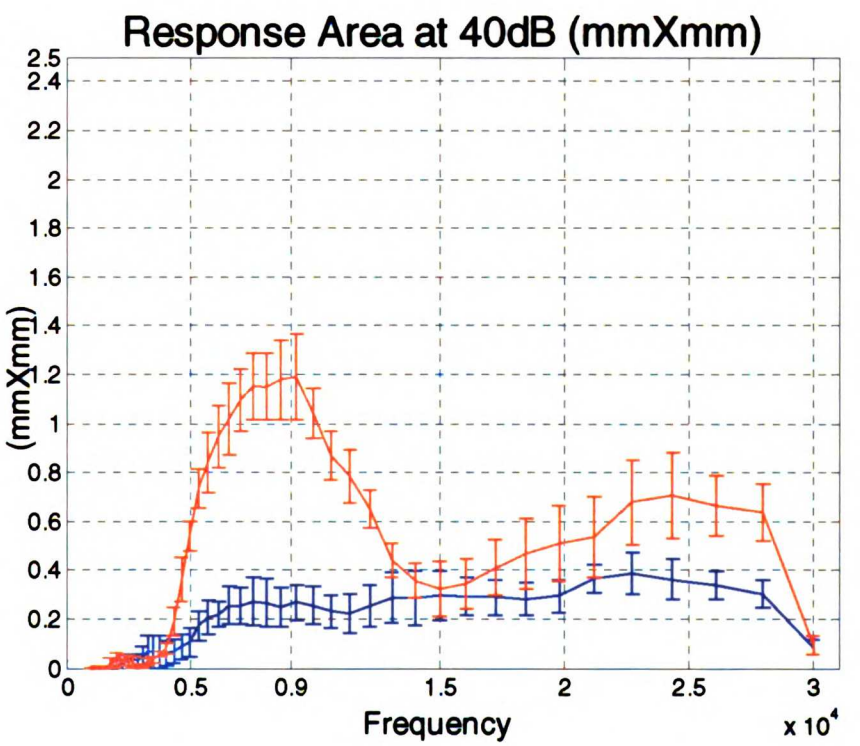
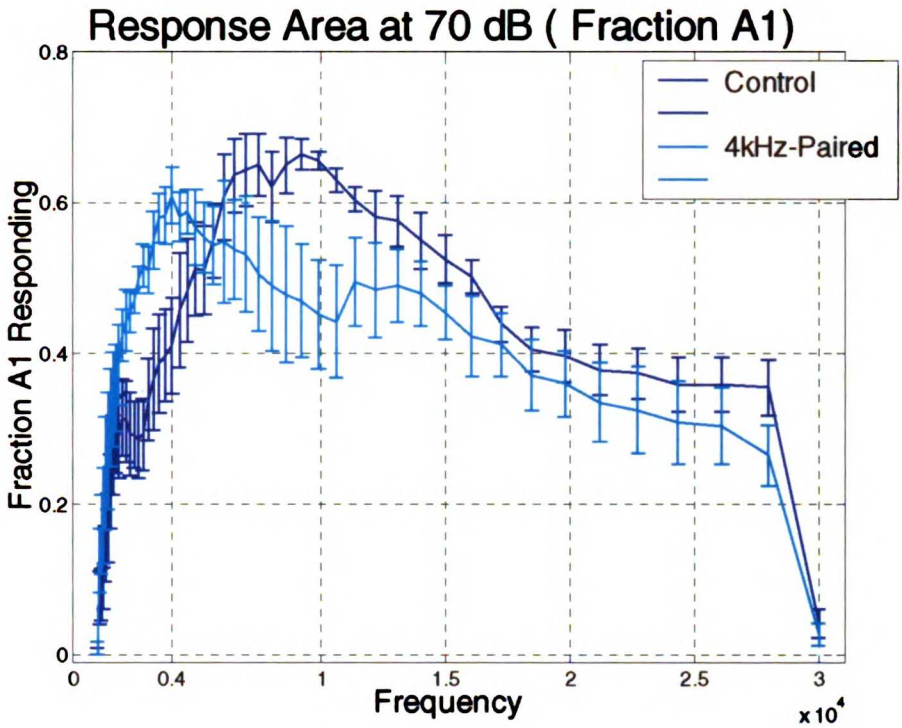


Figure 3.8a-d; Response Areas Naïve vs. 4kHz-Paired Groups (Fraction A1)



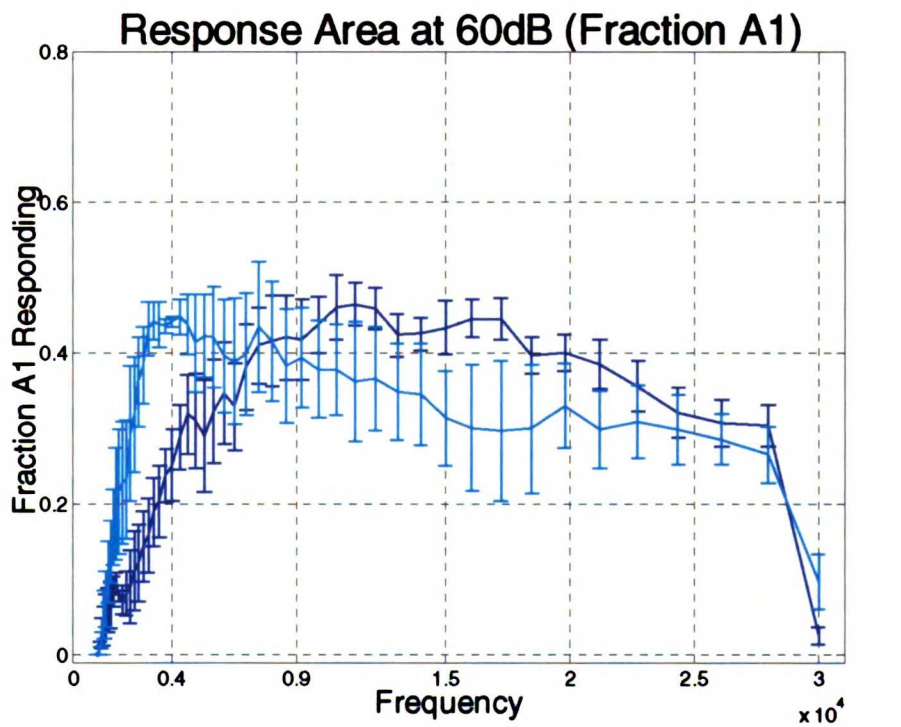
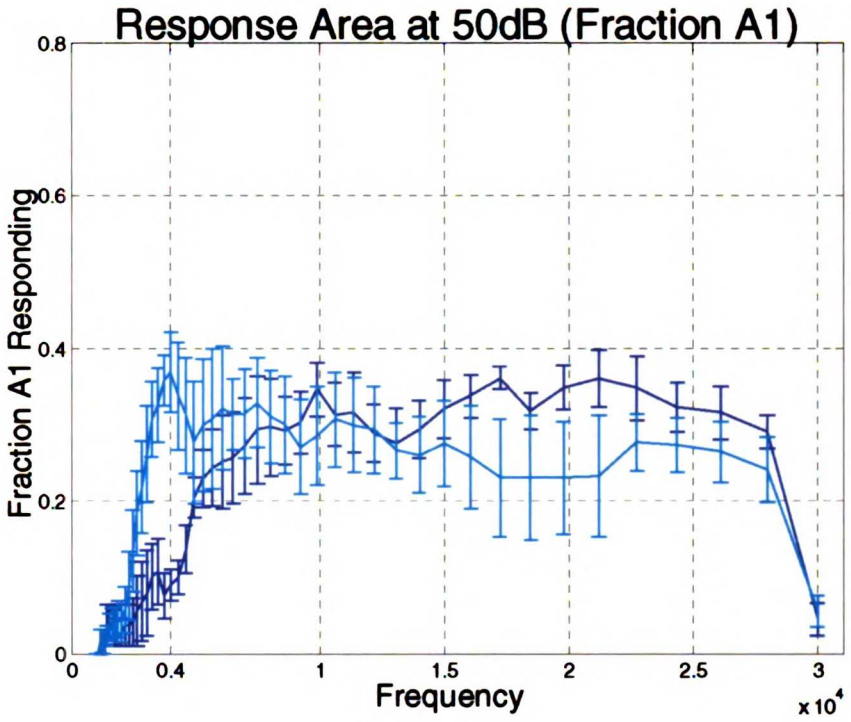


Figure 3.8a-d (cont.); Response Areas Naïve vs. 4kHz-Paired Groups (Fraction A1)



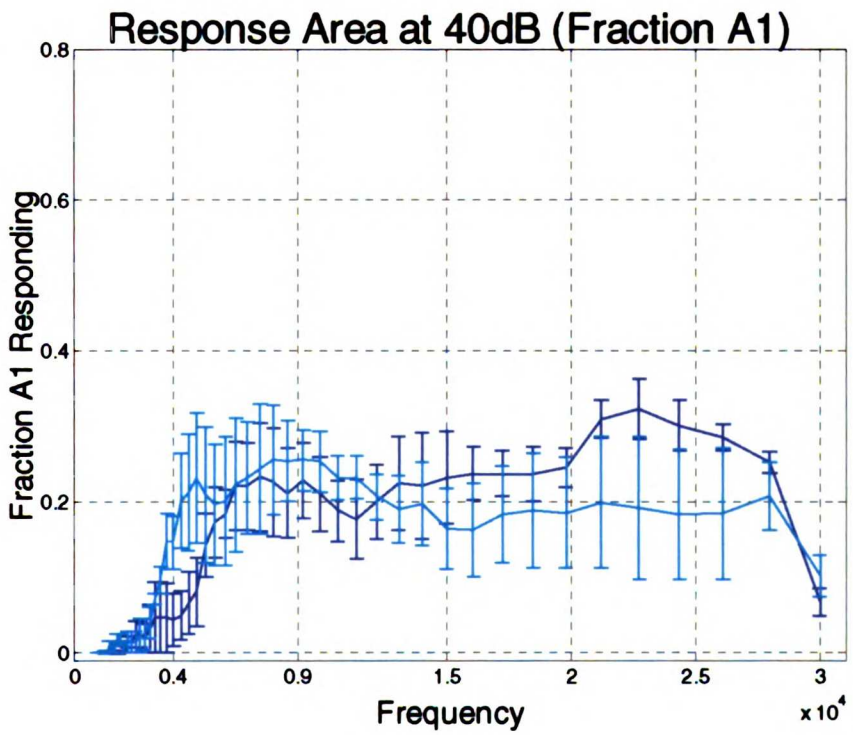
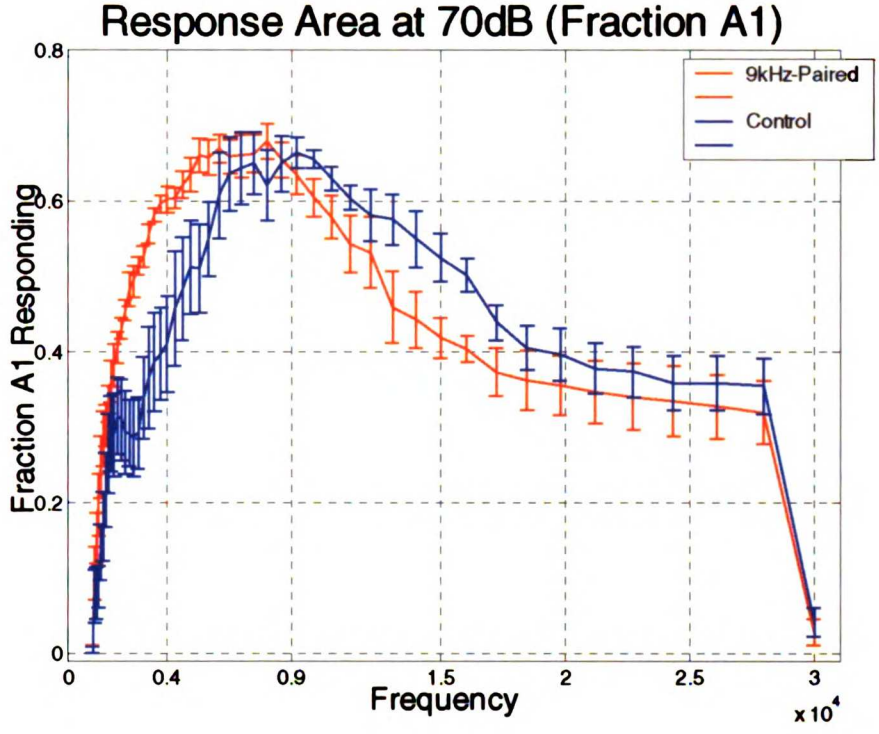


Figure 3.8e-h; Response Areas Naïve vs. 4kHz-Paired Groups (Fraction A1)



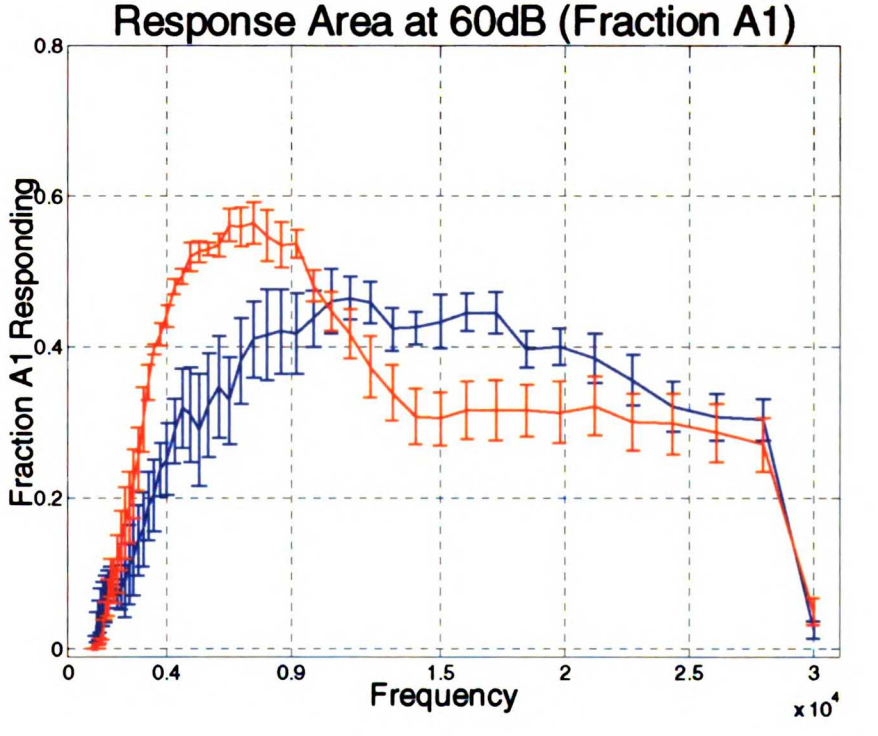


Figure 3.8e-h (cont.); Response Areas Naïve vs. 4kHz-Paired Groups (Fraction A1)

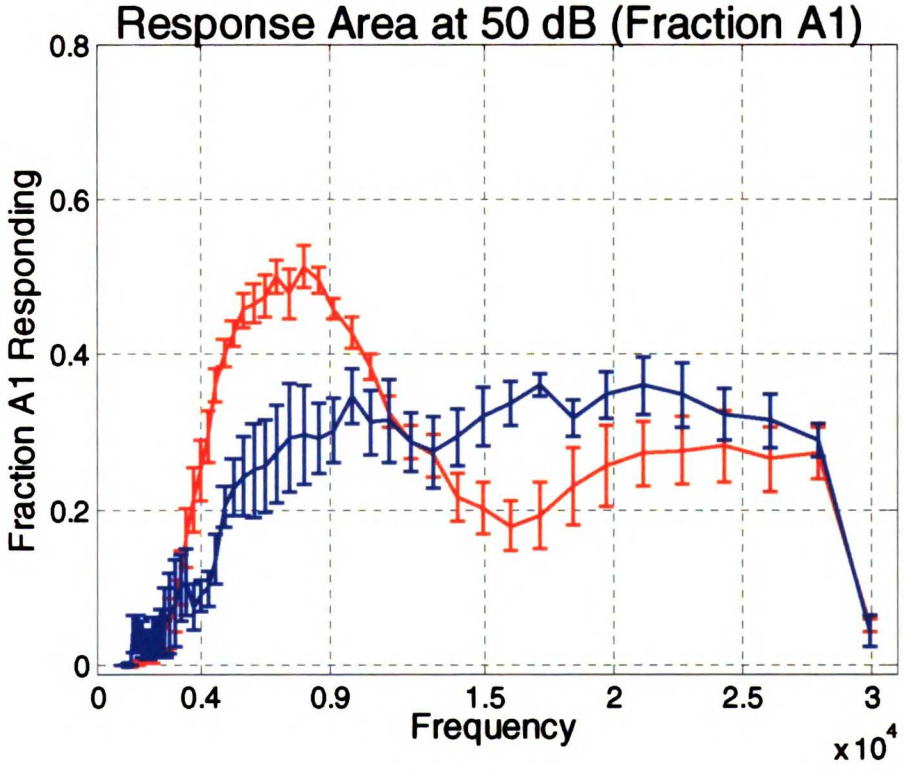


Figure 3.8e-h (cont.); Response Areas Naïve vs. 4kHz-Paired Groups (Fraction A1)

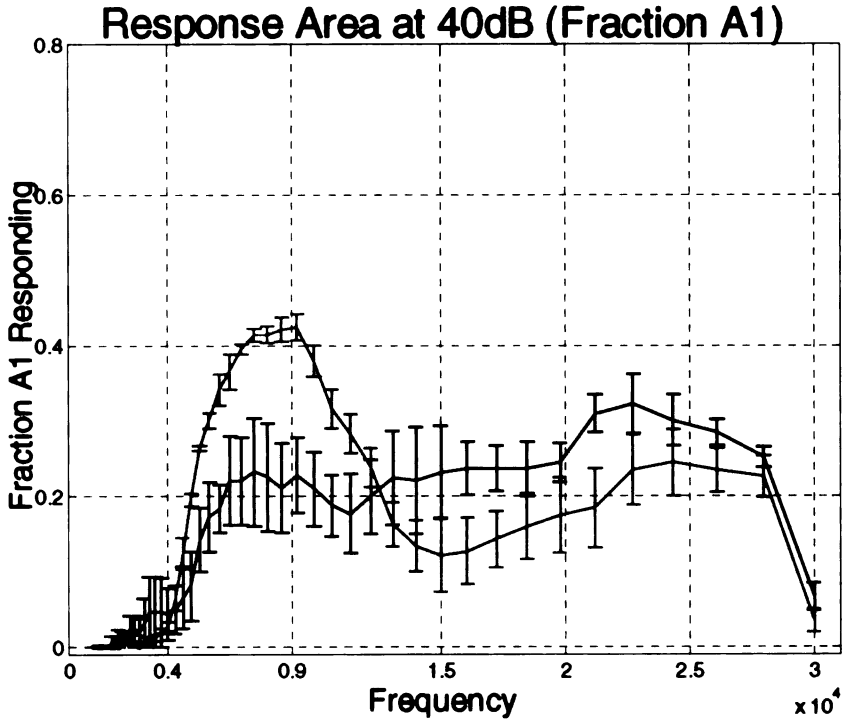
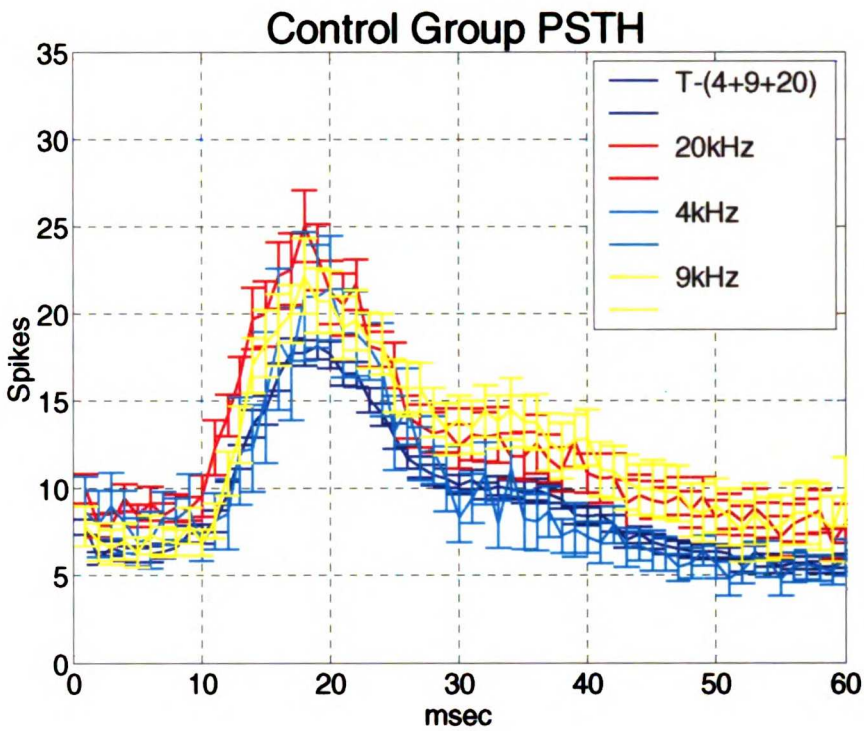


Figure 3.9a-b; Averaged PSTHs Naïve vs. 4kHz-Paired Groups



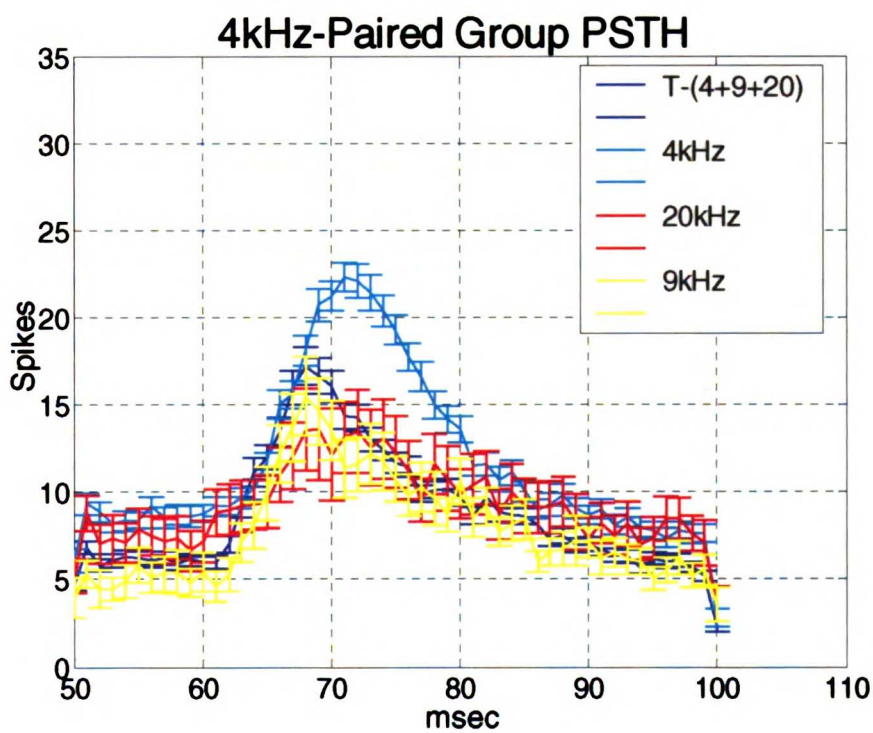
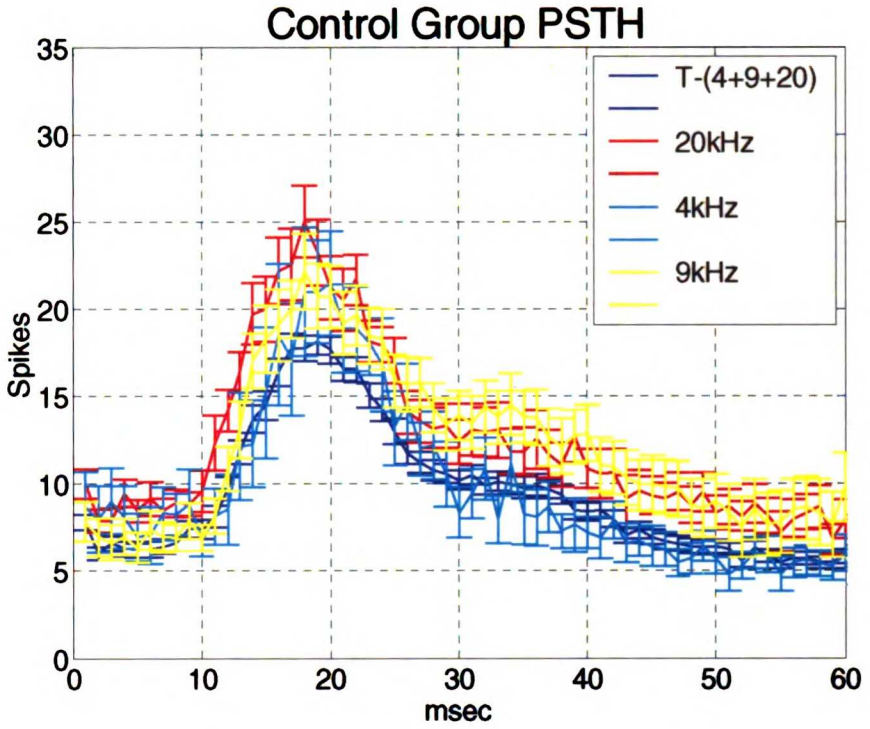
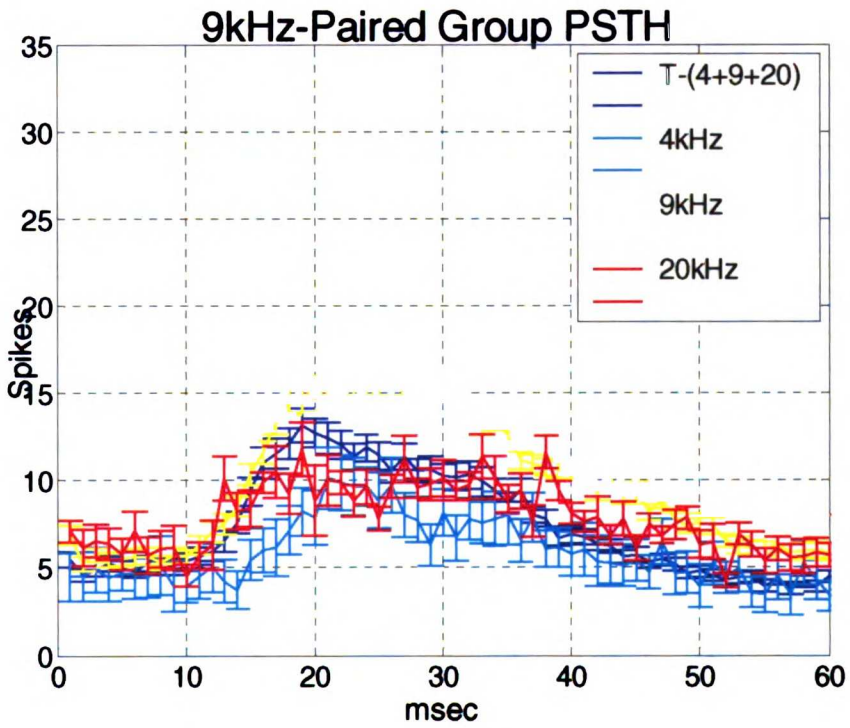


Figure 3.9c; Averaged PSTHs Naïve vs. 9kHz-Paired Groups





Tables 1 and 2; T-Test h values for within-group (4kHz-Paired, 9kHz-Paired) Averaged PSTHs (0.5 octave bins)

4kHz-paired group PSTH t-test; h values for alpha = 0.05

msec Bin	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
4k vs. 9k	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0
4k vs. 20k	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1
4k vs. Oth	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
9k vs. 20k	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9k vs. Oth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20k vs. Oth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

9kHz-paired group PSTH t-test; h values for alpha = 0.05

msec Bin	20	21	22	23	24	25	26	27	28	29	30	31	32
9k vs. 4k	1	1	1	1	1	1	1	1	1	1	1	1	1
9k vs. 20k	1	1	1	1	1	1	1	0	1	1	1	1	0
9k vs. Oth	1	1	1	1	1	1	1	1	1	1	1	1	1
4k vs. 20k	0	0	0	0	0	0	0	0	0	1	0	0	0
4k vs. Oth	0	0	0	0	0	0	0	0	0	0	0	0	0
20k vs. Oth	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3; T-Test h values for Averaged PSTHs Naïve vs. 4kHz-Paired vs. 9kHz-Paired Groups (20-30 msec after stimulus onset)

PSTH t-tests;
h values for alpha = 0.05

	msec Bin	20	21	22	23	24	25	26	27	28	29	30
Naive	9k vs. 4k	0	0	0	0	0	0	0	0	0	0	1
	9k vs. 20k	0	0	0	0	0	0	0	0	0	0	0
	4k vs. 20k	0	0	0	0	0	0	0	0	0	0	1
9kHz-paired	9k vs. 4k	1	1	1	1	1	1	1	1	1	1	1
	9k vs. 20k	1	1	1	1	1	1	1	0	1	1	1
	4k vs. 20k	0	0	0	0	0	0	0	0	0	1	0
4kHz-paired	4k vs. 9k	1	1	1	1	1	1	1	1	1	1	0
	4k vs. 20k	1	1	1	1	1	1	1	1	0	0	1
	9k vs. 20k	0	0	0	0	0	0	0	0	0	0	0

Figure 3.1 Graphical representation of the pairing protocol. X-axis is time and the y-axis is both microamperes in the case of stimulation (red) and dB in the case of stimulus presentation intensity (blue). Note that there are caveats to both of these measures (The Impedance of the animal for the actual amount of current passing and the animal's body and pinna positions for the sound intensity that actually reaches the cochlea) and therefore are approximate. There are 6 50 ms pips at either 9kHz or 4kHz and there are ten 1 ms pulses of approximately 100 microamperes of current. A pip train was chosen based on the auditory cortex's responsiveness to onsets and thus to drive as much of the isofrequency axis in the cortex as possible. Ten pulses of 100 microamperes of current were chosen to mimic a very large burst of activity which has been demonstrated to be optimal for maximal NE release in the cortex (see discussion). There is a 100ms delay between sound onset and stimulation onset meant to mimic the natural response to a behaviorally relevant stimulus as shown in previous studies (see discussion).

Figure 3.2 Schematic of the pairing protocol. A computer drives both the auditory stimulus and microstimulation in a precise temporal relationship; designed in LabView software. Note that the animal is actually in a wire cage that is transparent to sound not in a plexiglass cage as pictured. Animal and speaker are isolated from outside sound in a sound isolation chamber. Attenuation is estimated to be approximately 40 dB.

Figure 3.3a-b Histological confirmation of stimulating electrode placement.

An electrolytic lesion was done in order to identify electrode placement. 10 microamperes of current was delivered for 15 seconds with positive polarity. a) Nissl stains of brain slices from a stimulus-paired animal done in Cresyl violet. Also shown are slices 500 microns dorsal and ventral from the lesion site. b) Nissl stains of brain slices from a stimulus-paired animal done in neutral red. Also shown are slices 500 microns dorsal and ventral from the lesion site.

Figure 3.4 Representative CF map of a right primary auditory cortices of a naïve rat compared to that of a 9kHz-paired animal. Each polygon represents one penetration. Color represents each site's characteristic frequency. Non-responsive and auditory responding non-A1 sites are marked with O's and X's respectively. The arrow on the legend denotes the paired frequency. Note both the overall increase in size as well as the 9kHz-specific expansion (see discussion).

Figure 3.5 Representative CF map of a right primary auditory cortices of a naïve rat compared to that of a 4kHz-paired animal. Each polygon represents one penetration. Color represents each site's characteristic frequency. Non-responsive and auditory responding non-A1 sites are marked with O's and X's respectively. The arrow on the legend denotes the paired frequency. Note both the overall increase in size as well as the 4kHz-specific expansion (see discussion).

Figure 3.6 Bar graph of the primary auditory cortical areas (mm^2) from the naïve, 4kHz, and 9kHz-paired groups. Graph is an average of four representative naïve maps, four 4kHz-paired maps, and four 9kHz-paired maps. Error bar represents standard error. Notice the significant overall increase in the size of A1 in the paired groups.

Figure 3.7a-h a) Square millimeters of A1 responding in 4kHz-paired versus naïve groups across frequencies at 40dB b) at 50dB c) at 60dB d) at 70dB. Notice that between 40 dB and 60 dB, there was a significant increase in the area of A1 neurons representing 4kHz. e) Square millimeters of A1 responding in 9kHz-paired versus naïve groups across frequencies at 40dB f) at 50dB g) at 60dB h) at 70dB. Note that between 40 dB and 70 dB, there was a significant increase in the areas of A1 neurons representing 9kHz. Error bars are standard errors.

Figure 3.8a-h a) Fractions of A1 responding in 4kHz-paired versus naïve groups across frequencies at 40dB b) at 50dB c) at 60dB d) at 70dB. Notice that between 40 dB and 60 dB, there was a significant increase in the fraction of A1 neurons representing 4kHz. e) Fractions of A1 responding in 9kHz-paired versus naïve groups across frequencies at 40dB f) at 50dB g) at 60dB h) at 70dB. Note that between 40 dB and 70 dB, there is a significant increase in the fraction of A1 neurons representing 9kHz. Error bars are standard errors.

Figure 3.9a-c a) Averaged PSTHs split into 4kHz, 9kHz, 20kHz, and all bins from the naïve group (as seen earlier). b) Averaged PSTHs split into 4kHz, 9kHz, 20kHz, and all bins from the 4kHz-paired group c) Averaged PSTHs split into 4kHz, 9kHz, 20kHz, and all bins from the 9kHz-paired group. Notice the prolonged response of the paired frequency bins (9kHz bin for the 9kHz-paired group and 4kHz bin for the 4kHz paired group). All other responses seem similar, or are depressed from naïve measures.

Chapter 4: Examination of A1 Properties After LC Stimulation Without Tone-Pip Pairing

Chapter 4: Examination of A1 Properties After LC Stimulation Without Tone-Pip Pairing

Abstract

The following experiment was designed to remove one variable (the auditory stimulus) and examine the cortical effects of chronic phasic activation of the LC-NE system and likely elevated NE levels in cortex. It is difficult to absolutely deprive the animal of auditory stimuli in this experimental environment. Self-generated sounds occur as well. At the same time, it is the repeated exposure of stimulus-paired LC activity that provides the specificity of the documented plasticities. In the following cases we investigate elevated LC activity that was not reliably linked with any particular auditory stimulus.

Introduction

Schizophrenics are unable to ignore distracting or irrelevant sensory stimuli and therefore cannot attend to relevant aspects of the environment. This and other disturbances can be viewed as a defect in gating of afferent sensory information, a disturbance that has been proposed to result in sensory overload of the brain as an information processor with limited capacity. Many schizophrenics are in a continuous state of hyper-arousal and have elevated plasma NA levels. NA levels are also 300% of control values in the ventral septum of schizophrenics. Such symptoms may be the result of an aberrant LC-NAergic system that has been implicated in sensory and attentional filtering (Hornykiewicz 1986).

Results

LC stimulation alone induces a general expansion of A1

In the second experiment, LC stimulation was delivered in the absence of a paired pip train. This treatment induced a general expansion of A1, although not to the degree seen in the LC-stimulation tone-pip paired groups. Figure 4.1 shows a comparison between the maps of a naïve animal and an animal treated with an LC stimulation-alone regimen. The average size of A1 in the stimulation-only group was 1.5499 +/- 0.02555 mm^2 ($p < 0.01$). The A1 expansion was significant in comparison to naïve animals, but was more modest than the changes induced in paired animals (Fig. 4.2).

The A1 expansion in LC-stimulation alone animals is frequency nonspecific.

The A1 expansion due to an LC-stimulation-alone regimen was frequency nonspecific. The fractions of A1 responding at the various intensities tested, are quantified in figures 4.4a-d. The trajectory of response across frequency is remarkably similar between the simulation-alone and naïve group at all intensities.

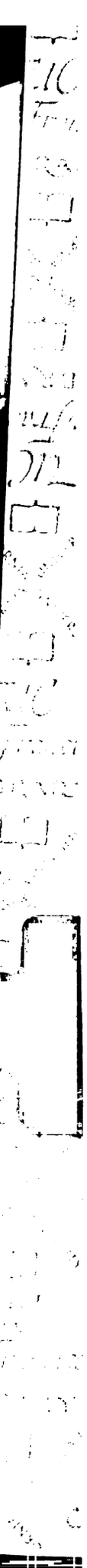
Although the fraction of A1 responding was remarkably similar between stimulation-alone and naïve groups, the raw area responding across frequencies at the different intensities tested was significantly different. This was shown in the previous chapter, but is examined across frequencies and intensities in figures 4.3a-d. Although the trajectories are quite similar, there was a modest but clear increase in the raw area of A1 responding across frequencies and intensities.

LC stimulation alone induces alterations in neuronal spiking properties across A1

LC stimulation in the absence of a consistently paired stimulus (pip train) caused an increase in the stimulus-driven spiking across A1. Examination of the PSTHs of multiunits within A1 in these animals reveals an overall increase in excitability of neurons in all frequency bins. This analysis is shown in figure 4.5a-b; the increase in excitability was significant across all frequencies in comparison to adult naïve and paired groups. (Note that the time scales are different because there was a 50 msec window prior to stimulus presentation in the stimulation only group causing the actual onset to be delayed by 50 msec; the results are comparable as shown.) As will be shown in later chapters, this increase in stimulus-driven activity is not simply due to an increase in bandwidth. Rather, this increase appears to reflect an increase in the number of spikes per pip within the tuning curve stimulus set. Onsets and times to peak were not significantly altered in any of these frequency bins.

Discussion

The expansion of A1 within the stimulation-alone group fits in well within the model for the expansion seen in the paired groups. Although the increased excitability due to the presence of NE in the cortex is present, the peripheral input is absent. Therefore there is little or no auditory cortical activity during the presence of NE and the propagation of Hebbian plasticity is due only to spontaneous activity. This induces a more modest increase in cortical size.



The absence of peripheral input to the cortex likely also explains the frequency non-specificity seen in the stimulation-alone group. Due to the lack of NE-correlated stimulus-driven activity in the cortex the plasticity is uniform, and not exaggerated towards any particular isofrequency region.

There appears to be a peak in the stimulation-group at around 2kHz. This might be viewed as an effect due to the stimulation alone. I argue that this alteration is likely due to sounds that the animal generates during the stimulation protocol, or lack thereof. If the general auditory environment excludes certain spectral ranges and is temporally aligned with the NE coding, the excluded spectra and their influence on the further propagation of cortical activity are possibly downgraded in the long term. The exclusion of propagation allows more privileged information to be unfettered in its activation and prevents noise, therefore increasing the fidelity of representation.

The behavior of animals during and after LC stimulation is discussed further in later chapters.

A microphone was placed in the pairing chamber with a naïve animal. Background noise was recorded in various conditions using a Bruel and Kjaer conditioning amplifier and Digidesign hardware and software. At first during silence and talking as would occur on a normal experimental day, no spectrally specific background noise was detected. However, as I left the room, I noticed a blip on the oscilloscope time locked to the lab door closing behind me. I then repeated this noise numerous times and generated a spectrogram. There appears to be a peak in the sound intensity at 2kHz in the sound chamber as the sound of the lab door closing penetrated the animal's soundchamber. Figure 4.6a shows the sound wave form and

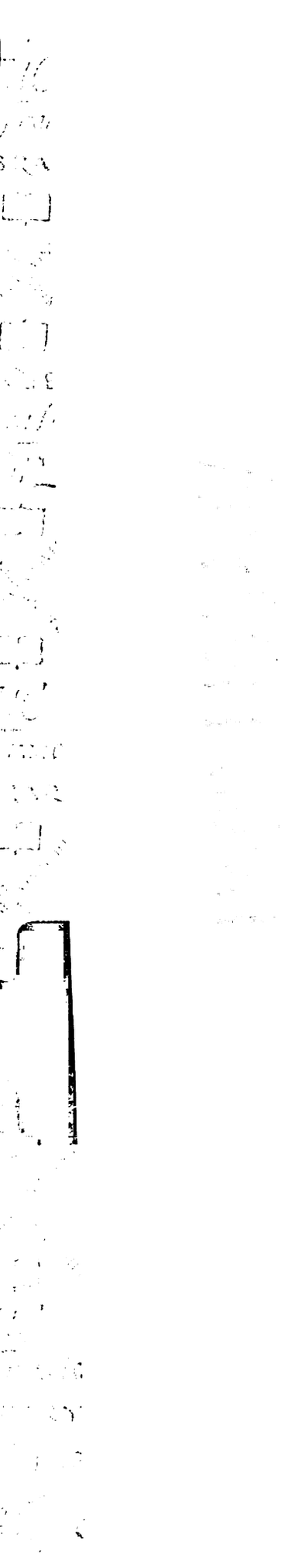


figure 4.6b shows the spectrogram. Perhaps this peak in response at around 2kHz is due to a chance pairing of LC stimulation with the animals' movement in the cage (i.e. the experimenter in the lab closing the door). Or perhaps stimulation of the LC induces a state in which the animal actively seeks out the most advantageous response to the most reliable stimulus. The lack of any identifiably reliable stimulus might cause the animal to look for the most consistent or prominent stimulus, which might be the door closing. Or perhaps the plasticities are a combination of all stimuli occurring at or near the LC stimulation. This is further evidenced as this effect at approximately 2 kHz is present in the other stimulated groups as well although not to the same degree. Perhaps this difference in degree is because the chance pairing of the door closing is not as reliable as the pip pairing that is extremely consistent. This difference in consistency is potentially reflected in the observed plasticity as this plasticity is theoretically a manifestation of the prioritizing of behaviorally relevant stimuli.

The increased excitability of multiunits in all bins in the stimulation- only group compared to the naïve and paired groups implies that LC activity in the absence of an auditory stimulus induces a general hyper-excitability of cortical neurons. This aberrant excitability is apparently attenuated by stimulus-driven cortical activity, as is exemplified in the paired groups previously discussed. Perhaps these effects occur as NE lowers thresholds in the cortex while the stimulus-driven inhibition is absent. As this excitability is manifested, it can be self-propagating as normal auditory stimuli in the absence of LC activity, reach the cortex, and induce a very large and temporally narrow response. This large synchronized response might cause Hebbian

strengthening of nearby synapses and induce a further increase in subsequent excitability. In addition, as the spiking thresholds across the cortex are progressively lowered across the stimulation protocol and in the absence of stimulus-driven inhibition, would allow internal noise to run rampant and further increase this aberrant excitability. A model for these effects will be discussed in later chapters.

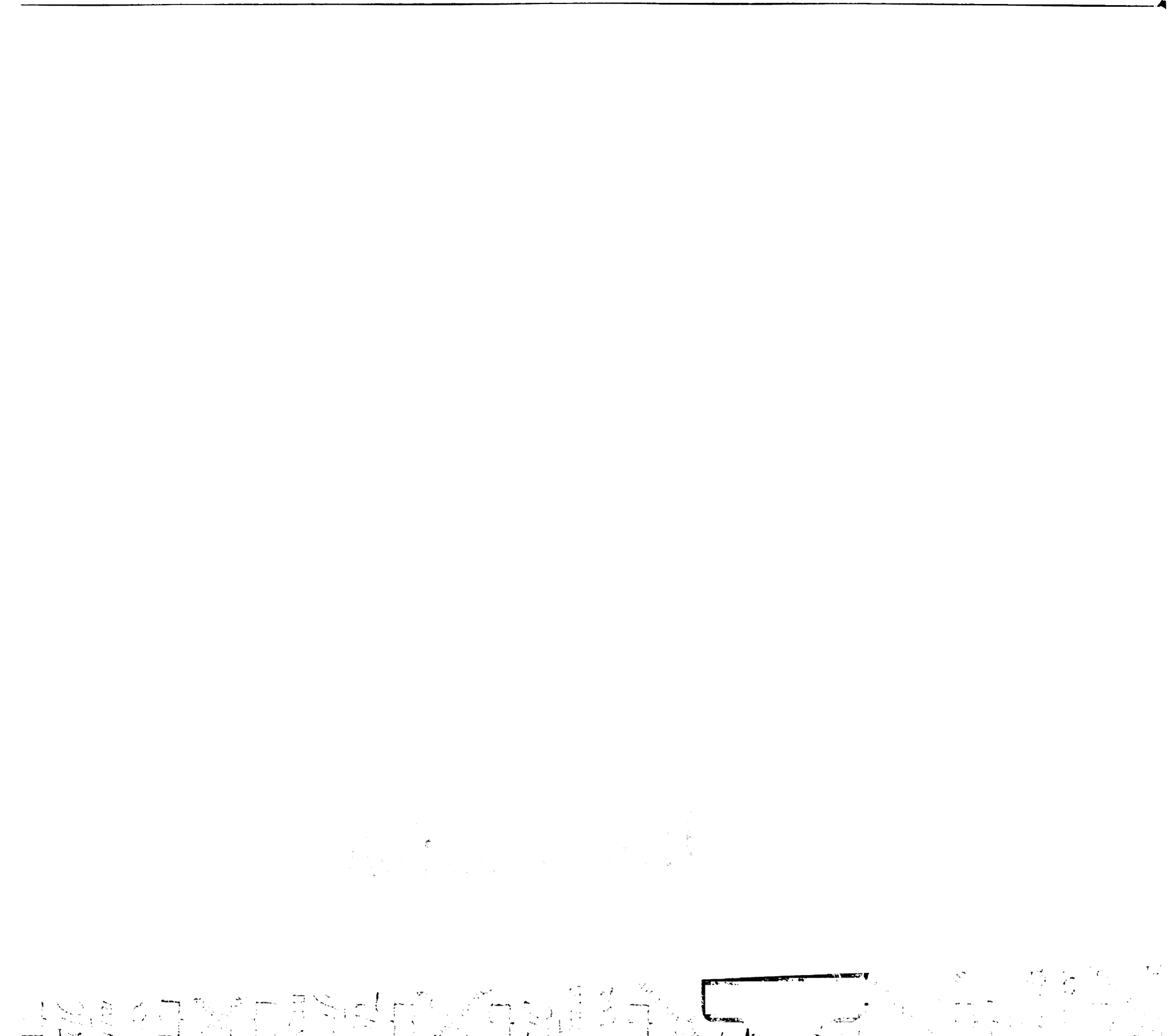
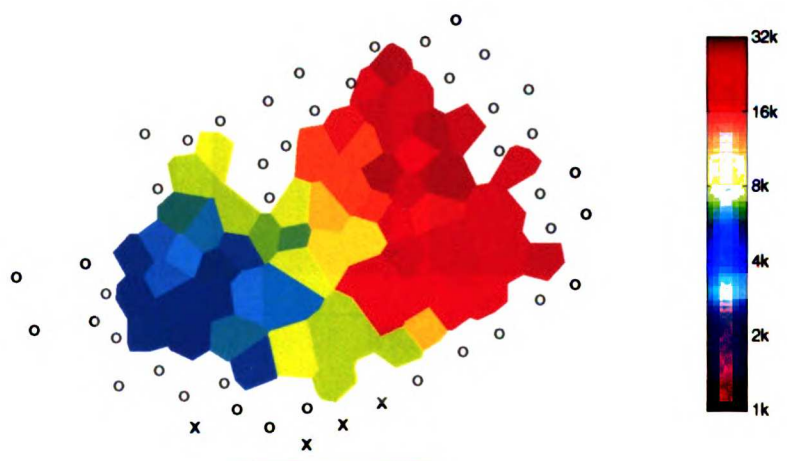


Figure 4.1; Naïve vs. Stimulation Only Maps



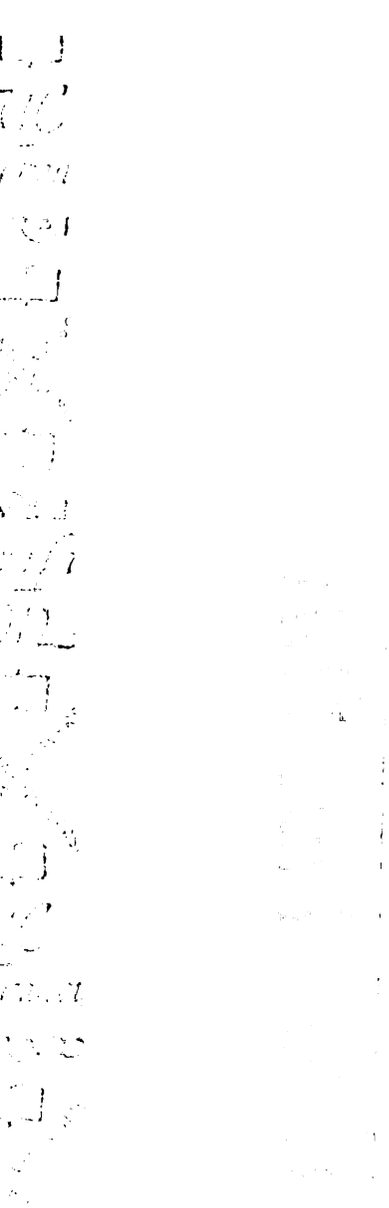
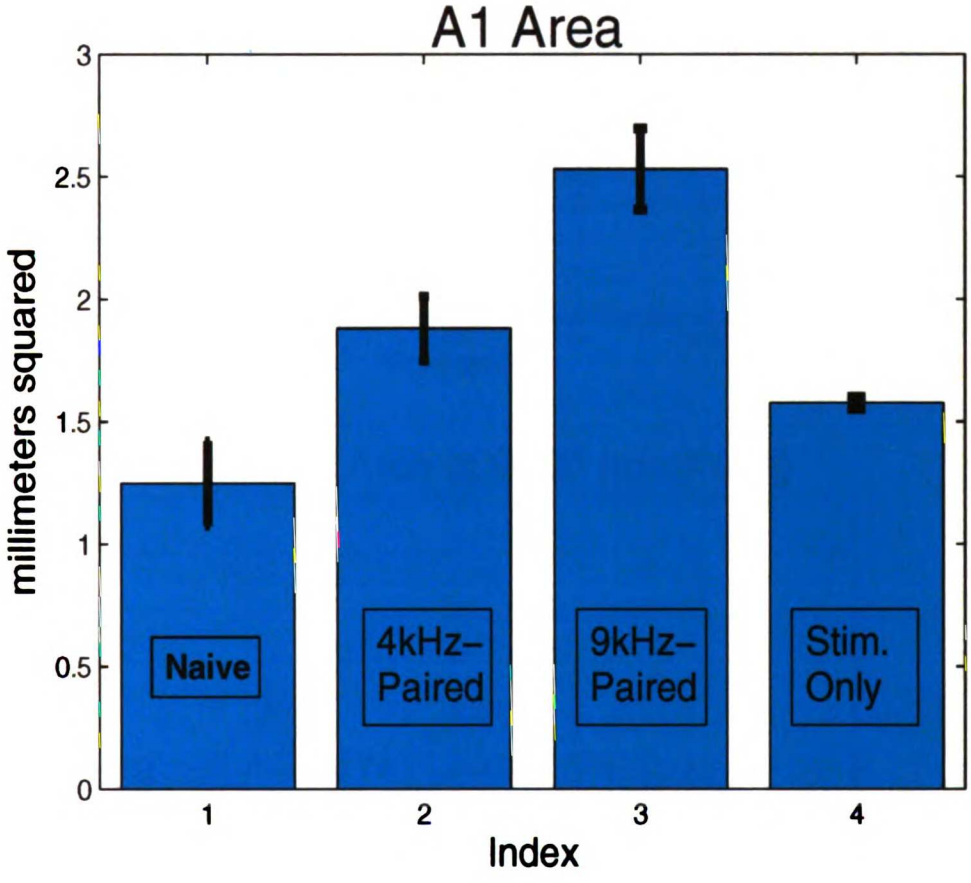


Figure 4.2; A1 Areas Naïve vs. Paired vs. Stim Only Groups



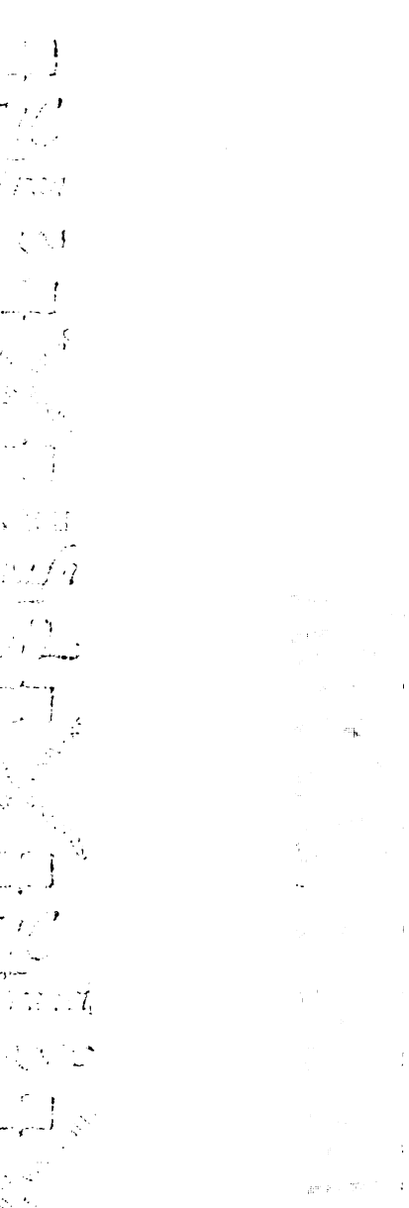
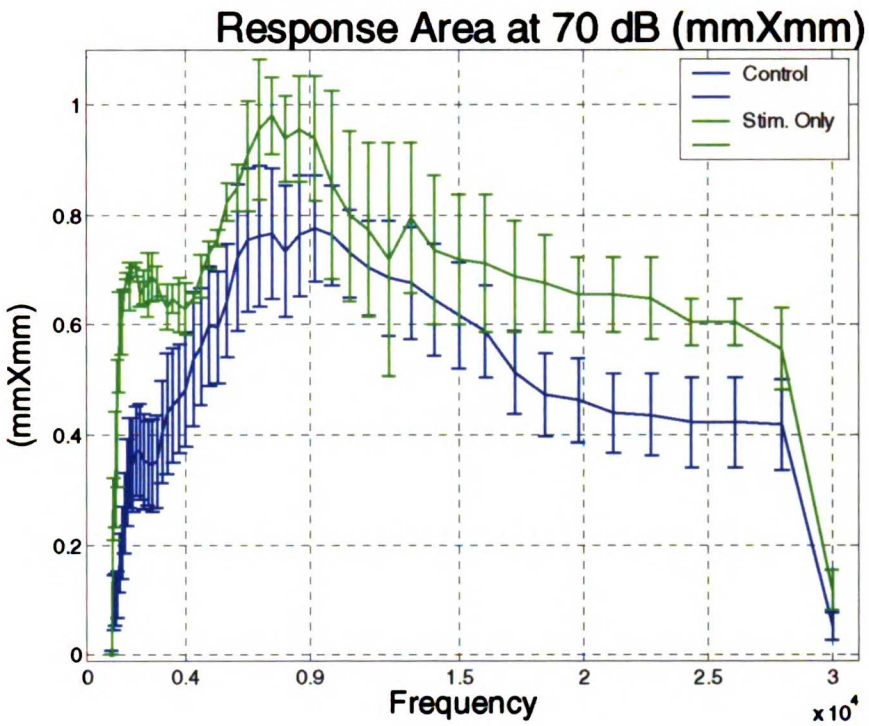


Figure 4.3a-d; Response Areas Naïve vs. 4kHz-Paired Groups (mm^2)



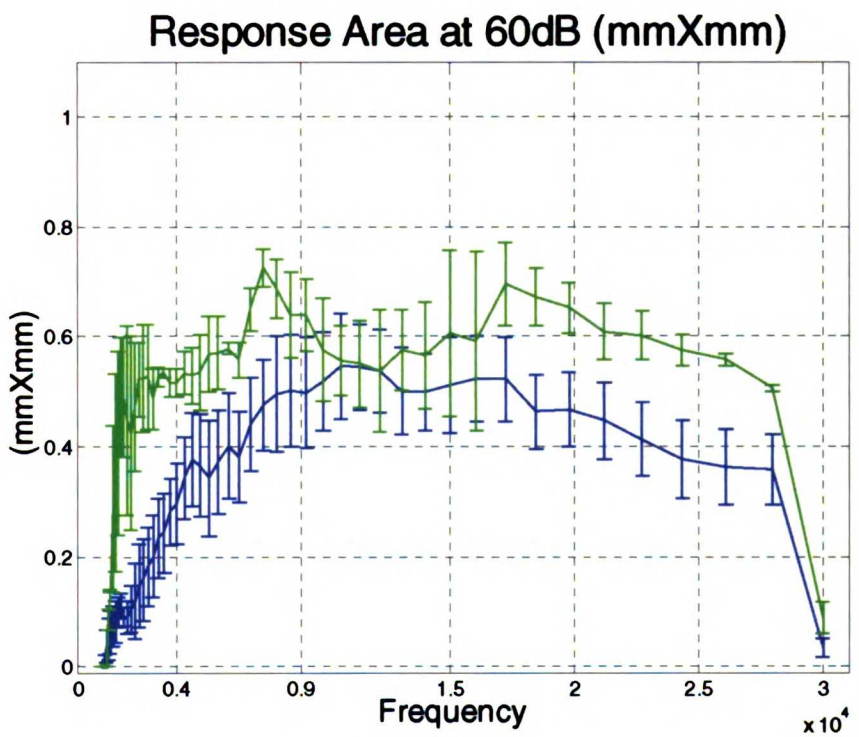
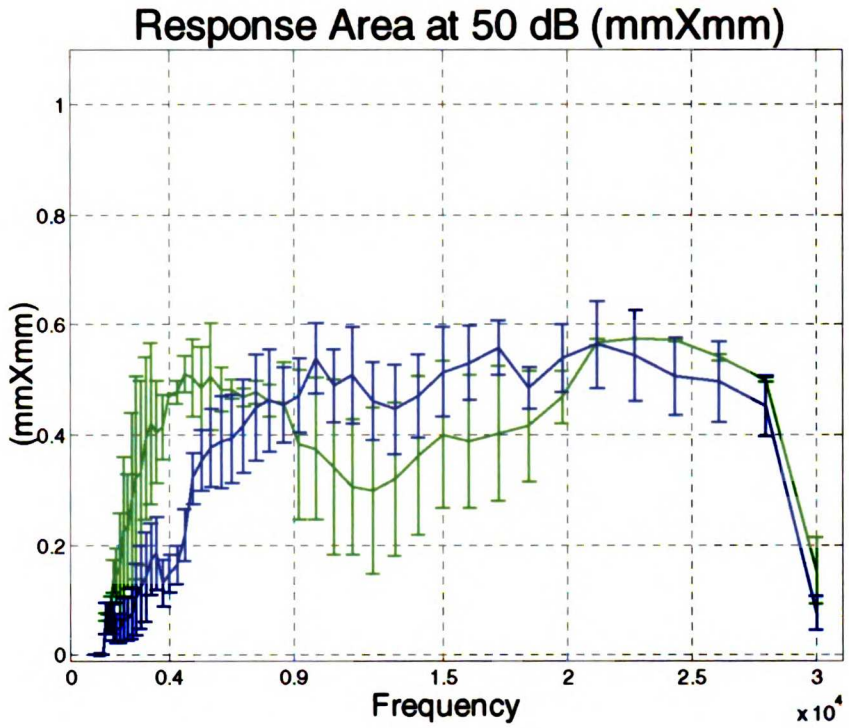


Figure 4.3a-d (cont.); Response Areas Naïve vs. 4kHz-Paired Groups (mm^2)



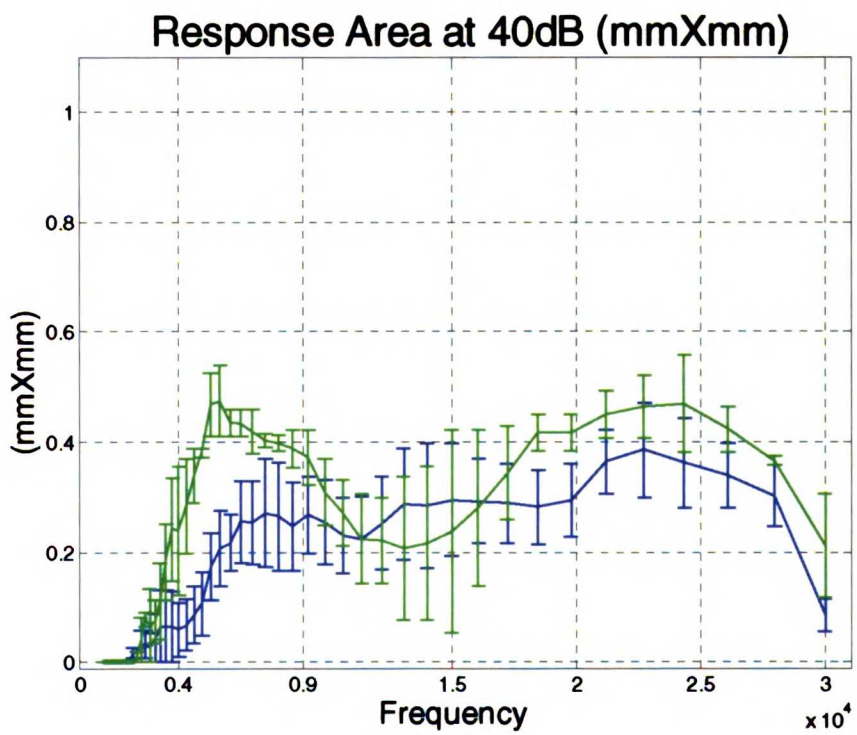
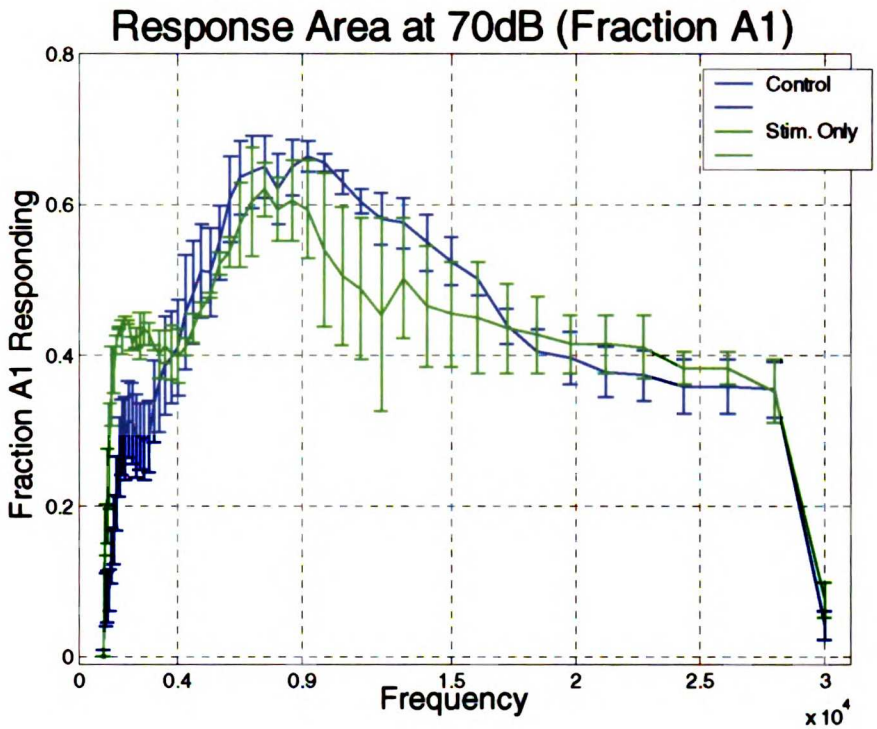
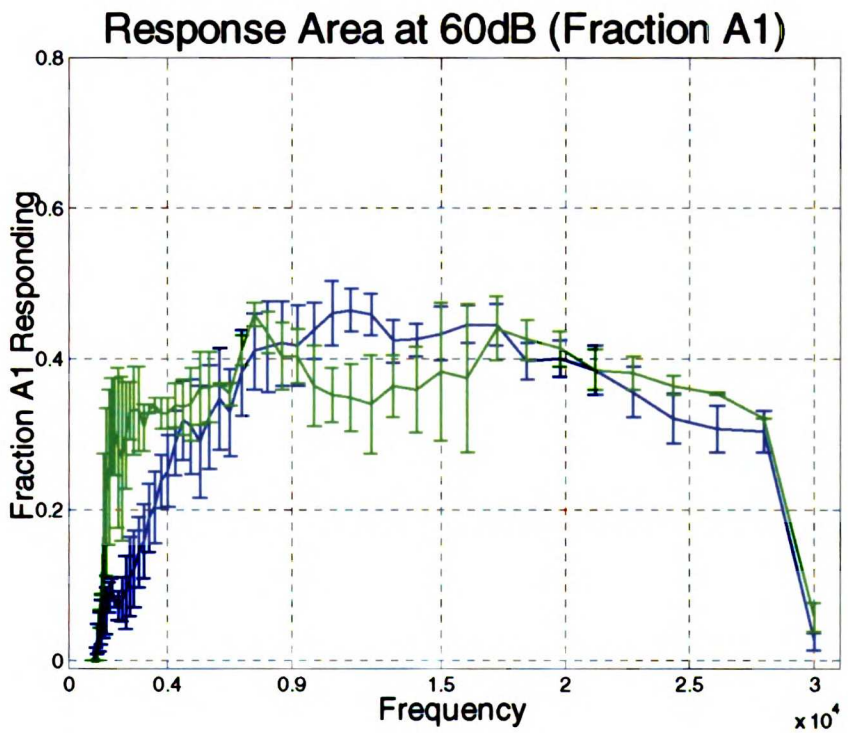


Figure 4.4a-d; Response Areas Naïve vs. 4kHz-Paired Groups (Fraction A1)





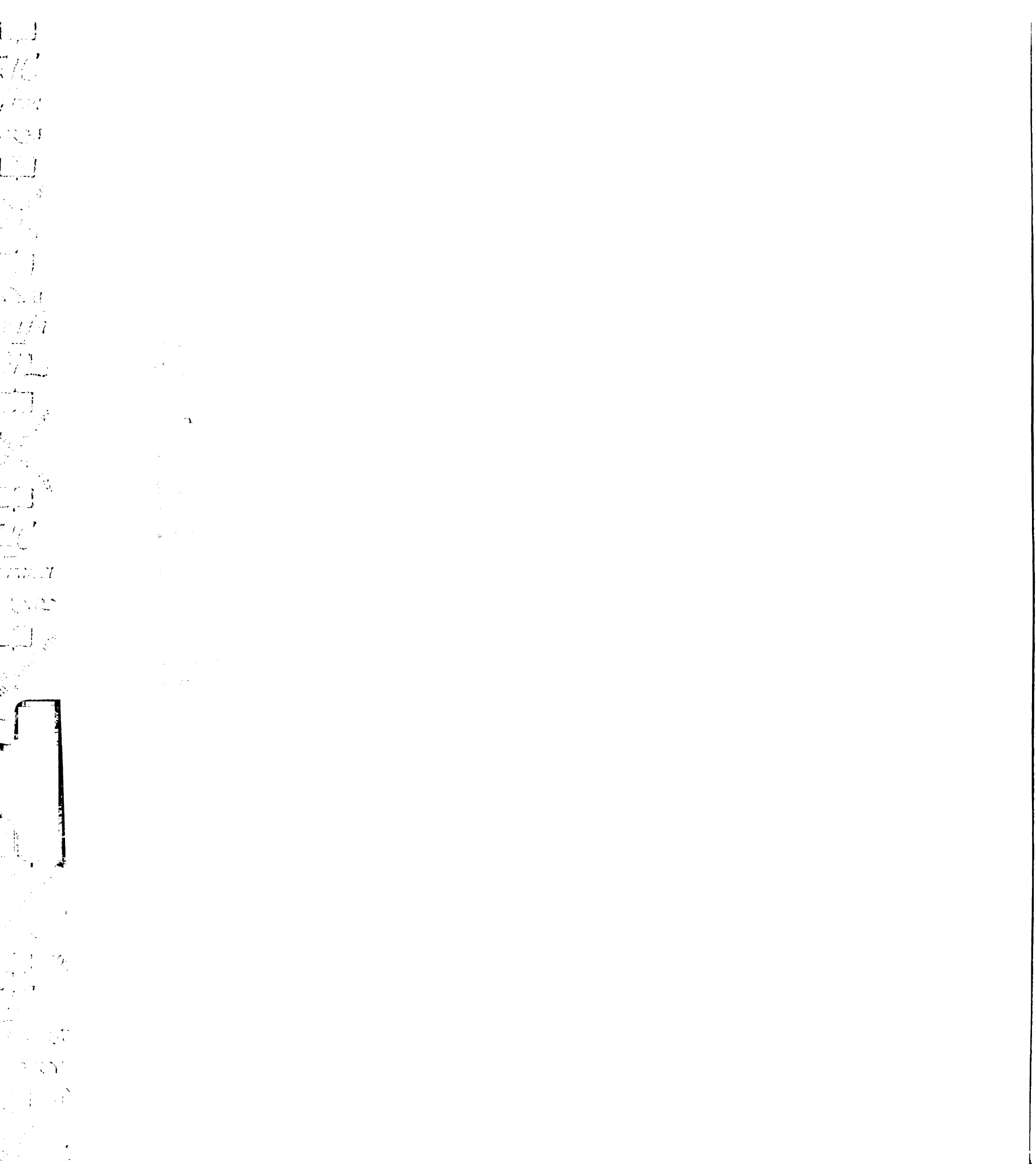


Figure 4.4a-d (cont.); Response Areas Naïve vs. 4kHz-Paired Groups (mm^2)

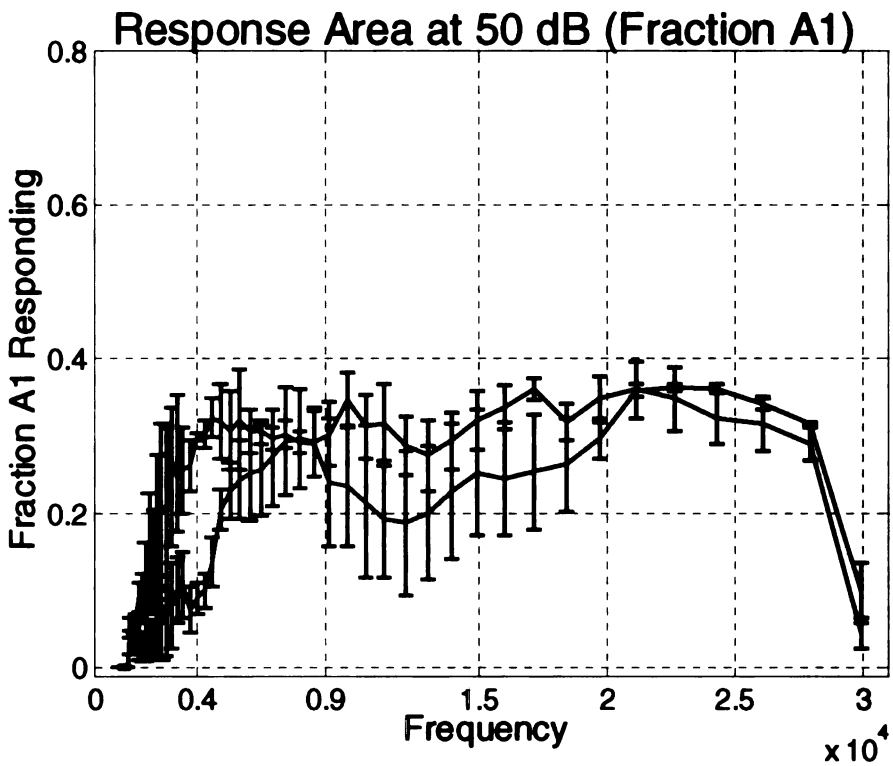


Figure 4.4a-d (cont.); Response Areas Naïve vs. 4kHz-Paired Groups (mm^2)

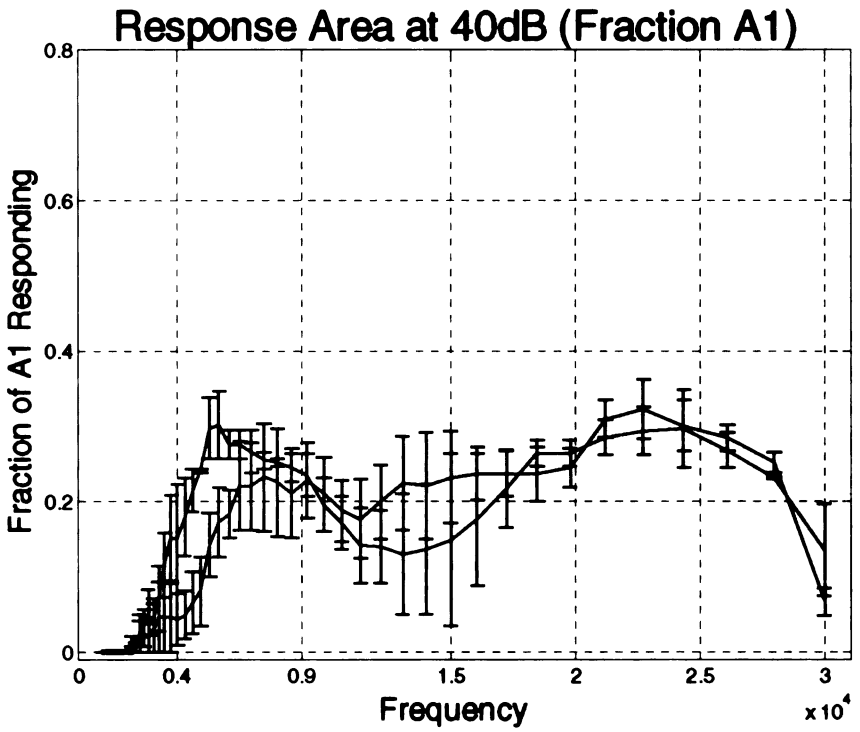
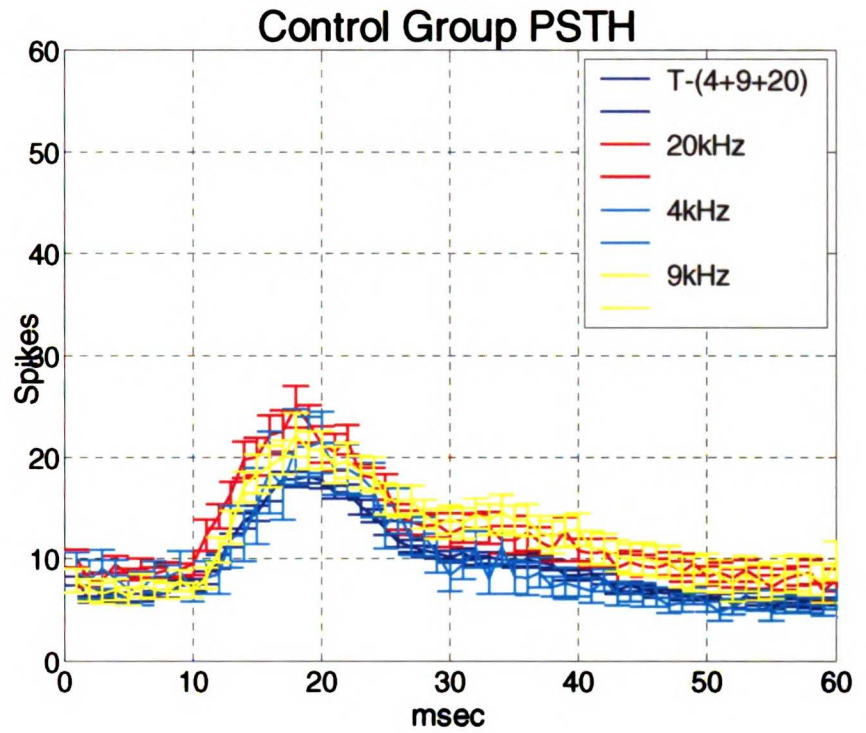


Figure 4.5a-b; Averaged PSTHs Naïve vs. Stim. Only Groups



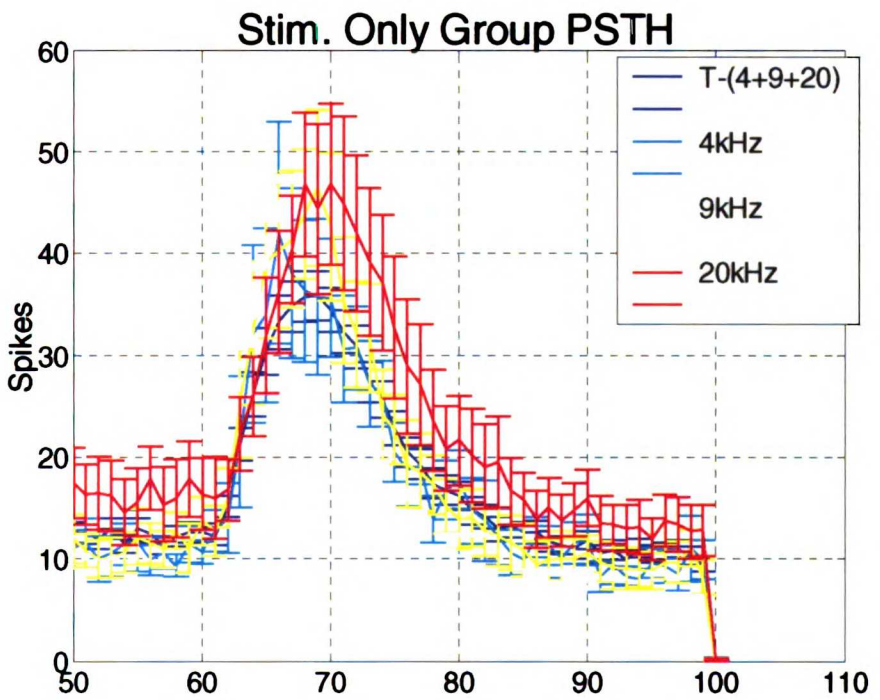


Figure 4.5c-d; Averaged PSTHs Stim. Only vs. 4kHzPaired Groups

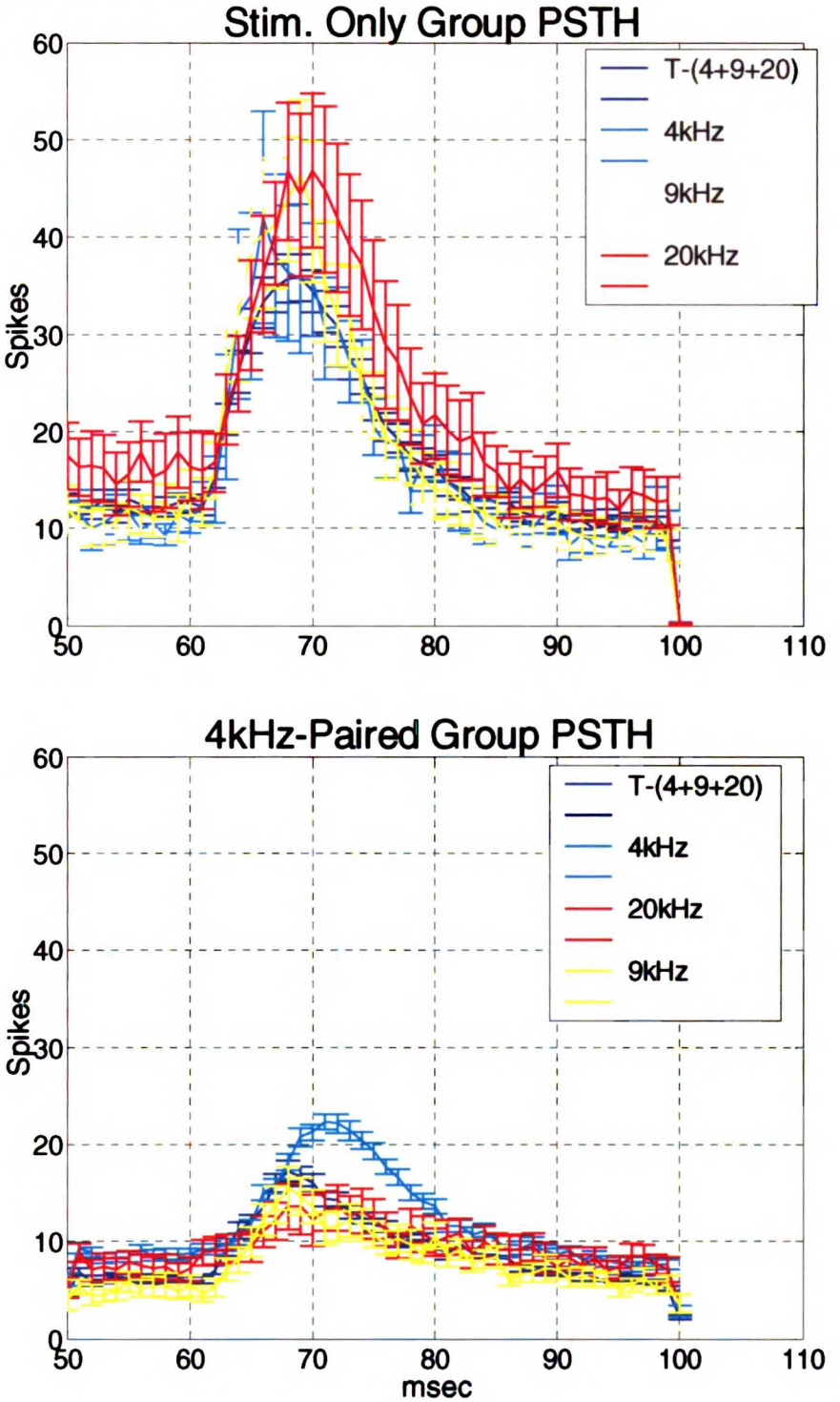


Figure 4.5e; Averaged PSTHs Stim. Only vs. 9kHz-Paired Groups

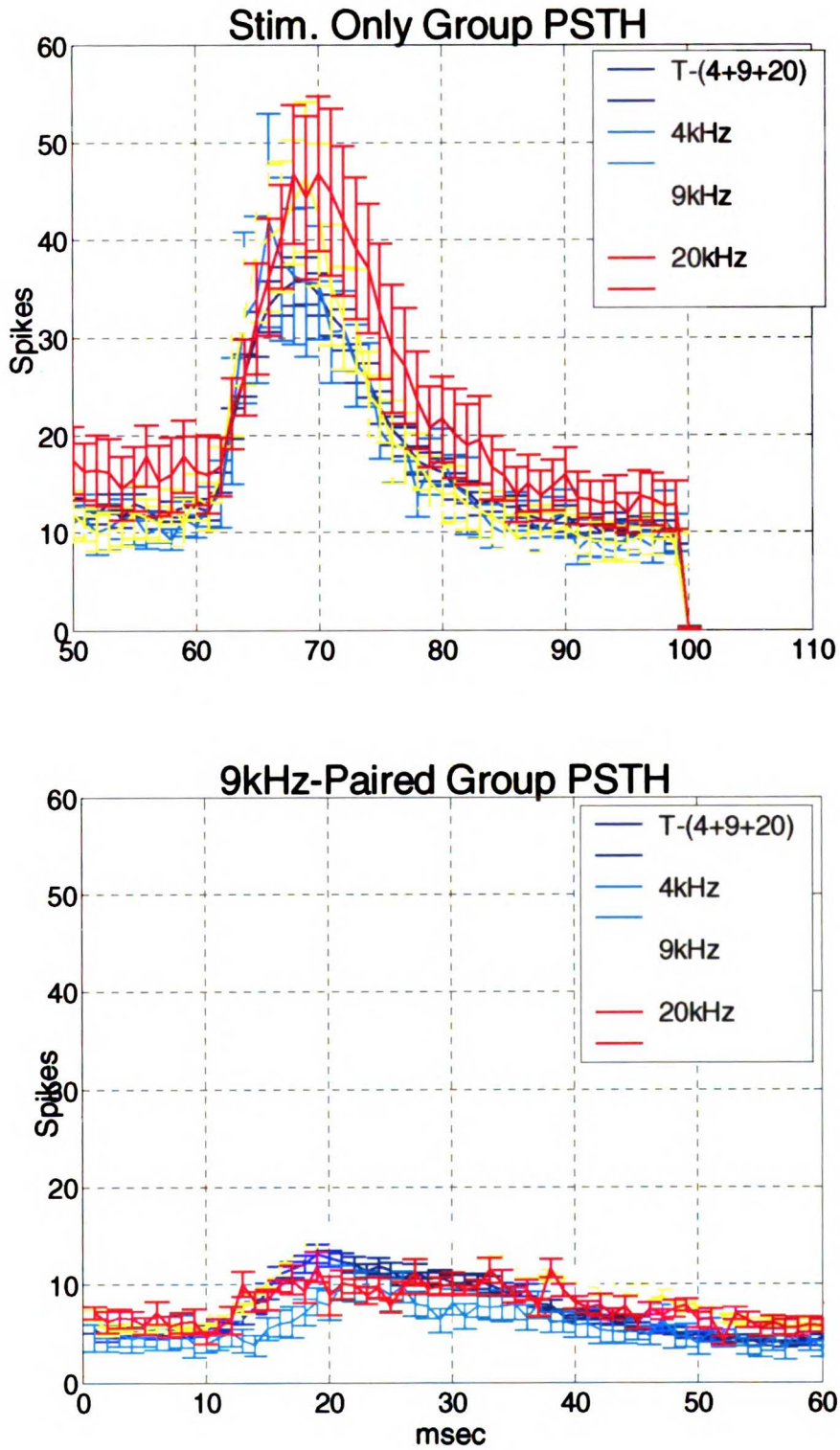
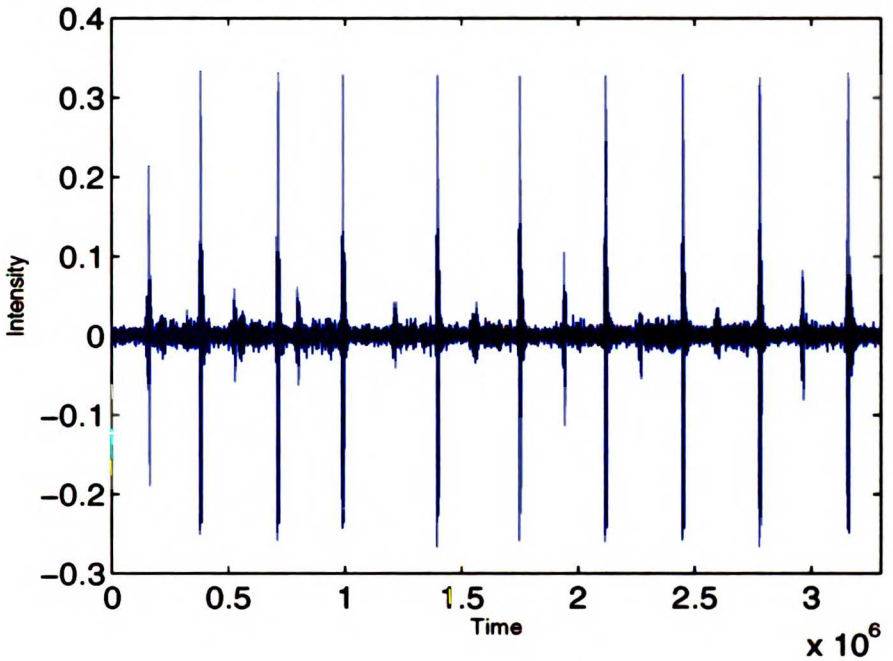


Figure 4.6a-b; Background Noise Experiment; Signal and Power Spectral Density



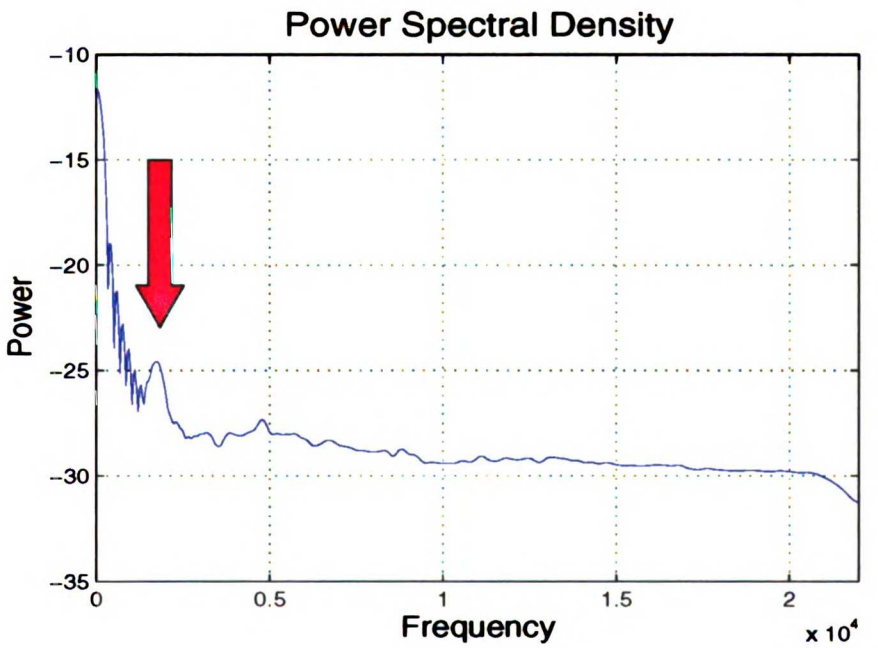


Figure 4.1 Representative CF map of a right primary auditory cortex of a naïve rat compared to that of a LC stimulation-alone animal. Each polygon represents one penetration. Color represents each site's characteristic frequency. Non-responsive and auditory responding non-A1 sites are marked with O's and X's respectively. Note that overall increase in size of A1 in the LC simulation-alone animal (see discussion).

Figure 4.2 Bar graph of the A1 areas (mm^2) from naïve, 4kHz, 9kHz-paired, and LC stimulation-alone groups. Graph is an average of four representative naïve maps, four 4kHz-paired maps, four 9kHz-paired, and two LC stimulation-alone maps. Error bars are standard errors. Note the significant overall increase in the size of A1 in the paired and stimulation-alone groups.

Figure 4.3a-d a) Millimeters of A1 responding in LC stimulation-alone versus naïve groups across frequencies at 40dB b) at 50dB c) at 60dB d) at 70dB. Note that between 40 dB and 70 dB, there was an increase in the overall size in the stimulation-alone group that was fairly consistent across frequencies. Note the peak near 2kHz (see discussion for a possible explanation).

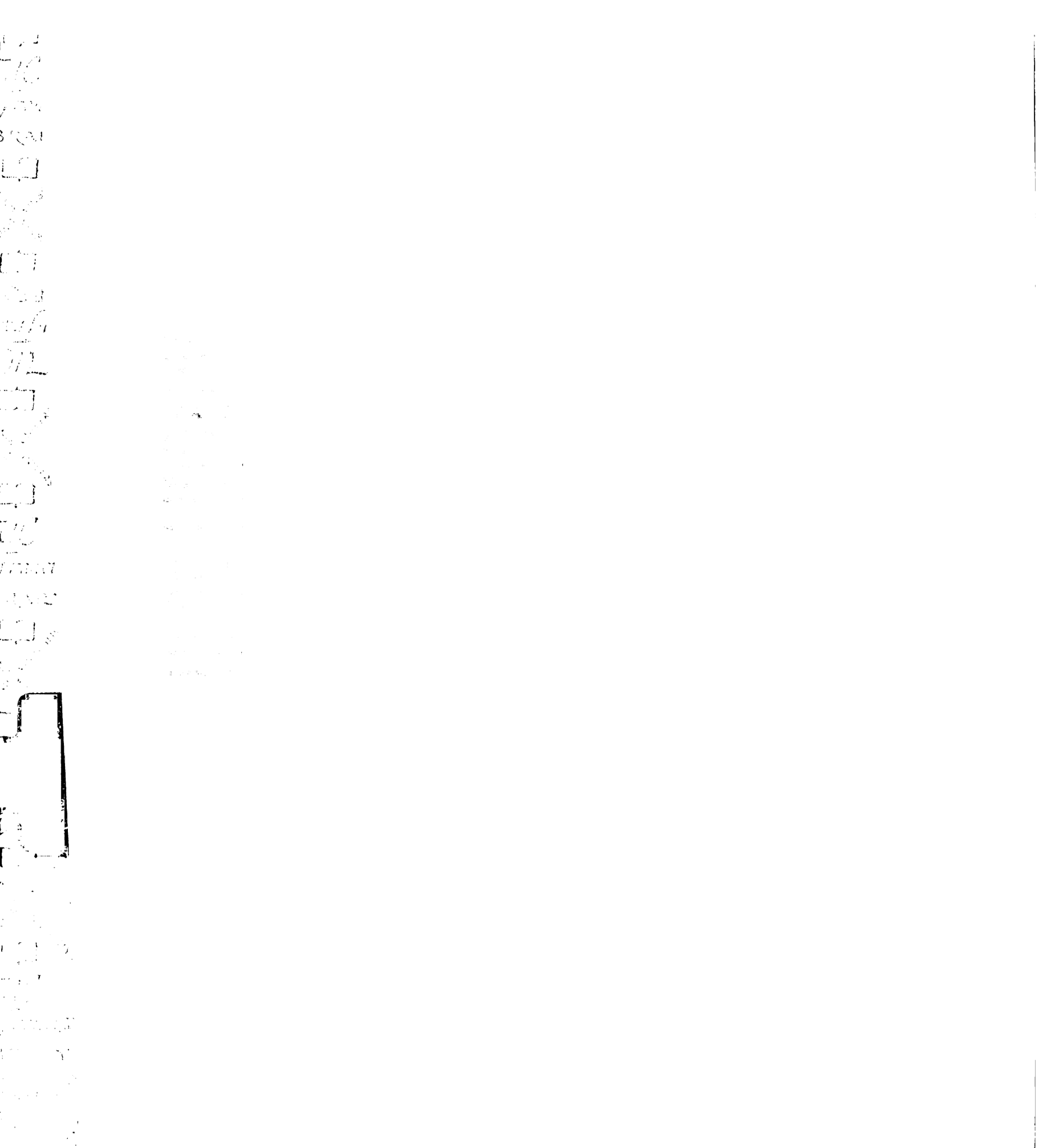
Figure 4.4a-d a) Fractions of A1 responding in LC stimulation-alone versus naïve groups across frequencies at 40dB b) at 50dB c) at 60dB d) at 70dB. Note that between 40 dB and 70 dB, there was reliable consistency between naïve and

stimulation-alone groups. Note the peak near 2kHz (see discussion for a possible explanation).

Figure 4.5a-b a) Averaged PSTHs split into 4kHz, 9kHz, 20kHz, and "all" bins from the naïve group (as seen earlier). b) Averaged PSTHs split into 4kHz, 9kHz, 20kHz, and all bins from the stimulation-alone group. Note the very large increase in response amplitude across frequency bins in the LC stimulation-alone group (see discussion).

Figure 4.5c-e Summary of PSTH affects in a) stimulation-alone b) 4kHz-Paired c) 9kHz-paired groups. Note the different y-axis scale; this adjustment was necessary to graphically represent and directly compare all PSTH effects.

Figure 4.6a-b Results of Background Noise experiment. a) Signal plot of the laboratory door closing repeatedly while the sound chamber is closed. b) Spectral analysis of signal plot in a; Red arrow denotes peak of 1767 Hz.



Chapter 5: Examination of A1 Properties in DSP-4 Treated Animals

Chapter 5: Examination of A1 Properties in DSP-4 Treated Animals

Abstract

The various forms of plasticity described above identify the LC as an important modulator of the cortical retention of experience. To prove that LC-NE activity was required for these changes to occur, the LC was lesioned in a group of animals prior to implantation, pairing, and then subsequent mapping.

There are many established means of lesioning the LC. In the following experiments we used a PA injection regimen of (n-)2-chloroethyl)-n-ethyl-2-bromobenzylamine hydrochloride (DSP-4) as is outlined in previous publications. The concentrations of NA in the hippocampus and neocortex were shown to be markedly reduced by treatment with DSP4 while the concentrations of DA and 5HT were unaffected (Al-Zahrani et al. 1998).

Introduction

NA-depleted rats extinguish much more slowly than controls after the behavioral contingency is removed. Lesions of the dorsal NA-bundle produce a similar extinction deficit known as "the dorsal bundle extinction effect" or DBEE. Results of such studies implicate the NA system in three limbic forebrain functions, each of which may be regarded as a component of attention: limiting, stimulus identification, and mismatch detection. Mason (1980) argued that the most satisfactory explanation from the literature is that the DBEE results from impairment of sensory attention, or in the ability to notice changes in salient cues.

In a five-choice serial retention paradigm used to evaluate visuospatial attention in rats, lesions of the LC impaired performance only when additional attentional demands are placed upon the subject, e.g., distracters or temporally unpredictable targets.

Methods

DSP-4 was intraperitoneally injected into eight 15 month old rats in four weekly doses of 50mg/kg. All animals were monitored daily for food and water consumption, body mass and overall health. One animal died in the third week of treatment and one animal was sacrificed due to an upper respiratory infection.

Animals were subsequently implanted in the LC, allowed to recover, paired with an identical regimen of pairing with the 9 kHz pip train, and then mapped.

Results

DSP-4 Treatment Induced a Reduction in Size of the Area representing A1

To prove that these effects require activation of LC neurons, the LC was lesioned while all other experimental conditions were unaltered. DSP-4 is a proven method for lesioning the LC. Animals were treated with DSP-4, allowed to recover, implanted, and then exposed to the same pairing conditions as in the 9 kHz pairing experiment. This is essentially a sham pairing in that current is delivered to the area of the LC subsequent to its lesioning, and a 9 kHz pip train was delivered in the same temporal relationship as in previous experiments. In effect, the animal was exposed to a 9 kHz pip train without an ascending NE system. This treatment caused a reduction

in the size of A1 compared with adult naïve animals. Figure 5.1 shows an example of an A1 map from a DSP-4 treated animal compared with that of an adult naïve animal. This is quantified in Figure 5.2 and is significant compared to paired, and stimulation-only groups, but not compared to the naïve group. The average size of A1 in the DSP-4-treated group was 0.9168 +/- 0.1023 mm^2 ($p < 0.11$ when compared to naïve controls).

The Reduction of A1 Size in DSP-4 Treated Animals is Frequency Nonspecific.

The A1 reduction due to the DSP-4 regimen was frequency non-specific. The fractions of A1 responding at the various intensities tested, are quantified in figures 5.4a-d. The trajectory of response across frequency is remarkably similar between the DSP-4 treated and naïve groups at all intensities. Although the fraction of A1 responding analysis is remarkably similar between DSP-4 treated and naïve groups, the raw area responding across frequencies at the different intensities tested is significantly different. This was shown in the previous chapter, but is examined across frequencies and intensities in figures 5.3a-d. Although the trajectories are quite similar, there is a modest but clear decrease in the raw area of A1 responding across frequencies and intensities.

DSP-4 Treatment does not induce changes in neuronal spiking properties across A1

Despite the alteration in A1 size due to the DSP-4 regimen, the neuronal spiking properties across A1, as revealed in PSTH analysis, appeared to be unaltered. PSTH analysis between the DSP-4 and naïve groups is shown in Figure 5.5. Spike onsets

and time to peak are nearly identical and, although there is a slight increase in the over amplitudes of responses in individual DSP-4 treated animals, this is not a significant effect upon comparison between DSP-4 and naïve groups. Thus the only apparent difference between DSP-4 and naïve groups is the overall change in A1 size.

Discussion

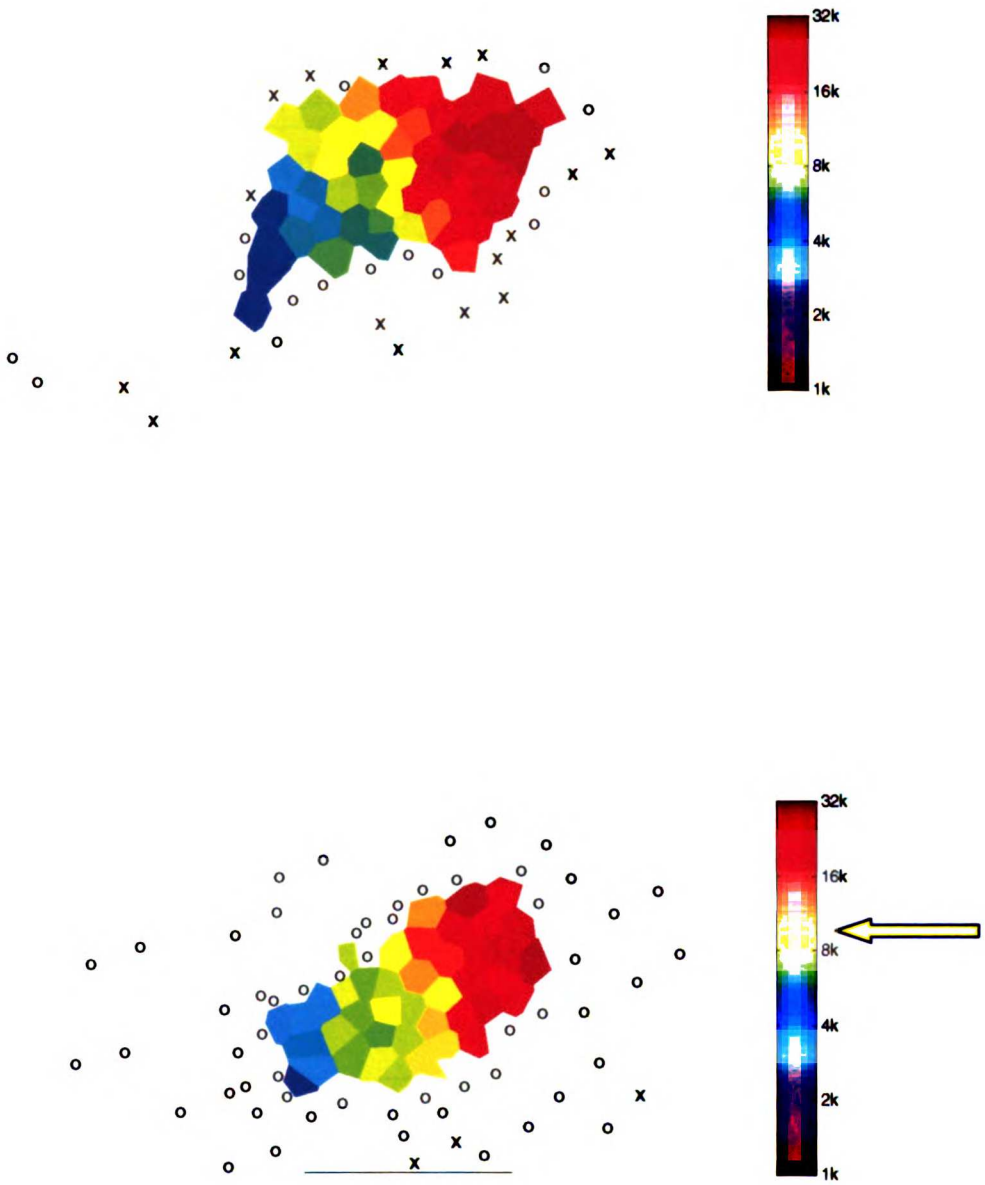
These results are once again nicely explained by the model. The elimination of noradrenergic fibers via DSP-4 lesion decreases the stability of A1 be removing a natural source of excitability in the brain. The lack of this source decreases the likelihood that spontaneous activity will lead to spiking, propagation, and Hebbian plasticity. The overall results are a less-stable A1, the elevation of neuronal spiking thresholds, and an increase in latencies of certain neurons. This increase in latency would cause these neurons to be excluded from A1 even if they are still driven be auditory stimuli.

Because LC noradrenergic fibers are lesioned, there is a lack of excitatory impact of the LC throughout the entire brain and therefore the effect of intrinsic noise and extrinsic input to A1 is uniformly lessened. The stability of A1 is bolstered less by the inhibition of leak K+ conductance therefore spike-rendering input (spontaneous or elicited) will be unlikely to cause a downstream spread of Hebbian plasticity because there will only be a few neurons firing synchronously; the bulk of neurons will either fire more randomly or not at all.

It seems plausible based on the literature that these animals might have difficulty adapting away from any previous behavioral association to the experience of the 9

kHz frequency presented. In other words, these animals have trouble extinguishing prior associations because of their LC lesions and this might result in a type of plasticity on its own. Instead it seems that other neuromodulatory centers may compensate for the lack of NA-ergic input. Even more interestingly, the spread of plasticity may require not only the general impact of NA but also the extrinsic input and neuraxis firing to combine with this hyper-excitability. Both may be required.

Figure 5.1 Comparison of Naïve and DSP4-treated maps



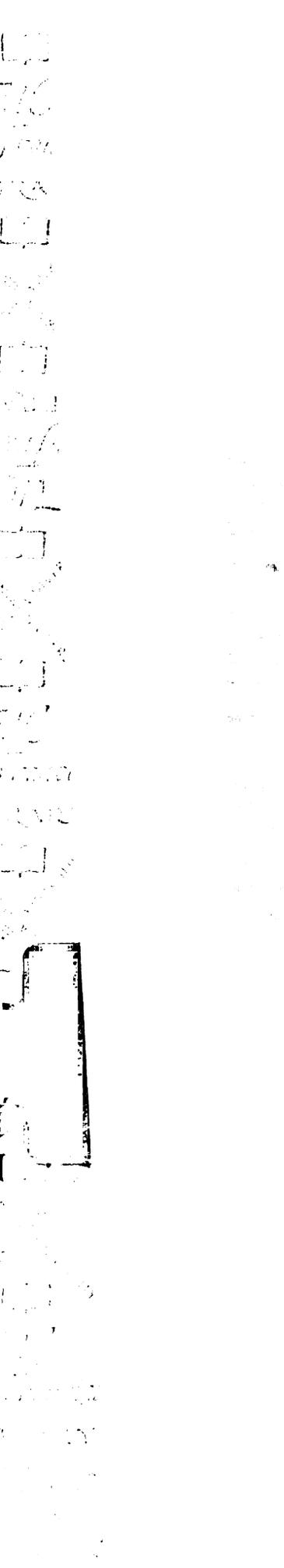


Figure 5.2 A1 Areas Naïve, Paired, Stim.-Only, and DSP4-treated groups

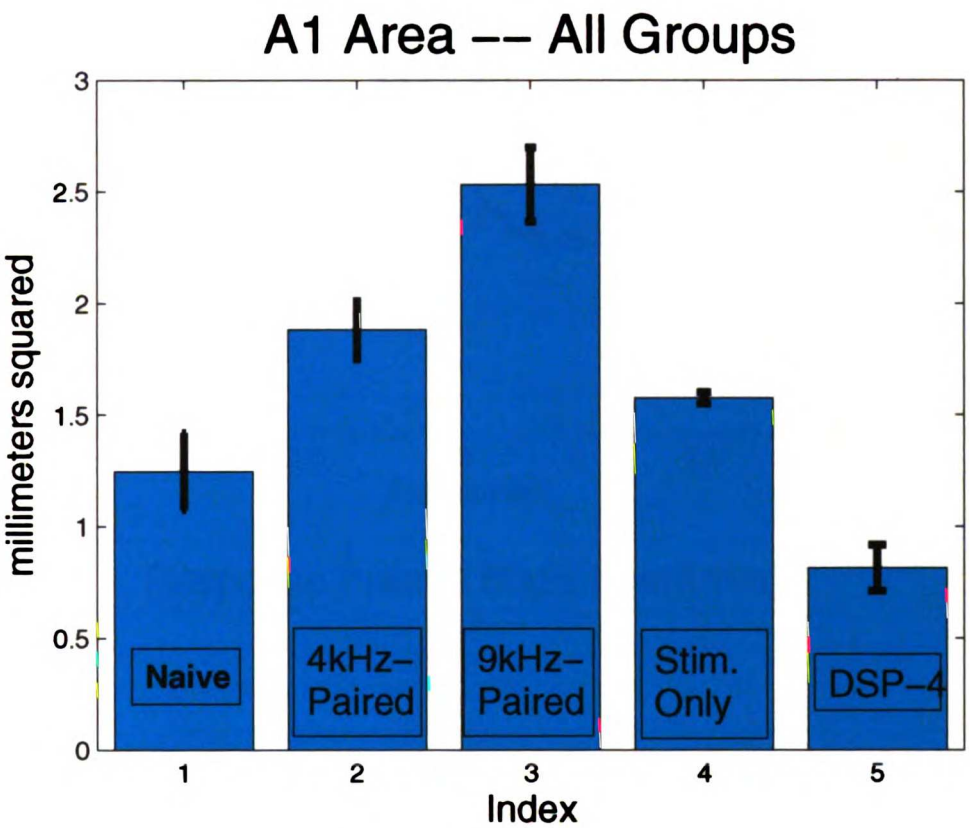
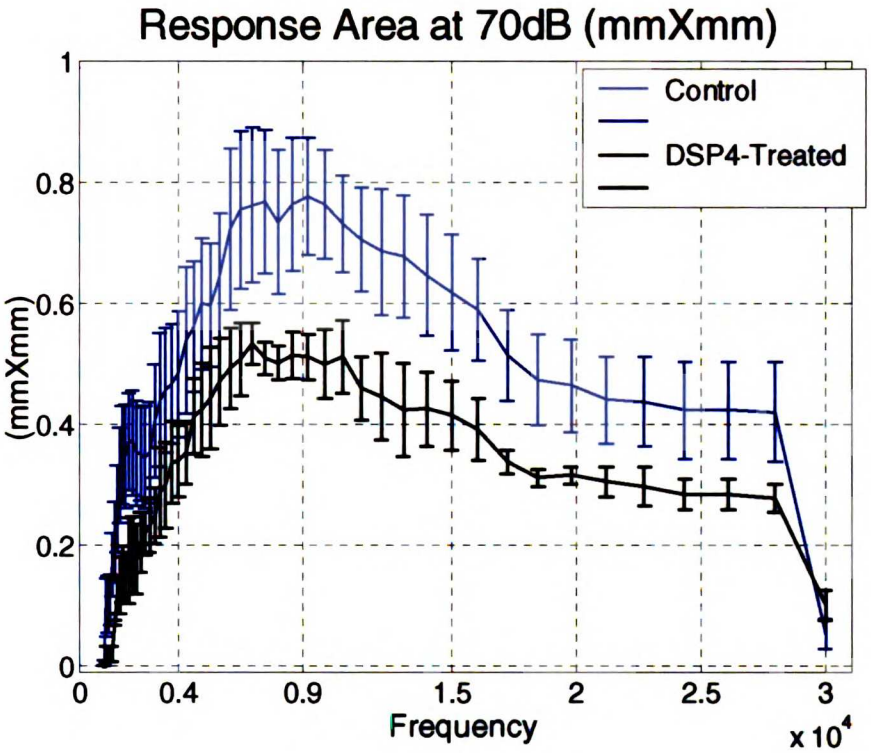


Figure 5.3a-d; Response Areas Naïve vs. DSP4-Treated Groups (mm^2)



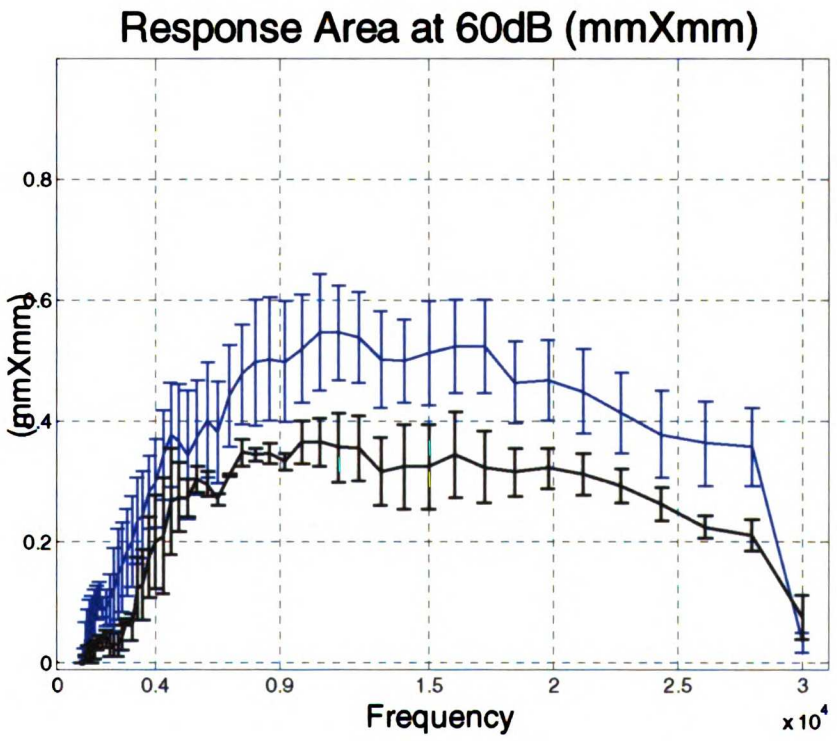


Figure 5.3a-d (cont.); Response Areas Naïve vs. DSP4-Treated Groups (mm^2)

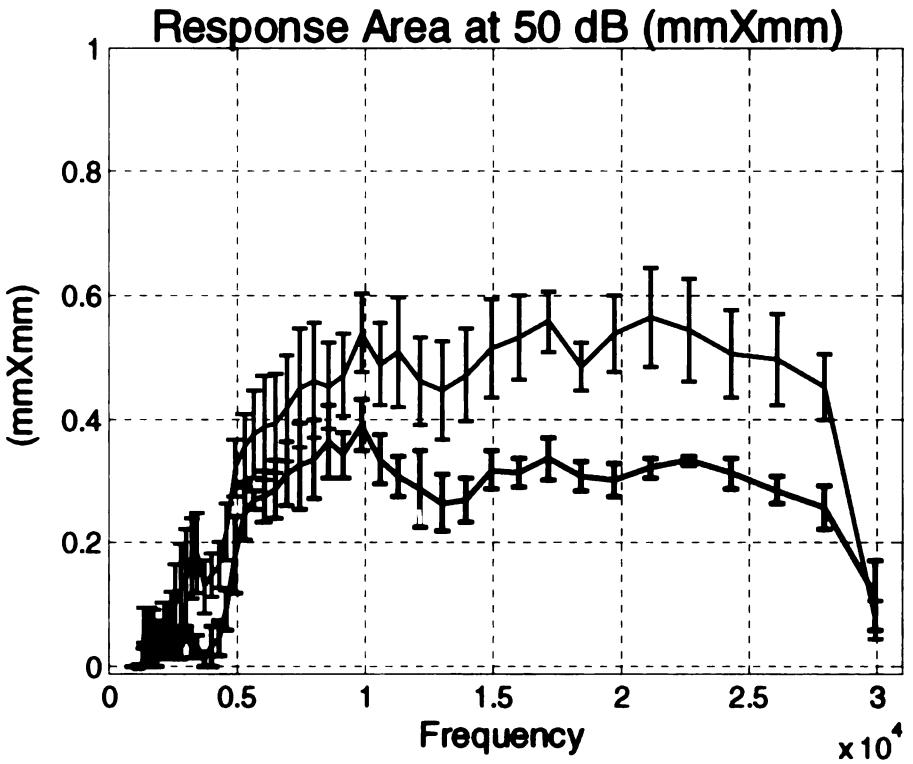


Figure 5.3a-d (cont.); Response Areas Naïve vs. DSP4-Treated Groups (mm^2)

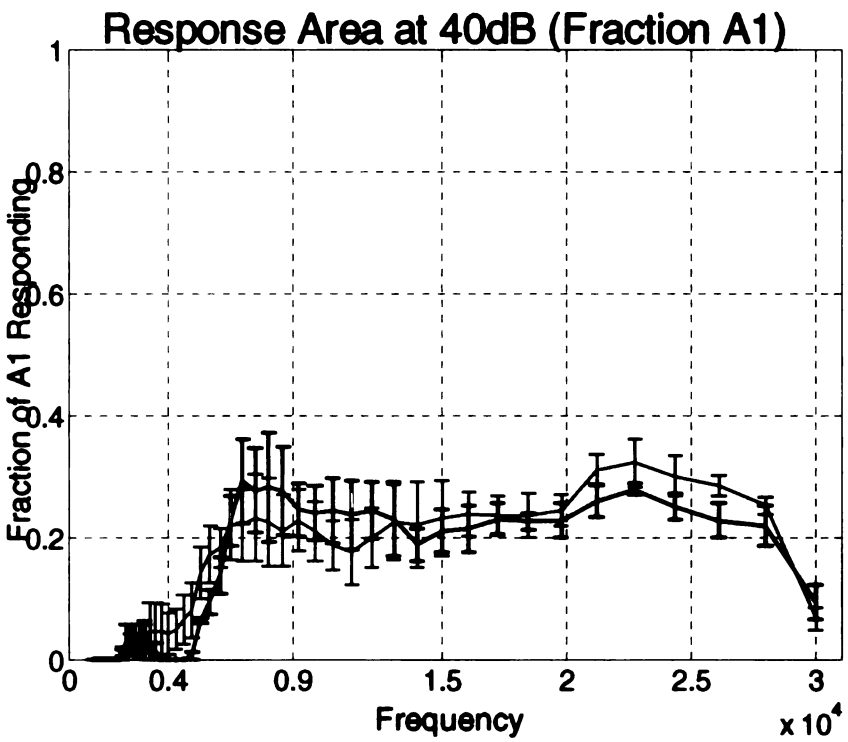
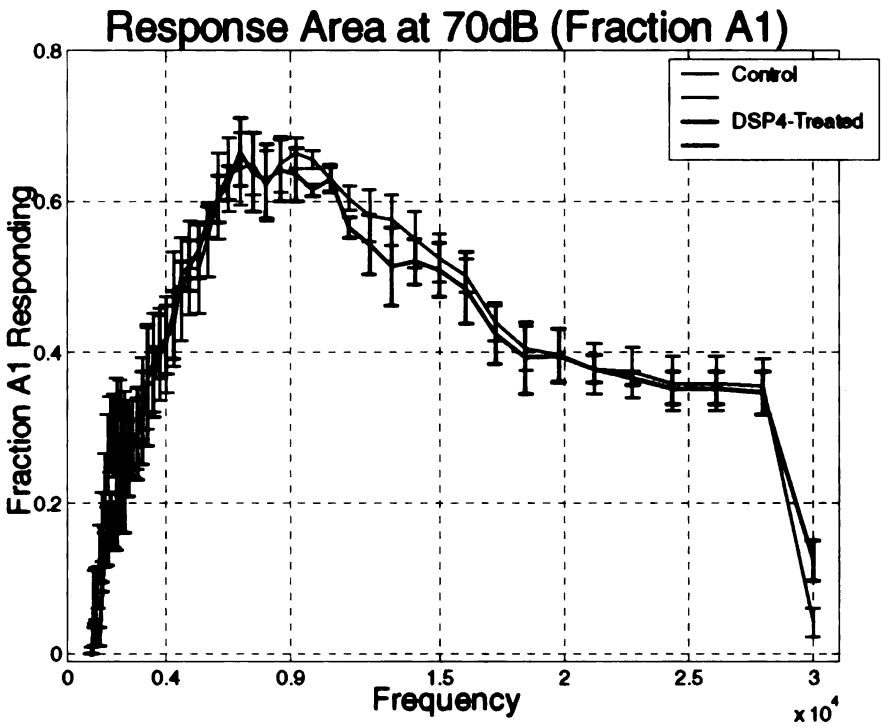


Figure 5.4a-d; Response Areas Naïve vs. DSP4-Treated Groups (Fraction A1)



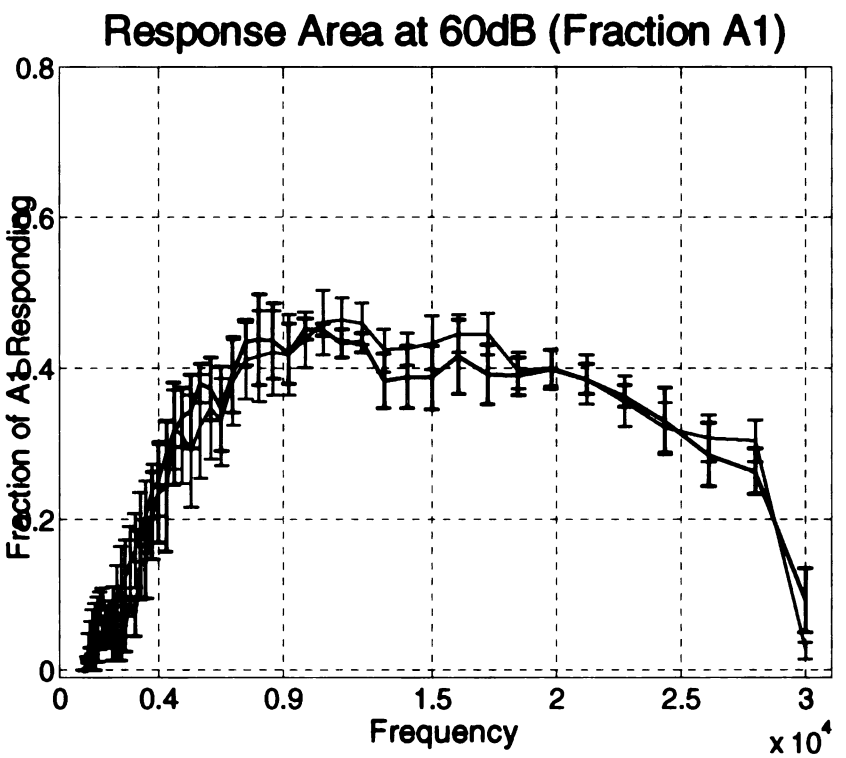
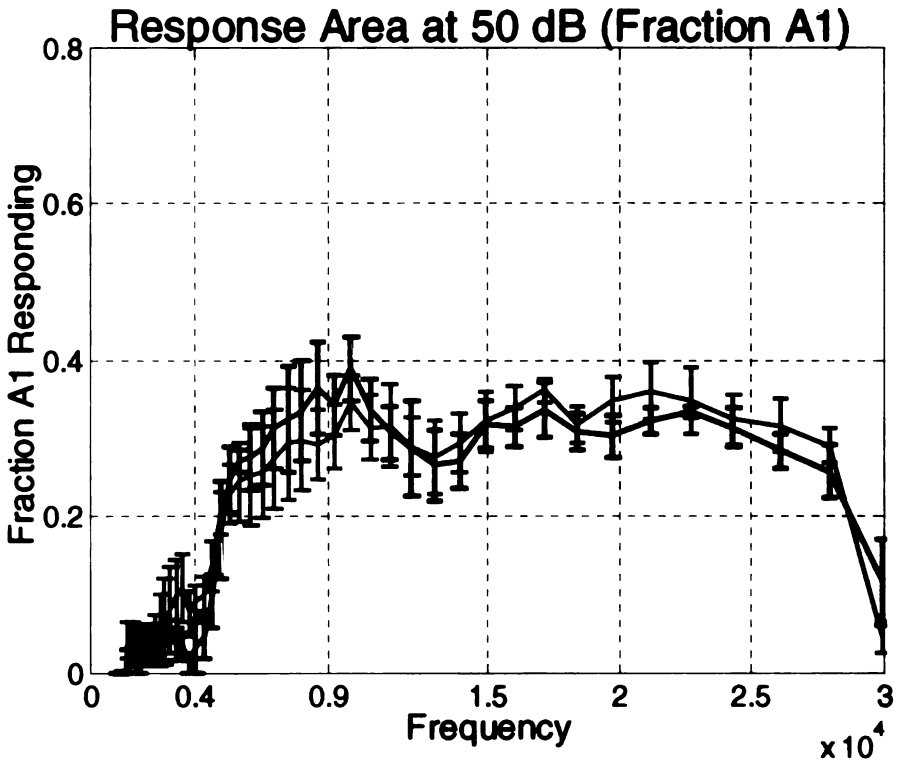


Figure 5.4a-d (cont.); Response Areas Naïve vs. DSP4-Treated Groups (Fraction A1)



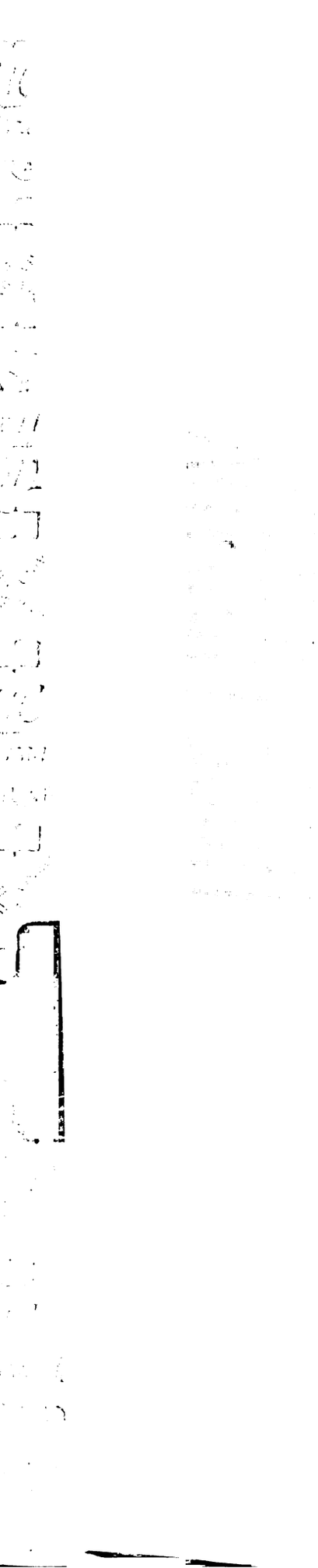


Figure 5.4a-d (cont.); Response Areas Naïve vs. DSP4-Treated Groups (Fraction A1)

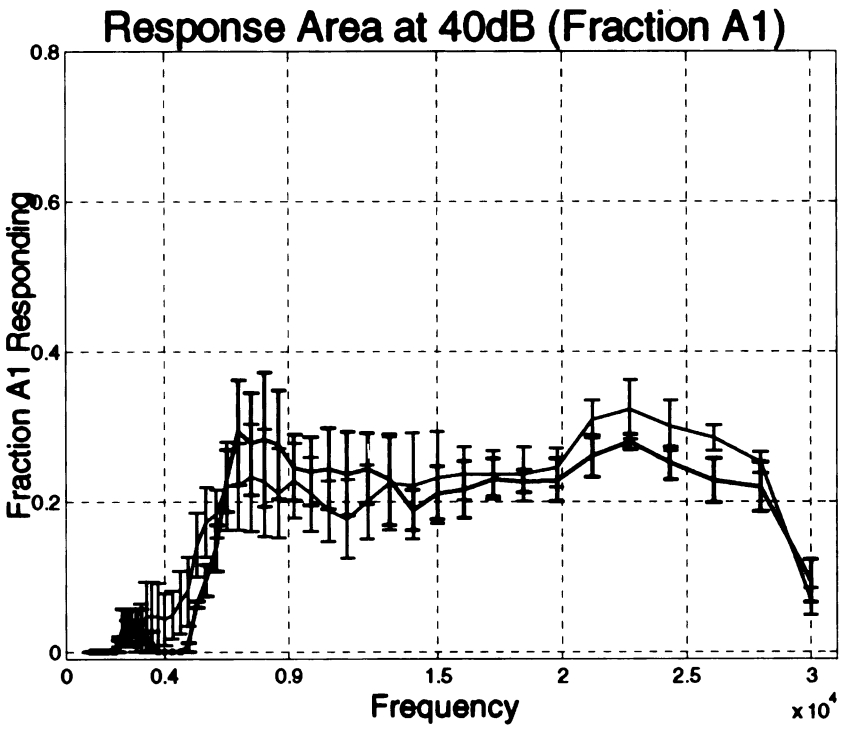
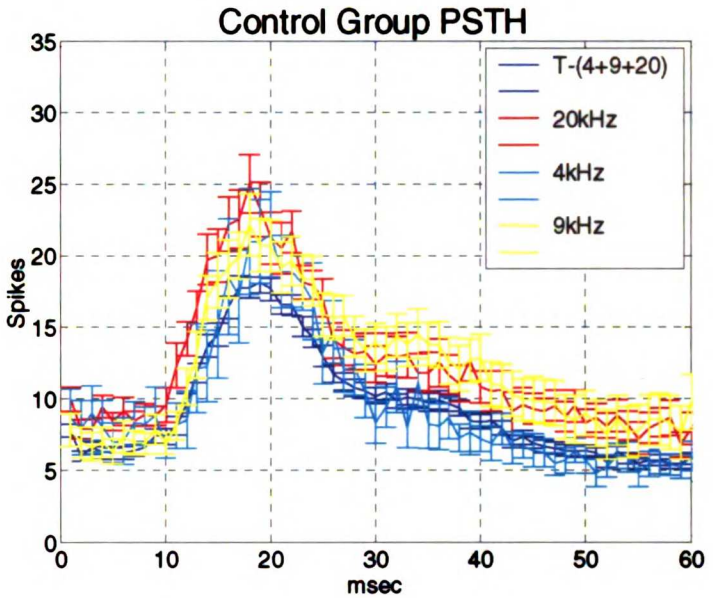


Figure 5.5; Averaged PSTHs Naïve vs. DSP4-Treated Groups



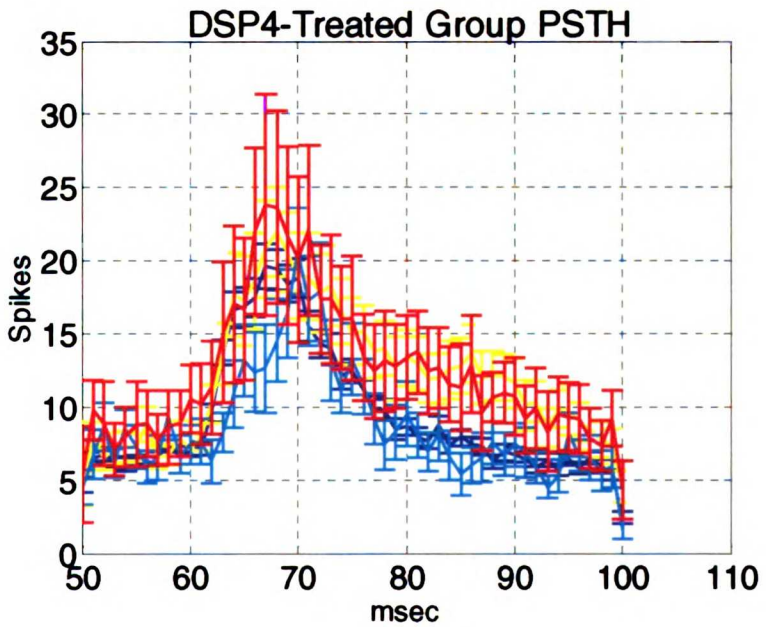


Figure 5.1 Representative CF map of right primary auditory cortices of a naïve rat compared to that of a DSP-4-pretreated animal. Each polygon represents one penetration. Color represents each site's characteristic frequency. Non-responsive and auditory responding non-A1 sites are marked with O's and X's respectively. Notice the overall decrease in size of the DSP-4 pretreated Animal (see discussion).

Figure 5.2 Bar graph of the primary auditory cortical areas (mm^2) from the naïve, 4kHz, 9kHz-paired, Stimulation-Alone, and DSP-4-pretreated groups. Graph is an average of four representative naïve maps, four 4kHz-paired maps, four 9kHz-paired, two Stimulation Alone, and three DSP-4 pretreated maps. Error bar represents standard error. Notice the significant overall increase in the size of A1 in the paired and stimulation-alone groups and the significant decrease in the size of A1 in the DSP-4-pretreated group.

Figure 5.3a-d a) Millimeters of A1 responding in DSP-4-preteated versus naïve groups across frequencies at 40dB b) at 50dB c) at 60dB d) at 70dB. Notice that between 40 dB and 70 dB, there is a decrease in the overall size in the DSP-4-pretreated group that is fairly consistent across frequencies.

Figure 5.4a-d a) Fractions of A1 responding in DSP-4-pretreated versus naïve groups across frequencies at 40dB b) at 50dB c) at 60dB d) at 70dB. Notice that between 40

dB and 70 dB, there is reliable consistency between naïve and stimulation-alone groups.

Figure 5.5a-b a) Averaged PSTHs split into 4kHz, 9kHz, 20kHz, and "all" bins from the naïve group (as seen earlier). b) Averaged PSTHs split into 4kHz, 9kHz, 20kHz, and all bins from the DSP-4-pretreated. Notice the nearly indistinguishable profiles between the naïve and DSP-4-pretreated groups.

Chapter 6: Behavior

Chapter 6 : Behavior

Animals displayed certain stereotyped behaviors after their daily pairing sessions that previous studies may explain. First, as the animals were placed in the cage than connected to the stimulator, there was an initial phase of exploratory activity in which the animal moved around in the cage somewhat and there was often some sniffing around the cage. After several pairings the animals seemed to find one place in the cage where it would often remain in what seemed to be some sort of daze for the remainder of the pairing session. There was often a small head bob and a wink of the ipsilateral eye or blinking of both eyes time locked to the stimulation of the LC. During this "daze" phase, the animals were also easily startled by even a snap of the fingers.

After the pairing session, animals were replaced in their housing cage at which time the animal, almost without fail, would run in ipsiversive motions for thirty seconds or so, after which they seemed to assume one position and remain there in a state of what appeared to be rest. Previous studies have indicated that unilateral activation of the VTA with morphine elicits contraversive circling in rats (Jenck, Bozarth, and Wise 1988). Perhaps this observed behavior is somehow related. All of these behaviors were similar in the paired and stimulation-only groups.

The DSP-4-pretreated group had different behaviors. First, the treatment with DSP-4 was obviously taxing to the animals; one animal died due to this treatment. Animals were generally more lethargic, and during the sham pairing sessions, displayed little exploratory activity. They were not obviously aroused by stimulation.

After the sham pairing sessions, they did not exhibit the ipsiversive running and remained lethargic.

Chapter 7: General Discussion

Chapter 7: General discussion

As animals interact with their environments, their perceptions of and reactions to these environments indicate their enduring memories of it as these perceptions and reactions pass through several interacting filters, before the "important" information is identified. Brain processes (the animal) then react to it in a hypothetically advantageous manner, guiding it to positive and not adverse consequences. These processes are not accomplished by any one system, and our attempt here to isolate a single mechanism of event retention is necessarily limited. At the same time, this study further elucidates the role of a particularly important modulator, NE delivered by the LC.

Once a natural stimulus is perceived by an animal, it might be identified as predicting harm or benefit, and on that basis, engage the LC and the amygdala. The stimulus-driven neural activity is also passed on to the thalamus which if prepared, possibly by LC-induced burst mode allowance (which allows input to be faithfully transferred to the cortex via large rhythmic bursts in response to sensory input, see ref.) would potentially more selectively activate this sensory-driven activity. This is another filter.

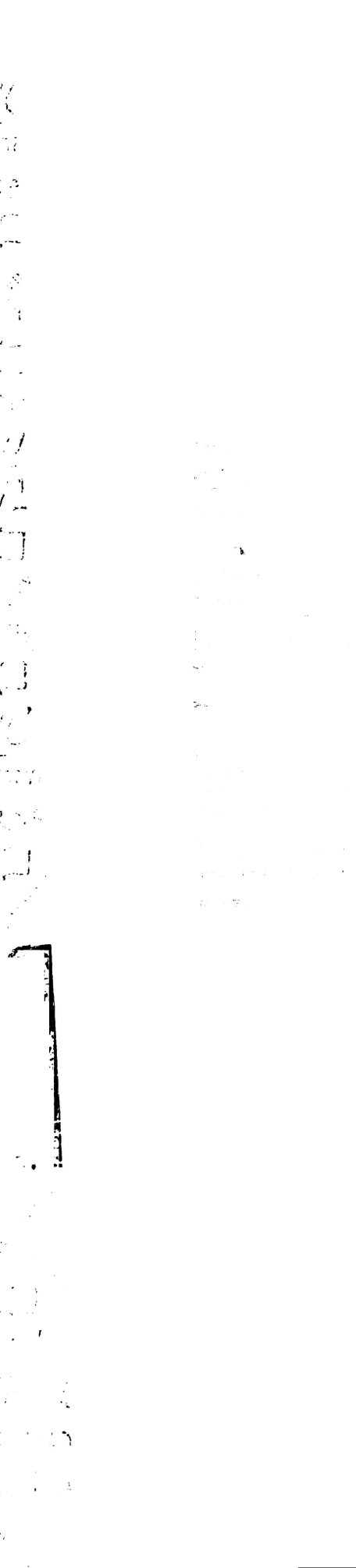
If the sensory information does indeed induce bursting in the thalamus, it will likely result in cortical activation at least in layer IV but potentially activating horizontal systems and potentially cortical inhibitory nets. Some of these cortical systems might already be NE-primed by the initial sensory-driven LC activity. There are many assumptions so far which shouldn't be forgotten, but allow me to continue. This NE-priming potentially decreases the threshold for spiking in cortical neurons

through G-protein-mediated inhibition of leak-K+ channels allowing neurons to spike more reliably when driven by sensory input (and/or potentially by noise/spontaneous activity). Below, I present a cortical model that can explain the aforementioned effects on A1 tonotopy and multiunit response properties.

As we have already discussed, the LC activity in response to an incoming stimulus provides the animal with a significance tag to the ensuing activity across the neuraxis. How does the LC accomplish this tagging? And how does this tag allow the brain to retain the necessary information in its cortical representations? Finally, how can the differences in plasticities between the paired, stimulation-alone, and DSP-4-pretreated groups be explained? Figures 7.5-7.7 presents a hypothesis that represents my initial attempt to answer these questions.

An auditory stimulus that activates the isofrequency regions across the neuraxis and that also activates the LC will likely be passed faithfully into the cortex via the mechanisms discussed above. But how does the representation of this stimulus then become modified as shown in the data above? In Figure 7.5, I hypothesize that the incoming LC-activating stimulus activates the isofrequency region of A1. As this is occurring, the LC send its NE signal in an autocrine and general fashion, but only the areas activated by stimulus-driven activity will make use of this NE priming which, as we have discussed previously, likely lowers the threshold for spiking by inhibiting leak potassium channels. Though internal noise might also activate NE-primed regions that are not specific to the auditory stimulus, this activity will likely not be synchronized across any substantial portion of the cortex, and therefore will likely not be propagated via activation of horizontal connections in layer III, or not to the same

degree as the stimulus driven activity. However, the stimulus driven, NE-primed horizontal activity will induce neurons immediately adjacent to the isofrequency region representing the paired stimulus to take on a response profile that will now include the paired or behaviorally relevant stimulus. The NE-primed, stimulus-driven horizontal activity can then drive flanking inhibitory nets that prevent substantial activity in isofrequency regions flanking the paired and/or LC-activating stimulus, thus preventing aberrant plasticity in these regions that do not already have representations related to the relevant stimulus while also damping the future response of these areas to stimuli that do not activate the LC. One can in turn imagine how this might be occurring in a step-wise manner: As the horizontal connections allow neurons that previously did not represent the incoming stimulus to take on a response profile that now includes this stimulus, they can now be included in this ever-changing and in this case continuously growing isofrequency region. In future epochs of NE-primed, stimulus-driven activity, this activity will spread further horizontally still further expanding the isofrequency region. While such a process may explain the frequency-specific expansion, how can it explain the general expansion of A1? This model also allows for a general increase in the activity of A1 neurons, via NE-primed spontaneous activity. The lowering of thresholds across A1 increases the likelihood that spontaneous activity will drive spiking as well. This increased likelihood might allow neurons previously classified as "non-A1", to decrease their response latencies and therefore meet our criteria for A1 inclusion. This NE-primed spontaneous activity is unrelated to any particular stimulus-driven activity, and might therefore be considered aberrant.



To this point, we have hypothesized how this experimental manipulation might generate an A1 that has more neurons responding to auditory stimuli in general, and a larger fraction of all A1 neurons responding to the paired stimulus. How can this model explain the alterations in response properties in paired animals? Figures 3.8a-c show averaged PSTH traces for the four frequency bins in the naïve, 4kHz-paired, and 9kHz paired groups. As previously discussed the PSTH traces for the paired bin indicate that the activity in A1 induced by the paired stimulus is prolonged in duration, while responses are similar in onset latency compared to the corresponding responses in the naïve group. More over, all other bins (non-paired and overall) within the paired groups show decreased overall response amplitudes. I hypothesize that the prolongation of activity within the paired bin is due to convergence or horizontal strengthening, and to decreased thresholds. As the horizontal connections are strengthened across the pairing regimen, the propagation of this horizontal activity has further to travel and several synapses to course to encompass the isofrequency region representing the paired frequency; this might arguably account for the temporal widening of the response window. The onset latencies of previously non-A1 neurons are decreased via decreased thresholds and over the course of pairing, thus increasing the number of neurons that meet our A1 criteria. In summary, the decreased onset latencies of the neurons within the isofrequency region representing the paired frequency and the long-distance horizontal connections between these neurons could account for the spread of isofrequency plasticity, general A1 enlargement (as A1 is defined by our physiological criteria), and the widening of the temporal response window of the neurons within the paired bin.

This class of model iterates the importance of LC-NE-priming, as well as stimulus-driven activity in the propagation of the plasticity described in the paired animal groups. How can this model explain the different effects displayed by the stimulation-only animals? Figure 7.6 shows the same model, but without peripheral auditory input. As we've seen in the above Results sections, there is a general, frequency-nonspecific enlargement of A1. There is also a general increase in the response profiles of multiunits within this group, such that when a neuron responds to an incoming stimulus, its responses are strongly amplified. In other words, the neurons respond with several times more spikes (versus the naïve and paired groups) for any given supra-threshold auditory stimulus. How can the model in figure 7.6 account for these results?

The LC is activated in a similar manner to the paired groups, so the inhibition of leak K+ channels and therefore the decreased thresholds and increased excitability will theoretically still be present. However, stimulus- driven activity will not be present, leaving only the spontaneous noise to take advantage of the NE-priming of neurons throughout the neuraxis including the auditory cortex. In turn, this lack of stimulus driven activity will not engage horizontal systems in a frequency specific manner, but only in a manner driven by spontaneous activity (which will likely not be synchronous in character), and in the absence of coordinated activation, inhibitory nets will not be equivalently engaged. It does seem though that this increased excitability and spontaneous activity are enough to promote the stability of the tonotopy within the auditory cortex, while leading to increased excitability which

might in turn promote the general, but modest increase in the area of the auditory cortex containing neurons that meet our criteria for A1 inclusion.

The increase in reactivity of A1 neurons is a result that seems counter-intuitive to the results of the paired groups, in which activity unrelated to the paired stimulus is either inhibited or depressed in a way that leads to neurons being less reactive (than naïve or stimulation alone animals) to the identical stimulus. The general hyper-excitability of auditory cortical neurons in the stimulation-alone group occurs without any corresponding increase in either threshold or bandwidth (Measured 30dB above threshold; BW30) and thus appears to reflect a system that is begging for attention through large synchronized epochs of responsivity; but because there isn't an identifiable behavioral strategy or even stimulus by which to develop a strategy, there is no limiter on the system. This lack of limiting could potentially lead to catastrophic perpetuation of hyper-excitability. Without an identifiably relevant stimulus by which to develop a behavioral strategy, the activity enters what might be viewed as a revolving door with no means by which to dissipate. Based on these results, I propose that there is a stimulus-driven activation of inhibitory nets that serves as a limiter of cortical neuronal responsiveness. These results might prove to be very important in the understanding of mental diseases in which there is generally elevated LC activity without a predictable and precise temporal relationship with behavioral stimuli. Amelioration of this over-responsiveness through behavioral therapy might be a useful therapeutic strategy.

Figure 7.7 describes a model in which LC NE influences were removed, while leaving the auditory stimulation intact. Although the stimulus is intact, the stimulus-

driven activity will likely be different in character because not only is the stimulus-paired NE-priming absent which is likely accomplished by phasic LC activity time-locked to the stimulus, but also the background or tonic LC activity which likely serves to maintain a background level of NE influence throughout the neuraxis is removed. Though this background level likely varies depending on the state of vigilance and mental acuity of the animal, it possibly serves a role to create stability of representations that are already present in the brain. By lowering spiking thresholds, this tonic LC activity likely allows some spontaneous noise to induce spiking and in turn creating a stable tonotopy within A1. This lack of tonic activity may explain the modest shrinking of A1 size within the DSP-4-pretreated group. The lack of tonic activity might plausibly increase the threshold for spontaneous-noise induced firing and stability and thus decrease the number of auditory cortical neurons that meet our A1 criteria. Also, the potential increases in spiking thresholds across the auditory cortex due to the lack of NA-mediated decreases in leak K+-channel conductances, might decrease the number of neurons responding at latencies short enough to meet our A1 criteria and therefore decrease the size of our physiologically-defined A1.

Perhaps there are other compensatory mechanisms at work in these animals that no longer have an LC. Perhaps acetylcholine from the nucleus basalis or dopamine from the ventral tegmental area serve to bolster the responsiveness of individual mulitunits in A1 as there seems to be no change in the response profiles of mulitunits between the naïve and DSP-4-pretreated groups. Animals without an LC tend to extinguish conditioned learning more slowly than animals with an intact LC. Perhaps

this is due to the inability of the new conditioned stimulus to induce NE-priming across the neuraxis. The new stimulus will have to engage other compensatory mechanisms in order to gain an advantage to be reliably transferred through the neuraxis.

I will now turn my attention to these other compensatory mechanisms and how plasticity that has been previously related to these nuclei (NB, VTA) relates and compares to the LC-related data presented here.

Figure 7.1; Averaged PSTHs 9kHz Bin All Groups

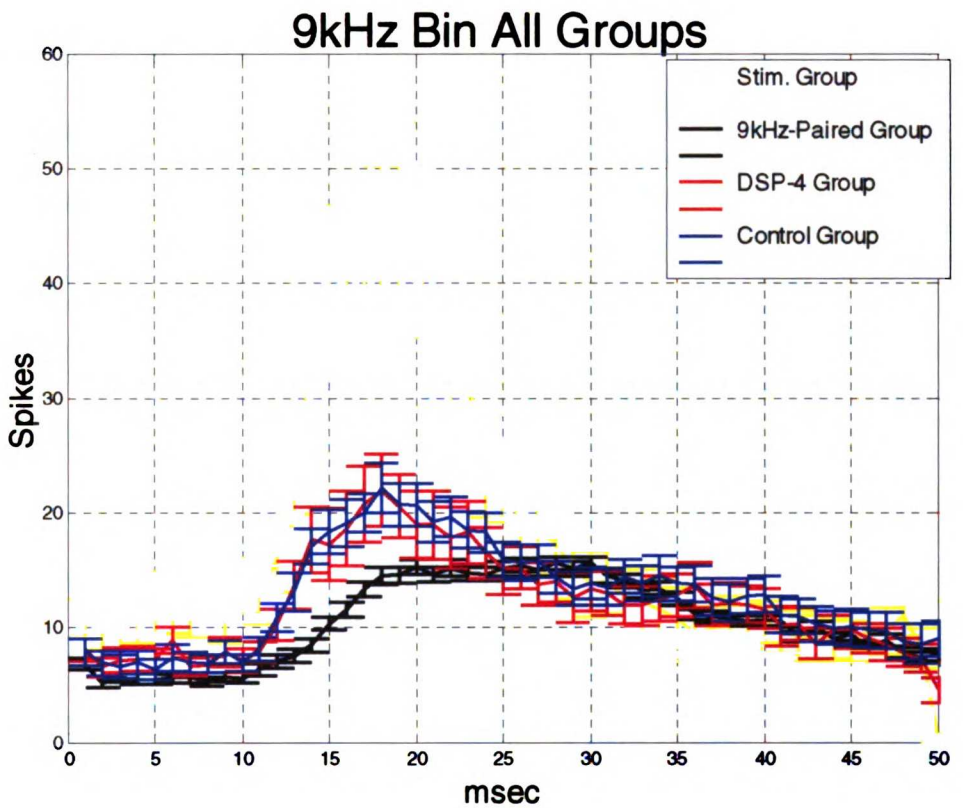


Figure 7.2; Averaged PSTHs 4kHz Bin All Groups

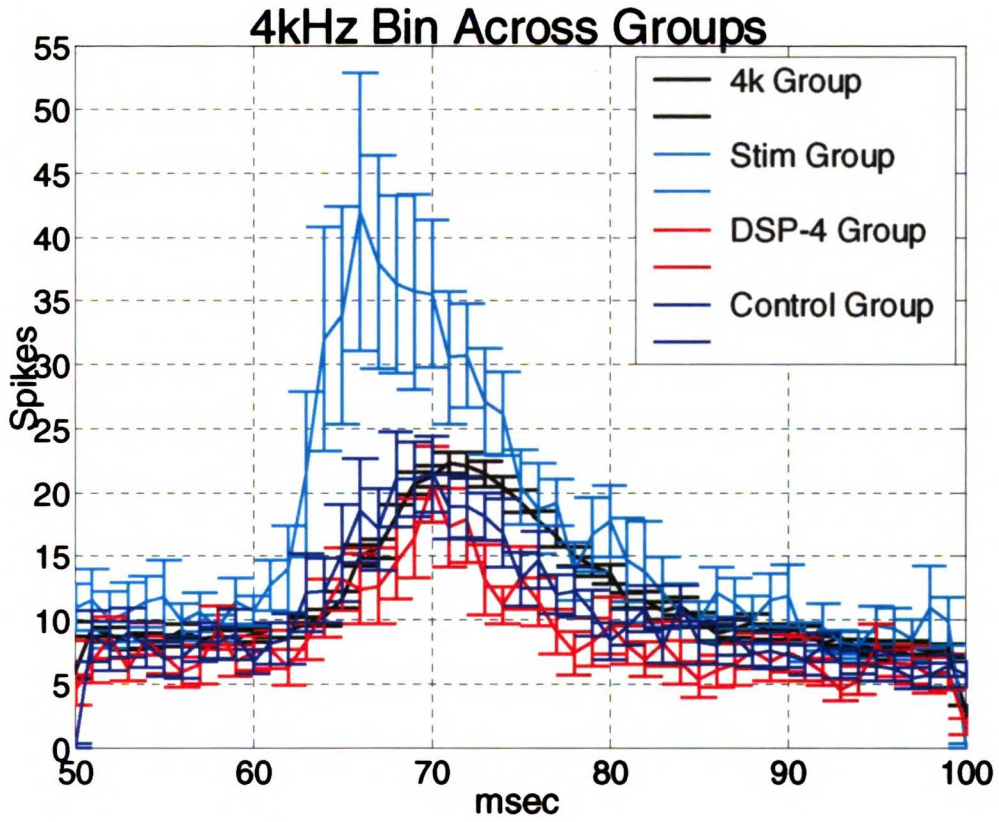


Figure 7.3 Bandwidths (BW30) in 0.5 octave bins all groups

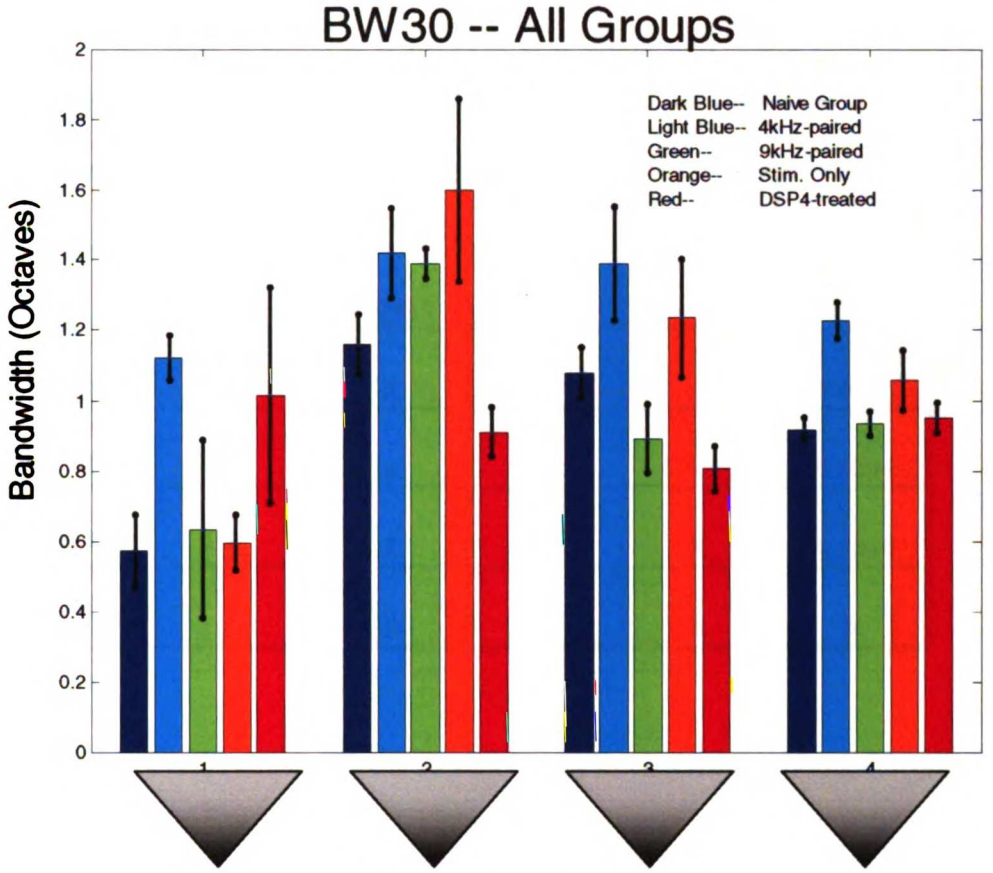
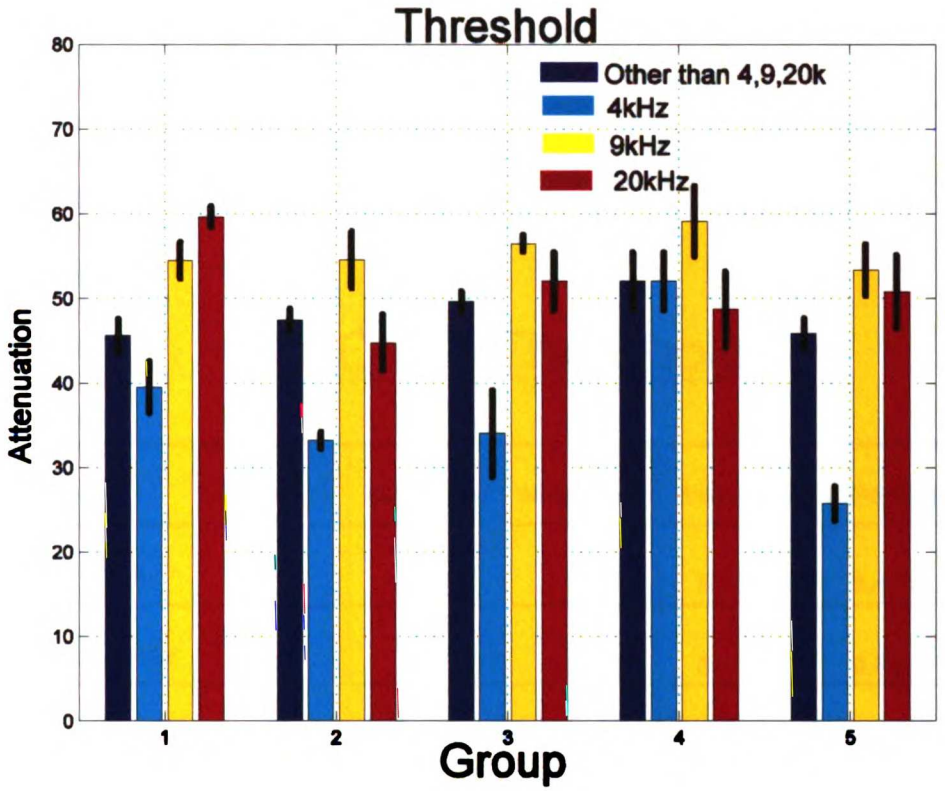


Table 4; BW30 between-group ANOVA values

ANOVA; Bandwidth (BW30)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naïve	X	0.0523	0.8905	0.4729	0.9345
4kHz-paired	X	X	0.152	0.4727	0.0043
9kHz-paired	X	X	X	0.5648	0.8094
Stim. Only	X	X	X	X	0.3844
DSP4-treated	X	X	X	X	X

Figure 7.4 Thresholds in 0.5 Octave Bins All Groups



Group 1 – Naïve

Group 2 – 4kHz-Paired

Group 3 – 9kHz-Paired

Group 4 – Stimulation Only

Group 5 – DSP4-Treated

Table 5; Threshold between-group ANOVA values

ANOVA; Threshold

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naïve	X	0.4753	0.7959	0.5540	0.4735
4kHz-paired	X	X	0.6637	0.1596	0.8923
9kHz-paired	X	X	X	0.3926	0.6241
Stim. Only	X	X	X	X	0.2224
DSP4-treated	X	X	X	X	X

Figure 7.5 Model of Plasticity for 9kHz-Paired Group

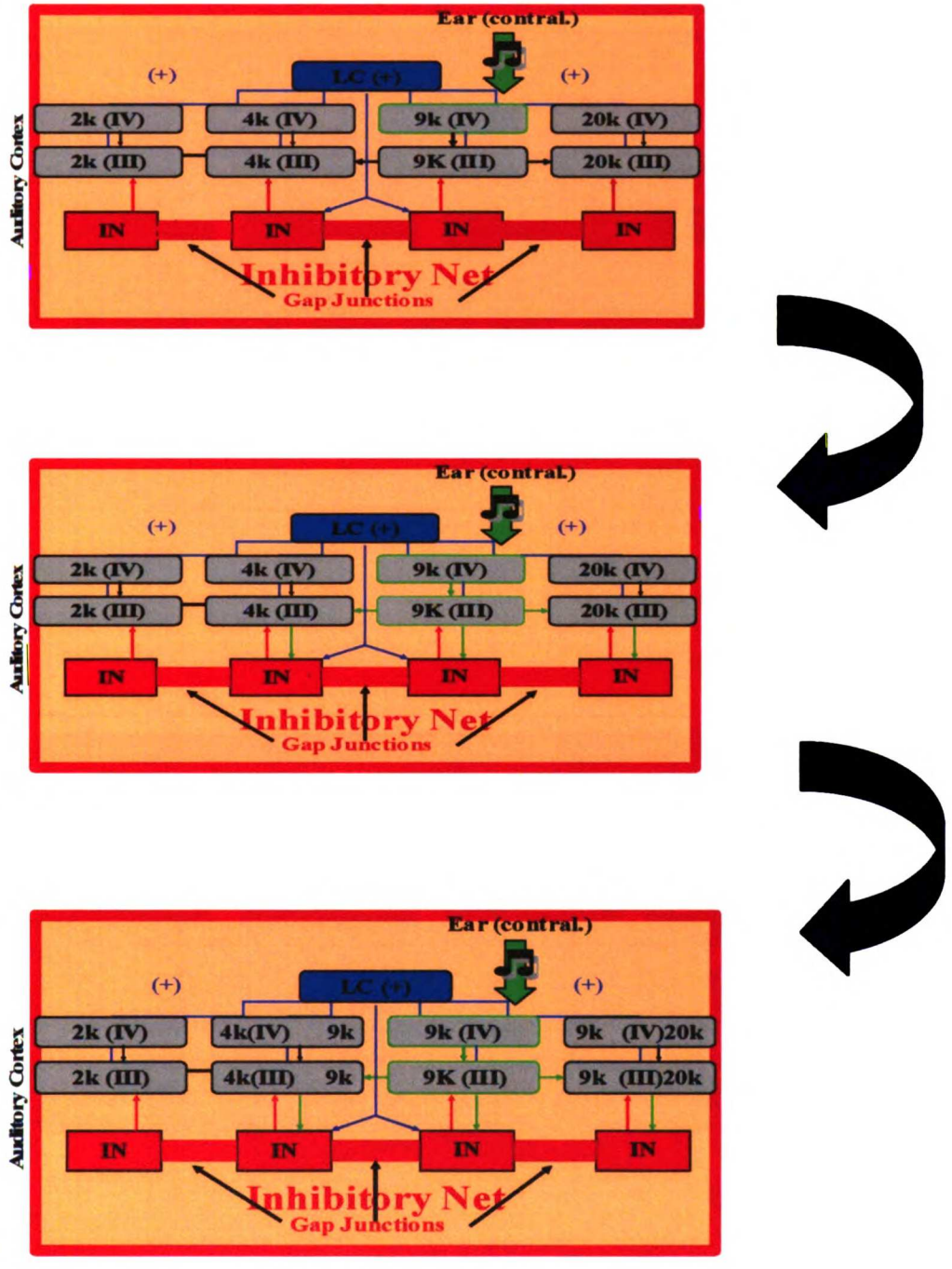


Figure 7.6 Model of Plasticity for Stim. Only Group

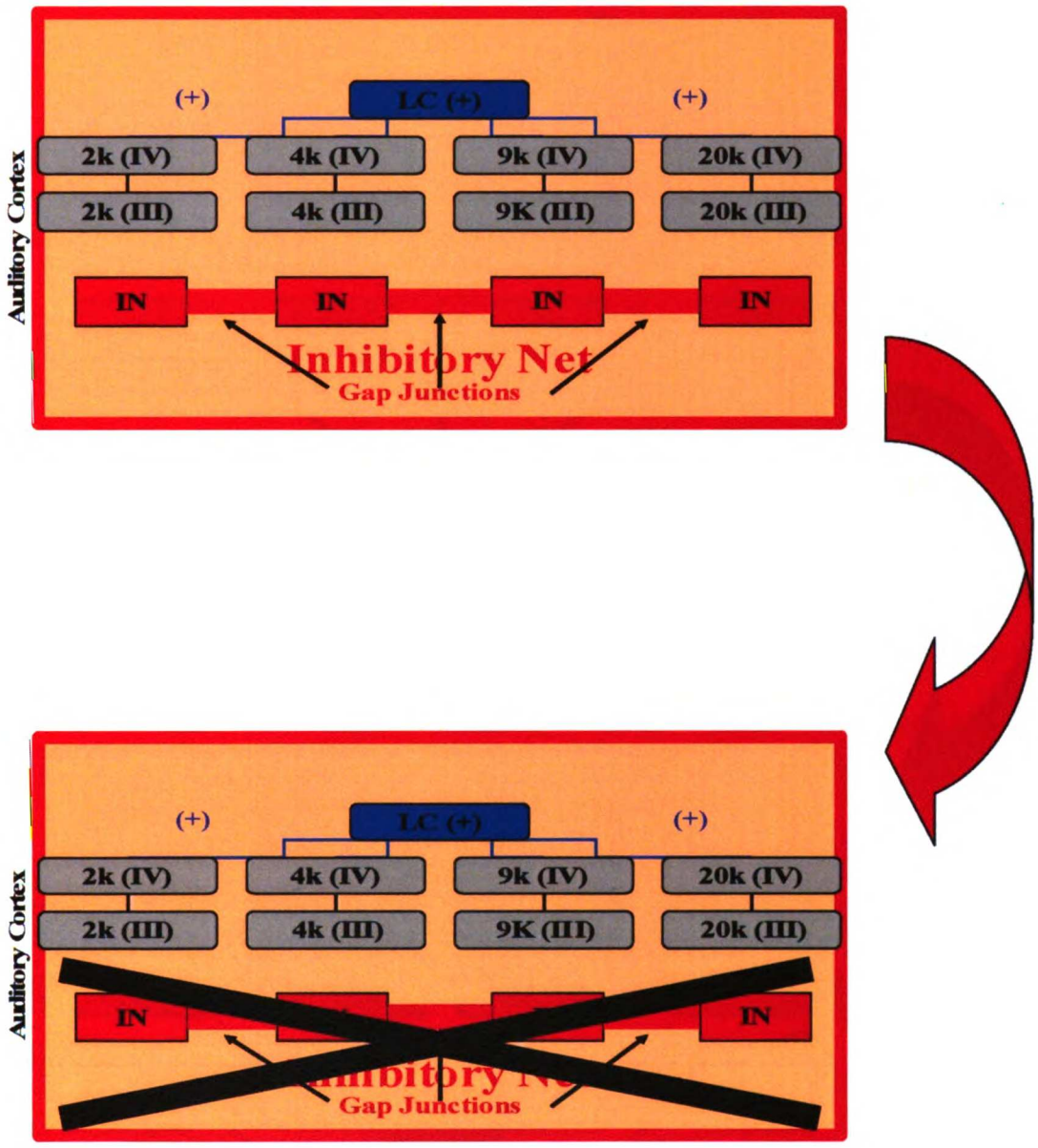
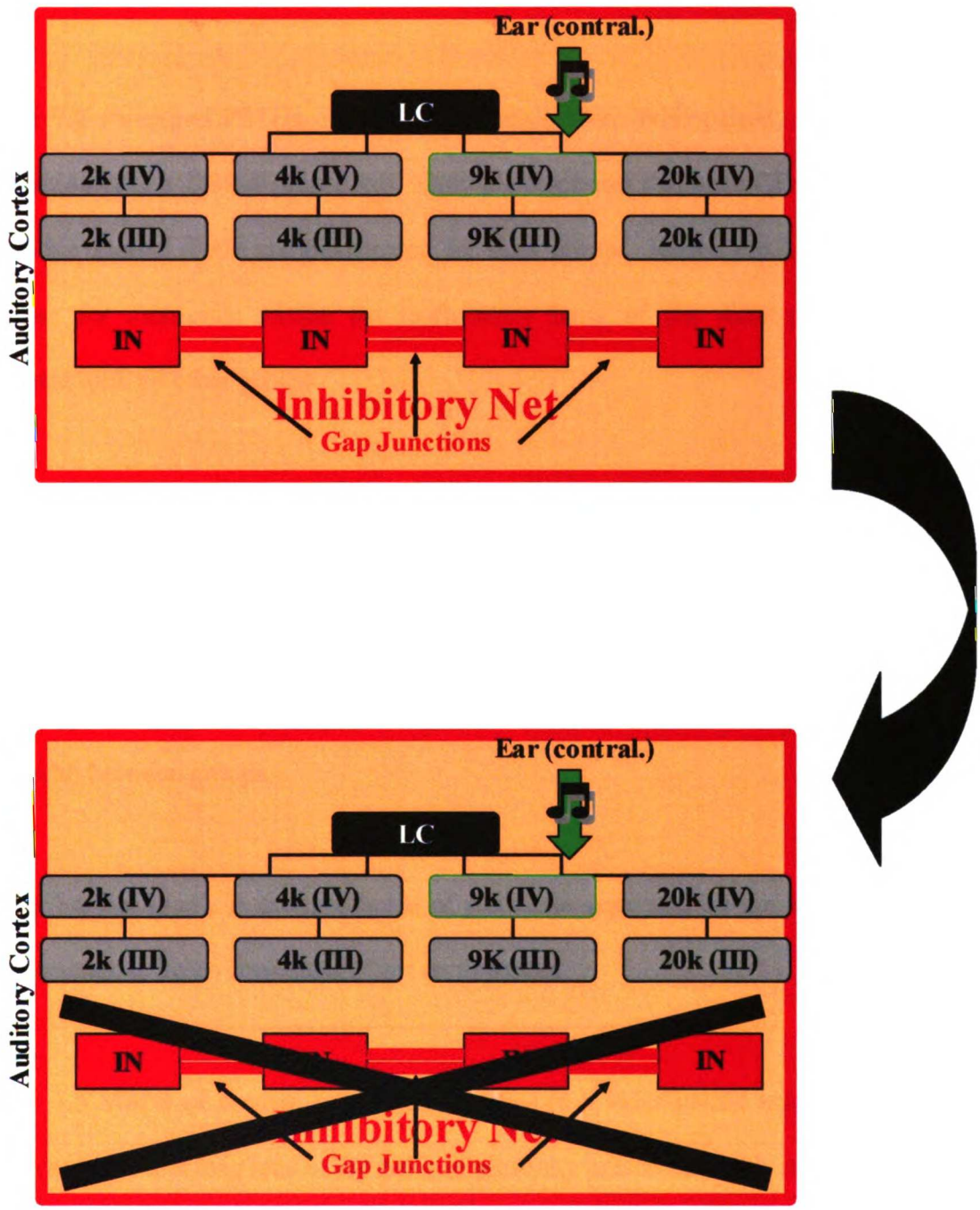


Figure 7.7 Model of Plasticity for DSP4-Treated Group



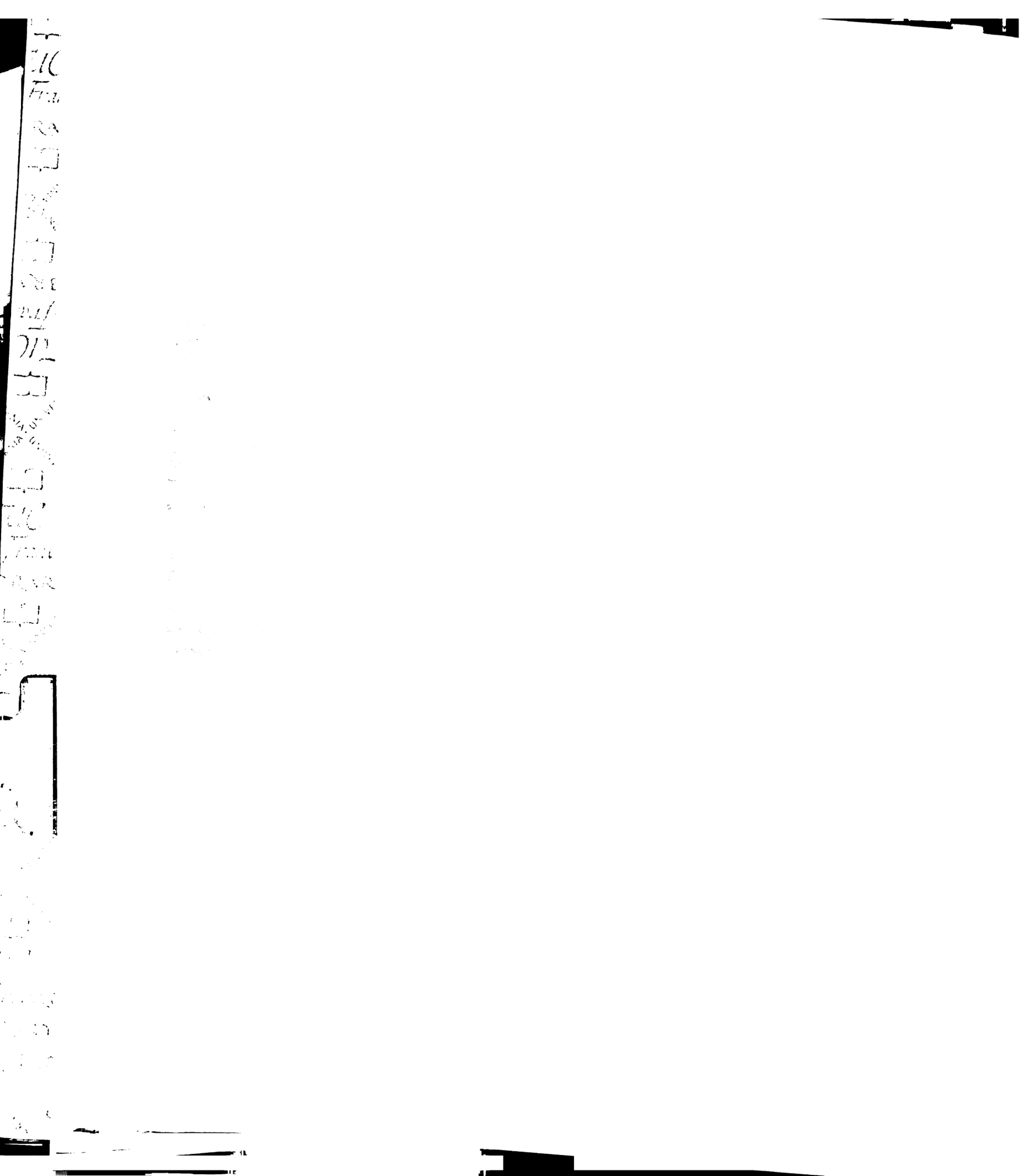


Figure 7.1 Averaged PSTHs of the five groups (naïve, 4kHz-paired, 9kHz-paired, Stimulation-Alone, DSP-4-pretreated). Only the responses that have CFs within a 0.5 octave bin around 9kHz are represented for each group. Notice the larger relative response for multiunits within the isofrquency band of the 9kHz-paired group compared with all other groups.

Figure 7.2 Averaged PSTHs of the five groups (naïve, 4kHz-paired, 9kHz-paired, Stimulation-Alone, DSP-4-pretreated). Only the responses that have CFs within a 0.5 octave bin around 4kHz are represented for each group. Notice the larger relative response for multiunits within the isofrquency band of the 4kHz-paired group compared with all other groups.

Figure 7.3 Bar graph of the average BW30s of neurons within the same bins as analyzed in the PSTH section, across all groups. There might be a small effect in which the BW30s of mulitunits with CFs within the frequency bin corresponding to the paired frequency are a bit larger. There appears to be no significant effect on bandwidth between groups.

Figure 7.4 Bar graph of the thresholds of responses separated by the same criteria. There appears to be no consistent effect on threshold.

Figure 7.5 Model of cortical response properties in a 9kHz-paired animal during a pairing session, that may lead to the types of plasticity presented in this study.

Figure 7.6 Model of cortical response properties in a Stimulation-Alone animal during a pairing session, that may lead to the types of plasticity presented in this study.

Figure 7.7 Model of cortical response properties in a DSP-4-pretreated animal during a pairing session, that may lead to the types of plasticity presented in this study.

Chapter 8: Integrative Theories

Chapter 8: Integrative Theories

The mechanisms underlying behavioral modification and the plasticities related to it are numerous and interacting. This very diversity that allows flexibility and ingenuity in the face of novelty, stress, and reward, makes difficult and complex the study of the processes underlying it. As mentioned previously, the VTA, the NB, and now the LC have been investigated in some detail, and their relevance to adult learning and adaptation have begun to be elucidated. In the following paragraphs, I will compare and contrast recent studies involving these three nuclei in particular and their relationship to adult auditory cortical plasticity. Subsequently, I will propose a broader view of just how the LC might be accomplishing relevance tagging and storage and directing the sculpting of advantageous behavioral responses to the relevant stimulus.

In the primary auditory cortex, dopamine (DA) release presumably from terminals of VTA neurons, has been observed during auditory learning that remodels spectral representations (Stark and Scheich 1997; Bakin, South and Weinberger 1996).

Acetylcholine-producing nucleus basalis neurons are activated as a function of the behavioral significance of stimuli, and because it receives input for both limbic and paralimbic structures, the NB is uniquely positioned to provide the cortex with information about the behavioral significance of incoming stimuli. Several forms of learning and memory are impaired by cholinergic antagonists and NB lesions (Butt and Hodge 1995; Leanza et al 1996; Baskerville et al. 1997).

Table 1 provides a summary of auditory cortical effects of pairing auditory stimuli with VTA, NB, and LC stimulation.

LC neurons co-release galanin, a peptide, during large bursts of LC activity. Galanin receptors are located in the amygdala and galanin microinjections into the lateral ventrical or amygdala induce anxiolytic and anxiogenic effects, respectively; aberrant galanin release can induce symptoms of anxiety (Wrenn and Crawley 2001). Immediately following a pairing session, animals exhibited signs of anxiety including increased startle responses and running in circles. These behavioral effects of our experiments might be the result of similar galanin influences. Galanin recepters also exist in the VTA, and activation of these receptors produce an inhibitory effect on DAergic VTA neurons (Wrenn and Crawley 2001). This might also contribute to the behavioral effects we observed following pairing sessions.

In rats, the NA content is highest in the cingulate and frontal areas, followed by visual, auditory, and sensorimotor cortices. Clinical observations suggest that the cingulate cortex may be involved in the modulation of orientation to novel stimuli, acquisition of spatial memory tasks, and discriminative avoidance. Damage to the cingulate gyrus results in cognitive impairment, emotional lability, depression, and loss of awareness and "emotive dynamics" as described by Papez. Several neurosurgical studies demonstrate amelioration of depressive symptoms and chronic pain following cingulotomy.

The distribution of NA fibers in the cingulate cortex of rodents is very interesting. Area 24 has the lowest density, while area 29 has the highest density of DBH-reactive fibers in the neocortex. NA-fiber density in area 24 increases from rat to monkey to

human. In area 29 of the rat and monkey, layers Ib,c show dense, while II and IV show moderate, staining. Deeper layers are largely devoid of DBH+ fibers.

In the cingulate cortex, beta-R binding is among the highest in the brain. There is no change or a slight increase in this binding after lesions of the LC. In the rodent, beta 1-R sites are dense in layer Ib,c and II of areas 24 and 29, as well as in layer IV of area 29. In contrast, postsynaptic beta 2-Rs are fewer in number, and enriched primarily in layer I of area 29.

Chemical stimulation of the mPFC phasically activates LC neurons sometimes followed by long lasting oscillatory activity. Direct projections from the mPFC to the LC are involved in tonic and phasic activations of the LC (Jodo et al.1997). Dual labeling experiments suggest that NA interacts with cortical GABAergic interneurons primarily via beta-Rs. However, electrophysiological studies indicate that fast spiking, late spiking, and somatostatin+ cortical interneurons are depolarized by NA acting at alpha-Rs. NA showed heterogenous actions on CCK+ cells via alpha-Rs. Further experiments are required to elucidate the differential actions of NA on cortical interneurons.

One important finding that has emerged from recent work is that the phasic and tonic modes of LC activity can also be produced by changes in baseline excitatory drive to the LC in the absence of any change in coupling. Decreases in baseline afferent drive promote the phasic mode of LC activity, whereas increases favor the tonic mode. Afferent drive can originate from subcortical inputs and also descending "top-down" influences, primarily from the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC), structures known to be involved in executive functions and

evaluation of behavioral salience and response evaluation and evaluating rewards and costs, respectively.

There are many disorders in which direct or indirect aberrations of the LC are implicated. Data obtained from multiple models of epilepsy converge to conclude that endogenous NA is an antiepileptic agent. It was found that intact NA innervation is necessary for the antiepileptic effects of vagus nerve stimulation in patients with refractory epilepsy. In addition NA loss increases neuronal damage following focally induced status epilepticus, confirming the protective effect of NA on the brain (Browning et al. 1998).

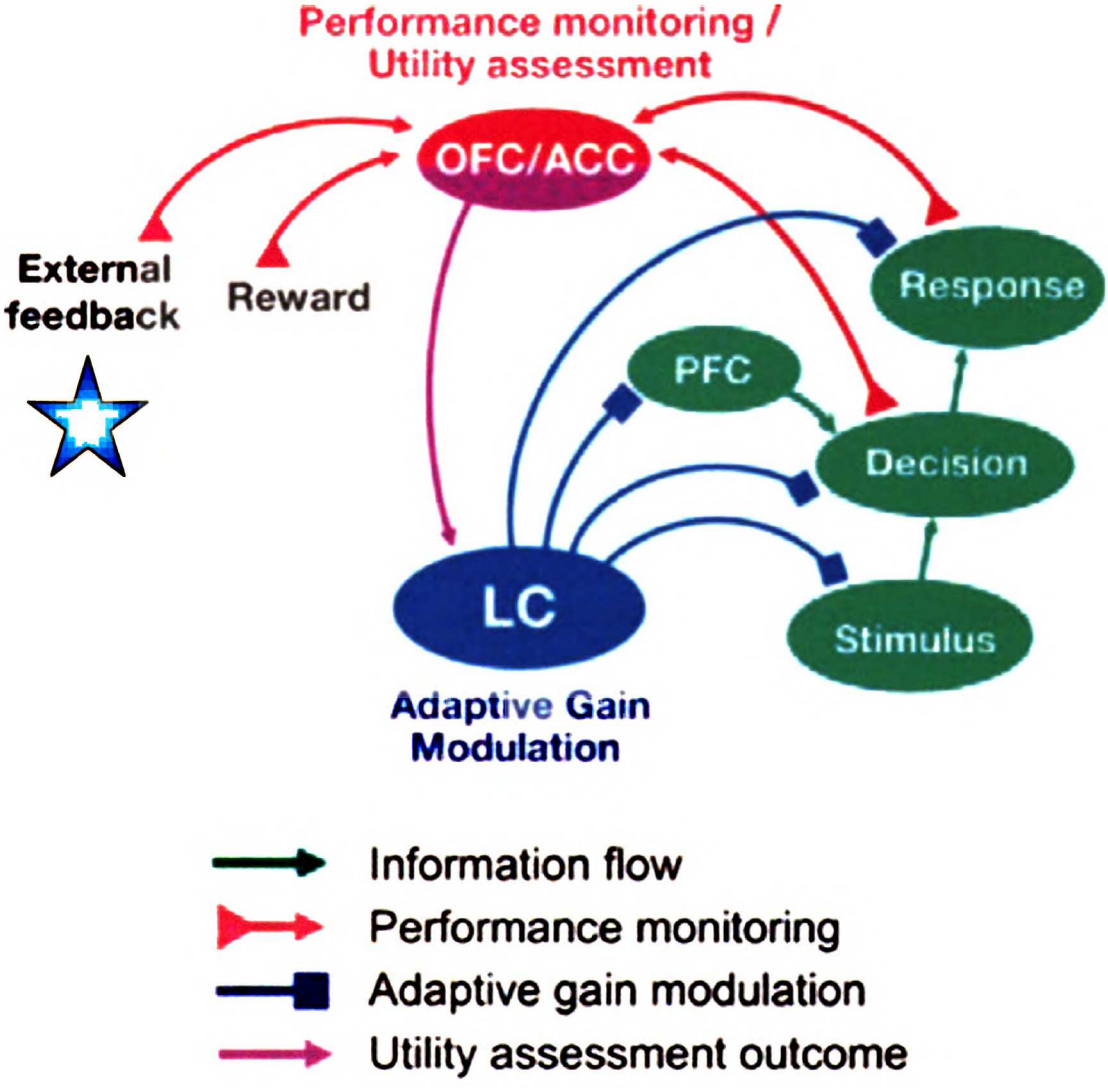
With the aforementioned information in mind, I propose a model of LC-mediated plasticity that is essentially an extension of a model presented by Aston-Jones and Cohen (2005). This model is outlined in figure 8.1 and is entitled the adaptive gain theory of locus coeruleus function. This theory proposes that:

> "the outcome of decision processes associated with high potential utility are represented in frontal structures (OFC and ACC) that drive LC responses...Brief lapses in performance, in the context of otherwise high utility, augment the LC phasic mode, improving task performance. In contrast, enduring decreases in utility drive transitions to the tonic mode, promoting disengagement from the current task and facilitating the exploration of behavioral alternatives. Taken together, these mechanisms constitute a self-regulating system by which the LC—informed by evaluations of utility in frontal structures—can control behavioral strategy through adjustments of gain in its global efferent targets."

The experimental results outlined in this paper potentially extend this model. These results suggest the addition of a stimulus-driven inhibition of responsivity in cortical targets of the LC. In other words, a stimulus that is time-locked to the LC

phasic activity might maintain the resulting cortical activity within a normal gain range. If, however, there is no stimulus that is temporally associated with the LC response, the resulting response will lack this stimulus-driven inhibition and allow aberrant cortical activity, as is evidenced by response profile analysis in this paper. These data suggest that a reliable stimulus is required in association with phasic LC activity in order for the adaptive gain theory to hold true. This suggests potential mechanisms by which these "evaluations of utility" occur (as denoted by the blue star in figure 8.1). The utility must be accompanied by a means to identify it (i.e. a stimulus). Without the stimulus to identify the utility, the mechanisms by which behavioral utility is identified and stored break down, potentially leading to hyper-excitability and aberrant activity in cortical structures which can have catastrophic consequences.

Figure 8.1 Modified Model of LC Performance Monitoring (Aston-Jones and Cohen 2005)



Aston-Jones, G and Cohen, JD. 2005
Annu. Rev. Neurosci. 28:403–50

Table 6; Characteristics of Auditory Cortical Plasticity (nucleus basalis, ventral tegmental area, and LC influences)

Characteristics of Auditory Cortical Plasticity

	VTA*	NB**	LC
*Size of functional A1	**No Change**	Increased	Increased
*Stimulus Frequency Representation	Increased	Increased	Increased
*Adjacent Frequency Representation (%)	Decreased	Increased	Decreased
*Frequency Specificity of the effects	**Sharper**	**Broader**	**No Change**
*Temporal Asymmetry Of the effects	**Yes**	**No**	**Undetermined**
*Magnitude Paired Freq.	**No Change**	**No Change**	**No Change**
*Magnitude Unpaired Freq.	**No Change**	**No Change**	Decreased

*Values of Ventral Tegmental Area are from Bao et al. 2001
**Values from Nucleus Basalis are from Kilgard and Merzenich 1998; Bakin and Weinberger 1996; Van Essen et al. 1992

Figure 8.1 Model of LC performance monitoring Aston-Jones and Cohen 2005. This is entitled the adaptive gain theory of locus coeruleus function. The blue star denotes the portion of the model extended by the results included in this paper in which performance monitoring requires stimulus association.

Table 1 Characteristics of auditory cortical plasticity. Previous results on nucleus basalis and VTA influences on auditory cortical plasticity are compared and contrasted with the results outlined in this paper related to LC influences on auditory cortical plasticity.

Chapter 9: Future Directions

Chapter 9: Future Directions

The above experimental results extend the previous literature in several significant ways. First, the LC must now be included as a powerful influence on the cortical manifestations of memory and the behavioral modifications associated with those memories. In addition, the mechanisms utilized by the cortex to store such memories must be amended to account for the alterations in response properties examined herein. However, these results are not all-inclusive, and do not provide a full explanation of LC cortical influences. Instead, they are the first few steps on the ladder that may lead to an understanding of the complex, interacting, and potentially redundant modules that are permissive and also motivating to an animal's behavioral success. Below I discuss some of the possible extensions of the above experiments.

Experiments performed by Bao and Merzenich (2002) revealed that the VTA's influence on cortical plasticities similar to those discussed above for the LC, is temporally asymmetrical. That is, forward pairing of the auditory stimulus with VTA stimulation induced stimulus-specific expansion, while backwards pairing induced a corresponding reduction. One future experiment might be to similarly examine the temporal symmetry of LC-induced plasticities. In fact, it is possible that the asymmetry of the VTA results might be induced via LC-mediated influences over the VTA. Perhaps stimulation of the VTA induces antidromic activation of the LC, and thus it is the asymmetry of LC influence over the cortex that is the deciding factor in the cortical manifestation of this asymmetry. Experiments designed to examine the temporal symmetry of LC cortical influence with and without VTA lesions, and an

examination of the VTA's influence on cortical plasticity with and without LC lesions would answer these questions.

Another possibly interacting nucleus is the NB. Perhaps the LC-induced plasticities discussed above are all caused by the LC activation of the NB. It has been previously shown that the LC exerts its desynchronizing influence over the cortex via excitation of F-cells in the NB (see introduction and review). An additional future experiment would repeat the above experiments, but with an NB lesion. Is the LC a powerful influence over cortical representations without an intact NB? Is the NB an powerful influence over cortical plasticity without an intact LC or is the NB mediating its effect via antidromic activation of the LC?

I would also like to propose a future experiment that is designed to assess the scope of the response-profile modification examined in the experiment in which animals received LC stimulation without the presentation of any reliable stimulus. How far reaching is this increase in responsivity? If the animal receives LC stimulation without a reliable stimulus to relate it to, the modality-specific neurons seem to be hyper-responsive to any stimulus within that modality (see chapter 4). Does this hyper-responsivity extend to the somatosensory (or other) domain(s)? Repeating the experiment in chapter 4, but mapping the somatosensory (or other) cortex would address this question.

Another, rather complex extension of the above findings might occur in a series of experiments that more closely replicate what occurs in nature. In a natural setting, it is not likely that LC activity would be repetitively paired with a simple pip train. Rather, LC activity would likely be paired with a more temporally and spectrally

complex stimulus. How might this be represented? Potentially these mechanisms could be uncovered by amplifying these more spectrally and temporally complex stimuli by the mechanism employed in the above experiments. One of the difficulties here is finding the means to identify the amplification. In the preceding experiments this was made much easier due to the simplicity of the stimulus. A MEG study in humans examining the effects of the subject's mother's voice compared to that of a stranger is one suggestion of how to uncover these likely complex mechanisms

Finally, I would like to propose an experiment designed to test the extension of the adaptive gain theory of LC function outlined in the previous chapter. Specifically, this experiment is designed to examine the possibility that it is the activity within the primary auditory cortex due to the paired stimulus that actually drives the stimulus-driven gain control. In this experiment, the LC would be implanted as done in previous experiments. In addition, the auditory cortex would be chronically implanted with an array of electrodes with resistances is the range necessary for both recording and stimulation. The cortex would then be mapped in order to identify a group of penetrations that constitute an isofrequency band within A1. The animal would then receive a daily regimen pairing LC stimulation with A1 isofrequency stimulation for 20 days as done in previous experiments. A1 would then be mapped.

There are various possibilities for the results of this experiment. Perhaps all of the cortical neurons would be maintained within the normal gain range, suggesting that A1 stimulation is sufficient to account for the newly proposed facet of the adaptive gain theory. Alternatively, and perhaps more interestingly, only the neurons

within the stimulated isofrequency band would be within the normal gain range and neurons outside this isofrequency band might have varying degrees of hyper-excitability, depending upon their proximity to the stimulated isofrequency region. This would suggest that there are various and interacting systems (including A1) that work in concert to accomplish the stimulus-driven inhibition of LC efferents necessary to keep these targets within a normal and healthy range of excitability.

This is not an all-inclusive list of future experiments. However, it is clear that the above results have far reaching implications; the answers to the basic questions in the preceding experiments allow the examination of more complex issues underlying behavioral modification.

ANOVA Statistics

Table 7; Within Group Averaged PSTHs (0.5 octave bins)

ANOVA; Within Group Comparisons

Bins: Group	4kHz vs. 20kHz	9kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. Others	9kHz vs. Others	20kHz vs. Others
Naïve	**0.0043**	0.06725	**0.0004**	0.2987	**$2.33e^{-6}$**	**$9.38e^{-5}$**
4kHz-Paired	**0.0253**	**$1.79e^{-14}$**	**$5.01e^{-13}$**	**$2.22e^{-7}$**	**0.0083**	**$3.29e^{-6}$**
9kHz-Paired	**$9.37e^{-8}$**	**0.0079**	**$3.78e^{-10}$**	**0.0084**	**0.0001**	0.06
Stim. Only	**$5.28e^{-5}$**	**0.0004**	0.843	0.763	0.948	**$6.78e^{-5}$**
DSP4-treated	**$1.60e^{-5}$**	0.137	**0.0089**	**0.0403**	**0.0019**	**$2.96e^{-6}$**

Table 8; Naïve vs. 4kHz-Paired Groups Averaged PSTHs (0.5 octave bins)

ANOVA; Naïve vs. 4kHz-paired Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0.2047	**2.08e-5**	**0.0071**	**0.033**
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	0.0008	**1.78e-15**	0.346	**1.76e-9**
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	0.0155	**9.87e-12**	0.663	**5.27e-7**
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	0.0054	**0.0006**	**0.0001**	0.276

Table 9; Naïve vs. 9kHz-Paired Groups Averaged PSTHs (0.5 octave bins)

ANOVA; Naïve vs. 9kHz-paired Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0.0004	0.366	7.27e-12	4.25e-6
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	4.26e-14	5.82e-6	0	2.22e-16
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	2.44e-10	0.0002	0	1.62e-12
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	0.0097	0.901	2.82e-10	0.0001

Table 10; Naïve vs. Stim. Only Groups Averaged PSTHs (0.5 octave bins)

ANOVA; Naïve vs. Stim. Only Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0	5.17e-10	1.35e-11	1.84e-14
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	0	1.63e-5	3.55e-6	6.01e-8
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	0	9.49e-6	2.18e-6	4.49e-8
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	0	8.34e-12	7.32e-14	0

*All Comparisons are Significant

Table 11; Naïve vs. DSP4-Treated Groups Averaged PSTHs (0.5 octave bins)

ANOVA; Naïve vs. DSP4-treated Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0.0011	0.079	0.5216	0.191
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	0.810	0.0816	3.43e-6	6.53e-7
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	0.844	0.2318	0.0002	3.46e-5
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	9.76e-6	0.0043	0.601	0.774

Table 12; 4kHz-Paired vs. 9kHz-Paired Groups Averaged PSTHs (0.5 octave bins)

ANOVA; 4kHz-paired vs. 9kHz-paired Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	1.41e-11	0.0002	0	6.71e-14
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	0.184	0.0005	0.0008	0.5891
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	2.36e-13	0.011	0	1.55e-15
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	0.0097	0.901	2.82e-10	0.0001

Table 13; 4kHz-Paired vs. Stim. Only Groups Averaged PSTHs (0.5 octave bins)

ANOVA; 4kHz-paired vs. Stim. Only Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0	1.28e-6	1.59e-7	1.24e-9
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	0	0	0	0
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	0	9.40e-10	1.10e-11	3.11e-15
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	0	1.00e-13	2.22e-16	0

*All Comparisons are Significant

Table 14; 9kHz-Paired vs. Stim. Only Groups Averaged PSTHs (0.5 octave bins)

ANOVA; 9kHz-paired vs. Stim. Only Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0	0	0	0
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	0	1.59e-11	1.73e-13	1.11e-16
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	0	5.55e-16	0	0
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	0	0	0	0

*All Comparisons are Significant

Table 15; 4kHz-Paired vs. DSP4-Treated Groups Averaged PSTHs (0.5 octave bins)

ANOVA; 4kHz-paired vs. DSP4-treated Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0.492	0.3916	**0.0002**	**3.41e-5**
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	2.81e-14	**1.74e-9**	**1.08e-5**	**0.0018**
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	0.003	0.306	**0.0108**	**0.0017**
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	1.34e-6	**5.72e-5**	0.072	0.438

Table 16; 9kHz-Paired vs. DSP4- Treated Groups Averaged PSTHs (0.5 octave bins)

ANOVA; 9kHz-paired vs. DSP4-treated Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	0	**0**	**5.99e-15**	**1.93e-9**
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	2.23e-5	**0.007**	0.7085	0.6847
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	7.20e-13	**4.70e-8**	**0.0003**	**0.024**
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	3.89e-15	**2.77e-10**	**1.62e-6**	**0.0004**

Table 17; Stim. Only vs. DSP4-Treated Groups Averaged PSTHs (0.5 octave bins)

ANOVA; Stim. Only vs. DSP4-treated Groups

Bins:	4kHz vs. 20kHz	4kHz vs. 9kHz	4kHz vs. 4kHz	4kHz vs. Others
	1.95e-6	1.50e-8	9.34e-14	2.40e-14
Bins:	9kHz vs. 20kHz	9kHz vs. 9kHz	9kHz vs. 4kHz	9kHz vs. Others
	9.70e-6	1.66e-7	1.36e-11	3.32e-12
Bins:	20kHz vs. 20 kHz	20kHz vs. 9kHz	20kHz vs. 4kHz	20kHz vs. Others
	0	0	0	0
Bins:	Others vs. 20kHz.	Others vs. 9kHz	Others vs. 4kHz	Others vs. Others
	3.02e-8	8.62e-11	0	0

*All Comparisons are Significant

Table 18; Response Area at 40dB (%A1) All Groups

ANOVA; Response Area at 40dB (%A1)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naïve	X	0.7434	0.4537	**0.0497**	0.7630
4kHz-paired	X	X	0.6070	0.5787	0.5232
9kHz-paired	X	X	X	0.9605	0.3218
Stim. Only	X	X	X	X	0.2706
DSP4-treated	X	X	X	X	X

Table 19; Response Area at 50dB (%A1) All Groups

ANOVA; Response Area at 50dB (%A1)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naïve	X	**0.2149**	**0.2534**	**0.169**	**0.771**
4kHz-paired	X	X	**0.8874**	**0.9199**	**0.1366**
9kHz-paired	X	X	X	**0.9472**	**0.1746**
Stim. Only	X	X	X	X	**0.1046**
DSP4-treated	X	X	X	X	X

Table 20; Response Area at 60dB (%A1) All Groups

ANOVA; Response Area at 60dB (%A1)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	0.1078	0.1861	0.0619	0.5890
4kHz-paired	X	X	0.9774	0.8220	0.0352
9kHz-paired	X	X	X	0.8352	0.0772
Stim. Only	X	X	X	X	0.0181
DSP4-treated	X	X	X	X	X

Table 21; Response Area at 70dB (%A1) All Groups

ANOVA; Response Area at 70dB (%A1)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	0.6096	0.1509	0.3948	0.4686
4kHz-paired	X	X	0.2797	0.7203	0.2036
9kHz-paired	X	X	X	0.4010	0.0370
Stim. Only	X	X	X	X	0.1039
DSP4-treated	X	X	X	X	X

Table 22; Response Area at 40dB (mm^2) All Groups

ANOVA; Response Area at 40dB (mm^2)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	0.0009	7.30e-5	0.0183	0.0107
4kHz-paired	X	X	0.0314	0.3012	1.38e-7
9kHz-paired	X	X	X	0.0057	9.56e-7
Stim. Only	X	X	X	X	6.76e-6
DSP4-treated	X	X	X	X	X

Table 23; Response Area at 50dB (mm^2) All Groups

ANOVA; Response Area at 50dB (mm^2)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	1.31e-6	5.05e-7	0.0003	0.1598
4kHz-paired	X	X	0.0191	0.0517	7.46e-9
9kHz-paired	X	X	X	0.0005	3.38e-8
Stim. Only	X	X	X	X	1.02e-6
DSP4-treated	X	X	X	X	X

Table 24; Response Area at 60dB (mm^2) All Groups

ANOVA; Response Area at 60dB (mm^2)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	9.67e-10	2.38e-10	1.97e-6	0.0008
4kHz-paired	X	X	0.0032	0.0119	1.11e-16
9kHz-paired	X	X	X	1.05e-5	6.95e-14
Stim. Only	X	X	X	X	8.99e-15
DSP4-treated	X	X	X	X	X

Table 25; Response Area at 70dB (mm^2) All Groups

ANOVA; Response Area at 70dB (mm^2)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	2.65e-10	0	2.08e-6	1.60e-5
4kHz-paired	X	X	4.23e-7	0.003	0
9kHz-paired	X	X	X	8.33e-12	0
Stim. Only	X	X	X	X	0
DSP4-treated	X	X	X	X	X

Table 26; Spontaneous Activity (50ms) All Groups

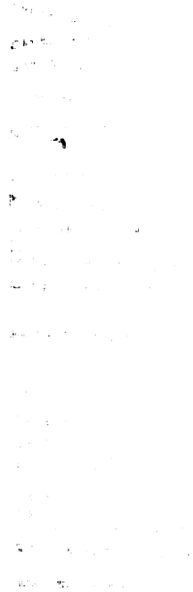
ANOVA; Spontaneous (50ms)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	0.5684	0	0	1.635e-9
4kHz-paired	X	X	0	0	1.756e-9
9kHz-paired	X	X	X	0	0
Stim. Only	X	X	X	X	6.392e-5
DSP4-treated	X	X	X	X	X

Table 27; Spontaneous Activity (10ms) All Groups

ANOVA; Spontaneous (10ms)

	Naive	4kHz-paired	9kHz-paired	Stim. Only	DSP4-treated
Naive	X	0.7495	0.0065	0.0009	0.0014
4kHz-paired	X	X	0.061	0.0069	0.0067
9kHz-paired	X	X	X	9.11e-5	0.0002
Stim. Only	X	X	X	X	0.6742
DSP4-treated	X	X	X	X	X



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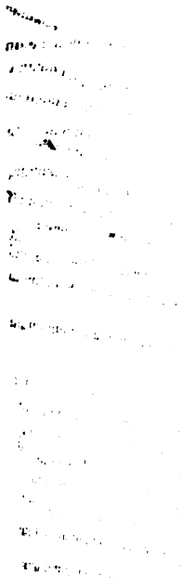

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