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Nucleus basalis activity enables spatial and temporal plasticity in rat auditory cortex

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Nucleus Basalis Activity Enables Spatial and Temporal Plasticity in Rat Auditory Cortex

by

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DISSERTATION

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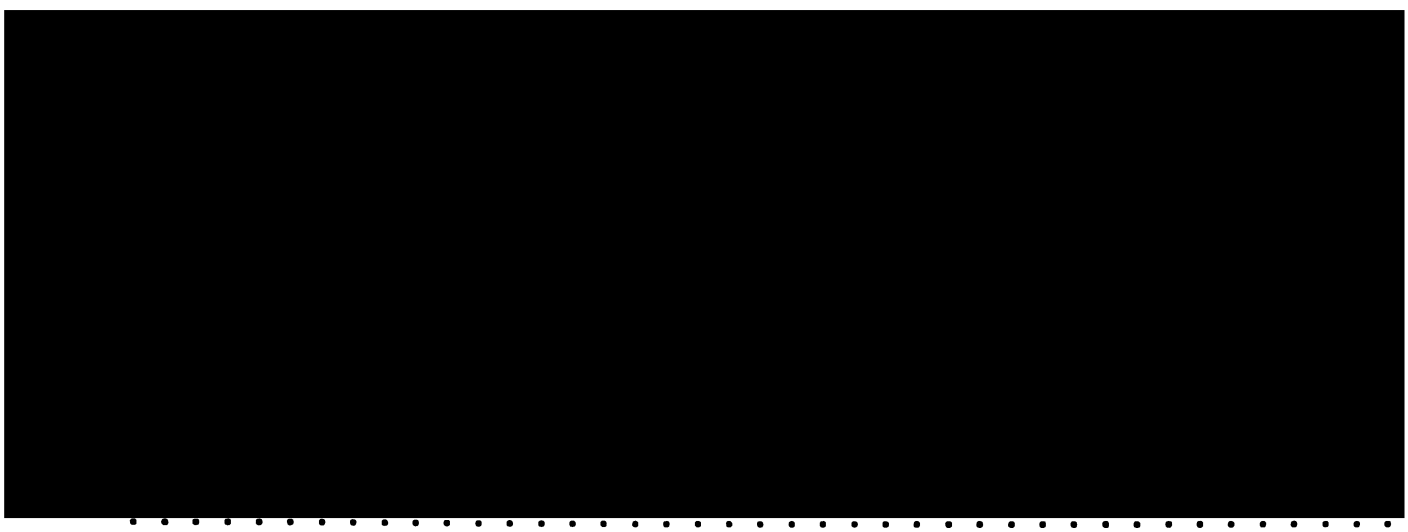

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For my parents, Chris and Brenda.

Thank you for the experience, strength, and hope

I know I need never pay back.

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"What fell was relaxed, owl-downy, soft…"

Nucleus Basalis Activity Enables Spatial and

Temporal Plasticity in Rat Auditory Cortex

by Michael P. Kilgard

ABSTRACT

The mammalian brain is a sophisticated self-organizing system. The cholinergic nucleus basalis (NB) may facilitate learning by allowing the cortex to differentiate behaviorally important stimuli from the tens of thousands of behaviorally irrelevant stimuli encountered each day. To explore the role of NB in long-term cortical plasticity, I developed a simple paradigm using electrical activation of NB and confirmed that activity of cholinergic NB neurons paired with sensory stimuli is sufficient to generate enduring reorganizations of cortical circuitry.

A chronic stimulating electrode was used to pair activation of NB with the presentation of an auditory stimulus several hundred times daily. Stimulation was delivered to adult rats that were awake and unrestrained. After four weeks of such pairing, a detailed map of the response properties of primary auditory cortex neurons was reconstructed from up to one hundred microelectrode penetrations. The reorganizations that resulted were among the largest recorded in primary sensory cortex and confirm the hypothesis that NB plays an active instructional role in representational plasticity.

Several classes of tonal stimuli were used to investigate the rules that relate sensory experience to changes in cortical response properties. The plasticity observed was specific to the stimulus paired with NB stimulation. For example, the region of the A1 map representing the paired tone frequency was selectively expanded, while stimuli presented as often but without NB activation did not result in cortical reorganization. Receptive field sizes (frequency selectivity) were narrowed, broadened or left unaltered depending on specific parameters of the acoustic stimulus paired with NB stimulation. This result parallels the differential receptive field remodeling that accompanied extended training of monkeys on tasks involving similar stimuli (Jenkins et al., 1990; Recanzone et al., 1993; Recanzone et al., 1992d). Temporal response properties were also altered by NB stimulation. The maximum following rates of cortical neurons were significantly increased after repeated pairing of NB activation with stimuli modulated at 15 Hz, and significantly decreased after pairing with 5 Hz stimuli.

These results substantiate the hypothesis that NB activity marks important stimuli and allows simple cortical rules to improve the representation of features likely to be useful based on the statistics of input.

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CHAPTER ONE:

Introduction

Primary sensory cortices for sight, sound and touch each represent their respective sensory epithelium in a topographic manner. For example, in somatosensory cortex neighboring locations in the cortex respond to inputs arising from neighboring locations on the body surface. Such local order results in a complete map of the body surface laid out on the cortical surface. Similar topography (retinotopic and tonotopic) exists in visual and auditory cortex. Despite their high degree of order, these maps have been shown to be dynamic constructs that are capable of substantial plasticity (Buonomano and Merzenich, 1998). Over the last twenty-five years experiments conducted in many laboratories have shown that primary sensory topography can be substantially reorganized, even in adults, by peripheral manipulations (such as digit amputation) (Kaas et al., 1983; Kaas, 1991; Merzenich et al., 1983a; Merzenich et al., 1984; Merzenich et al., 1990; Merzenich and Jenkins, 1993; Merzenich and Sameshima, 1993). For example, rapid reorganization of the somatotopic map in cortical field 3b and later progressive refinement were observed following median nerve transection and resulted in an expanded representation of hand inputs from the spared ulnar nerve (Merzenich et al., 1983b). Amputation of the "middle finger" of an adult owl monkey resulted in expansion of the cortical regions that respond to inputs from the neighboring "index" and "ring" fingers (Merzenich et al., 1984). Similar reorganizations have been observed in visual and auditory cortex following restricted retinal and cochlear lesions (Darian-Smith and Gilbert, 1995; Chino et al., 1995; Robertson and Irvine, 1989; Rajan et al., 1993).

Hebbian plasticity has been implicated in both cortical development and adult cortical reorganization. Hebbian mechanisms are correlation-based and maintain local

topography because neighboring receptor locations are more highly correlated than distant sites. To test this hypothesis, surgical connection of two digits (snydactyly) was used to alter the correlation statistics across the hand. Several months after digit fusion, the normally sharp border between the two digits had disappeared and many recorded neurons developed two-digit receptive fields (Clark et al., 1988; Allard et al., 1991).

Recent experiments have demonstrated that the processes that underlie these plasticity effects operate continuously to allow important experiences to remodel cortical representations. Jenkins, Recanzone and colleagues observed map reorganizations as a result of extended training on behavioral tasks that engage a limited sector of primary sensory cortex (Jenkins et al., 1990; Recanzone et al., 1992d; Recanzone et al., 1992b; Recanzone et al., 1993). The expanded cortical representations of the behaviorally important sensory input were well correlated with improved task performance. Cortical maps can also be substantially altered during natural activities such as nursing. Xerri and colleagues observed a two-fold increase in the region of the somatotopic map representing the nipple-bearing region of the rat ventrum in lactating rats compared to controls (Xerri et al., 1994).

Weinberger and colleagues have demonstrated that classical conditioning results in significant plasticity of frequency tuning in auditory cortex of adult rodents. Cortical responses to the tone frequency associated with footshock were reliable increased and shown to persist for several weeks. The conditioned neural response could still be measured while the animal was anesthetized and thus was not due to behavioral arousal.

The experiments described in this thesis were initially inspired by the elegant experiments of Gregg Recanzone and colleagues at UCSF, and Ehud Ahissar and colleagues at Hebrew University. Both groups demonstrated that cortical plasticity is not determined by sensory input alone. Recanzone and colleagues trained new-world monkeys on a simple limited-hold task. Two groups of monkeys received identical auditory and tactile stimulation. One group released when the tactile stimulus changed and ignored the auditory stimuli, while the other group attended to the auditory stimuli and ignored the tactile stimuli. Detailed maps of auditory and somatosensory cortex were constructed after several weeks of training. Cortical map reorganization was consistently documented in the cortical field corresponding to the attended modality and not in the map of the unattended modality (Recanzone et al., 1992d; Recanzone et al., 1992b). Ahissar and colleagues increased the correlation between auditory cortex neurons by generating stimuli that activated one neuron triggered on the firing of another. While little plasticity was observed when the auditory stimulus was ignored (not used in any behavioral task), the "functional connection" between the neural pairs was strengthened when monkeys attended to the stimulus as part of a simple behavioral task (Ahissar et al., 1992). These experiments strongly suggest that attention gates the correlation-based rules that modify connection strengths and network dynamics in the adult cortex.

Several features of the central cholinergic system support the hypothesis that acetylcholine is critically important for modulating plasticity. Nucleus basalis (NB) is located in the basal forebrain and is the source of extrinsic cholinergic input to the neocortex. Cholinergic antagonists and NB lesions have been shown to block plasticity

in different cortical fields in both young and adult animals of several species. (Bear and Singer, 1986; Gu and Singer, 1993; Juliano et al., 1991; Webster et al., 1991a; Sachdev et al., 1998; Baskerville et al., 1997; Zhu and Waite, 1998). These experiments provide strong evidence that acetylcholine is required for cortical map reorganizations. The cholinergic system is also important in a variety of learning and memory tasks in humans and rats (Hasselmo, 1995; Steckler et al., 1995; Riekkinen et al., 1992; Winkler et al., 1995; Butt and Hodge, 1995).

Single-unit recordings and microdialysis studies have shown that NB neurons are activated and acetylcholine is released in the cortex in response to novel and behaviorally arousing stimuli (Orsetti et al., 1996; Acquas et al., 1996; Pirch, 1993; Richardson and DeLong, 1991; Whalen et al., 1994). Pairing of electrical stimulation of NB or iontophoresis of acetylcholine with sensory stimuli is sufficient to generate cortical plasticity (Hars et al., 1993; Edeline et al., 1994b; Edeline et al., 1994a; Bakin and Weinberger, 1996; Metherate and Weinberger, 1990; Metherate and Weinberger, 1989; McKenna et al., 1989; Tremblay et al., 1990; Webster et al., 1991b; Howard and Simons, 1994). All of these properties support the hypothesis that acetylcholine released by NB neurons serves as a reinforcement signal to guide cortical plasticity (Singer, 1986; Weinberger, 1993).

My thesis work has extended the above experiments in several important ways. First, I quantified plasticity guided by NB activity at the level of the cortical map by recording from up to 100 locations in a single animal. Second, I recorded complete frequency-intensity tuning curves at every site and repetition rate transfer functions at most sites. Third, I demonstrated that the recorded plasticity was progressive over the

course of several weeks and endured for at least twenty-four hours after ending NB stimulation. Fourth, I immunolesioned NB to demonstrate that the cholinergic neurons of the NB were required for NB stimulation-induced plasticity. Fifth, I paired seven different classes of stimuli with NB activation to explore how the statistics of the sensory input determine the nature, degree, and direction of cortical reorganization.

CHAPTER TWO:

Distributed Representation of Spectral and Temporal Information in Rat Primary Auditory Cortex

ABSTRACT

Modulations of amplitude and frequency are common features of natural sounds, and are prominent in behaviorally important communication sounds. The mammalian auditory cortex is known to contain representations of these important stimulus parameters (e.g. Gaese and Ostwald, 1995). This study describes the distributed representations of tone frequency and modulation rate in the rat primary auditory cortex. Detailed maps of auditory cortex responses to single tones and tone trains were constructed from recordings from fifty to sixty microelectrode penetrations introduced into primary auditory cortex (A-1) in each of nine barbiturate-anesthetized rats. Recorded data demonstrated that the cortex uses a distributed coding strategy to represent both spectral and temporal information in the rat, as in other species (Langner, 1992). Just as spectral information is encoded in the firing patterns of neurons tuned to different frequencies, temporal information appears to be encoded using a set of filters covering a range of behaviorally important repetition rates. Although the average A-1 repetition rate transfer function (RRTF) was low-pass, with a sharp drop-off in evoked spikes per tone above 9 pulses per second (pps), individual RRTF's exhibited significant structure between 4 and 10 Hz, including substantial facilitation or depression to tones presented at specific rates. No organized topography of these temporal filters could be determined.

INTRODUCTION

Cortical neurons are often precisely tuned for relevant features of sensory stimuli. This strategy of analyzing sensory information with neural filters distributed across the cortical surface results in the representation of individual stimuli by patterns of activity in large populations of neurons. In many sensory systems, neurons with varying filter properties are organized into "maps" (Pubols and Pubols, 1971; Hubel and Wiesel, 1968; Merzenich and Brugge, 1973) . Receptor surfaces, for example, are commonly systematically mapped in the cortex (Schreiner, 1992).

The auditory sensory epithelium, the cochlea, has a one-dimensional surface, representing sound frequency. In every mammalian species investigated to date, the topography of the cochlea is maintained in its representation within the primary auditory cortex (Merzenich and Brugge, 1973; Merzenich et al., 1975; Merzenich et al., 1976; McMullen and Glaser, 1982; Aitkin et al., 1986; Kelly 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). Neurons tuned to particular sound frequencies are organized from low to high across the cortex. Because the cortex is a two-dimensional structure with a columnar organization, stimulus features can be mapped along the other ("isofrequency") representational dimension (Scheich, 1991; Schreiner, 1992; Ehret, 1997). In the cat, for example, tuning curves are narrower at the center of the isofrequency contour and broader near the ends. In this study we examined the topography of both spectral and temporal filters in the rat auditory cortex.

The rat auditory system has been used in a number of neuroanatomical studies that have indicated that the thalamocortical system is similar to that of other mammals (Roger and Arnault, 1989; Clerici and Coleman, 1990; Winer and Larue, 1987; Arnault and Roger, 1990; Shi and Cassell, 1997). Despite detailed descriptions of cytoarchitecture and connectivity in the rat, few detailed electrophysiological studies have been conducted in the rat auditory cortex. Sally and Kelly investigated the organization of the frequency map in rat A-1 and demonstrated that high to low frequencies are represented from anterior to posterior (Sally and Kelly, 1988). This organization parallels that defined in detailed mapping studies conducted in A-1 of the gray squirrel (Merzenich et al., 1976). Rat A-1 neurons were found to respond to tones with phasic short latency responses. Unlike cats and monkeys, rat A-1 neurons exhibit little non-monotonicity (Sally and Kelly, 1988).

Rat vocalizations exhibit modulations in the 2-20 Hz range (Kaltwasser, 1990). Gaese and Ostwald used sinusoidal amplitude modulated (SAM) stimuli to investigate temporal coding in rat auditory cortex (Gaese and Ostwald, 1995). The majority of responses had band-pass transfer functions for modulation rate, preferring rates between 8 and 12 Hz. The sinusoidal nature of the stimuli used makes it difficult to determine whether the cortex is band-pass for repetition rates of other simple stimuli because amplitude slope and repetition rate cannot be varied independently with SAM stimuli (Eggermont, 1991). The auditory cortex responds briskly to transients (Heil, 1997b; Heil, 1997a). It is possible that the band-pass modulation transfer functions (MTF's) observed resulted because the rise time at low modulation rates was too slow to be detected by auditory cortex neurons.

In this study, the neural responses to single and repeated tones were investigated in detail to define the fundamental aspects of the distributed representations of basic spectral and temporal information in rat auditory cortex.

METHODS

This study is based on neural responses collected from 440 microelectrode penetrations into the right primary auditory cortex in nine adult female Sprague-Dawley rats. Surgical anesthesia was induced with sodium pentobarbital (50 mg/kg). Throughout the surgical procedures and during the recording session, a state of areflexia was maintained with supplemental doses of dilute IP pentobarbital (8mg/ml). The trachea was cannulated to ensure adequate ventilation and to minimize breathing-related noises. The skull was supported in a head holder. The cisternae magnum was drained of CSF to minimize cerebral edema. After reflecting the temporalis muscle, auditory cortex was exposed via a wide craniotomy and the dura mater was resected. The cortex was maintained under a thin layer of viscous silicon oil to prevent desiccation. The location of each penetration was reproduced on a 40X digitized image of the cortical surface, and sited with reference to the surface microvasculature.

The primary auditory cortex was defined on the basis of its short latency (8-20 msec) responses and its continuous topography of "best frequency" (BF, frequency to which neurons respond at lowest intensity). Responsive sites that exhibited clearly discontinuous best frequencies AND either long latency responses, unusually high thresholds, or very broad tuning were considered to be non-A1 sites. Penetration sites were chosen to avoid damaging blood vessels while generating a detailed and evenly spaced map. The boundaries of the map were functionally determined using non-responsive and non-A1 sites.

Recordings were made in a shielded, double-walled sound chamber (IAC). Action potentials were recorded simultaneously from two Parylene-coated tungsten microelectrodes (FHC, 2MΩ at 1kHz) that were lowered orthogonally into the cortex to a depth of ~550 μm (layers IV/V). The neural signal was filtered (0.3 to 8 kHz) and amplified (1000X). Action potential waveforms were recorded whenever a set threshold was exceeded, allowing off-line spike sorting using Autocut software. Although most responses in this study represented the spike activity of several neurons, single units were separated when possible, confirming that single units exhibited tuning that was qualitatively similar to multi-unit response samples.

Monaural stimuli were delivered to the left ear via a calibrated ear phone (STAX 54) positioned just inside the pinnae. Frequencies and intensities were calibrated using a B&K sound level meter and a Ubiquitous spectrum analyzer. Two types of stimuli were generated using Brainwave (Datawave). Auditory frequency response tuning curves were determined by presenting 45 frequencies spanning 3-4.5 octaves centered on the approximate best frequency of the site. Each frequency was presented at 15 intensities ranging between 0 and 75dB (675 total stimuli). Tuning curve tones were randomly interleaved and separated by 500msec. In four animals, RRTF's were derived at all recording sites by randomly interleaving 12 repetitions of 16 different tone repetition rates (3-25 Hz). A two second silent period separated the tone trains. The frequency of the RRTF tones was set to the frequency that resulted in consistent vigorous responses at both of the recording sites. In a few cases, tuning curves did not overlap and two different tone frequencies were used. RRTF stimuli were presented at 70dB SPL. All tonal stimuli used in this study were 25msec long, including 3 msec rise and fall times.

Tuning curve parameters were defined by an experienced blind observer using custom software that displayed raw spike data without reference to the frequencies and intensities which generated the responses. For each tuning curve, best frequency, threshold, bandwidth (10, 20, 30 and 40 dB above threshold), and latency data were recorded. The minimum latency was defined as the time from stimulus onset to the earliest consistent response for all 15 intensities, for the three frequencies that were nearest the BF (45 stimuli). The peak latency was defined as the time of the peak in the histogram created from all 675 stimuli. The signal to noise ratio is the number of spikes evoked by a 70dB tone near the best frequency within a 35 msec window divided by the number of spikes expected due to spontaneous activity.

RRTF data was quantified by determining the number of spikes that arrived within a fixed window (4-39 msec) after tone onset. In this study the RRTF is the average number of spikes for each of the last five tones of the six tone train plotted as a function of repetition rate. To allow for comparisons across sites, normalized spike rates were generated by dividing the number of spikes per tone by the number of spikes in response to a single tone presented in isolation (first tone). Normalized spike rates above one indicate facilitation, while rates less than one indicate adaptation of the neural response relative to the response to an isolated tone. The maximum rate that results in an average mulit-unit response (4-39 msec after tone onset) of one spike per tone plus spontaneous is defined as the maximum following rate.

Voronoi tessellation was used to generate polygons from a set of non-uniformly spaced points such that every point in the polygon was nearer to the sampled point than to any other. These polygons served two purposes. 1) They provided an easy method of

visualizing the cortical topography. 2) They allowed area information to be estimated from discretely sampled penetrations, by assigning each point on the cortical surface the qualities of the closest sampled point. For example, this simple measure generates reliable estimates of the percent of the cortex that responds to a given frequency-intensity combination. The percent of the cortical surface responding to a given stimulus was estimated by adding all of the areas of the penetrations that responded, divided by the total area of A-1. This measure allows higher sampling of cortical regions of particular interest without introducing bias into group data because densely sampled regions result in smaller polygons that contribute less to this measure. By contrast, the percentage of sites exhibiting a particular response characteristic can be easily biased by non-uniform sampling densities.

RESULTS

Size and location of A-1. Auditory cortex in the rat can be reliably located using the lateral suture and underlying blood vessels as landmarks (Sally and Kelly, 1988). Primary auditory cortex (A-1) is located ~1 mm dorsal to the horizontal portion of the suture and ~1.5 mm posterior to the vertical portion of the suture. The widest anterior-posterior extent was 1.9 ± 0.2 mm (mean ± standard error). The widest dorsal-ventral extent was 1.5 ± 0.2 mm. The average A-1 area was 1.92 ± 0.16 mm^2.

Tuning curves. Rat A-1 frequency-intensity tuning curves derived for almost all neuronal samples were V-shaped, like most tuning curves recorded in rodents (Sally and Kelly, 1988). Figure 2-1a illustrates a representative tuning curve and the parameters derived from it, including threshold and bandwidth. A range of tuning curve shapes were observed, although the basic V-shape predominated (figure 2-1b). The "best frequency" is the frequency that evokes a consistent neural response at the lowest tone intensity. We recorded from units at 440 sites in nine animals with best frequencies ranging from 0.8 to 60 kHz. Both behavioral thresholds and neural thresholds were higher near the extremes of the hearing range (Kelly and Masterton, 1977). The distribution of best frequencies was observed to be fairly regular across the entire rat hearing range (figure 2-1c). Spectral information is represented by neurons with a significant range of spectral responsivity. The average bandwidths were 0.92 ± 0.41, 1.42 ± 0.54, 1.73 ± 0.62, and 2.08 ± 0.68 octaves (mean ± standard deviation) at 10, 20, 30 and 40 dB above threshold, respectively. Tuning curves with high BF's tended to be more sharply tuned, compared

to lower frequency tuning curves (Figure 2-1d). The average bandwidth 20 dB above threshold for sites above 20 kHz was 1.09 octaves, compared to 1.53 for sites below 20 kHz (t-test, $p<.00001$).

The best frequencies of A-1 neurons increased from posterior to anterior, as illustrated in maps from two representative rats (Figure 2-2 a&b). Each polygon in a map represents one penetration; the color indicates the best frequency of neurons sampled at that site. Continuous topography of frequency tuning was observed in every animal. Figure 2-2c illustrates the relationship between the distance from the posterior border of A-1and the best frequency of each penetration from six animals. Figure 2-3a shows all of the tuning curve tips from the map shown in Figure 2-2b. The reproducibility of these maps and the relatively even distribution of frequency tuning make the rat a useful species for studying the effects that behavioral training and other plasticity paradigms have on cortical representations of sound frequency.

Although A-1 topography is well ordered for tones presented near threshold, highly overlapping populations of neurons are activated by loud tones. For example, more than one-fourth of the cortical surface is activated by a 40 dB 8 kHz tone. The average percent of the cortex responding to any frequency-intensity combination was derived by overlaying each of the tuning curve outlines weighted by the area of the polygons (Figure 2-3b). This measure is useful in plasticity studies because it can be used to quantify changes in the percent of the cortex representing stimulus features (see chapters three and five).

Topography: . The only recorded response property that varied systematically across the isofrequency contour was the response strength, quantified as the signal to noise ratio. The median (10-90 percentile) signal to noise ratio within 0.25 mm of the A-1 midline was 18 (8- 59), compared to 12 (5-42) for sites greater than 0.25 mm from the midline. We observed no consistent mapping of response latency, stimulus threshold, or frequency tuning bandwidth.

Repetition rate transfer functions: RRTF's were derived at 142 sites from responses to trains of six short tone pips presented at repetition rates ranging from 3 to 25 Hz. The carrier frequency of the RRTF tones was selected to be near the best frequency of each site. On average, 1.9 +/- 0.9 (mean +/- SD) spikes were evoked by a tone in isolation, with a latency of 15+/- 5 msec. Spontaneous rates ranged from about 1 to 10 spikes/second.

Many sites responded with approximately the same number of spikes per tone at repetition rates less than 8 Hz, with sharply decreasing spikes per tone from 10 to 14 Hz, and few spikes to tones presented at more than 15 Hz (figure 2-4a). The RRTF was quantified by measuring the number of spikes occurring in a fixed window (35 msec) after each stimulus. The plot next to the dot rasters shows the average number of spikes for each of the five tone pips after the first (60 stimulus presentations/symbol). The solid line shows the average number of spikes for the first stimulus in the train (168 presentations), and the dotted line shows the number of spikes expected in a 35msec sampling window due to spontaneous activity. The average RRTF's from different sites were normalized by dividing the number of spikes for each tone by the number of spikes

in response to the first tone. The mean response of the 142 A-1 RRTF's derived in this study clearly shows that the average normalized response of the cortex to repeated stimuli was a low pass function that fell off rapidly above 10 Hz (figure 2-5a).

Although simple low-pass RRTF's were common, it is important to recognize that a substantial diversity of temporal response properties was observed across sites. In general cortical RRTF's had a significant amount of structure from 2-9 Hz, but reliably fell off above about 10Hz. The standard deviation of the normalized spike rate for repetition rates near 8 Hz were as large as the mean, indicating that a range of temporal filter functions are used to represent time-varying inputs within A-1. Responses to 8.4 Hz trains, for example, ranged from substantial adaptation to strong facilitation (more than twice as many spikes for each tone in the train compared to single tones in isolation) (figure 2-5c). Some sites were simply slow and followed poorly at repetition rates faster than 8 Hz (figure 2-4b), while other sites could respond to each event at 15 Hz and followed every other stimulus at 20 Hz (figure 2-4c). In addition to these examples that represent simple shifts in the maximum following rate of cortical neurons, many sites had temporal response properties that included strong adaptation or facilitation at specific repetition rates (figure 2-6). In a few cases, the number of spikes per tone was changed two-fold by increasing the interval between tone pips by as little as 10 milliseconds.

Both notched and band-pass RRTF's were observed in rat A-1 (Figure 2-7a&b). In the example of a notched RRTF, only half as many spikes were evoked per tone at 7 Hz compared to 9 Hz. Such temporal filters transform information about repetition rate into modulation of the number of evoked spikes per tone. Thus temporal information is expressed in both the timing of neural discharges and the number of action potentials

evoked by each stimulus event. In approximately 60% of A-1 sites, the response to some range of repetition rates slower than the best rate resulted in less than 70% as many spikes per tone as for the best rate (Figure 2-8a). Thus, although the mean RRTF has no peaks, most individual sites prefer particular repetition rates.

The distribution of best rates (rate that drives the maximum number of spikes per tone pip) was fairly even; there were approximately equal numbers of sites preferring repetition rates ranging from about 12 Hz to less than 3 Hz (figure 2-8b). It is interesting to note that best repetition rates also appeared to be roughly evenly distributed when plotted as a function of best frequency, suggesting that any band of cortex representing a significant range of frequencies contains neurons that "represent" all rates less than 12 Hz (figure 2-8c). Figure 9 illustrates the "topography" of best repetition rate in relation to best tone frequency. Although similar repetition rates were sometimes clustered together in individual animals, there were no consistent patterns of such clustering across animals.

Maximum following rate was correlated with both minimum latency and number of spikes in response to an isolated tone (Figure 2-10). The average minimum latency for sites that were able to follow tones presented at rates above 11 pps was 15.3 ± 0.2 msec, compared to 17.3 ± 0.3 msec for the sites with maximum rates less than 11 pps ($p < 0.00001$). The mean driven response to an isolated tone was 2.3 ± 0.1 spikes for the fast sites compared to 1.6 ± 0.1 for the slower sites ($p < 0.00001$). Thus, as reported in cat A1, vigorous, short latency responses tended to be able to follow repeated stimuli at faster rates (Schreiner et al., 1997; Raggio and Schreiner, 1994; Brosch and Schreiner, 1997).

In notched RRTF's, the notch was commonly located halfway between two peaks, suggesting that notches may be formed when responses consistently arrive out of phase with a neural oscillator. For example if the site shown in figure 2-7a has a 10 Hz neural oscillator, a 5 Hz stimulus would also be expected to be facilitated, while a 7 Hz stimulus would not. Consistent with this role of oscillators in temporal filtering, some sites exhibited multiple cycles of repetitive spiking after stimulus trains of 10 to 15 Hz (figure 2-11a). Although oscillators would be an interesting strategy to implement temporal filtering, non-oscillatory processes are also likely to be important. For example in a few sites, a burst of spikes at a fixed latency relative to the first tone was evoked when the first tone is followed closely by a second tone (figure 2-11b).

Non-A1 responses. Several auditory fields surrounding A-1 have been shown to receive projections from regions of the medial geniculate other than the ventral division (Arnault and Roger, 1990; Romanski and LeDoux, 1993; Winer and Larue, 1987). The posterior field was the easiest to identify because neurons in that region responded fairly well to tones. Additionally, this field appeared to have a tonotopic organization that was a mirror image of A-1. Neurons in this field had longer latencies and adapted to repeated stimuli even at low stimulus repetition rates (figure 2-11c). Additionally responses were more sustained compared to A-1, where neurons usually responded with only a short burst of spikes at tonal onsets. As observed in several other rodent species, tuning curves in the other non-primary fields generally responded poorly to tones and had high thresholds, long latencies, and/or broad tuning (Redies et al., 1989; Stiebler et al., 1997; Thomas et al., 1993; Sally and Kelly, 1988). No clear representational topography of

DISCUSSION

The aim of this study was to elaborate how the primary auditory cortex in the rat represents spectral and temporal features of auditory stimuli. Detailed maps were constructed with data collected from nine adult animals. Tuning curves were derived at every penetration to investigate the organization of tuned responses for tone frequency. Trains of short tone pips presented at various rates were used to investigate the nature of the distributed representations of repetition rate.

Rat A-1 neurons have V-shaped tuning curves and respond to tones with short latency, phasicresponses. Minimum thresholds were consistent with published behavioral thresholds (Kelly and Masterton, 1977). Bandwidths at 20dB above threshold ranged from .4 to 2.5 octaves. Rat A-1 exhibited an orderly map of increasing tone frequency from posterior to anterior (Sally and Kelly, 1988).

The majority of rat A-1 neurons responded well to trains of tone pips presented at rates below 10 Hz. However, substantial variability exists in temporal response properties. A few sites did not follow well at rates above 5 Hz, while others responded well to each tone presented at 15 Hz. Some sites exhibit response profiles that were indicative of oscillatory processes. The variability in RRTF's may represent an important coding strategy for temporal information. These results suggest that any frequency band of substantial width contains neurons that selectively prefer stimulus rates ranging from about 2 to 15 Hz. Although nearby cortical penetrations often have similar RRTF's, the resolution of even this fine-grained data sample make it impossible to definitively demonstrate whether or not repetition rate is mapped in auditory cortex.

If individual neurons expressed the same degree of variability locally as was observed from penetration to penetration across A-1, individual multi-unit RRTF's should exhibit the low-pass property observed in the population RRTF. The high degree of structure in the RRTF's recorded in this study provide evidence that local groups of neurons have similar temporal response properties. It remains unclear whether this is due to local similarity of intrinsic cellular properties, local network interactions, or both.

The low-pass nature of the RRTF's described in this study are different from the predominately band-pass MTF's observed by Gaese and others (Gaese and Ostwald, 1995). Two significant differences between the studies apparently account for much of the differences. The most important is that sinusoidally amplitude modulated (SAM) tones were used in the previous study, while tones with a constant amplitude ramp were used in the present study. Primary auditory cortex responds well to intensity transients, while it responds poorly to stimuli with slowly increasing amplitude. Thus A-1 neurons may be tuned for 10 Hz SAM stimuli not because they cannot follow slower rates but because they prefer steeper amplitude ramps. When a constant amplitude ramp is used, the mean RRTF for A-1 neurons is clearly low-pass.

Additionally, Gaese and colleagues quantified MTF's using either a synchronization measure or a spike rate that was not normalized to the number of stimulus cycles (Gaese and Ostwald, 1995). The shallow amplitude ramps of slow SAM's provide the cortex with an impoverished time mark for stimulus onset and result in a wider distribution of spike latencies that leads to lowered synchronization scores (Eggermont, 1991). The spike rate measure used in the previous study was normalized to stimulus power and not to number of stimulus cycles. Thus, in addition to having a

steeper amplitude ramp, the 10 Hz SAM stimulus had twice as many onsets as the 5 Hz stimulus. Our stimuli allowed spikes per tone onset to be measured as a function of repetition rate, while maintaining constant stimulus power. Both studies clearly demonstrate that most rat A-1 neurons do not respond well to stimuli above about 15 Hz.

Phillips et al. recorded the responses of cat A-1 neurons to trains of short tone pips, and observed exclusively low-pass responses. Interestingly they did not observe any facilitation of spike rate to tones during a train compared to isolated tones. In one-fourth of the sites in our study, stimulus repetition resulted in more than a 130% increase in spikes per tone compared to a single tone in isolation. Phillips adjusted the frequency at each site precisely to that site's best frequency, while in the current study, frequencies were selected that evoked the strongest responses in the two simultaneously recorded penetrations. Phillips suggested that facilitation may only occur when off-BF frequencies are used. Systematic studies are needed to determine whether tones delivered to different regions of the tuning curve can have different RRTF's.

An important parameter that was not varied in our study was stimulus duration. It will be interesting to determine the contribution of stimulus off-time in cortical RRTF's by increasing stimulus duration.

The reduction in the ability of cortical neurons to follow repetitive stimuli compared to subcortical auditory nuclei can be explained by strong inhibition following excitatory input. Inhibitory influences would create a refractory period and generate a low-pass temporal response profile. The richness of RRTF's observed in this study likely results from interactions between a number of factors shaping cortical processing of temporal information (Buonomano et al., 1997; Buonomano and Merzenich, 1995).

Paired pulse facilitation and depression clearly play a role. Rebound from inhibition could provide facilitation over a specific range of repetition rates. Intrinsic properties have also been implicated in shaping the timing of cortical responses (Langner, 1992).

Oscillatory responses have been observed in auditory cortex in a number of studies in rats, cats, and monkeys (Schreiner and Urbas, 1986; Eggermont and Smith, 1995; Sally and Kelly, 1988; Dinse et al., 1997). These oscillations appear to influence cortical responses for up to several hundred milliseconds after an initial excitatory input. Oscillations are commonly observed in the auto-correlations of spontaneous activity, and can be reset by single tone pips. Two studies have shown that best modulation frequencies are correlated with intrinsic oscillation rates (Kenmochi and Eggermont, 1997; Dinse et al., 1997). A prevalence of notched RRTF's in our data may be further evidence that oscillatory processes shape temporal information processing. This relationship warrants further study, including correlating intrinsic and spontaneous oscillatory response properties with RRTF notch location and width.

Rats have been used in a wide range of learning paradigms using auditory stimuli. To infer neural mechanisms involved in this learning, it is important to better understand the representation of auditory stimuli in normal animals. This study demonstrates that rat auditory cortex has a distributed representation of both spectral and temporal information. Each isofrequency contour contains neurons that can act as temporal filters that respond well to rates ranging from two to fifteen Hz. This data is consistent with the progressive reduction in the maximum following rate of temporal modulations from auditory nerve to higher stations of the auditory pathway (Langner, 1992). Bilateral lesions of rat auditory cortex have been shown to abolish differentiation between

unmodulated and 5 Hz AM modulated tonal stimuli in a simple classical conditioning paradigm, while leaving differentiation of 50 and 500 Hz modulations intact (Grigor'eva and Vasil'ev, 1981). These results indicate that the relatively long integration time of cortical neurons is useful for extracting temporal stimulus features below 20 Hz.

In the present study, we have shown that rat primary auditory cortex is similar to other mammalian species. Spatial and temporal information is encoded in the firing patterns of neurons exhibiting a wide range of response tuning profiles. These results suggest that the representation of stimulus parameters of complex auditory signals are likely to involve spike rate, place and temporal coding strategies.

Figure 2-1. (**A**) Tuning curve for representative rat A-1 penetration. BF is the frequency that elicits a consistent neural response at the lowest intensity, threshold. BW is the range of frequencies the neurons are responsive to at the specified intensity above threshold. (**B**) Eleven tuning curve outlines from A-1 in a single animal. (**C**) Thresholds to elicit excitatory responses at BF, for neurons sampled from nine animals. (**D**) Bandwidths at 20dB above threshold as a function of BF.

Figure 2-2. (**A & B**) Representative BF maps of primary rat auditory cortex from two adult rats. Each polygon represents one penetration. Color represents each site's best frequency. Non-responsive and auditory responding non-A-1 sites are marked with O's and X's, respectively. (**C**) Best frequency as a function of distance from the posterior border of A-1. Each color represents a different animal.

Figure 2-3. (**A**) Tuning curve tips for all of the penetrations from one rat (figure 2-2B). The tip of each V depicts minimum threshold for each site. Width of the V represents tuning curve width 10dB above threshold. (**B**) Mean percent of the cortical surface that responds to a tone of any frequency/intensity combination.

Figure 2-4. (**A**) Dot raster and repetition rate transfer function for a site representative of the median response. Short horizontal lines mark time windows used for RRTF quantification. Vertical solid lines in panels to the right mark the average response to first tone. Vertical dotted line marks spontaneous rate. (**B**) RRTF with 7 Hz cutoff. C) RRTF with unusually fast (high repetition rate) following.

Figure 2-5. (**A**) Mean normalized spike rate as a function of repetition rate with standard errors of the mean. (**B**) Standard deviation of normalized spike rate as a function of repetition rate. (**C**) Distribution of normalized spike rates at 8.4 Hz. Examples shown in Figures 2-4 & 2-6 have ratios of .97, .22, 1.07, 1.83, and 1.9 at 8.4 Hz, respectively.

Figure 2-6. RRTF's for six penetrations with strong tuning for specific repetition rates. Note that the sum of these RRTF's has a low-pass shape.

Figure 2-7. (**A**) Example of a notched RRTF. (**B**) Example of a band-pass RRTF.

Figure 2-8. (**A**) Distribution of RRTF modulation depth, expressed as the minimum response for repetition rates less than the best rate divided by the response to the best rate. The examples in figures 2-4 & 2-7 have depths of 8, --, 26, 47 and 63%, respectively. Note that only sites with best repetition rates greater than 4 Hz are shown. (**B**) Distribution of best repetition rates (rate that evokes the most spikes per tone). (**C**) Best repetition rate as a function of BF.

Figure 2-9. Representative cortical map labeled with best tone frequency and best repetition rate. Reliable RRTF's could not be generated from sites labeled zero.

Figure 2-10. (**A** & **B**) Scatter plots of maximum following rate as a function of minimum latency and spikes evoked per tone, with best linear fits.

Figure 2-11. (**A**) An RRTF that exhibits oscillatory responses after trains of greater than 10 Hz. (**B**) Unusual A-1 RRTF. Note the fixed-latency response following the second tone presented at greater than 14 Hz. (**C**) Representative RRTF for the posterior auditory field. Note sustained discharge and strong adaptation to repeated stimuli.

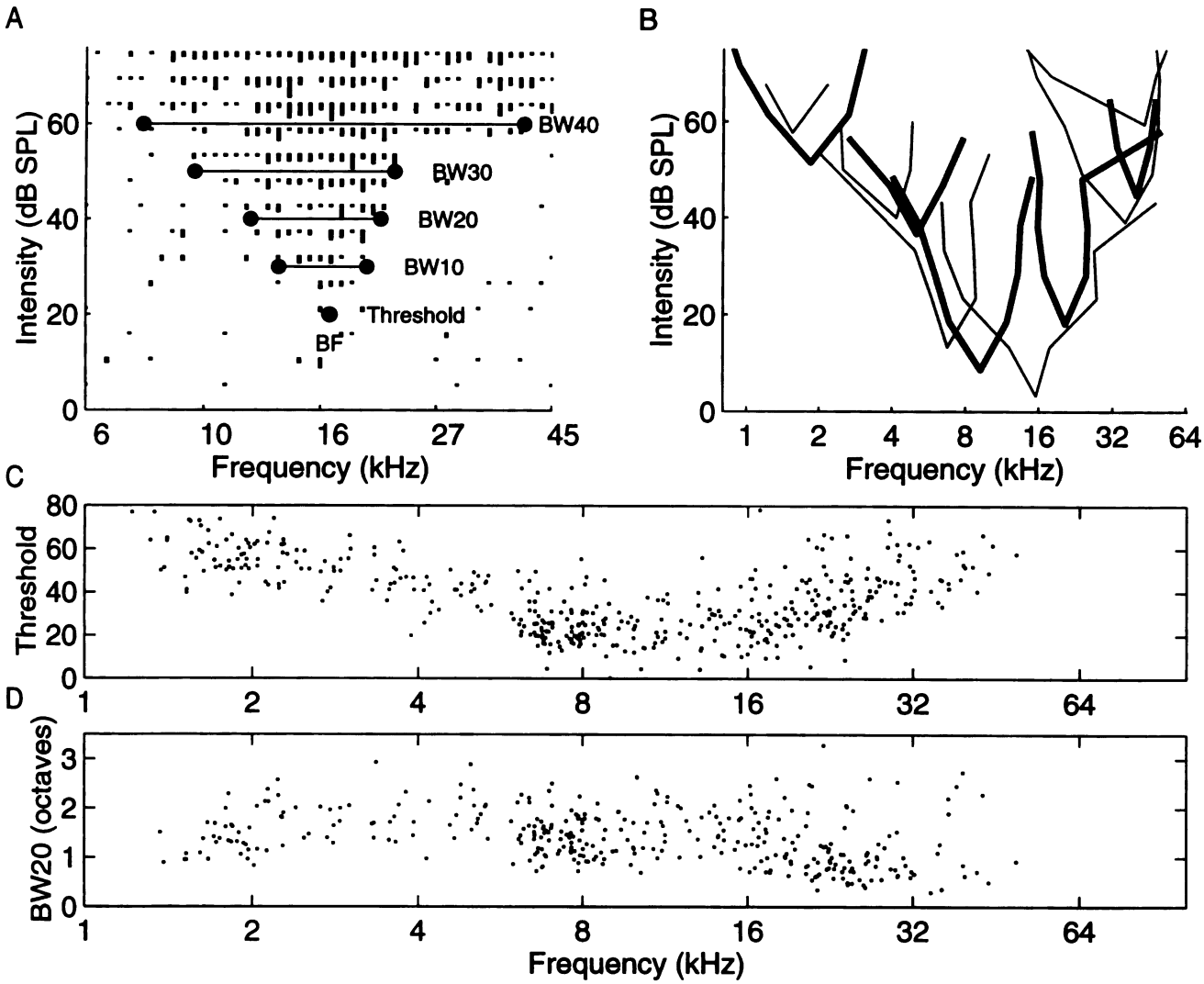

A
B
C
D
Intensity (dB SPL)
Frequency (kHz)
BW40
BW30
BW20
BW10
Threshold
BF
Threshold
BW20 (octaves)
0
20
40
60
80
1
2
4
8
16
32
64
6
10
16
27
45
0
1
2
3

FIGURE 2-1

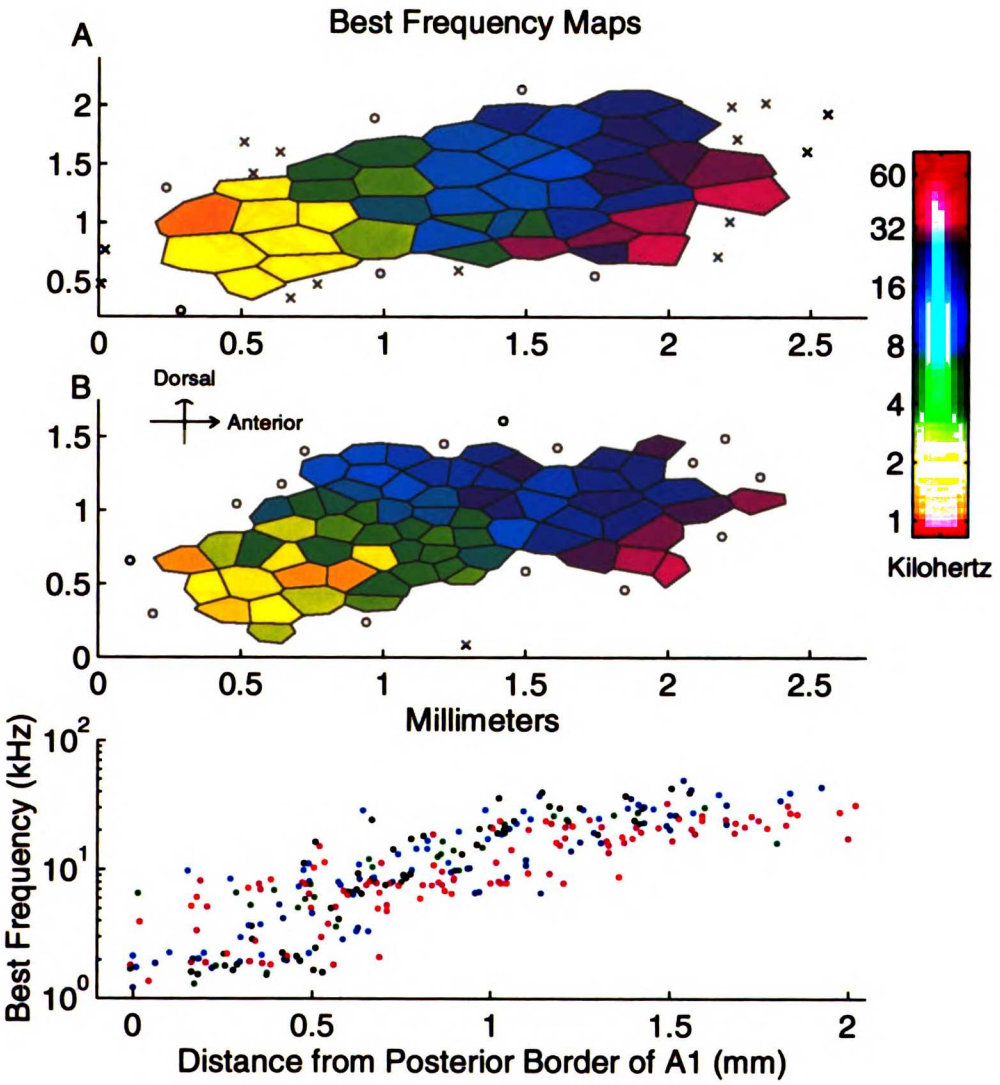

Best Frequency Maps
A
B
Dorsal
Anterior
Millimeters
Kilohertz
60
32
16
8
4
2
1
Best Frequency (kHz)
Distance from Posterior Border of A1 (mm)

FIGURE 2-2

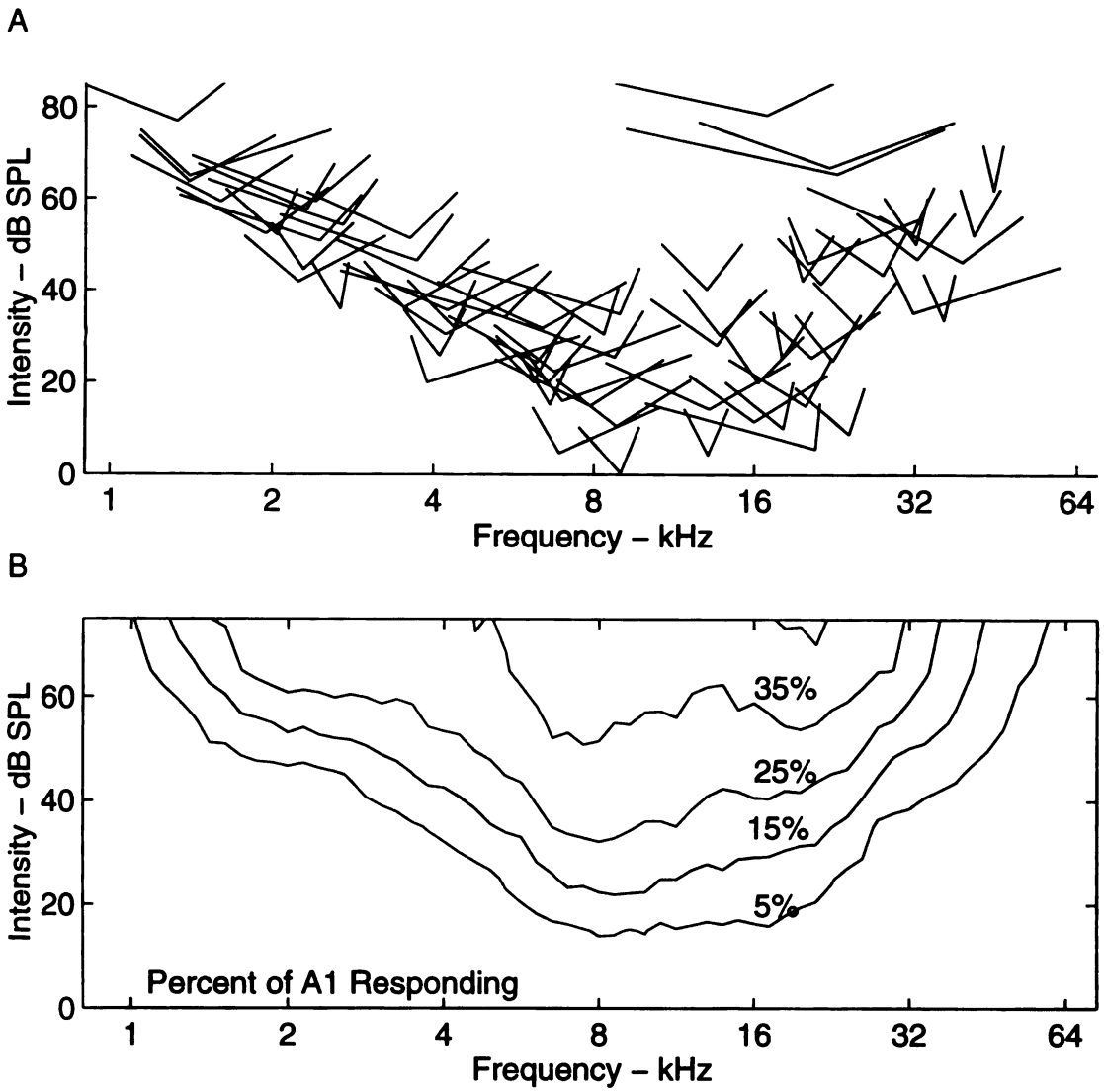

A
Intensity – dB SPL
80
60
40
20
0
1
2
4
8
16
32
64
Frequency – kHz
B
Intensity – dB SPL
60
40
20
0
35%
25%
15%
5%
Percent of A1 Responding
1
2
4
8
16
32
64
Frequency – kHz

FIGURE 2-3

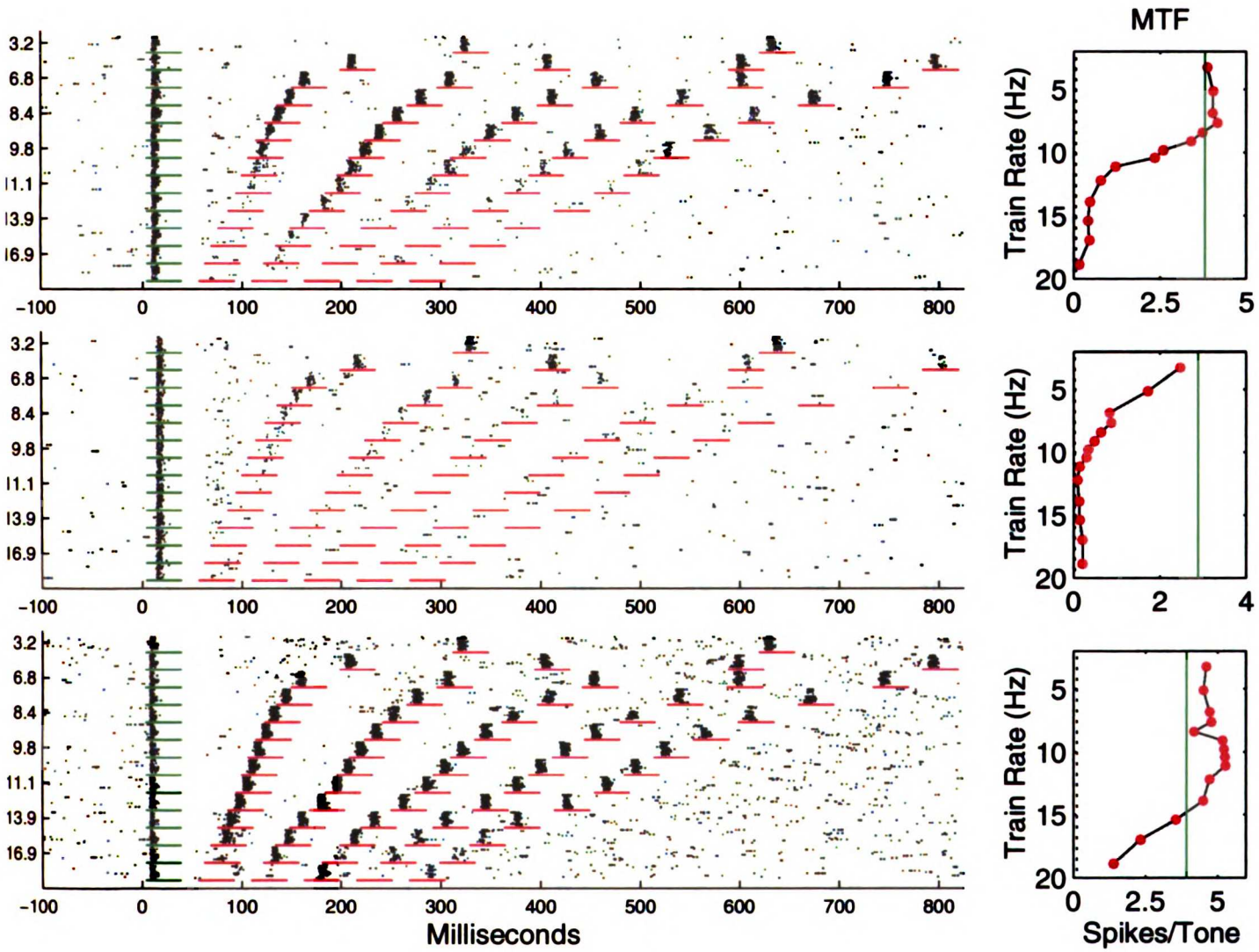

MTF
3.2
6.8
8.4
9.8
11.1
13.9
16.9
-100
0
100
200
300
400
500
600
700
800
Train Rate (Hz)
5
10
15
20
0
2.5
5
2
4
Milliseconds
Spikes/Tone

FIGURE 2-4

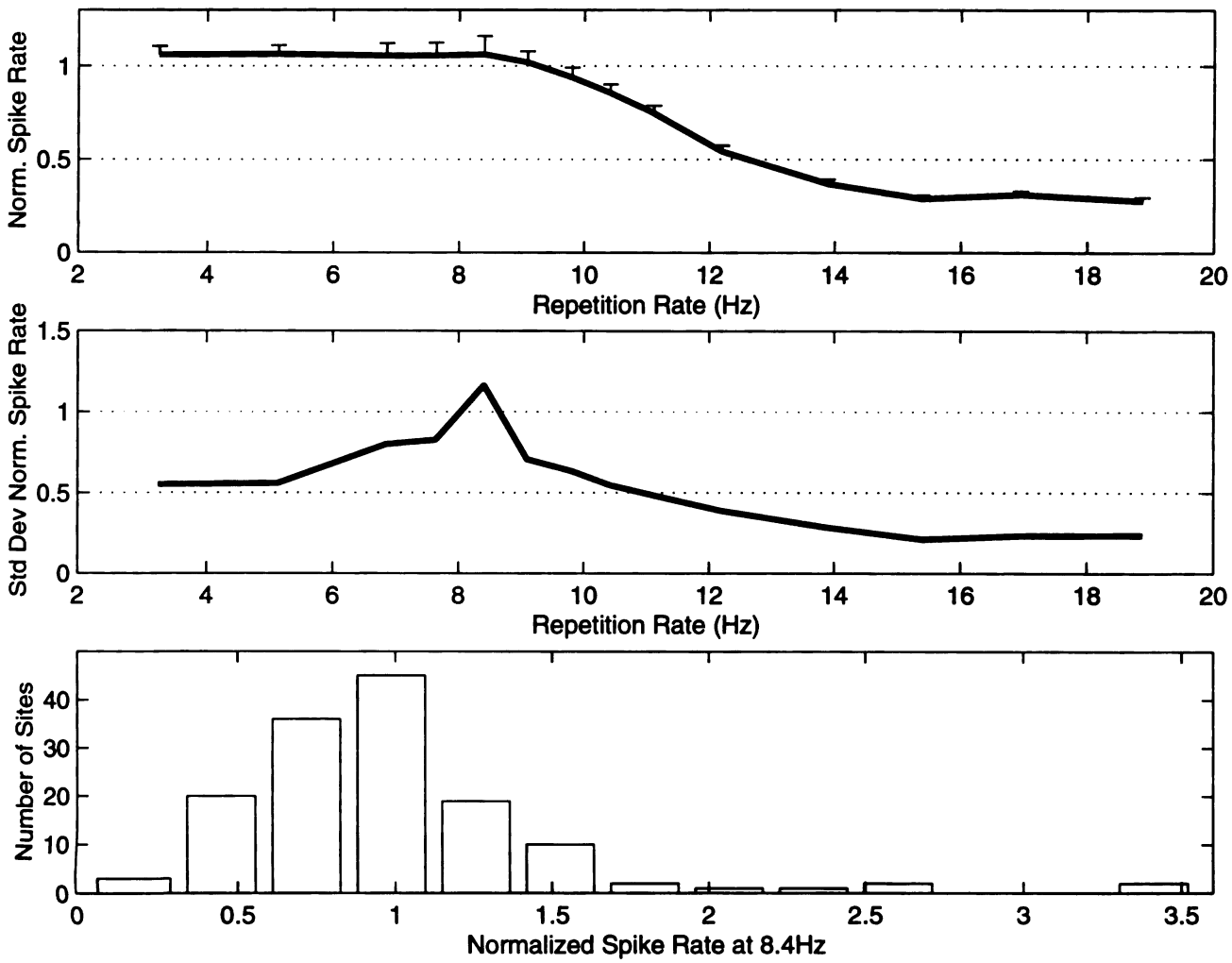

Norm. Spike Rate
1
0.5
0
2
4
6
8
10
12
14
16
18
20
Repetition Rate (Hz)
Std Dev Norm. Spike Rate
1.5
1
0.5
0
2
4
6
8
10
12
14
16
18
20
Repetition Rate (Hz)
Number of Sites
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
Normalized Spike Rate at 8.4Hz

FIGURE 2-5

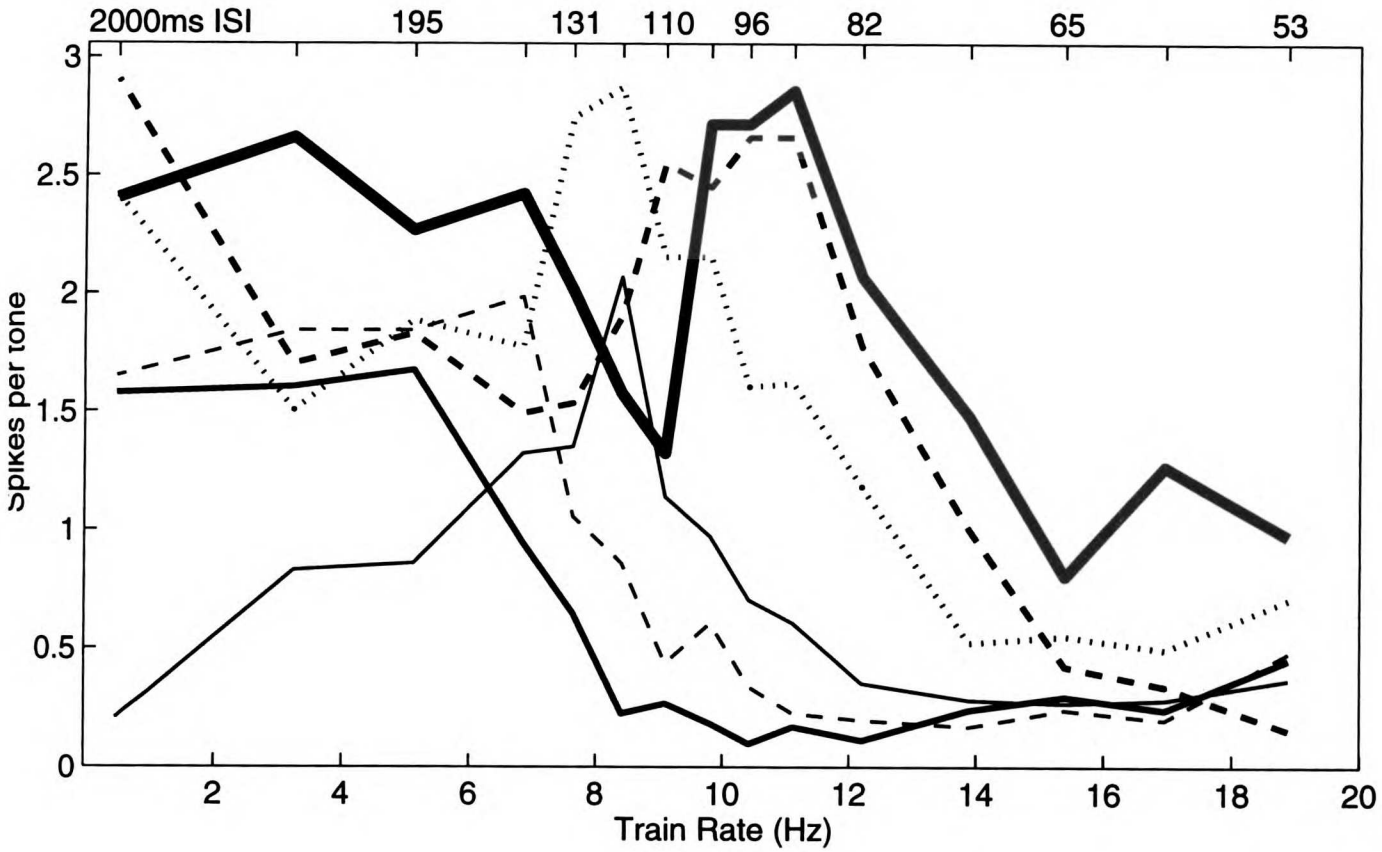


FIGURE 2-6

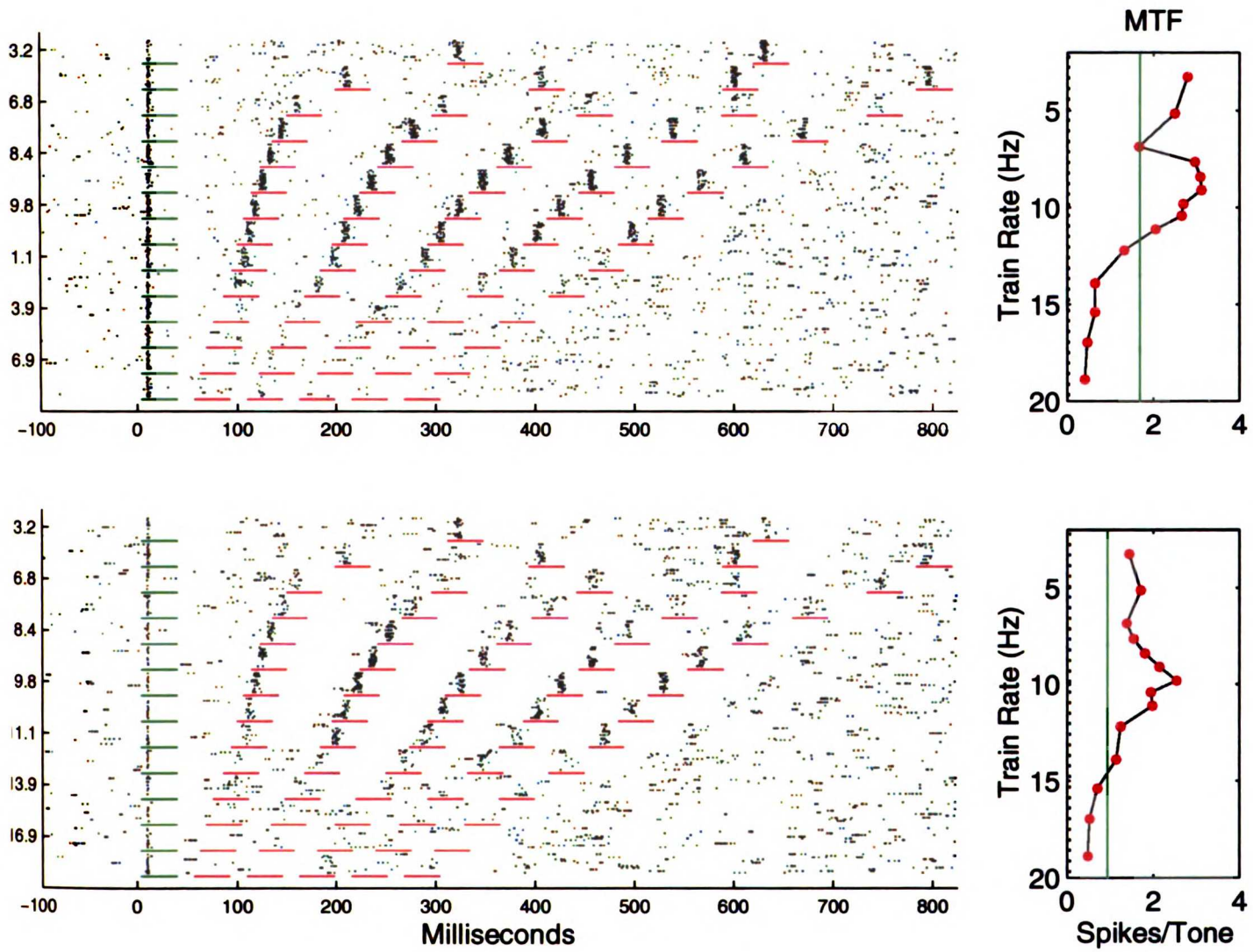


FIGURE 2-7

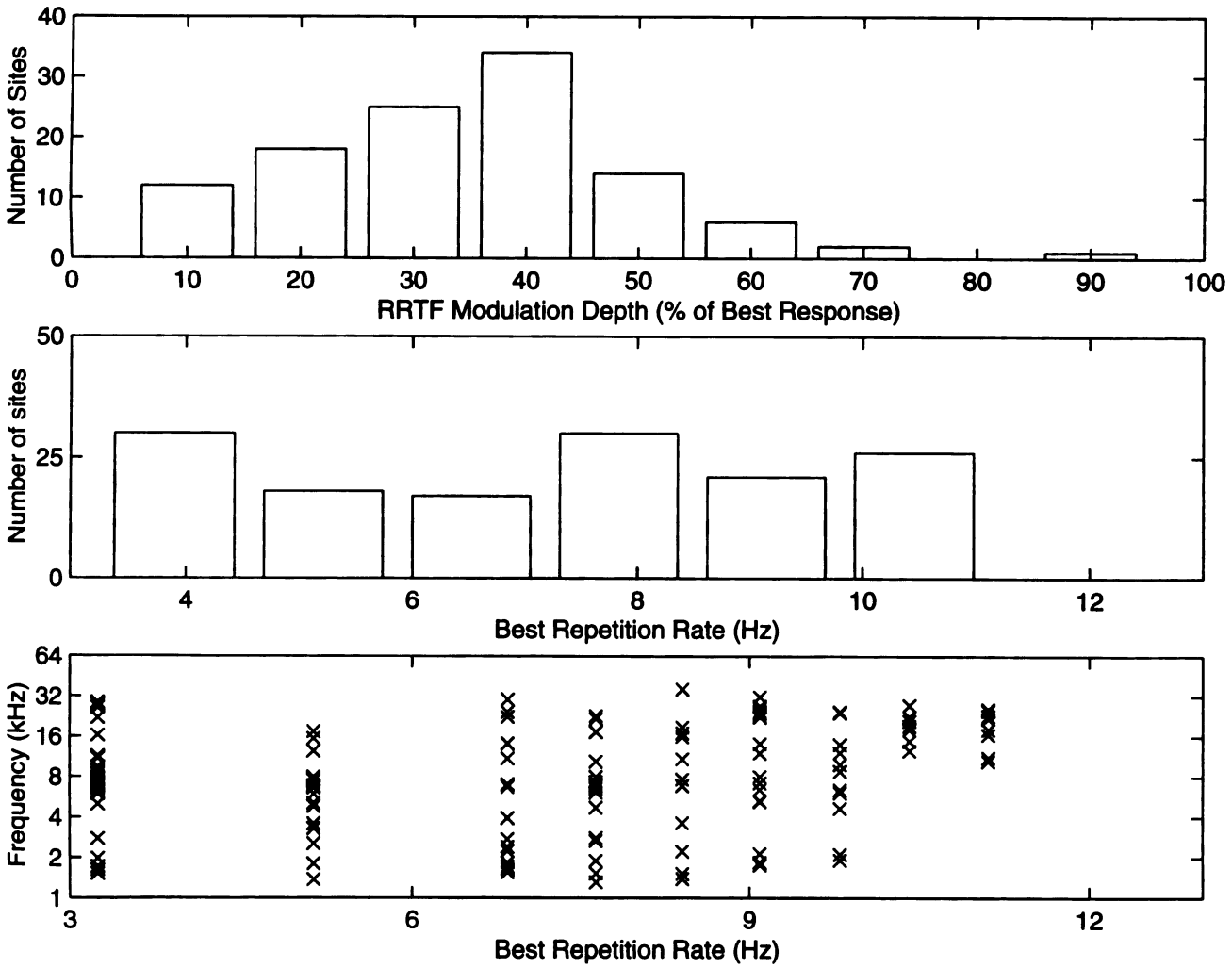

Number of Sites
RRTF Modulation Depth (% of Best Response)
Number of sites
Best Repetition Rate (Hz)
Frequency (kHz)
Best Repetition Rate (Hz)

FIGURE 2-8

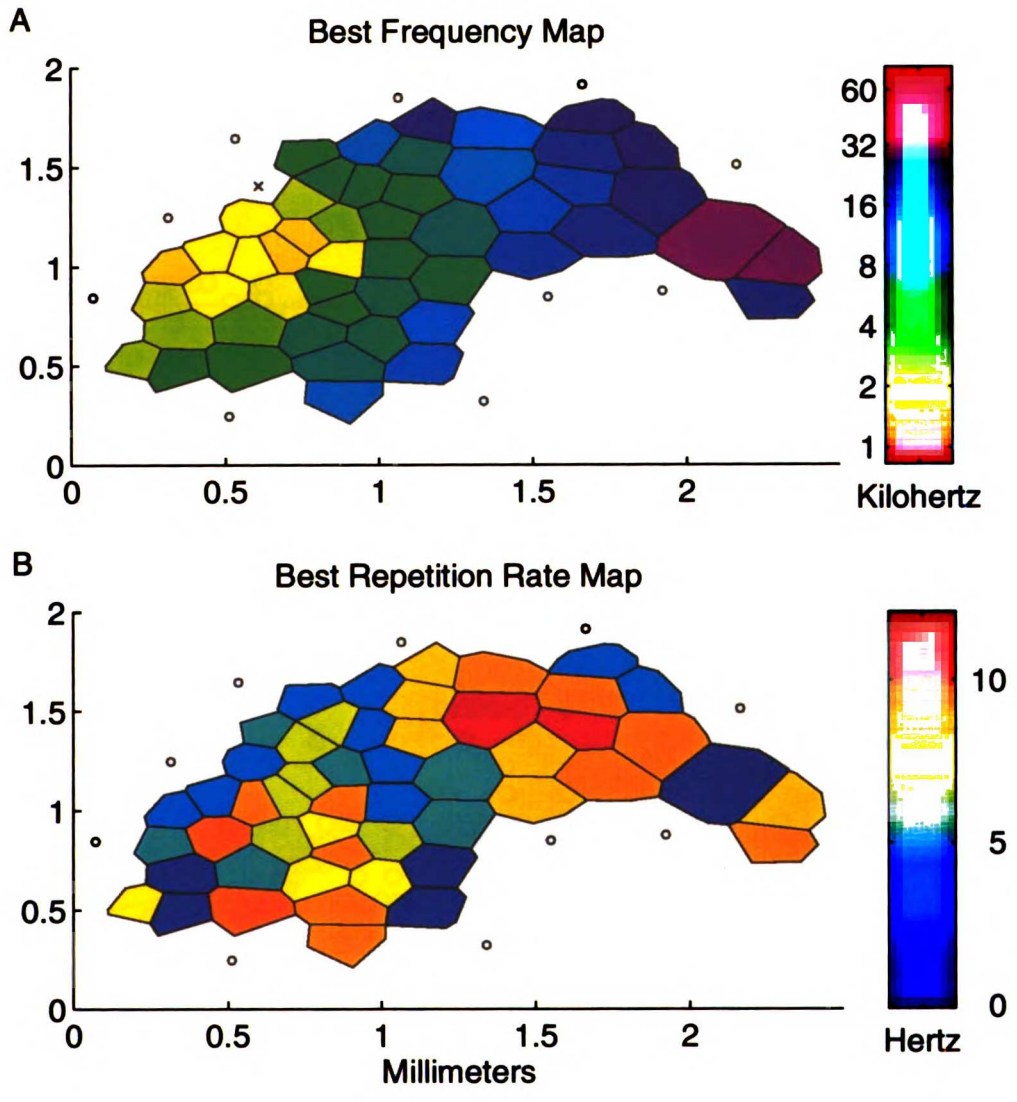


FIGURE 2-9

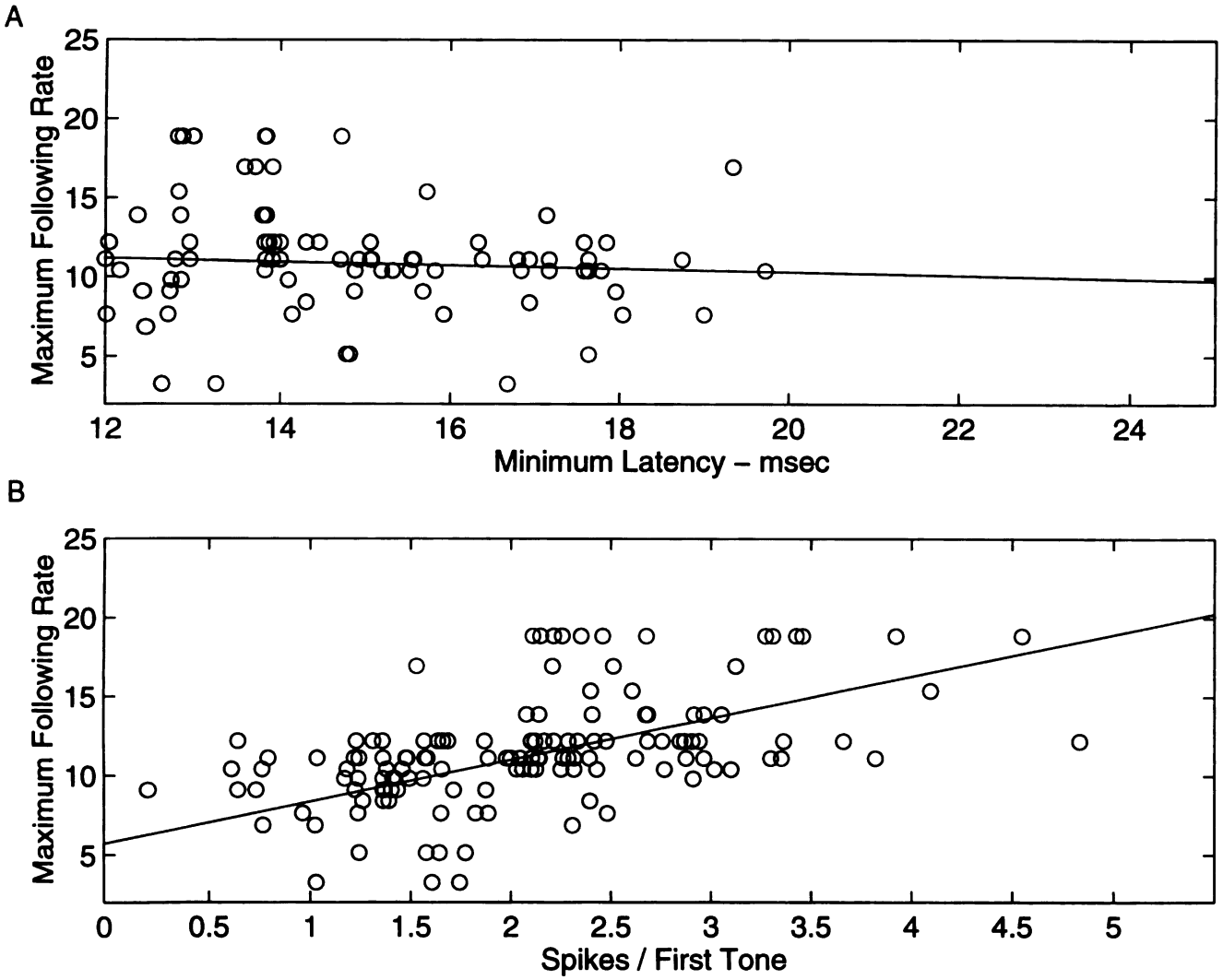

A
Maximum Following Rate
25
20
15
10
5
12
14
16
18
20
22
24
Minimum Latency – msec
B
Maximum Following Rate
25
20
15
10
5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Spikes / First Tone

FIGURE 2-10

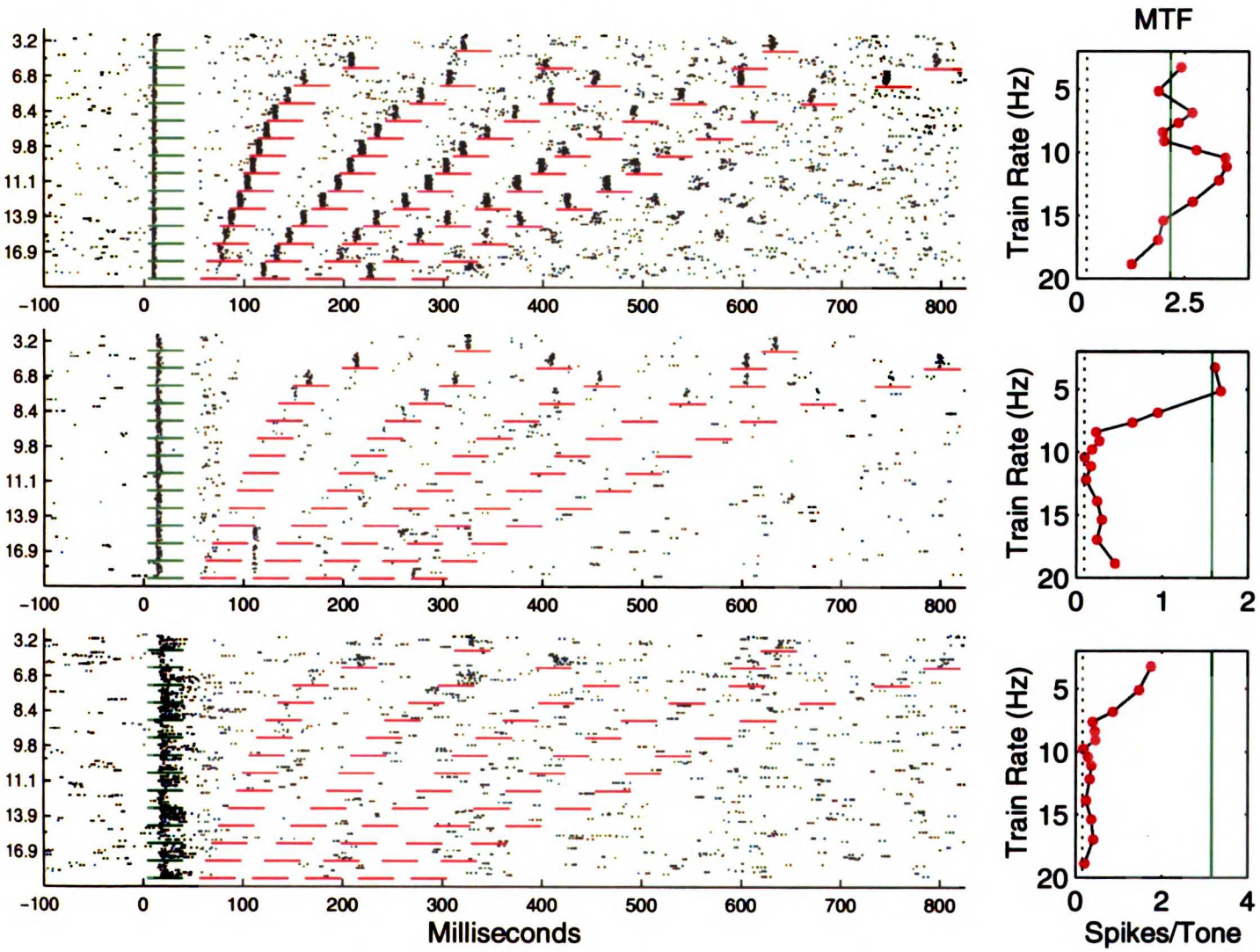

MTF
3.2
6.8
8.4
9.8
11.1
13.9
16.9
-100
0
100
200
300
400
500
600
700
800
Milliseconds
Train Rate (Hz)
5
10
15
20
0
2.5
1
2
4
Spikes/Tone

FIGURE 2-11

CHAPTER THREE:

Nucleus Basalis Activity Enables Cortical Map Reorganization

ABSTRACT

Little is known about the mechanisms that allow the cortex to selectively improve the neural representations of behaviorally important stimuli while ignoring irrelevant stimuli. Diffuse neuromodulatory systems may facilitate cortical plasticity by acting as a teacher to mark important stimuli. This study demonstrates that episodic, electrical stimulation of nucleus basalis paired with an auditory stimulus results in a massive progressive reorganization of the primary auditory cortex in the adult rat. This simple paradigm causes receptive field sizes to be narrowed, broadened, or left unaltered depending on specific parameters of the acoustic stimulus paired with nucleus basalis activation. This differential plasticity parallels the receptive field remodeling that results from different types of behavioral training, suggesting that input characteristics may be able to drive appropriate receptive field alterations independent of explicit knowledge of the task. These findings suggest that the basal forebrain plays an active instructional role in representational plasticity.

INTRODUCTION

The mammalian cerebral cortex is a highly sophisticated self-organizing system (Singer, 1986). The statistics of sensory inputs from the external world are not sufficient to guide cortical self-organization because the behavioral importance of inputs is not strongly correlated with their frequency of occurrence. The behavioral value of stimuli has been shown to regulate learning in experiments conducted over more than a century (Thorndike, 1911). Recently, behavioral relevance has been shown to directly modulate representational plasticity in cortical learning models (Merzenich et al., 1990; Ahissar et al., 1992; Ahissar and Ahissar, 1994; Weinberger, 1993). The cholinergic nucleus basalis (NB) has been implicated in this modulation of learning and memory. The NB is uniquely positioned to provide the cortex with information about the behavioral importance of particular stimuli, because it receives inputs from limbic and paralimbic structures and sends projections to the entire cortex (Mesulam et al., 1983; Steriade and Biesold, 1990). Consistent with this notion, NB neurons are activated as a function of the behavioral significance of stimuli (Richardson and DeLong, 1991; Butt and Hodge, 1995) . Several forms of learning and memory are impaired by cholinergic antagonists and by NB lesions (Hasselmo, 1995; Steckler et al., 1995; Riekkinen et al., 1992; Winkler et al., 1995; Butt and Hodge, 1995). Even the highly robust cortical map reorganization that follows peripheral dennervation is blocked by NB lesions (Juliano et al., 1991; Webster et al., 1991a; Sachdev et al., 1998; Baskerville et al., 1997; Zhu and Waite, 1998).

Many studies using acute preparations have shown that electrical stimulation of the NB (Metherate and Ashe, 1991; Metherate and Ashe, 1993; Edeline et al., 1994b;

Edeline et al., 1994a) or local administration of acetylcholine (ACh) (Metherate and Weinberger, 1989; Metherate and Weinberger, 1990; McKenna et al., 1989), can modulate stimulus-evoked single-unit responses. The variability across studies in the direction, magnitude, and duration of the modulation has made it difficult to relate these effects to long-term cortical map plasticity. Although studies using stimulation of NB reported mostly facilitation of the response to the paired stimulus, in several studies using local administration of ACh to alter receptive field organization the opposite effect was reported. In these studies ACh most often caused a significant stimulus-specific decrease in cortical responsiveness following the pairing procedure. The average duration of the plasticity also varied across studies from less than ten minutes to more than an hour.

METHODS

To clarify the role of NB in representational plasticity, we investigated the consequences of the long-term pairing of tones with episodic NB stimulation. A chronic stimulating electrode was implanted in the right NB of 21 adult rats. Platinum bipolar stimulating electrodes were lowered 7mm below the cortical surface 3.3 mm lateral and 2.3 mm posterior to bregma in ~300 g barbiturate anesthetized rats, and cemented into place using sterile techniques approved under UCSF animal care protocols. After two weeks of recovery, 250 ms (or a 15 Hz train of six 25 msec) 50 dB SPL tones were paired with 200 ms of NB electrical stimulation in a sound-shielded, calibrated test chamber (five days/week). Electrical stimulation began either 50 ms after tone onset (n=15) or 200 ms before (n=6). The two timings did not appear to affect plasticity and data from both groups were pooled. The current level (70-150 μAmp) was chosen to be the minimum necessary to desynchronize the EEG during slow wave sleep for 1-2 seconds. Stimulation consisted of 100 Hz capacitatively coupled biphasic pulses of 0.1ms duration. Tonal and electrical stimuli did not evoke any observable behavioral responses (i.e. did not cause rats to stop grooming or if sleeping, awaken).

After recovery, animals were placed in a sound attenuation chamber and a pure tone was paired with brief trial-by-trial epochs of NB stimulation during daily sessions. The tone paired with NB stimulation occurred randomly every 8-40 seconds. Pairing was repeated three to five hundred times per day for 20-25 days. The rats were unanesthetized and unrestrained throughout this procedure.

Twenty-four hours after the last session, each animal was anesthetized and a detailed map of primary auditory cortex (A1) was generated from 70-110 microelectrode

penetrations. Twenty-four hours after the last pairing, animals were anaesthetized with pentobarbital and the right auditory cortex surgically exposed. Parylene-coated tungsten microelectrodes (2MΩ) were lowered 550 μm below the pial surface (layer 4/5), and complete tuning curves were generated with 50 ms pure tones (with 3 ms ramps) presented at 2 Hz to the contralateral ear. The evoked spikes of a small cluster of neurons were collected at each site. To determine the effects of conditioning on the bandwidth of individual neurons, spike waveforms were collected during eight experiments and sorted off-line using software from Brainwave Technologies. Penetration locations were referenced using the cortical vasculature as landmarks. Primary auditory cortex was defined on the basis of its short latency (8-20 msec) responses and continuous tonotopy (BF increases from posterior to anterior). Responsive sites that exhibited clearly discontinuous best frequencies AND either long latency responses, unusually high threshold, or very broad tuning were considered to be non-A1 sites. Penetration sites were chosen to avoid blood vessels while generating a detailed and evenly spaced map. The edges of the map were estimated using a line connecting the non-responsive and non-A1 sites. Effect of conditioning on mean bandwidths across all conditions was determined using ANOVA; pairwise comparisons were analyzed by Bonferroni post-hoc testsDuring this cortical mapping phase, experimenters were blind to the tone frequency that had been paired with NB stimulation. Frequency-intensity response characteristics of sampled neurons were documented in every penetration by presenting 45 pure tone frequencies at 15 sound intensities. Tuning curves were defined by a blind, experienced observer. The set of tone frequencies presented at each site was approximately centered on the BF of each site. Thus, during analysis each tuning curve is approximately

centered in the stimulus space and simply blanking the axes and analyzing the sites in random order allowed for tuning curve characterization to be completely blind. Using custom analysis software the tuning curve edges for each site were defined and recorded without the possibility of experimenter bias. (See also Chapter Five Methods)

RESULTS

Figure 3-1A illustrates the organization of A1 in a representative naive rat. The color on each polygon denotes each penetration's best frequency (BF), the frequency that evoked a neuronal response at the lowest stimulus intensity. The frequency representation is complete and regular in control rats. Each frequency is represented by a band of neurons that extends roughly dorso-ventrally across A1. The 9 kilohertz (kHz) isofrequency band, for example, is shaded light blue in Figure 3-1A and penetrations with a BF within a third of an octave of 9 kHz are hatched with white. Figure 3-1B shows the tips of the tuning curves recorded in every penetration. The tip of the "V" marks the BF; the width of the "V" denotes the range of frequencies the neurons at the site responded to at 10 dB above threshold. In naive rats, BF's were evenly distributed across the entire hearing range of the rat, in accordance with the well-known tonotopic organization of A1 (Sally and Kelly, 1988).

Pairing a specific tonal stimulus with NB stimulation resulted in remodeling of cortical area A1 in all 21 experimental rats. In the representative example shown in figure 3-1C, a 50 dB 9 kHz tone was paired with NB stimulation approximately 300 times per day over a period of 20 days. This paradigm produced a clear expansion of the region of cortex that represented frequencies near 9 kHz (Fig 1C). Figure 3-1D illustrates the clustering of tuning curve best frequencies near the frequency that was paired with NB stimulation. After pairing, neurons from twenty of the penetrations into the conditioned map shown in Figure 3-1C had BF's within a third of an octave of 9 kHz, compared to only six in the equivalently sampled control map. The increase in 9 kHz representation resulted in a clear decrease in the area of A1 that responded to lower

frequencies. In the control map, twenty-two penetrations had BF's less than 5 kHz, compared to only four penetrations in the conditioned map. It should be noted that the decrease in low frequency responses is not a consistent finding. In other examples the low frequency responses appeared unaltered and the representation of higher frequencies was decreased.

Because the tone paired with NB stimulation was well above threshold, it was important to examine not only the shifts in the tuning curve tips, but also the responses of cortical neurons to tones at the conditioned intensity. During pairing many of the neurons with BF's different from 9 kHz were excited by the auditory stimulus because most rat A1 tuning curves broaden as intensity is increased. In the naive map, less than 25% of neurons within A1 responded to 9 kHz presented at 50 dB. By contrast, almost 50% of the conditioned cortex responded to the same stimulus.

Figure 3-2 summarizes the magnitude of representational changes that resulted from pairing one frequency with NB stimulation in ten animals. Figure 3-2A represents data from seven naïve controls and illustrates the average percent of the surface of A1 that responded to tones at any combination of frequency and intensity. Figure 3-2C-D shows the percent change relative to controls after pairing NB stimulation with 4, 9, or 19 kHz tones, respectively. In each case the cortical area representing the paired stimulus nearly doubled. These results indicate that the responses of tens or hundreds of thousands of A1 neurons can be altered by pairing tones with NB stimulation in a passively stimulated animal.

In four animals, NB stimulation was paired with a train of six 9 kHz tones pips (25 msec) presented at 15 Hz to test the effects of increasing temporal structure in the

auditory stimulus (Fig 1E&F). Conditioning with this stimulus unexpectedly resulted in even greater cortical reorganization than conditioning with a 250 ms tone ($p<0.01$, Fig. 3C). In the example shown, the 9 kHz isofrequency band was increased from roughly 250 μm wide in a naive A1 to more than a millimeter wide. After pairing, over 85% of A1 responded to 9 kHz at 50 dB. Additionally, 50% of A1 penetrations had best frequencies within one-third of an octave of 9 kHz, compared to less than 15% in the control animals. It should be noted that the extent of cortical map reorganization generated using NB activation is significantly larger than the reorganization typically observed after several months of operant training (Recanzone et al., 1992d; Recanzone et al., 1993; Jenkins et al., 1990).

The six short tones presented at 15 Hz evoked less than 30 percent more spikes than a single tone, because most rat A1 neurons do not follow onsets presented faster than 12-14 Hz (unpublished observation). It seems unlikely that the larger reorganization evoked with stimulus trains is simply due to an increased cortical response to the stimuli.

Two animals were mapped after only one week of conditioning with the 15 Hz stimulus to examine the rate of cortical remodeling evoked by our paradigm. The 9 kHz representation was increased by 18% after one week of training. This reorganization was nearly halfway to the 44% increase that was recorded following a month of conditioning, indicating that the cortical remodeling generated by NB stimulation was progressive in nature (Fig. 3B).

To probe the competitive processes underlying cortical reorganization, five rats were conditioned with two different randomly interleaved tones that were over an octave apart. Two distinct classes of reorganizations resulted. The tuning curve tips were either

1) shifted toward a point between the two conditioned frequencies, so that both were within the receptive field at 50 dB (n=3), or 2) shifted toward only one of the two conditioned frequencies (n=2, Fig 1G&H). The two classes of results may be the consequence of subtle variations in A1 prior to NB pairing, which can have large effects when competitive processes are involved.

To document that NB activation is required for the cortical reorganizations observed in this study, during four of our experiments two additional frequencies were delivered on identical presentation schedules as the paired tones, but were not paired with NB stimulation. These stimuli, which never occurred within eight seconds of NB stimulation, did not measurably affect cortical responses or representations. In contrast to the large changes induced by pairing tones with NB stimulation, no significant cortical map reorganizations were observed in previous experiments after tens of thousands of behaviorally irrelevant stimuli were presented over three to five months (Recanzone et al., 1993; Recanzone et al., 1992d). Additionally, short-term repetition of one frequency without behavioral relevance (habituation) results in a dramatic decrease in A1 responses to that frequency (Condon and Weinberger, 1991). These studies suggest that stimulus presentation without behavioral importance does not result in significant map changes. Although unlikely to be a contributing factor, we acknowledge that we did not record from animals that experienced extensive stimulus presentation without any NB stimulation.

Microdialysis experiments have shown that electrical stimulation of the NB results in ACh release in the cortex (Casamenti et al., 1986; Dykes et al., 1990; Jimenez-Capdeville et al., 1997; Rasmusson et al., 1992). Additionally, both the short-term

plasticity and EEG desynchronization evoked by NB stimulation are blocked by atropine(Hars et al., 1993; Bakin and Weinberger, 1996). Thus the cortical plasticity demonstrated in this report likely involves the release of cortical ACh paired with tones. To test for the necessity of ACh release in our paradigm, 19 kHz was paired with electrical stimulation of the NB in animals with highly specific lesions of the cholinergic NB neurons. The cholinergic neurons of the NB were selectively destroyed by infusing 2.5μg of 192 IgG-saporin immunotoxin into the right lateral ventricle prior to the surgery to implant the chronic stimulating electrode. The toxin, an antibody to the low-affinity NGF receptor linked to a ribosome-inactivating toxin, has been shown to specifically destroy most of the cholinergic neurons of the basal forebrain projecting to the cortex, while sparing the parvalbumin-containing neurons as well as cholinergic neurons that project from the NB to the amygdala (Heckers et al., 1994). Electrical stimulation of NB and tone presentation was identical for lesioned and non-lesioned animals. The percent of the cortex responding to 19 kHz following pairing in lesioned animals was not significantly different from naïve controls (two tailed t-test, n=2). No significant increase in the 19 kHz representation was observed in lesioned animals. Even though ACh release is clearly important for NB function, it may be too simplistic to focus exclusively on ACh because only one-third of NB projection neurons are cholinergic (Gritti et al., 1997). One-third are GABAergic and the remaining third are uncharacterized. Future work is needed to elucidate the function of concurrent release of these transmitters in cortical plasticity.

Interestingly, the nature of the auditory stimuli paired with NB activation had a profound effect on the selectivity of cortical responses (Fig. 3D). Sharpness of tuning

was quantified as the width of the tuning curve 10 dB above threshold, BW10. When a 250 ms tone was used as the conditioning stimulus, the average BW10 was not significantly different from the average BW10 of control rats (0.93 vs. 1.02 octaves). Conditioning with a temporally modulated stimulus (a train of six short tones of the same frequency) resulted in a mean cortical response that was less selective than controls (1.46 octaves, $p<0.0001$). Conditioning with two tones engaging different spatial locations on the input array (cochlea) resulted in cortical responses that were more selective than controls (0.70 octaves, $p<0.0001$). Thus, our paradigm can result in receptive fields that are narrowed, broadened, or unaltered depending on specific parameters of the acoustic stimulus paired with NB stimulation.

DISCUSSION

Similar increases and decreases in receptive field sizes have been recorded in the somatosensory and auditory cortices of New World monkeys that have been trained at tactile or auditory discrimination, detection or time-order judgment tasks (Merzenich et al., 1990). A pure tone discrimination task or a task involving a stimulus that moved across several fingers decreased receptive field diameters by approximately 40% (Recanzone et al., 1993; Jenkins et al., 1990). In contrast, a task requiring detection of differences in the amplitude modulation rate of tactile stimuli delivered to a constant skin surface increased receptive field diameters by more than 50% (Recanzone et al., 1992d).

The mechanisms responsible for remodeling receptive fields in a manner that is appropriate for the particular task an animal practices are not well-defined. One possibility is that top-down instruction from a higher cortical field with explicit knowledge of the goals of the operant task directs cortical plasticity. The fact that our simple paradigm, without any behavioral task, can generate the same receptive field effects induced by extended periods of operant training suggests that the characteristics of the stimuli paired with subcortical neuromodulatory input are sufficient to determine the direction of receptive field alterations.

Adult cortical plasticity appears to be responsible for improvements in a variety of behavioral skills, maintenance of precise sensory representations, compensation for damage to sensory systems, and functional recovery from central nervous system damage (Merzenich et al., 1990). Our results suggest that activation of the NB is sufficient to guide both large-scale cortical map reorganization and receptive field reorganization to

Figure 3-1. **(A, C, E & G)** Representative maps of primary auditory cortex (A1) that show the effects of pairing 9 kHz tones with electrical stimulation of nucleus basalis (NB). **(A)** Representative map from an experimentally naive rat demonstrating the normal, orderly progression of best frequencies (BF) recorded in rat A1. Each polygon represents one electrode penetration. The color of each polygon indicates the BF in kHz. The polygons (Voronoi tessellations) were generated such that every point on the cortical surface was assumed to have the characteristics of the closest sampled penetration. Hatched polygons designate sites with BFs within 1/3 of an octave of 9 kHz, illustrating a typical isofrequency band. Penetrations that were either not responsive to tones (O) or did not meet the criteria of A1 responses (X) were used to determine the borders of A1. **(C)** Map of A1 after pairing a 250 ms 9 kHz tone with NB stimulation. **(E)** Map of A1 after pairing a train of six 9 kHz tones with NB stimulation. **(G)** Map of A1 after pairing both 9 and 19 kHz with NB stimulation. Note the expansion of the 9 kHz isofrequency band in **C**, **E**, and **G**. Scale bar=200 μm. **(B, D, F, H)** Distribution of tuning curve tips at every A1 penetration from each map, which indicate the BF, threshold, and receptive field width 10 dB above threshold for neurons recorded at each penetration. Threshold as a function of frequency matches previously defined behavioral thresholds. Solid vertical lines mark the frequency paired with NB stimulation. Dotted vertical lines mark frequencies presented as often, but not paired with stimulation.

Figure 3-2. (**A**) Percent of the surface of A1 that responds to pure tones at each combination of tone frequency and intensity, average of experimentally naïve animals (n=7). (**B-D**) Percent change in the percent of primary auditory cortex responding to tones after one month of 4, 9, or 19 kHz paired with NB stimulation. (n= 4, 4, 2). Each group showed a significant increase over controls in percent responding to the conditioned frequency at 50 dB above the minimum threshold ($p<0.005$, two tailed t-test). The percent of A1 responding is the sum of the areas of all of the Voronoi tessellations that responded to the particular frequency/intensity combination of interest divided by the total area of A1. The function is highly reproducible across naïve controls with an average standard error across frequencies of <3%. Tessellation was chosen to derive area measurements from discretely sampled points by assuming that each location on the cortical surface had the characteristics of the closest sampled penetration.

Figure 3-3. (**A**) Percent of the surface of A1 that responds to pure tones of any combination of tone frequency and intensity, average of seven experimentally naïve animals. (**B**) Percent change in the percent of primary auditory cortex responding after one week of pairing 9 kHz tone pip train (15Hz) with NB stimulation. Significant increase in the response to 9 kHz at 50 dB above the minimum threshold compared to controls (t-test; $n=2$, $p<0.05$). (**C**) Percent change in the percent of primary auditory cortex responding after one month of pairing 9 kHz tone pip train (15Hz) with NB stimulation. Significant increase in the response to 9 kHz at 50 dB above the minimum threshold compared to controls ($n=4$, $p<0.00001$). (**D**) Distribution of receptive field width, BW10, for every A1 penetration for each of the four classes of experiments. Pairing one frequency with NB stimulation did not significantly effect the BW10 distribution relative to naive animals, while pairing two frequencies (4 & 14 or 9 & 19 kHz,) or a 15 Hz train of stimuli caused receptive field width to be decreased and increased, respectively. The same effect is present in the distributions of BW20-BW40. The dashed vertical line marks the mean of each distribution. Single units were sorted from the multi-unit data derived from the four naive animals (15 units) and four train conditioned animals (33 units). The mean BW10 for single units was also increased by 15 Hz train conditioning (0.91 vs. 1.38 octaves, $p<0.005$). Note that this widening of tuning curves adds with the BF shifts to generate the large increase in percent of A1 responding after train conditioning.

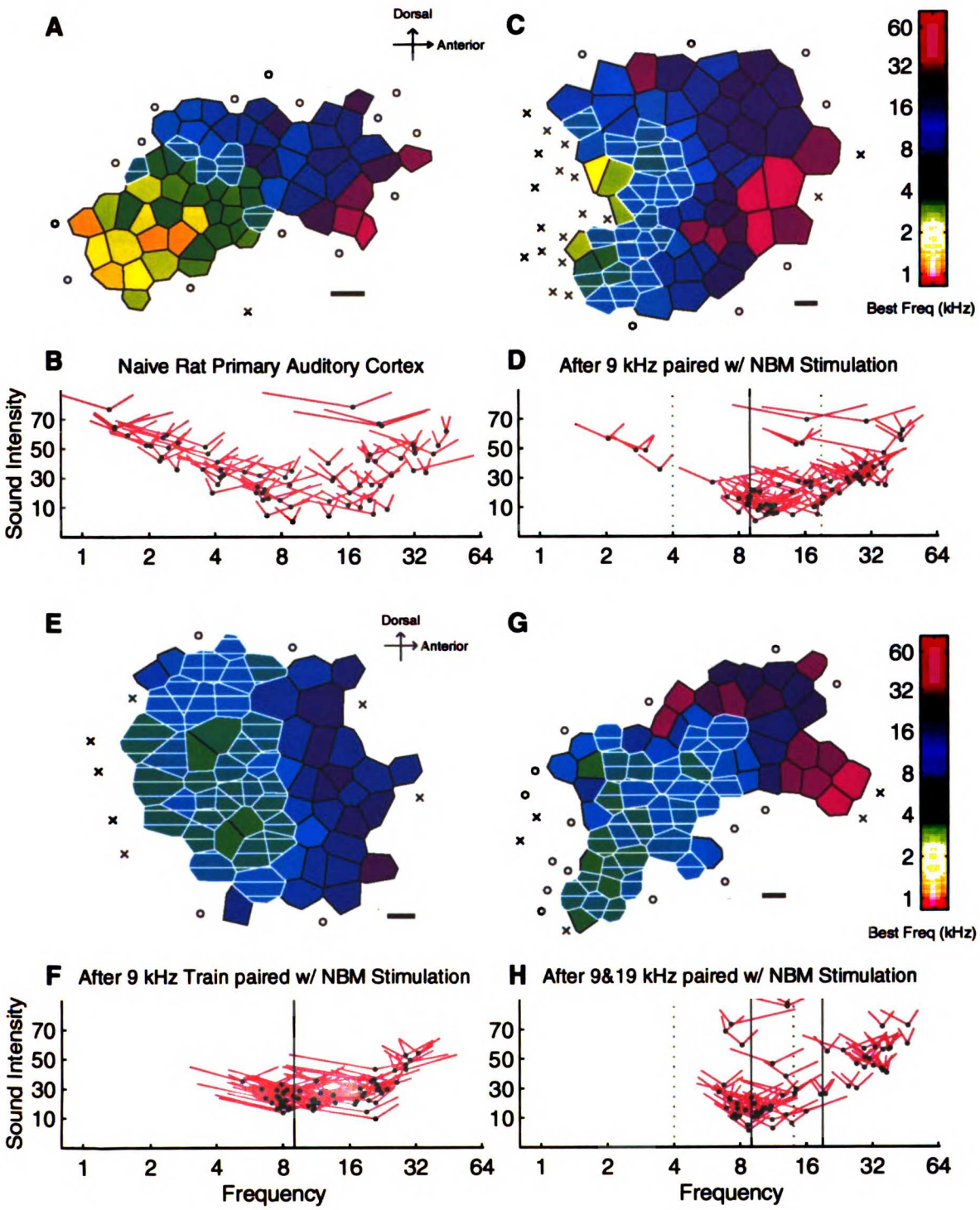

A
Dorsal
Anterior
C
60
32
16
8
4
2
1
Best Freq (kHz)
B
Naive Rat Primary Auditory Cortex
D
After 9 kHz paired w/ NBM Stimulation
Sound Intensity
70
50
30
10
1
2
4
8
16
32
64
E
Dorsal
Anterior
G
60
32
16
8
4
2
1
Best Freq (kHz)
F
After 9 kHz Train paired w/ NBM Stimulation
H
After 9&19 kHz paired w/ NBM Stimulation
Sound Intensity
70
50
30
10
1
2
4
8
16
32
64
Frequency
Frequency

FIGURE 3-1

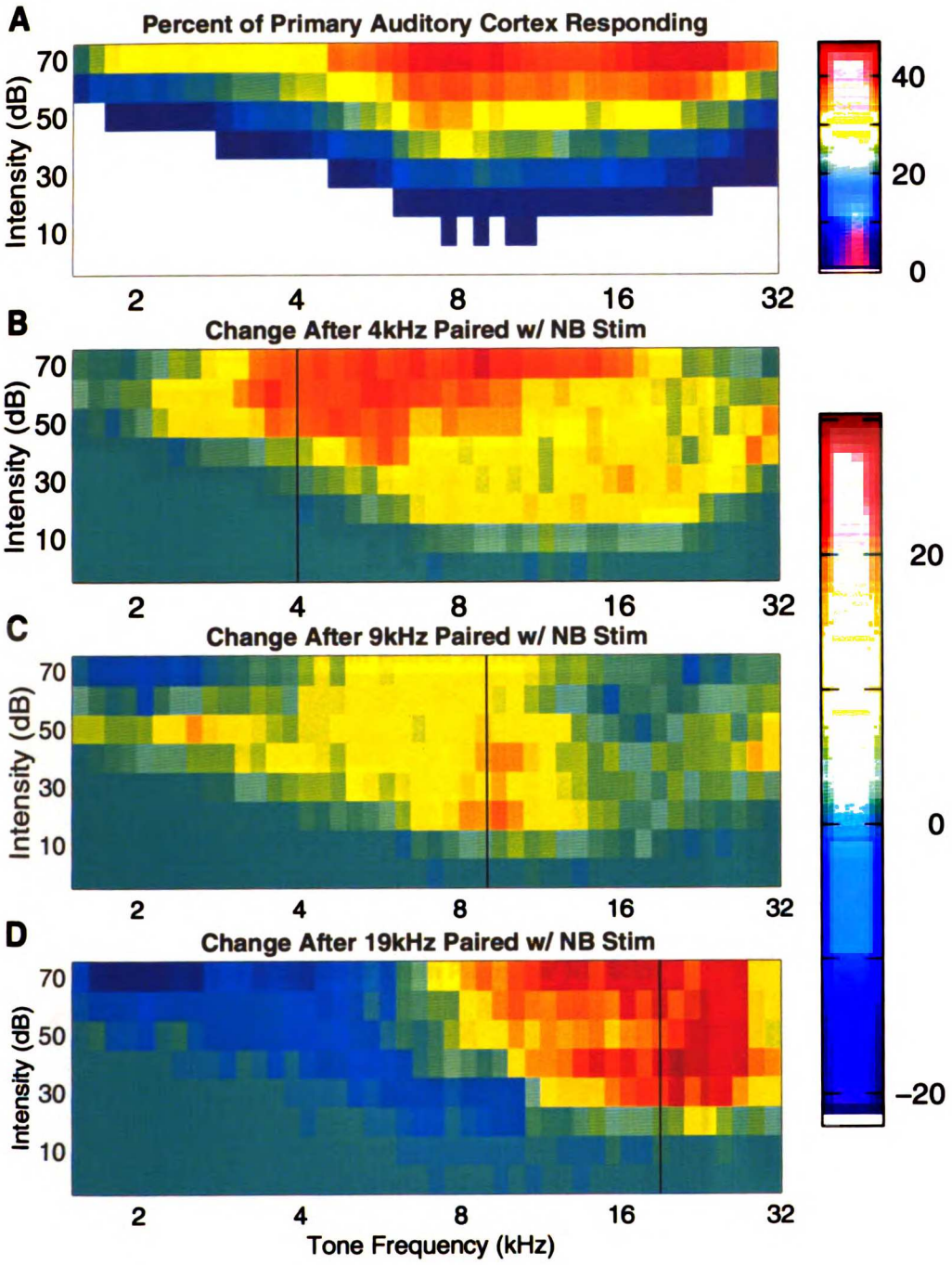
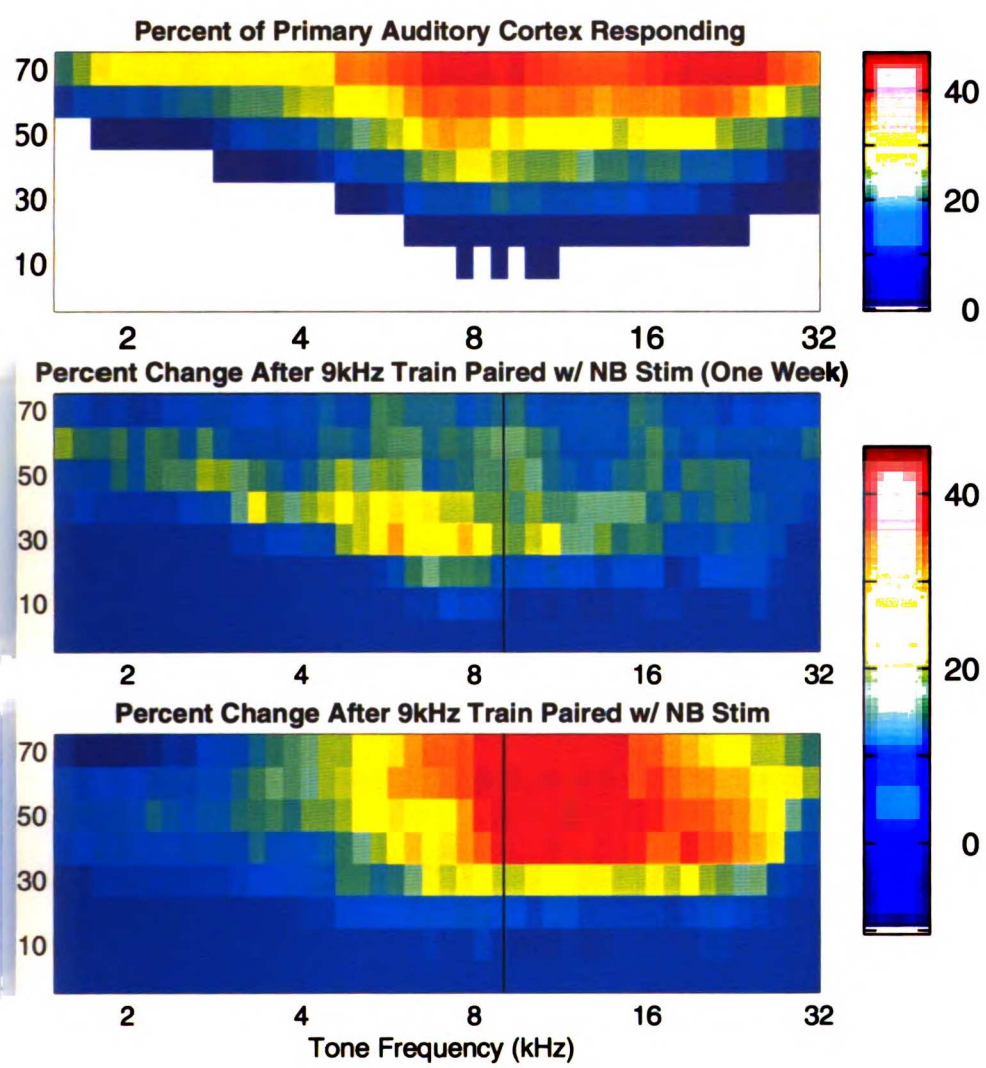

A
Percent of Primary Auditory Cortex Responding
Intensity (dB)
70
50
30
10
2
4
8
16
32
40
20
0
B
Change After 4kHz Paired w/ NB Stim
C
Change After 9kHz Paired w/ NB Stim
D
Change After 19kHz Paired w/ NB Stim
20
0
−20
Tone Frequency (kHz)

FIGURE 3-2


Percent of Primary Auditory Cortex Responding
70
50
30
10
2
4
8
16
32
40
20
0
Percent Change After 9kHz Train Paired w/ NB Stim (One Week)
70
50
30
10
2
4
8
16
32
40
20
0
Percent Change After 9kHz Train Paired w/ NB Stim
70
50
30
10
2
4
8
16
32
40
20
0
Tone Frequency (kHz)

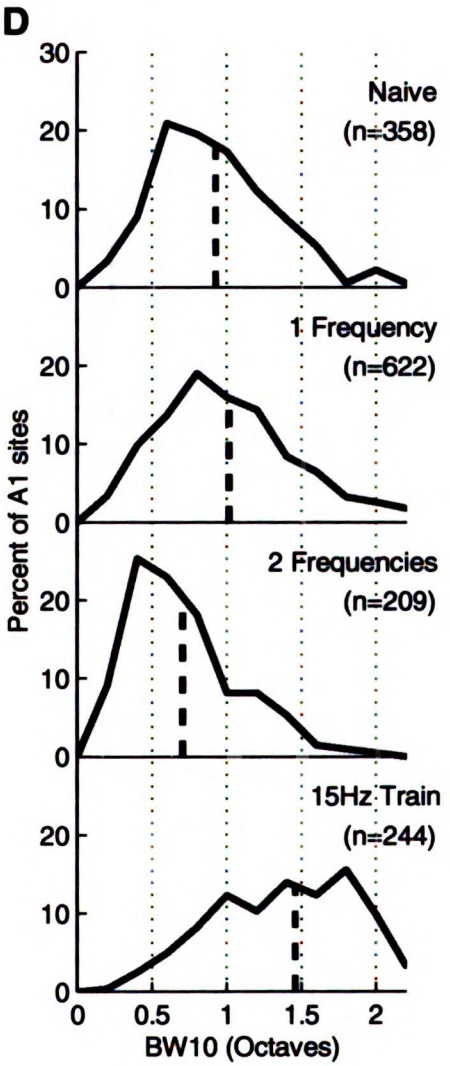

D
Naive
(n=358)
1 Frequency
(n=622)
2 Frequencies
(n=209)
15Hz Train
(n=244)
Percent of A1 sites
30
20
10
0
0
0.5
1
1.5
2
BW10 (Octaves)

FIGURE 3-3

CHAPTER FOUR:

Plasticity of Temporal Information Processing in the Primary Auditory Cortex

ABSTRACT

Neurons in the rat primary auditory cortex (A1) are generally unable to respond to tones presented at more than 12 pulses per second (pps). To test whether the maximum following rate of A1 neurons of adult rats can be modified by experience, trains of brief tones of random carrier frequency but fixed repetition rate were paired with electrical stimulation of the nucleus basalis (NB) 300 to 400 times per day for 20-25 days. This paradigm dramatically altered the temporal response properties of A1 neurons. Pairing NB stimulation with 5 pps stimuli was sufficient to decrease the cortical response to rapidly presented stimuli, while 15 pps pairing significantly increased the maximum cortical following rate. Although this NB pairing induced temporal plasticity, we observed no significant reorganization of cortical tonotopy. In striking contrast, when fixed carrier frequency 15 pps trains were paired with NB stimulation, the cortical representation of the carrier frequency was expanded, and the mean maximum following rate was not significantly increased.

These studies demonstrate that NB activation paired with tone trains elicits extensive cortical remodeling of temporal response properties, and that simple differences in spectral and temporal features of the sensory input can drive very different cortical reorganizations.

INTRODUCTION

Most studies of cortical plasticity have documented changes evoked by spatially or spectrally specific stimuli (Bakin and Weinberger, 1990; Bakin et al., 1996; Gilbert, 1996; Recanzone et al., 1992b; Recanzone et al., 1992c; Recanzone et al., 1993; Weinberger, 1993; Xerri et al., 1996). In a recent experiment of that class, we demonstrated that the representation of a given tone frequency can be greatly expanded in A1, and that large-scale remodeling of the spectral selectivity of A1 receptive fields (frequency-intensity tuning curves) is achieved by pairing NB activation with tonal stimulation in a non-behaving rat (see chapter three; also see studies by Weinberger and colleagues (Bakin and Weinberger, 1996; Bjordahl et al., **1998**)). NB neurons located in the basal forebrain send cholinergic and GABAergic projections to the entire cortical mantle (Figure 5-1a) (Mesulam et al., 1983). NB activation paired with sound stimulation failed to produce significant cortical reorganizations when the acetylcholine-containing cells in the NB were immuno-lesioned (see chapter three). Together, these studies support the long-standing view that the cholinergic projection from the NB is a primary modulatory input source for enabling experience-dependent cortical plasticity.

In the present study, we used NB stimulation to explore the principles governing the plasticity of cortical dynamics as they apply to the representation of the temporal features of rapid, successive stimulus events. One method of describing the capacity of cortical neurons to respond to successive inputs is to derive a "repetition rate transfer function" (RRTF) in which the neural discharge rate is defined as a function of the stimulus repetition rate. Depending upon the type of stimulus modulation used, RRTFs

derived for neurons in the primary visual, auditory or somatosensory cortices are low-pass or band-pass in form (De Ribaupierre et al., 1972a; Tolhurst and Movshon, 1975; Movshon et al., 1978; Schreiner and Urbas, 1988; Schreiner and Langer, 1986; Eggermont and Smith, 1995; Gaese and Ostwald, 1995; Hawken et al., 1996). In the primary visual and auditory cortical areas, the majority of neurons respond maximally to repeated stimuli when presented at 7-12 pps, responding progressively more poorly at higher repetition rates.

Because the RRTFs of cortical neurons reflect sequences of excitatory and inhibitory cortical circuit events that are set in motion when the cortex is abruptly engaged by a stimulus (De Ribaupierre et al., 1972b; De Ribaupierre et al., 1972a; Kenmochi and Eggermont, 1997; Eggermont and Smith, 1995; Chance et al., 1998; Brosch and Schreiner, 1997; Schreiner et al., 1997; Gaese and Ostwald, 1995; Cartling, 1997), one might predict that this elemental input sampling/recovery property of cortical circuits is immutable. However, a large body of evidence has long argued that temporal response properties of cortical neurons can be substantially altered by experience. For instance, the visual cortex of visually deprived animals responds poorly to stimuli repeated at rates above 5 pps (Beaulieu and Cynader, 1990; Pizzorusso et al., 1997). Furthermore, the results of several psychophysical studies demonstrating that the ability to discriminate differences in rate, stimulus duration, or interval separating successively presented stimuli can be greatly improved by training are consistent with progressive improvements in cortical processing of temporal information (Woodrow, 1935; Warm et al., 1975; Neisser and Hirst, 1974; Recanzone et al., 1992a; Nagarajan et al., 1998; Wright et al., 1997). Studies of auditory or visual signal recognition for stimuli that are

delivered in rapid succession have demonstrated that there can be large improvements in the ability to recognize briefly presented stimuli with progressively shorter times ensuing between the initial stimulus and the following masking stimulus, consistent with a several-fold training-induced decrease in cortical response recovery time (Karni and Sagi, 1991; Ahissar and Hochstein, 1993; Merzenich et al., 1996). Cortical plasticity studies in monkeys trained to detect changes in amplitude modulation rate recorded sharper and stronger responses to the trained modulation rate (Recanzone et al., 1992d). Importantly, this strengthening of cortical responses was strongly correlated with improvement in task performance.

Such changes in temporal properties of cortical responses probably result from plasticity of synaptic, intrinsic, or network time constants. For example, plasticity of excitatory synapses on inhibitory neurons (Oda et al., 1995; Charpier et al., 1995; Sastry et al., 1997; Grabauskas and Bradley, 1998; Hollrigel et al., 1998) and of inhibitory synapses themselves (Theodosis et al., 1995; Brussaard et al., 1997; Fischer et al., 1997) are likely to shape the responses of cortical neurons to rapidly successive stimuli *in vivo* (Buonomano et al., 1997; Buonomano and Merzenich, 1998; Buonomano and Merzenich, 1995). Additionally, pre-synaptic release probability contributes to the cortical response to successive inputs and is capable of experience-dependent plasticity (Markram and Tsodyks, 1996; Tsodyks and Markram, 1997). Together these experiments indicate that the capacity of the cortex to respond to successive events in time is shaped by a succession of excitatory and inhibitory processes, whose dynamics can be modified by experience.

RESULTS AND DISCUSSION

In this study, NB activation was paired with temporally modulated acoustic stimuli to investigate plasticity of the cortical representation of time-varying information. Stimulating electrodes were chronically implanted in the right NB of 15 adult rats. After recovery, animals were placed in a sound attenuation chamber and a train of six tone bursts paired with NB stimulation during daily sessions. Tone trains and NB stimulation occurred randomly every 8-40 seconds and was repeated three to four hundred times per day for 20-25 days. Rats were unanesthetized and unrestrained throughout this procedure. The train repetition rate for each rat was presented at a fixed rate of 5, 7.5 or 15 pps. The tonal (carrier) frequency was varied randomly trial by trial. The seven carrier frequencies that were applied extended across most of the frequency range represented in the primary auditory cortex (A1) of the rat. Twenty-four hours after the last pairing session, each animal was anesthetized, and the responses of A1 neurons were recorded from 30-60 microelectrode penetrations distributed evenly across A1. Frequency-intensity tuning curves and RRTFs were derived to characterize the spectral and temporal response properties of neurons in every penetration.

In naïve animals, at repetition rates up to about 9 pps, each brief tone generally evoked the same number of spikes from A1 neurons as did the first tone in the train (Figure 5-1c,d). At repetition rates from 9 to 14 pps, the number of spikes per tone fell off rapidly and only neurons at rare sites responded at all to rates above 15 pps.

In experimental rats in which 15 pps stimuli of variable carrier frequencies were paired with NB stimulation, there was a dramatic alteration of the temporal responses of

cortical neurons recorded all across A1. Although no specific response peak emerged at 15 pps, the cut-off frequency of low-pass RRTFs in A1 rose significantly in the majority of sampled neurons. In striking contrast to control rats, the average neuron in these samples exhibited strong responses to modulation rates between 10 and 20 pps (Figure 5-2a). The increase in the neural response to 15 pps trains after pairing was highly significant ($p<0.0001$; Figure 5-2c). The high-frequency slope of the average modulation transfer function (Figure 5-2a,c) shifted higher, reflecting a strong response improvement across a broad range of higher modulation frequencies.

To determine whether or not NB-induced temporal plasticity is specific to the repetition rate of the paired acoustic stimulus, 5 pps trains were paired with NB activation. The normalized evoked response to stimuli repeated at 5 pps was significantly increased ($p<0.01$), resulting in RRTF's with bandpass characteristics (maxima approximately corresponding to the 5 pps modulation rate applied during sound stimulus/NB pairing). Interestingly, 5 pps pairing resulted in two distinct classes of cortical responsiveness to faster rates. In three of four animals, the entire RRTF was shifted leftward causing significantly decreased maximum following rates (Figure 5-2b, c). In the remaining animal, 75% of sites exhibited strong facilitation to both 5 and 10 pps stimuli, but poor responses to stimuli repeated at 7.5 pps (data not shown). Thus NB-induced plasticity reliably increased the strength of the cortical response to stimuli presented at the paired rate, even though RRTF plasticity can take on at least two forms.

Pairing 7.5 pps stimuli of randomly-varied carrier frequency with NB stimulation resulted in a selective strengthening of the cortical response to stimuli repeated at rates near 7.5 pps. The mean RRTF again took on a bandpass form, with a substantially

stronger-than-normal response to modulated stimuli emerging at the paired stimulus rate (Figure 5-2c).

As reported previously, pairing tones presented at 15 pps with a constant carrier frequency of 9 kHz resulted in a dramatic enlargement of the region of A1 representing 9 kHz in every animal (see chapter three). Surprisingly, pairing trains of 9 kHz tones repeated at 15 pps did not result in the rightward shift in the mean RRTF that resulted from pairing NB stimulation with multiple frequency trains repeated at 15 pps. No significant alteration in the mean response to the paired repetition rate (15 pps) was detected in the population RRTF analysis (0.27 vs. 0.28, $p>0.5$; one-way ANOVA). Although it is unclear how topographic map reorganization and temporal plasticity are related, it is interesting to note that pairing random-frequency 15 pps trains with NB stimulation resulted in a significant increase in the maximum stimulus following rate of cortical neurons, but generated no systematic alterations of the A1 map of frequency.

Although no rightward shift in the mean cortical RRTF was observed after pairing 15 pps trains of 9 kHz tones, in one rat an unusual temporal response profile was observed in neurons sampled at about 1/4th of sampled A1 sites (16 sites) in a plasticity-expanded 9 kHz A1 representational zone. These neurons exhibited highly specific responses to 9 kHz and 15 pps. Four examples of these emergent temporal rate- (and frequency-) specific RRTFs recorded in this rat are shown in Figure 5-3. These RRTFs are the most selective responses recorded in a large group of more than six hundred penetrations from the eighteen other rats in this series. No responses of this class were recorded in rats in which the carrier frequency of the 15 pps trains was varied trial by trial. Of the 151 control penetrations only 3 exhibited responses that were qualitatively

similar. In contrast to the higher RRTF cut-off rates generated by 15 pps multiple-frequency trains paired with NB stimulation, these highly selective responses exhibited slower RRTF cut-off rates (i.e. poor responses to 9 pps) along with conspicuous responses at ~15 pps. Although these responses represent the extreme of the temporal plasticity effects observed in this study, comparable selectivity has been observed in song birds and echolocating bats in response to behaviorally important auditory stimuli (Suga, 1989; Esser et al., 1997; Doupe and Solis, 1997).

Paired NB and sensory stimulation provides a simple model with which to study the rules that operate in the cortex to transform sensory input structure and schedules into distributed cortical response patterns. Previous studies have focused on the plasticity of the cortical representation of spectral information, and have demonstrated that cholinergic modulation is sufficient to shift A1 tuning curves toward the frequency paired with NB stimulation (Chapter three; Bakin and Weinberger, 1996; Bjordahl et al., **1998;** Metherate and Weinberger, 1990; Metherate and Weinberger, 1989; McKenna et al., 1989). In this study, we have used NB activation to explore temporal information processing and have shown that the temporal response properties of A1 neurons can be dramatically altered to broadly refine or to degrade the capacity of the cortex to respond to rapidly successive input events. We have also shown that this plasticity manifests a large capacity to exaggerate the representation of specific, heavily presented sensory input rates. Finally, we have demonstrated that A1 neuronal networks a) can simultaneously generate spectrally and temporally selective responses; b) can reorganize topographically organized representations of tone frequency while generating no evident

change in the representation temporal information; c) or vice versa -- all as an apparent function of the spectrotemporal structures and schedules of sensory inputs.

This striking capacity for learning-based revision of the basic integration/segmentation times of the cortical processing machinery could result from plasticity of synaptic time constants, of intrinsic temporal characteristics, and/or network dynamics (Markram and Tsodyks, 1996; De Ribaupierre et al., 1972a; Kenmochi and Eggermont, 1997; Eggermont and Smith, 1995; Dinse et al., 1997). Paired-pulse facilitation and slow inhibitory potentials, for example, almost certainly play an important role in the cortical recovery of responsivity following any brief stimulation (Buonomano and Merzenich, 1995; Buonomano et al., 1997; Buonomano and Merzenich, 1998; Abbott et al., 1997; De Ribaupierre et al., 1972a; Markram and Tsodyks, 1996; Brosch and Schreiner, 1997; Schreiner et al., 1997). NB-induced plasticity stimulation will provide a powerful experimental approach for relating the cortical representation of temporal information to changes in basic cortical dynamics that shape the cortical responses to time-varying stimuli .

METHODS

Preparation: Platinum bipolar stimulating electrodes were lowered 7 mm below the cortical surface 3.3 mm lateral and 2.3 mm posterior to bregma in ~300 g barbiturate-anesthetized rats of either sex, and cemented into place using sterile techniques approved under UCSF Animal Care Facility protocols. After two weeks of recovery, trains of six 25 msec tones were paired with 200 msec of NB electrical stimulation in a sound-shielded, calibrated test chamber (five days/week). The frequency of the tone was either one of seven frequencies (1.3, 2, 3, 5, 9, 14, or 19 kHz) or was fixed (9 kHz). Tone amplitude was 20-30 dB above the minimum rat hearing threshold (Kelly and Masterton, 1977). In experiments using multiple carrier frequencies, the frequency of the tones within each train was constant, while the frequencies used from train to train were randomly varied. The tone pips in stimulus trains were presented in a given rat at either 5, 7.5 or 15 pps. Electrical stimulation began with the onset of the fourth tone. The stimulating current level (70-150 μAmp) was the minimum necessary to desynchronize the EEG during slow wave sleep for 1-2 seconds. Stimulation consisted of 100 pps capacitatively coupled biphasic pulses of 0.1 msec duration. Several microdialysis studies have shown that this stimulation paradigm results in the release of cortical acetylcholine (Casamenti et al., 1986; Jimenez-Capdeville et al., 1997; Rasmusson et al., 1992). Either cholinergic antagonists or lesions of the cholinergic cells in the NB with 192 immunoglobulin G-saporin are sufficient to block this plasticity generated by NB stimulation (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998). Tonal and

electrical stimuli did not evoke any observable behavioral responses (i.e. did not cause rats to stop grooming, or if sleeping, to awaken).

Electrophysiology: Twenty-four hours after the last pairing, animals were anaesthetized with sodium pentobarbital, the right auditory cortex surgically exposed, and neural responses were recorded with parylene-coated tungsten microelectrodes (FHC #070-02-01, 2MΩ). Penetration sites were chosen to evenly sample the cortical surface while avoiding blood vessels. To minimize the possibility of experimenter bias or response variability due to variable recording depth, at every penetration site the electrode was lowered to ~550 μm below the pial surface (layers 4/5), which yielded vigorous driven responses. Frequency/intensity response areas were reconstructed in detail by presenting 45 pure tone frequencies (50 msec duration, 3 msec ramps) at each of 15 sound intensities to the contralateral ear at a rate of 2 stimuli/sec The evoked spikes of a neuron or a small cluster of 2-5 neurons were collected at each site. Primary auditory cortex was defined on the basis of the short latency (8-20 msec) of its evoked neuronal spike responses and its continuous tonotopy (BF increases from posterior to anterior). Responsive sites that exhibited clearly discontinuous best frequencies *and* a) either long latency responses, b) high thresholds, or that c) had very broad tuning were considered to be non-A1 sample sites, and were not included in these sample data.

To determine the RRTF for each site, six tones (25msec with 5msec ramps, 70dB SPL) were presented twelve times at each of sixteen repetition rates. To minimize adaptation effects, repetition rates were randomly interleaved and two seconds of silence separated each train. The two second interval between trains allowed the response strength to 0.5 pps trains to be approximated. The frequencies of tones in the trains for

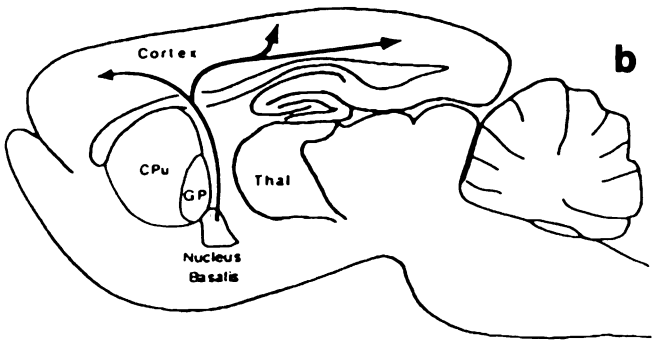
defining the RRTF in the mapping study were the frequency of the seven used in pairing that was closest to the best frequency for neurons sampled at each site. To reduce the variability resulting from different numbers of neurons included in different "multi-unit" responses recorded in this study, response amplitude was normalized using the number of spikes evoked at each site to a tone in isolation. The normalized RRTF was defined as the average number of spikes evoked for each of the last five tones in the train divided by the number of spikes evoked by the first tone in the train. Thus, a normalized spike rate of one indicates that at the given repetition rate each of the tones in the train, on average, evoked the same number of spikes as the first tone. Values greater than one indicate facilitation; values less than one indicate response adaptation. Only spikes occurring from 5-40 msec after each tone onset were used to calculate the RRTF. RRTF data could not be viewed on-line and were analyzed only after each experiment was completed. All analyses was automatized, and were therefore not subject to experimenter bias or error. The effect of NB pairing on mean RRTF across all conditions was determined with analysis of variance; pairwise comparisons were analyzed by Fisher's PLSD.

As a result of barbiturate anesthesia the modulation of responses recorded in this study may not be identical to the responses of awake animals.

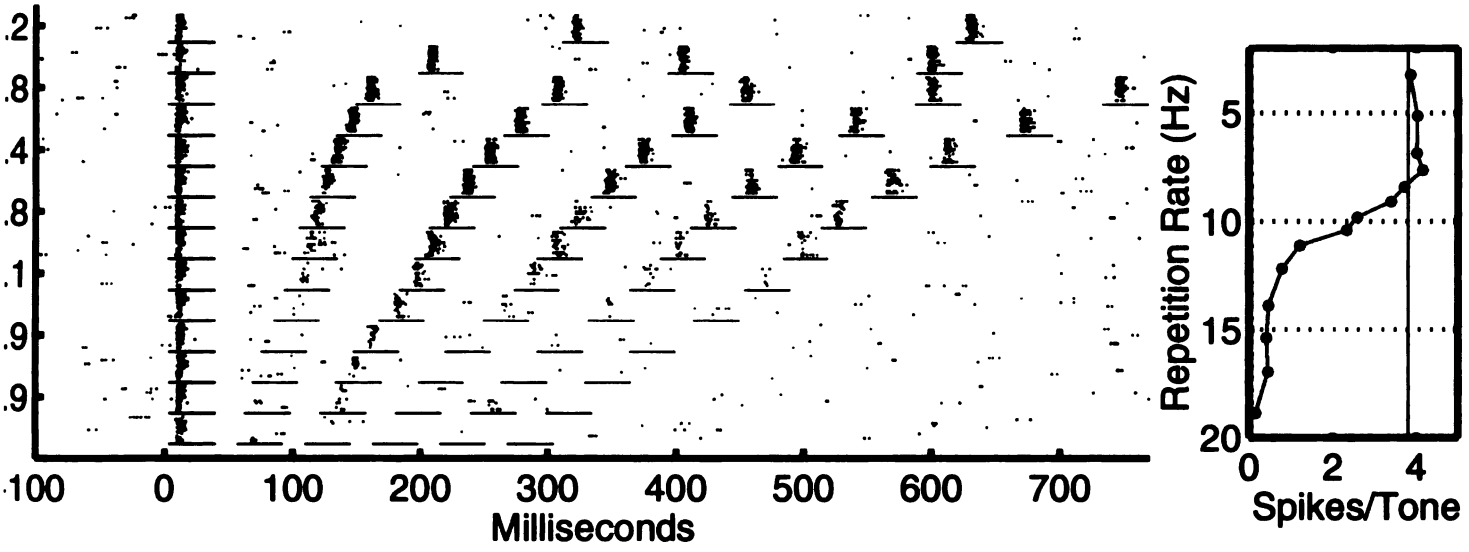
Figure 4-1. (**A**) Schematic diagram of the projection of nucleus basalis to the neocortex. (**B**) Number of rats and penetrations for each experimental condition. (**C**) Dot rasters and repetition rate transfer function (RRTF) of primary auditory cortex (A1) neurons from a naïve (control) rat. Each dot represents a single action potential. The short horizontal lines indicate the spike collection windows that were used to generate the RRTF. The RRTF quantifies the generally low-pass nature of A1 cortical neurons to these pulsed stimuli in the rat. The solid vertical line in the RRTF shows the average number of spikes evoked by the first tone in the train. (**D**) Mean normalized RRTF for all penetrations recorded from normal (control) rats. Error bars indicate standard errors.

Figure 4-2. (**A**) Response to repeated tones after pairing NB stimulation with 15 pps trains applied at 7 different carrier frequencies. The maximum following rate and the range over which strong stimulus-by-stimulus responses were evoked was increased all across A1 in this sample, compared to controls. (**B**) Response to repeated tones after pairing NB stimulation with 5 pps trains of tones at a randomly varied carrier frequency. The maximum following rate was significantly decreased compared to controls. (**C**) Mean normalized RRTFs for penetrations recorded from animals that received NB stimulation paired with 5, 7.5, 15 pps trains of tone (carrier) frequencies that varied from train to train, compared to control RRTFs. The RRTF of each site was normalized using the number of spikes evoked by the first tone in each train. Error bars indicate standard error. The rates that were significantly different from controls are marked with dots (one-way ANOVA, Fisher's PLSD, $p<0.05$).

Figure 4-3. **(A-D)** Four RRTFs representative of the neuronal responses recorded from 16 sample sites in the 9 kHz representational zone in a rat in which NB stimulation was paired with 9 kHz tonal stimulation modulated at 15 pps. In this rat, highly selective response remodeling in A1 for both 9 kHz and 15 pps was recorded broadly across this zone.



	# of Rats	# of Sites
Control	4	151
5 pps trains of multiple frequencies	4	153
7.5 pps trains of multiple frequencies	2	91
15 pps trains of multiple frequencies	5	139
15 pps 9 kHz trains	4	216



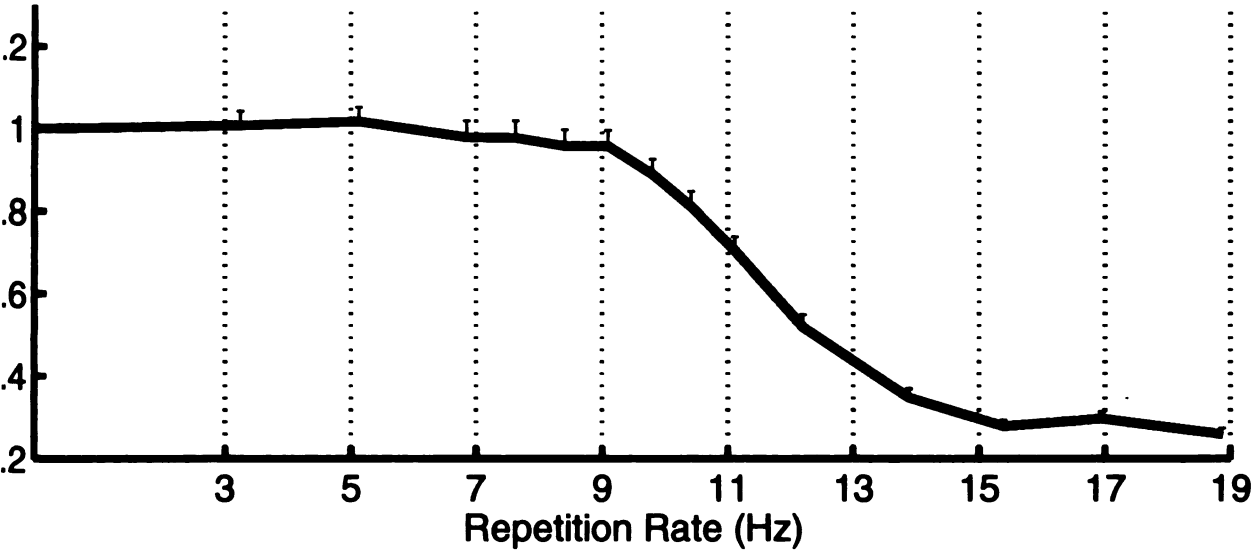
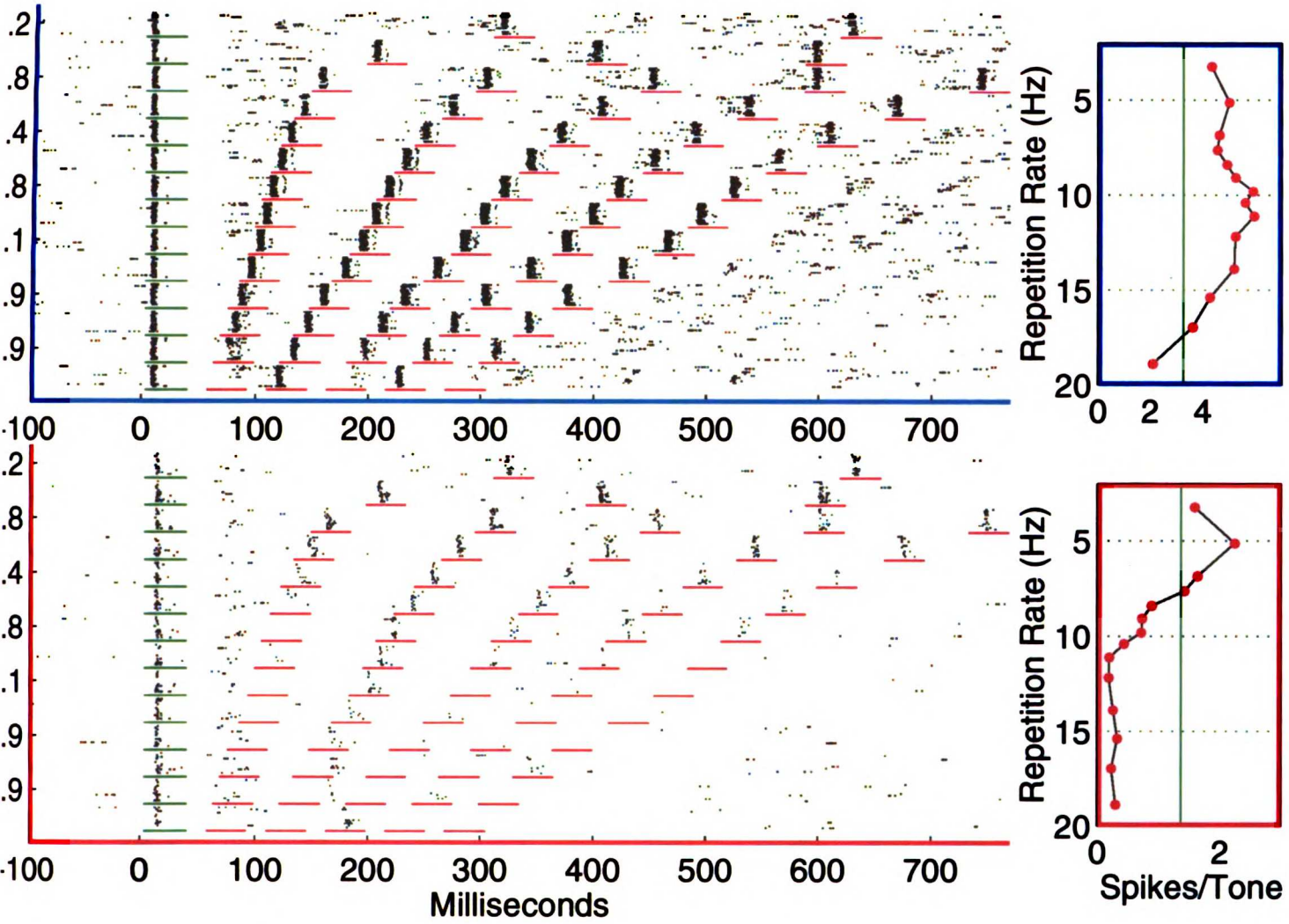


FIGURE 4-1


Repetition Rate (Hz)
5
10
15
20
0
2
4
-100
0
100
200
300
400
500
600
700
Milliseconds
Spikes/Tone

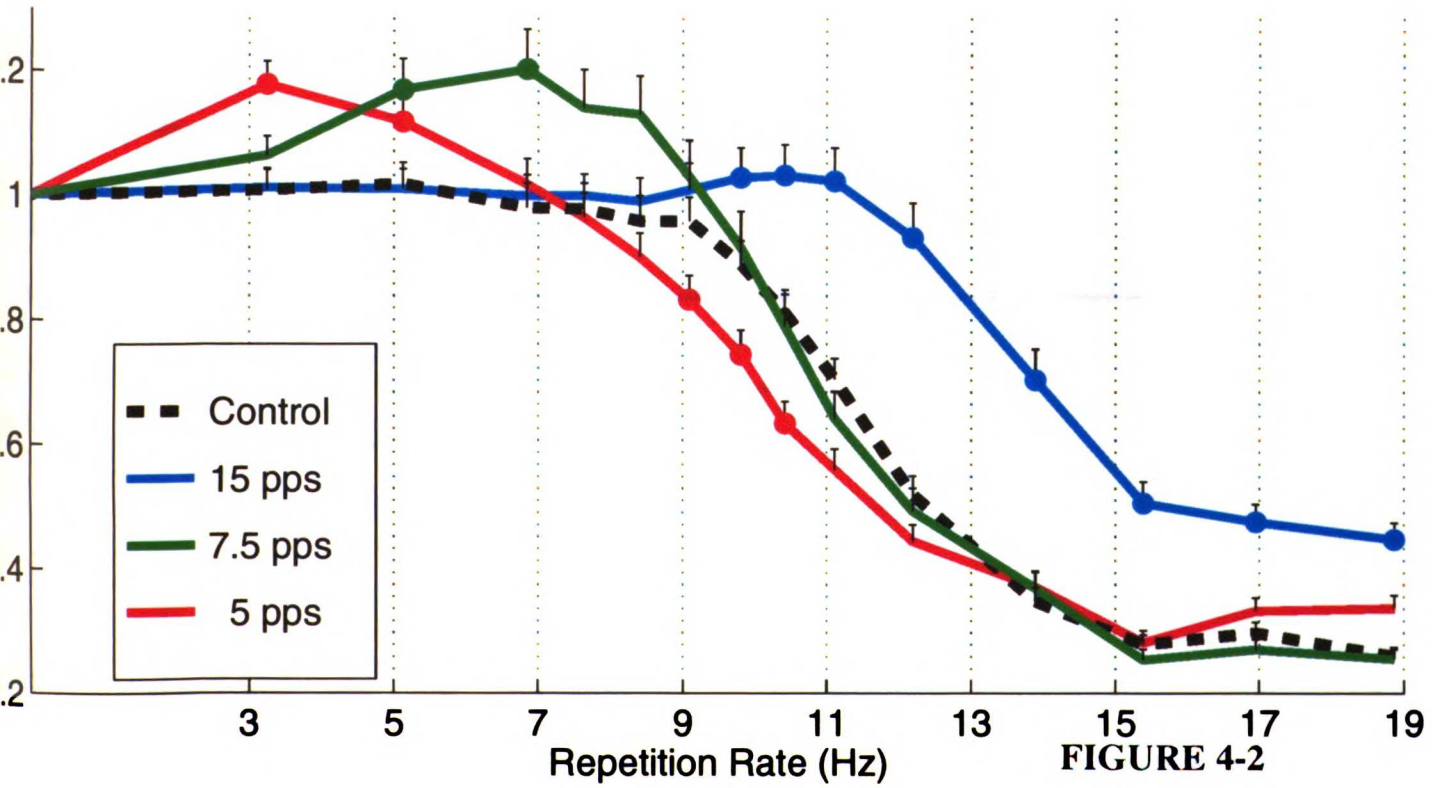

Control
15 pps
7.5 pps
5 pps
3
5
7
9
11
13
15
17
19
Repetition Rate (Hz)

FIGURE 4-2

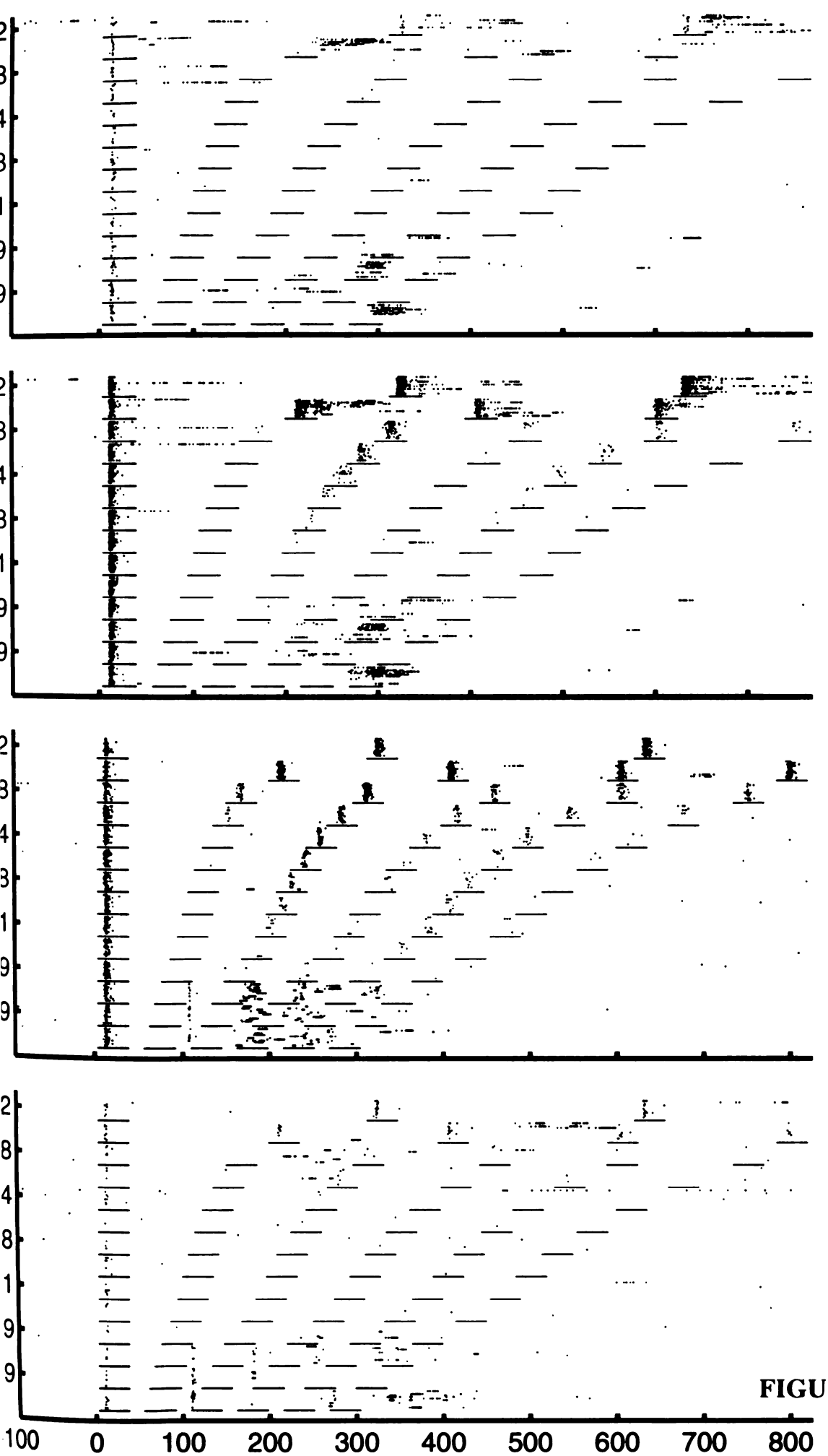


FIGURE 4-3

CHAPTER FIVE:

Features of Paired Auditory Input Determine Cortical Plasticity Induced by Nucleus Basalis Stimulation

ABSTRACT

Cortical plasticity is inhibited by lesions of the basal forebrain, the source of cholinergic input to the cortex. Repeated electrical stimulation of nucleus basalis (NB) paired with tonal stimuli is sufficient to generate large-scale changes in the representation of both spectral and temporal information in the primary auditory cortex (A1) of adult animals. In this study, we parametrically explored how cortical plasticity generated by NB stimulation is shaped by features of the paired auditory stimulus. For example, the region of the cortical representation that expanded was specific to the frequency of the paired tone. The introduction of a delay between NB stimulation and the paired auditory stimulus decreased the specificity of the reorganization. Both the direction and the degree of receptive field (frequency bandwidth) plasticity varied systematically as a function of the temporal modulation rate and of carrier-frequency variability. Frequency selectivity was decreased after pairing NB stimulation with a train of short 9 kHz tones presented at 15 pps, and increased after pairing unmodulated tones of different frequency. Expansion of the functionally-defined border of A1 and increased cortical excitability were also sensitive to features of the stimulus paired with NB activation.

These results suggest that simple rules operate in the cortex that guide cortical plasticity dependent on the statistics of inputs that co-occur with nucleus basalis activity.

INTRODUCTION

The major source of cholinergic projections to the neocortical mantle arises from neurons located in the basal forebrain, including the ventromedial aspect of the globus pallidus and the dorsal aspect of the substantia innominata. This region is called the Ch4 sector of the rodent central cholinergic system and includes the nucleus basalis (NB) (Mesulam et al., 1983). Only one third of NB projection neurons are cholinergic. One third are GABAergic, and the remaining third are uncharacterized. NB neurons project ipsilaterally to all of the neocortex, as well as the amygdala and the reticular nucleus of the thalamus (Mesulam et al., 1983; Levey et al., 1987). NB receives inputs from the amygdala, ventral tegmentum, frontal cortex, hypothalamus, and from a number of brainstem nuclei (Haring and Wang, 1986).

Several lines of evidence suggest that the cholinergic projections from NB to the cerebral cortex plays an important role in gating cortical plasticity. Both lesions of NB and cholinergic antagonists are sufficient to disrupt map plasticity in visual and somatosensory cortex. Bear and Singer showed that depletion of acetylcholine and norepinephrine by 6-hydroxydopamine (6-OHDA) prevents the ocular dominance (OD) shift that normally follows temporary eyelid suture (Bear and Singer, 1986). Continual application of muscarinic antagonists also blocked OD plasticity (Gu and Singer, 1993). Cholinergic modulation is also required for map plasticity in adult animals. Excitotoxic lesions of NB are sufficient to inhibit cortical reorganization following digit amputation or nerve section (Juliano et al., 1991; Webster et al., 1991a). Lesion quality has been substantially improved with the development of the cholinergic immunotoxin IgG 192-

saporin (a monoclonal antibody to the low-affinity NGF receptor linked to the ribosomal toxin saporin). These precise lesions prevent the plasticity that results from trimming all but two whiskers in adult rats (Sachdev et al., 1998; Baskerville et al., 1997) or from follicle removal in newborn rats (Zhu and Waite, 1998). These experiments provide strong evidence that NB is necessary for cortical map reorganizations in both young and adult animals.

Cholinergic lesions and antagonists disrupt learning and memory in both animals and humans (see (Hasselmo, 1995) for review). Cholinergic lesions in rats cause performance decrements in tasks involving short-term memory, spatial navigation, and passive avoidance (Steckler et al., 1995; Riekkinen et al., 1992; Winkler et al., 1995). Butt and Hodge showed that NB lesions have significant effect on the acquisition phase of a visual discrimination task, but only a modest effect on performance in pre-trained rats (Butt and Hodge, 1995). Consistent with an important role of cholinergic modulation in learning, microdialysis studies have shown that acetylcholine release is enhanced during acquisition of an operant behavior, but not during performance in pretrained animals (Orsetti et al., 1996). Acquas and colleagues demonstrated that extracellular acetylcholine levels are increased by novel stimuli and by stimuli associated with footshock, but are not increased by the same stimuli in habituated animals (Acquas et al., 1996).

Single unit recordings in rats, rabbits, and monkeys have shown that NB neurons respond to behaviorally arousing stimuli (Pirch, 1993; Richardson and DeLong, 1991; Whalen et al., 1994). Richardson and Delong demonstrated that an individual NB neuron can respond to both aversive and rewarding stimuli of different modalities. The response

of these neurons is often graded by the size of the reward. NB neurons can be conditioned to respond to innocuous stimuli that become associated with reward. The minimum latency for NB activation is approximately 50 msec. Thus acetylcholine released in the cortex follows behaviorally arousing stimuli. It has been proposed that acetylcholine serves as a reinforcement signal to guide cortical plasticity (Singer, 1986; Weinberger, 1993).

This hypothesis has been supported by a series of experiments which demonstrate that pairing of electrical stimulation of NB or iontophoresis of acetylcholine with sensory stimuli is sufficient to generate cortical plasticity. Hennevin and colleagues recorded the responses of auditory cortex neurons before and after NB stimulation paired with a pure tone chosen to evoke a consistent cortical response (Hars et al., 1993; Edeline et al., 1994b; Edeline et al., 1994a). Pairing increased the cortical response by up to 100% in the hemisphere ipsilateral to the stimulation site, without a significant increase in the contralateral hemisphere. In these experiments, NB-induced plasticity could be generated in awake, anesthetized or sleeping rats. Bakin and Weinberger showed that a similar stimulation paradigm results in systematic shifts of frequency receptive fields, which can last up to 30 minutes (Bakin and Weinberger, 1996). Metherate and Ashe showed that the plasticity is much decreased when cortical input and NB stimulation are separated by more than a second (Metherate and Ashe, 1991; Metherate and Ashe, 1993) . Atropine, a cholinergic antagonist, is able to block the plasticity generated by NB stimulation. Direct iontophoresis of acetylcholine or cholinergic agonists can also generate plasticity that is specific to the paired frequency (Metherate and Weinberger, 1990; Metherate and Weinberger, 1989; McKenna et al., 1989). NB stimulation has been

used to generate plasticity in somatosensory cortex of rats, cats and raccoons (Tremblay et al., 1990; Webster et al., 1991b; Howard and Simons, 1994). These results clearly demonstrate that cholinergic modulation can profoundly affect short term-plasticity.

Recent experiments have expanded these findings to explore the long-term effects of repeated daily pairing of NB stimulation with tonal stimuli (chapters three and four). Electrical stimulation of NB with tonal stimuli each day for a month generates long-term (>24 hours) reorganization of the cortical map of frequency. The maximum following rate of cortical neurons (~10 pps) is increased or decreased depending on the repetition rate paired with NB stimulation (5 vs. 15 pps). Interestingly, significant temporal plasticity was only observed when the spectral feature (carrier frequency) of the tone train was randomized. These results suggest that stimulus features do not shape cortical plasticity independently.

In this study several features of the auditory stimulus were systematically varied to further explore how the statistics of the sensory input paired with cholinergic modulation guide reorganization of A1 tuning curves and frequency maps.

METHODS

NB stimulating electrodes were implanted in thirty-four pentobarbital anesthetized (50 mg/kg) rats (~300 g). The rats received prophylactic treatment with ceftizox antibiotic (20 mg/kg), dexamethazone (4 mg/kg) and atropine (1 mg/kg). Platinum bipolar stimulating electrodes (SNE-200, Rhodes Medical) were lowered 7mm below the cortical surface 3.3 mm lateral and 2.3 mm posterior to bregma, and cemented into place using sterile techniques approved under UCSF animal care protocols. Three bone screws were used to anchor the electrode assembly. Leads were attached to screws over the cerebellum and cortex so that global EEG could be monitored.

After two weeks of recovery, one of four classes of tonal stimuli were paired with a 200 msec of NB electrical stimulation in a sound-shielded, calibrated test chamber (five days/week) for one month. Animals were placed in a 25 by 25cm wire cage in the middle of 60 by 70cm box lined with 3 inch acoustic foam. The cage was positioned 20 cm below the audio speaker. A small 4-pin connector attached to a swivel was used to record global EEG and deliver current. Each animal received three to five hundred pairings of tones and NB stimulation. Interstimulus intervals varied randomly from 10 to 30 seconds. Ten rats received NB stimulation paired with a 70dB SPL tone with a fixed frequency (4, 9, 19 kHz). In five rats, two different randomly interleaved tone frequencies were paired with NB stimulation (4 & 14 or 9 & 19 kHz). In four rats, a train of six short 9 kHz tones presented at 15 pulses per second (pps) were paired with NB stimulation. In ten rats, trains of short tones with varying tone frequency (1.3, 2, 3, 5, 9, 14, or 19 kHz) were interleaved. The tone frequency was constant within each

train. The repetition rate of the tones was fixed for each animal (5, 7.5, and 15 pps, n = 4, 2, and 4 rats, respectively). All tones had 3 msec onset and offset ramps.

To establish the specificity of NB pairing, many animals were also stimulated with other tones that were not paired with NB stimulation. Half of the animals in the one-frequency group were also presented, on the same schedule, with two other tone frequencies (see table). There were no unpaired stimuli delivered to the 9 kHz/15 pps rats. The multiple frequency train groups heard one of each of the multiple frequencies tone pips presented in isolation without NB stimulation, as often as they heard each train paired with NB stimulation.

When simple unmodulated tones were used as the auditory stimulus, electrical stimulation began 50 msec after tone onset in half the experiments and 200 msec before tone onset in the other half. When tone trains were used, stimulation occurred simultaneously with the onset of the fourth tone in trains. In four animals, 19 kHz tones were presented ten seconds after each NB stimulation. The current level (70-150 μAmp) was chosen to be the minimum necessary to desynchronize the EEG during slow wave sleep for 1-2 seconds. Stimulation consisted of 100 pps capacitatively coupled biphasic pulses of 0.1msec duration. Several microdialysis studies have shown that this stimulation paradigm results in release of cortical acetylcholine. Either cholinergic antagonists or lesions of the cholinergic cells in the NB with IgG 192-saporin are sufficient to block the plasticity generated by NB stimulation (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998). Tonal and electrical stimuli did not evoke any observable behavioral responses (i.e. did not cause rats to stop grooming or awaken, if sleeping).

Cholinergic neurons of the NB were selectively destroyed in two animals by infusion of 2.5 μg of 192 IgG -saporin (Chemicon) into the right lateral ventricle during the surgery to implant the NB stimulating electrode. Injections were made with a 10 μL hamilton syringe guided by stereotaxic coordinates (AP -1.0, ML +4.5, DV -4.5 mm relative to bregma). The 5 μL volume was delivered over 5 minutes and the syringe was kept in place for an additional five minutes. The 192 IgG monoclonal antibody binds to the low-affinity nerve growth factor receptor on the surface of cholinergic NB neurons and is internalized. The conjugated toxin leads to cell death. Lesioned animals were given 5-10 mL of saline i.p. daily during the recovery period. Standard AChE staining techniques were used to confirm cortical depletion.

This study is based on neural data collected from 2,359 microelectrode penetrations into the right primary auditory cortex in 46 adult female Sprague-Dawley rats. Surgical anesthesia was induced with sodium pentobarbital (50 mg/kg). Throughout the surgical procedures and during the recording session, a state of areflexia was maintained with supplemental doses of dilute IP pentobarbital (8mg/ml). The trachea was cannulated to ensure adequate ventilation and to minimize breathing-related noises. The skull was supported in a head holder which left the ears unobstructed. The cisternae magnum was drained of CSF to minimize cerebral edema. After reflecting the temporalis muscle, auditory cortex was exposed via a wide craniotomy and the dura mater was resected. The cortex was maintained under a thin layer of viscous silicon oil to prevent desiccation. The location of each penetration was reproduced on a 40X digitized image of the cortical surface, and sited with reference to the surface microvasculature.

The primary auditory cortex was defined on the basis of its short latency (8-20 msec) responses and its continuous tonotopy (preferred tone frequency increased from posterior to anterior). Responsive sites that exhibited clearly discontinuous best frequencies AND either long latency responses, unusually high thresholds, or very broad tuning were considered to be non-A1 sites. Penetration sites were chosen to avoid damaging blood vessels while generating a detailed and evenly spaced map. The boundaries of the map were functionally determined using non-responsive and non-A1 sites.

Recordings were made in a shielded, double-walled sound chamber (IAC). Action potentials were recorded simultaneously from two Parylene-coated tungsten microelectrodes (2MΩ at 1kHz) that were lowered orthogonally into the cortex to a depth of ~550 μm (layers IV/V). The neural signal was filtered (0.3 to 8 kHz) and amplified (1000X). Action potential waveforms were recorded whenever a set threshold was exceeded, allowing off-line spike sorting using Autocut software. Although most responses in this study represented the spike activity of several neurons, single units were separated when possible confirming that single units exhibited tuning that was qualitatively similar to mulit-unit response samples. To minimize experimenter-induced sampling bias the experimenter was blind to the frequency(ies) paired with NB stimulation.

Monaural stimuli were delivered to the left ear via a calibrated ear phone (STAX 54) positioned just inside the pinnae. Frequencies and intensities were calibrated using a B&K sound level meter and a Ubiquitous spectrum analyzer. Two types of stimuli were generated using Brainwave. Auditory frequency response tuning curves were determined

by presenting 45 frequencies spanning 3-4.5 octaves centered on the approximate best frequency of the site. Each frequency was presented at 15 intensities ranging between 0 and 75dB (675 total stimuli). Tuning curve tones were randomly interleaved and separated by 500msec. All tonal stimuli used in this study were 25msec long, including 3 msec rise and fall times.

Tuning curve parameters were defined by an experienced blind observer using custom software that displayed raw spike data without reference to the frequencies and intensities which generated the responses. For each tuning curve, best frequency, threshold, bandwidth (10, 20, 30 and 40 dB above threshold), and latency data were recorded. Best frequency (BF) is the frequency that evokes a consistent neural response at the lowest intensity. The minimum latency was defined as the time from stimulus onset to the earliest consistent response for all 15 intensities, for the three frequencies that were nearest the BF (45 stimuli).

Voronoi tessellation was used to generate polygons from a set of non-uniformly spaced points such that every point in the polygon was nearer to the sampled point than to any other. Tesselation allowed area to be estimated from discretely sampled penetrations by assuming that each location on the cortical surface had the response characteristics of the closest sampled point. The percent of the cortical surface responding to a given stimulus was estimated by adding all of the areas of the penetrations that included the stimulus within their receptive field, divided by the total responsive (A1) area. This measure is fairly insensitive to non-homogenous sampling densities because each penetration in a more densely sampled area has a proportionally smaller contribution, and vice versa.

RESULTS

Stimulation of NB paired with tonal stimuli is sufficient to significantly enhance cortical responses. These enhanced responses have been described in two ways: as an increase in the strength of the evoked response to the paired stimulus, and as an increase in the number of cortical neurons engaged by the paired stimulus (Hars et al., 1993; Edeline et al., 1994b; Edeline et al., 1994a; Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998). The number of neurons participating in the cortical representation of a conditioned tone can be increased by 1) receptive field plasticity that shifts the best frequency (BF) of primary auditory cortex (A1) neurons towards the paired frequency; 2) decreased selectivity of frequency tuning, and/or 3) expansion of primary auditory cortex boundaries. In this study, we examined the cortical plasticity generated by NB stimulation paired with nine different stimulus sets, and observed that cortical representations were enhanced by different combinations of all three of these effects, dependent on the nature of the auditory stimuli.

Detailed maps of A1 were reconstructed for 9 naïve rats and 37 NB-stimulated rats. A1 was distinguished from surrounding auditory fields by its tonotopy and characteristic short-latency responses. Continuous topography and an even distribution of preferred frequencies was observed in each control animal (Figure 5-2 & 5-4a-d). Rat A1 tuning curves were V-shaped and had monotonic intensity response functions (Sally and Kelly, 1988). The average bandwidths for A1 responses in naïve animals were 0.97

± 0.4 and 1.42 ± 1.0 octaves (mean ± SD) at 10 and 30 dB above threshold, respectively (see chapter two).

NB Stimulation Paired with Single Tone Frequency

Repeated electrical activation of NB paired with an unmodulated pure tone (4, 9, or 19 kHz) five days per week for one month was sufficient to generate substantial reorganization of the A1 frequency map (Figure 5-3 & 5-4 i-n). The cortical response to the paired frequency was enhanced in all ten animals. After pairing, an increased number of penetrations had BF's near the paired frequency, compared to naïve controls. Clustering of BF's indicates that the frequency tuning of cortical neurons were reorganized, such that BF's shifted toward the paired frequency. The pattern of BF shifts varied substantially from animal to animal. In two of the 9 kHz paired animals, neurons predominately representing higher frequencies appeared to shift their tuning curves lower, while the opposite appears to have occurred in the other two rats. Thus, the cortical representation of the paired frequency was reliably increased as a result of BF shifts toward the conditioned frequency, while there was significant variability in the specific map changes that led to the distortions.

The number of cortical neurons activated by the paired stimulus was also increased by decreasing the selectivity of frequency tuning. Pairing NB stimulation with a fixed tone frequency increased receptive field width by up to 20% (Figure 5-5). Plasticity of best frequency and bandwidth combined to increase the percent of A1 that responded to the paired tone frequency (Table 2). The percent of cortex responding to the paired frequency was increased by up to two-fold, compared to naïve controls. The

BF shifts that resulted in increases in the percent of cortex responding were greatest for frequencies near the paired frequency. Interestingly, increased bandwidth changes in A1 were not frequency-specific.

In addition to increases in the relative proportion of A1 that responded to a specific tonal stimulus paired with NB activation, the absolute area of A1 was also significantly increased by pairing NB activation with tonal stimuli (Figure 5-6). This increase in A1 area was based on the well-established functional definition that the primary auditory field has phasic, short-latency responses to tones and a continuous tonotopy. The three observed classes of cortical reorganization combined to result in an up to three-fold increase in the cortical area activated by the paired stimulus compared to controls (Table 2).

In four of the ten single tone experiments, two frequencies were presented with equal probability as the paired frequency, but were not paired with NB stimulation (Figure 5-7). These additional frequencies did not appear to affect the tuning curves shifts toward the paired frequency or the expansion of the borders of A1, but prevented the 20% decrease in frequency selectivity that followed single-tone pairing without unpaired frequency stimulation in A1 in all recorded rats (Figure 5-5).

Nucleus Basalis Lesions

Specific immunolesions of neurons within NB were used to demonstrate that the plasticity evoked by NB stimulation was specific to the cholinergic system. The toxin, 192 immunoglobulin G-saporin, destroys the cholinergic NB neurons that project to the

cortex. The lesion does not kill the GABAergic or other neurons that make up two-thirds of the projection from NB to cortex in the intact animal, and also spares the cholinergic neurons that project to the amygdala. Electrical stimulation of the immunolesioned NB paired with 19 kHz did not result in a significant increase in the number of neurons responding to 19 kHz (32 ± 0.3% versus 28 ± 4%, p> 0.5) and did not significantly increase the functionally defined area of A1 (2.4 ± .1 mm^2). Frequency tuning bandwidths were somewhat increased following NB immunolesioning and basal forebrain stimulation.

Relative Timing of Electrical and Auditory Stimulation

To investigate the importance of the relative timing between NB activation and tone onset, two different timings were used (stimulation onset 50 msec after or 200 msec before tone onset). These two timings resulted in indistinguishable plasticity effects, and the two timings were grouped in all further analyses. Four animals received NB stimulation that was separated from the auditory stimulus by ten seconds. In these animals, the area of A1 was not significantly different from controls and tuning curves shifted much less compared to the shifts observed following nearly simultaneous pairing of NB stimulation and tonal stimuli (Figures 5-6&5-8). Mean bandwidth at 10dB above threshold was increased by 20% compared to controls. Although this decrease in selectivity resulted in an increase in the percent of cortex that responded to the paired stimulus, this increase was not selective for the paired frequency (TABLE 2). Thus, a

delay between NB stimulation and auditory stimulation significantly degraded the magnitude and selectivity of the NB-induced reorganization.

NB Stimulation Paired with Two Tone Frequencies

In five animals, two different tone frequencies were randomly interleaved and each paired with NB stimulation (4&14 and 9&19 kHz). In three animals, tuning curves appeared to shift preferentially toward one of the two paired frequencies (Figure 5-7). In the other two animals, many tuning curves appear to have shifted such that they had BF's were ultimately centered between the two paired frequencies. Pairing with two frequencies significantly increased the selectivity of frequency tuning. Mean bandwidth was decreased to 75% of control values ($p < 0.0001$, Figure 5-5). A1 Area was not significantly different from naïve controls. The mean percent responding to each of the paired frequencies was not significantly different from controls.

NB Stimulation Paired with Modulated Auditory Stimuli

Pairing a train of six 9 kHz tones presented at 15 pps with NB stimulation resulted in a much larger effect on bandwidth compared to an unmodulated tone paired with NB stimulation. The average bandwidth at 10dB above threshold was almost 60% greater than naïve controls (Figure 5-5). This significant decrease in frequency selectivity led to a plasticity effect that was less selective for 9 kHz compared to the unmodulated tone pairing. Most of the tuning curve shifts towards 9 kHz occurred as a result of increases in the preferred frequencies of previously low frequency neurons

(Figure 5-9). The combination of wide tuning curves with significant BF shifts resulted in a very high percent of cortex responding to a 50dB 9 kHz tone (72% compared to 43% and 32% for unmodulated 9 kHz [$p < 0.005$] and controls [$p < 0.0001$] , respectively). The mean surface area of A1 was also significantly increased compared to controls (Figure 5-6), resulting in a more than three-fold increase in the number of neurons responding to the paired frequency.

To examine the time course of NB-facilitated plasticity, two animals were mapped after only one week of pairing, instead of the month of pairing delivered in all of the other experiments in this study. The tuning curves shifts toward 9 kHz were somewhat less than was observed after a month of pairing with the 9kHz 15 pps train, but were comparable to the shifts observed after a month of pairing with an ummodulated 9 kHz tone (Figure 5-10). One week of pairing increased the percent of cortex responding to a 50dB 9 kHz tone to 51% ($p < .05$, compared to controls). Interestingly, the mean bandwidth at 10dB above threshold was only increased by only 13%. Thus, most of the increase in percent responding resulted from tuning curve shifts, not tuning curve widening. A1 area was not affected (2.0 ± 0.3 mm^2)

In ten animals, stimuli with both temporal modulation and spectral variability were paired with NB activation. Tone frequency was selected randomly from seven possibilities, but was constant within each train. Each animal heard tones at only one repetition rate (5, 7.5, or 15 pps). Pairing multiple frequencies with NB stimulation had no systematic effect on the organization of the A1 frequency map (Figure 5-11). The area of A1 was not increased by NB pairing with trains of multiple frequencies. The plasticity of frequency selectivity tuning was significantly influenced by the repetition

rate of the train paired with NB stimulation. Bandwidth was increased by 16, 27 and 35% after pairing with 5, 7.5 and 15 pps trains of multiple frequencies (Figure 5-5). The decrease in spectral selectivity resulting from pairing multi-frequency 15 pps trains was significantly less than resulted from pairing 15 pps trains with a constant 9 kHz carrier frequency ($p < 0.0005$). Thus, plasticity of tuning curve bandwidth induced by NB stimulation is dependent on both spectral variability and temporal modulation of the paired auditory stimuli.

Plasticity of Other Response Characteristics

Minimum response latency was increased by three of the seven classes of stimuli paired with NB stimulation. The mean minimum neural response latency for naïve animals was 15.1 ± 0.2 msec. Pairing NB stimulation with two frequencies or with multiple frequencies modulated at 5 or 7 pps increased latencies to 16.4 ± 0.3, 16.2 ± 0.3, and 16.6 ± 0.4 msec, respectively ($p < 0.0005$). These three classes all had variable tone frequencies and slow modulation rates. NB stimulation paired with stimuli with either a rapid modulation rate or invariant carrier frequency did not significantly alter the minimum latency (15.0 ± 0.3, 14.8 ± 0.3, $15.0 \pm .2$ msec for single, 9 kHz train at 15 pps, and multiple frequency 15 pps train, respectively).

The mean number of spikes evoked per tone was statistically different from controls after pairing NB stimulation with trains of 9 kHz tones presented at 15 pps (2.1 ± 0.1 versus 3.6 ± 0.9; mean $\pm$ standard error, $p < 0.05$), but not after pairing any of the

other stimulus sets used in this study. Evoked response to tonal stimuli was also increased when NB stimulation was separated from the auditory stimuli by ten seconds (2.1 ± 0.1 versus 3.0 ± 0.4; mean $\pm$ standard error, $p < 0.05$). Previous studies using cholinergic modulation observed highly specific changes in the number of spikes evoked by different tones within a neuron's receptive field. In some cases the neural response to frequencies within one-fourth of an octave were facilitated, while the responses to other nearby frequencies were inhibited (Bakin and Weinberger, 1996; Metherate and Weinberger, 1990; Metherate and Weinberger, 1989; McKenna et al., 1989). The above analysis is focused on the receptive field as a unit and would not pick up changes in the response strength to frequencies within the tuning curve. To determine if such precise effects resulted from our long-term pairing of NB activation with tonal stimuli, we also examined the number spikes evoked as a function of frequency for every tuning curve. We observed no consistent peak at the paired frequency in individual sites or in the population as a whole (data not shown). Minimum stimulus thresholds showed no consistent change as a result of pairing NB stimulation with any of the auditory stimuli used in this study.

DISCUSSION

Merzenich and colleagues observed plasticity in auditory and somatosensory cortex that was highly dependent on the nature of behaviorally relevant stimuli encountered during extended operant training of owl monkeys (Recanzone et al., 1992d; Recanzone et al., 1992b; Jenkins et al., 1990). In this study, we used electrical stimulation of NB to activate cortical plasticity mechanisms, and varied the paired sensory stimuli to explore the relationship between the statistics of the sensory input and the class, direction, and magnitude of cortical reorganization. We observed that stimulus repetition rate and spectral variability systematically altered a number of cortical response parameters, including "best" frequency (BF), tuning curve width (frequency tuning bandwidth), area of functionally defined A1, cortical excitability (spikes/tone), neuronal response following rate, and minimum response latency (Table 3).

NB-Induced Cortical Map Reorganizations

Electrical activation of nucleus basalis paired with tonal stimuli was sufficient to generate significant reorganization of the A1 map of frequency. The preferred frequency of cortical neurons systematically shifted toward the paired frequency, such that the number of cortical neurons responding to the stimulus paired with NB stimulation was doubled. Our results indicate that the responses of tens of thousands of A1 neurons are altered by NB stimulation in passively stimulated animals.

Although the NB-induced increase in the size of the cortical representation was quite reliable, the direction of the tuning curve shift appears to be a "free parameter". In some cases high BF neurons shifted lower and in others low BF neurons shifted higher. This variability was particularly clear when two different frequencies were paired with NB stimulation, where tuning curves either shifted to one of the two frequencies or to a point between them. Small differences in initial starting conditions due to individual experiential histories may have significant effects when competitive processes are involved, and may be responsible for this variability across animals.

To demonstrate that cholinergic mechanisms are involved in this expansion, identical stimulation was delivered to rats with bilateral saporin-induced lesions of the cholinergic neurons that project from NB to the cortex. Consistent with previous reports that an intact NB is required for cortical map reorganization, no increase in the representation of the paired stimulus was observed in these cases (Juliano et al., 1991; Webster et al., 1991a; Sachdev et al., 1998; Baskerville et al., 1997; Zhu and Waite, 1998). This result does not rule out the possibility that the electrical stimulation of the basal forebrain used in this study resulted in a global arousal state that was not specific to NB activity, and that no plasticity occurred as a non-specific effect of NB lesions. We do not favor this explanation, however, because there is no evidence that the currents used in this study were behaviorally arousing. The rats used in our experiments did not stop grooming, eating or sleeping when the basal forebrain was stimulated. Weinberger and colleagues have also reported that similar stimulation produced no behavioral or autonomic responses (Bakin and Weinberger, 1996).

The NB-induced plasticity could also be reduced by separating NB stimulation and tone onset by ten seconds. This result has confirmed previous reports that NB activity must occur within a second of a sensory stimulus to effectively enhance the cortical representation of the stimulus (Metherate and Ashe, 1991; Metherate and Ashe, 1993).

Surprisingly, NB stimulation was sufficient to increase the total functioanlly defined area of the primary auditory cortex. The magnitude of this expansion was determined by the particular auditory stimuli that were paired with NB stimulation. It appears that this apparent expansion of A1 was dependent on the degree of spectral variability. The estimated area of A1 was fifty percent larger in rats in which only one frequency (modulated or unmodulated) was paired with NB stimulation; pairing two or seven different tone frequencies resulted in no significant increase. Merzenich and colleagues also observed that the absolute size of a cortical area (3b) could be apparently expanded following some types of behavioral training (Recanzone et al., 1992d; Jenkins et al., 1990; Merzenich et al., 1990). Recanzone, et al. recorded no increase in the size of the hand map in cortical area 3b as a result of extensive experience with a highly modulated stimulus (Recanzone et al., 1992d). In contrast, the hand map was increased by more than fifty percent after monkeys were trained to maintain light contact with a rotating disk (Jenkins et al., 1990). These results support previous findings that responses to important stimuli can emerge in cortical sectors that were initially unresponsive to these stimuli, and confirm that information in the sensory input shapes cortical reorganization.

NB-Induced Plasticity of Frequency Selectivity

Receptive field size was very sensitive to the auditory stimulus paired with NB activation. Frequency tuning bandwidth was increased by up to 60% or decreased by up to 25% simply by pairing different classes of tonal stimuli with identical NB stimulation. The seven classes of stimuli increased bandwidth as a function of both increased repetition rate and decreased spectral variability. Recanzone, Merzenich, and colleagues observed a similar relationship following operant training of monkeys. Cortical receptive field size were decreased by practicing tasks involving stimuli delivered to different locations on the receptor surface (cochlea or skin), and were increased by training on a task requiring detection of changes in the modulation rate of stimuli delivered to an invariant skin location (Jenkins et al., 1990; Recanzone et al., 1992d; Recanzone et al., 1992b). Our results extend these observations by independently varying spectral (spatial) variability and repetition rate. The finding that the statistics of the sensory input paired with NB stimulation generates analogous differential receptive field plasticity without behavioral training indicates that simple rules operate in the cortex to generate "useful" changes in circuitry based on the statistics of sensory stimuli marked by NB activity.

Recanzone and colleagues observed that training-induced changes in receptive field size were consistent with the operation of Hebb-like synapses driven to change by temporally coherent inputs in a competitive cortical network. They argued that increased receptive fields result from temporally synchronous activity generated in response to low-frequency (10-20 Hz) stimulation at an invariant location of the

receptor surface, while decreased receptive fields resulted from asynchronous cortical activity in response to stimuli that move across or are applied at inconsistent receptor locations (skin or cochlea). By systematically varying both spectral variability (1, 2 or 7 frequencies) and repetition rate (1, 5, 7.5, and 15 pps), our study strengthens the argument that receptive field size is determined by the structure of temporal correlations across the cortical surface.

The observation that bandwidth plasticity was not specific to the paired frequency is also consistent with plasticity induced by behavioral training in monkeys. The expansion of somatosensory receptive fields following training on a task involving vibration of only one digit was observed on several neighboring digits as well. Thus, in that model receptive field plasticity was not necessarily limited to neurons engaged directly by the sensory stimulus, but can influence the tuning properties of neurons located up to a millimeter away.

NB stimulation has been shown to increase the number of stimulus evoked spikes (Bakin and Weinberger, 1996; Edeline et al., 1994a; Edeline et al., 1994b; Tremblay et al., 1990; Webster et al., 1991b). In our paradigm, this increase was sensitive to the features of the auditory stimulus paired with NB stimulation. Of the seven classes of stimuli paired with NB stimulation, only 15 pps trains of 9 kHz tones resulted in a significant increase in evoked response strength (spikes/tone). Recanzone, et al. also observed an increase in evoked response after training monkeys on a task that involved the analogous tactile stimulus (a 20 Hz vibration of a single digit segment). Thus, our findings are consistent with previous demonstrations that cortical plasticity is strongly dependent on particular features of sensory inputs.

The maximum following rate of A1 neurons were decreased or increased by pairing NB stimulation with 5 or 15 pps tone trains, respectively (Chapter Four). In agreement with the documented correlation between following rate and minimum response latency, we observed that latency was significantly increased following pairing with 5 or 7.5 pps multiple frequency tone trains (Chapter Two; Brosch and Schreiner, 1997; Schreiner et al., 1997; Raggio and Schreiner, 1994).

Using an acute preparation, Metherate and Ashe observed that short-term cortical plasticity was dependent on the relative timing of NB stimulation and cortical input (Metherate and Ashe, 1993; Metherate and Ashe, 1991). Little or no plasticity was observed in those experiments if NB activity and stimulus were separated by more than a second. By delivering NB stimulation at three different times relative to tone onset, we have confirmed the importance of the timing of NB activity. Plasticity was not obviously different when NB stimulation began either two hundred milliseconds before or fifty milliseconds after tone onset. When three different tones were presented but only one paired with NB stimulation, tuning curves shifted toward the paired frequency and not toward the frequencies that occurred as often but were separated from NB stimulation by 8-30 seconds. When only one frequency was presented, the observed BF reorganization was decreased by the introduction of a ten second delay between NB stimulation and tone onset. Thus our results support previous observations that indicate that the effect of NB stimulation on cortical plasticity lasts at least a few hundred milliseconds, but less than several seconds.

In contrast to the transient effects of a single episode of NB stimulation delivered in most acute preparations, chronic NB stimulation resulted in cortical plasticity that was

long-lasting. All of the data presented in this study was collected from twenty-four to forty-eight hours after the last electrical activation of NB. Thus it appears that NB activity is sufficient to enable short-lived plasticity effects that become long-lasting with extended repetition over the course of days to weeks.

Behavioral Relevance

Nucleus basalis neurons respond vigorously to behaviorally important stimuli, either aversive or rewarding. Our data is consistent with the hypothesis that an important function of this activity is to mark individual events as behaviorally relevant so that cortical plasticity mechanisms can improve the representations of the important stimulus (Ahissar and Ahissar, 1994; Singer, 1986; Weinberger, 1993; Richardson and DeLong, 1991). The temporal precision of NB-induced plasticity would prevent frequent and irrelevant stimuli from interfering with the learning of novel, relevant stimuli. This function is analogous to supervised neural network models used in a variety of applications (i.e. back-propagation), with the exception that this hypothesized "teacher" only provides information about which stimuli are important, not about how to alter connection weights and network dynamics to improve the quality of the sensory representation.

Although it seems obvious that the representation of a pure tone would be improved by increasing the number of neurons tuned for the tone's frequency, in fact the "ideal solution" depends entirely on what information is needed from the stimulus. If an animal is conditioned that a pure tone predicts footshock, there is no way for it to know

what features of the stimulus predict reward (duration, rise time, bandwidth, intensity, frequency, modulation rate, etc.). The fact that animals generalize indicates that they do not assume that all of the features are required. Evolution appears to have shaped brain circuitry such that its default guesses are appropriate based on the evolutionary history of the species (i.e. phyletic memory, see Edelman's Neural Darwinism). These "guesses" may take the form of rules that operate within the brain to extract features of a stimulus that are most likely to contain relevant information based on other features of the stimulus and/or the context the stimulus appears in.

Although the relationship between stimulus representation and information processing is far from understood, different behavioral tasks clearly result in different types of representational plasticity in the cortex (Jenkins et al., 1990; Recanzone et al., 1992d; Recanzone et al., 1992b). The cortical reorganizations observed in this study closely parallel the reorganizations that result from extended behavioral training. The similarity suggests that the sensory input itself provides much of the information about how to improve sensory representations. In this initial study we focused on two stimulus features, repetition rate and spectral variability, and observed that each affected cortical plasticity in a systematic manner. These results indicate that the cortex uses these features to guide several forms of cortical plasticity, including reorganization of feature maps, plasticity of feature selectivity, expansion of a primary sensory cortical field, and increased strength of evoked responses. Additional experiments are needed to further determine how other stimulus parameters shape representational plasticity.

Table 5-1. Summary of Experiments. Brackets denote stimuli that were played in some experiments and not others (see text). Numbers separated by parentheses include two 15 pps 9 kHz animals that were stimulated for only one week.

Table 5-2. Size of cortical sector engaged, expressed as percent of A1 surface responding and as area in mm^2 in NB stimulated animals and naïve controls. Mean ± standard error. * means $p < 0.05$. The numbers in parenthesis is the ratio of paired to control.

Table 5-3. Summary of experimental results. Pluses denote parameters that were increased by NB stimulation paired with a given stimulus class, minuses denote decreases, and a zero indicates no significant difference from naïve controls. The number of symbols indicates the size of the plasticity effect.

Figure 5-1. A) Projections from Nucleus Basalis (NB) to neocortex, reticular thalamus, and amygdala. B) Schematic of auditory/NB pairing paradigm. C) Diagram of A1 tuning curve with relevant features labeled. BF is the frequency that elicits a consistent neural response at the lowest intensity, threshold. BW is the range of frequencies the neurons are responsive to at the specified intensity above threshold.

Figure 5-2. A) Representative BF maps of primary auditory cortex from a naïve adult rat. Each polygon represents one penetration. Color represents each site's best frequency. Non-responsive and auditory responding non-A1 sites are marked with O's and X's, respectively. B) Tuning curve tips for the rat in A).

The tip of each V depicts minimum threshold for each site. Width of the V represents tuning curve width 10dB above threshold. Scalebar = 250 μm.

Figure 5-3. Maps of best frequency with tuning curves for representative rats paired with 4, 9, and 19 kHz tones for A), B) and C) respectively.

Figure 5-4. Every tuning curve for each single frequency experiment, and control data from naïve animals. A-D) Naïve controls. E-H) 4 kHz paired with NB stimulation. I-L) 9kHz paired with NB stimulation. M-N) 19kHz paired with 19 kHz.

Figure 5-5. Mean bandwidth 10 dB above threshold with standard errors. Similar trends were observed for BW20-40.

Figure 5-6. Mean A1 area in mm^2 with standard errors. * means $p < 0.05$.

Figure 5-7. Every tuning curve for each two frequency experiment.

Figure 5-8. Every tuning curve for each NB out of phase (10 seconds) experiment.

Figure 5-9. Every tuning curve for each experiment that paired NB stimulation with trains of short 9 kHz tones presented at 15 pps (one month of pairing).

Figure 5-10. Every tuning curve for each experiment that paired NB stimulation with trains of short 9 kHz tones presented at 15 pps (after only one week of pairing).

Figure 5-11. Maps of best frequency with tuning curves for representative rats that had NB stimulation paired with A) 9 kHz tone pips presented at 15 pps or B) multiple frequency tone pips presented at 15 pps each day for one month.

TABLE 5-1

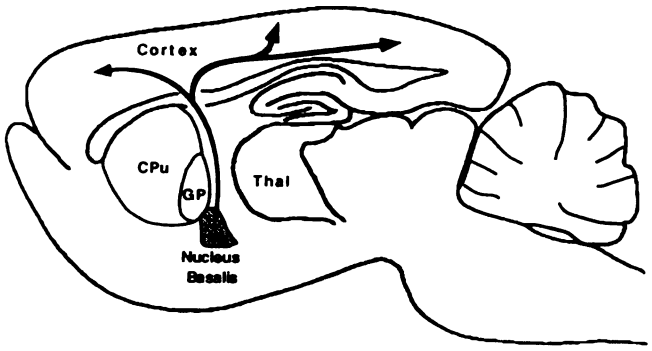
Experiment Group	Auditory stimuli Paired with NB Stimulation	Unpaired stimuli	Number of Rats	Number of A1 Sites
Naive	∅	∅	9	440
One Freq	4 kHz - 200msec 70dB	[2 & 9 kHz]	4	242
One Freq	9 kHz - 200msec 70dB	[4 & 19 kHz]	4	233
One Freq	19 kHz - 200msec 70dB	[4 & 9 kHz]	2	112
Out of phase	∅	19 kHz - 200msec 70dB - 10 sec before NB stimulation	4	206
Two Freq	4 & 14 kHz - 200msec 70dB	9 & 19 kHz	2	90
Two Freq	9 & 19 kHz - 200msec 70dB	4 & 14 kHz	3	119
Lesion	19 kHz - 200msec 70dB - NB immunolesion	∅	2	95
15 pps 9kHz	15 pps train of six tones, 9 kHz - 25msec	∅	4 (6)	224 (332)
15 pps multi	15 pps train of six tones, multiple frequency - 25msec	single tones of multiple frequency	4	223
7.5 pps multi	7.5 pps train of six tones, multiple frequency - 25msec	single tones of multiple frequency	2	92
5 pps multi	5 pps train of six tones, multiple frequency - 25msec	single tones of multiple frequency	4	175

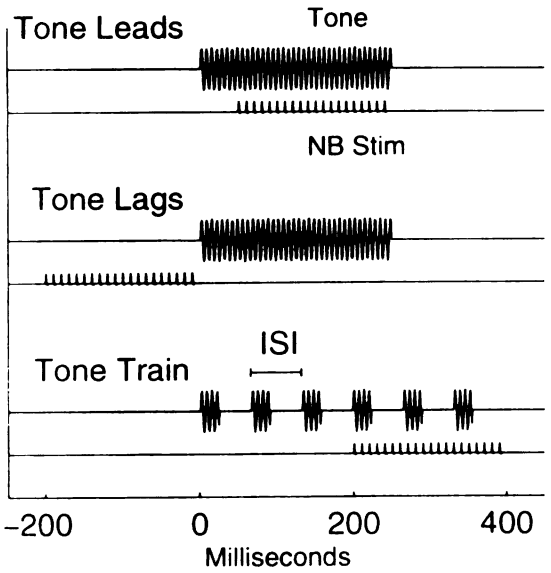
TABLE 5-2

Group	Percent of A1 Responding					A1 Area Responding - mm^2			
	2 kHz	4 kHz	9 kHz	19 kHz		2 kHz	4 kHz	9 kHz	19 kHz
Control	18 ± 4.1	17.6 ± 3.4	18.9 ± 1.8	27.8 ± 3.7		.35 ± .09	.34 ± .08	.36 ± .06	.52 ± .10
4 kHz	23.4 ± 9.1 (1.3)	35.4 ± 4.0* (**2.0**)	28.7 ± 5.2 (1.5)	37.9 ± 3.2 (1.4)		.62 ± .14 (1.8)	1.15 ± .37* (**3.4**)	.98 ± .35* (2.7)	1.19 ± .28* (2.2)
9 kHz	16.2 ± 7.0 (0.9)	26.4 ± 10.4 (1.5)	32.7 ± 5.5* (**1.7**)	30.1 ± 10.5 (1.1)		.45 ± .16 (1.3)	.75 ± .24 (2.2)	1.07 ± .27 * (**3.0**)	1.12 ± .47 (2.1)
19 kHz	9.5 ± 4.7 (0.5)	8.6 ± 4.9 (0.5)	15.1 ± 8.4 (0.8)	47.0 ± 4.7* (**1.7**)		.20 ± .07 (0.5)	.17 ± .08 (0.5)	.31 ± .13 (0.9)	1.07 ± .29* (**2.0**)
19 kHz Out	25.9 ±3.4 (1.4)	28.6 ±5.6 (1.6)	32.9 ±6.9* (1.7)	44.5 ±4.2* (1.6)		.61 ±.13 (1.7)	.71 ±.25 (2.1)	.82 ± .30 (2.3)	1.05 ± .19* (2.0)

TABLE 5-3

Experiment Group	BF Shift	Bandwidth	A1 Area	Spikes/ Tone	Maximum Repetition Rate	Minimum Latency
One Freq	++	+	++	0	n.d.	0
One Freq (w/ unpaired)	++	0	++	0	n.d.	0
Out of phase	+	+	0	+	n.d.	++
Two Freq	++	--	0	0	n.d.	0
Lesion	0	+	0	0	n.d.	0
15 pps 9 kHz	+++	+++	+	++	0	0
15 pps multi	0	++	0	0	+++	0
7.5 pps multi	0	+	0	0	-	++
5 pps multi	0	+	0	0	---	++


Cortex
CPu
GP
Thal
Nucleus
Basalis


Tone Leads
Tone
NB Stim
Tone Lags
ISI
Tone Train
-200
0
200
400
Milliseconds

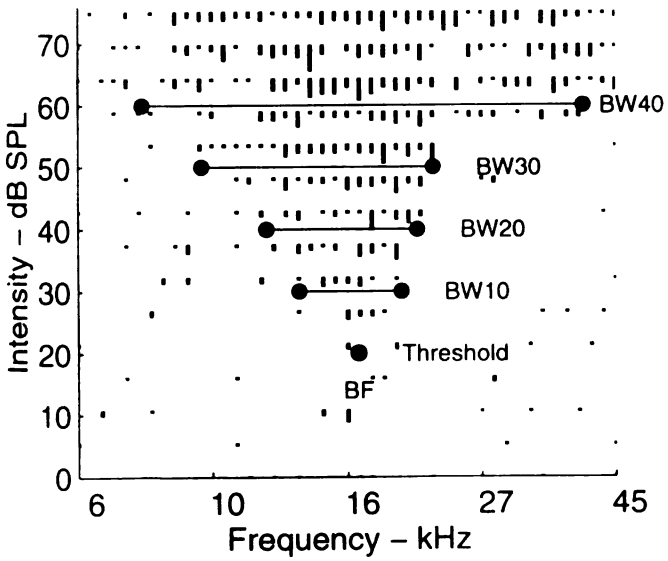

70
60
50
40
30
20
10
0
Intensity – dB SPL
BW40
BW30
BW20
BW10
Threshold
BF
6
10
16
27
45
Frequency – kHz

FIGURE 5-1

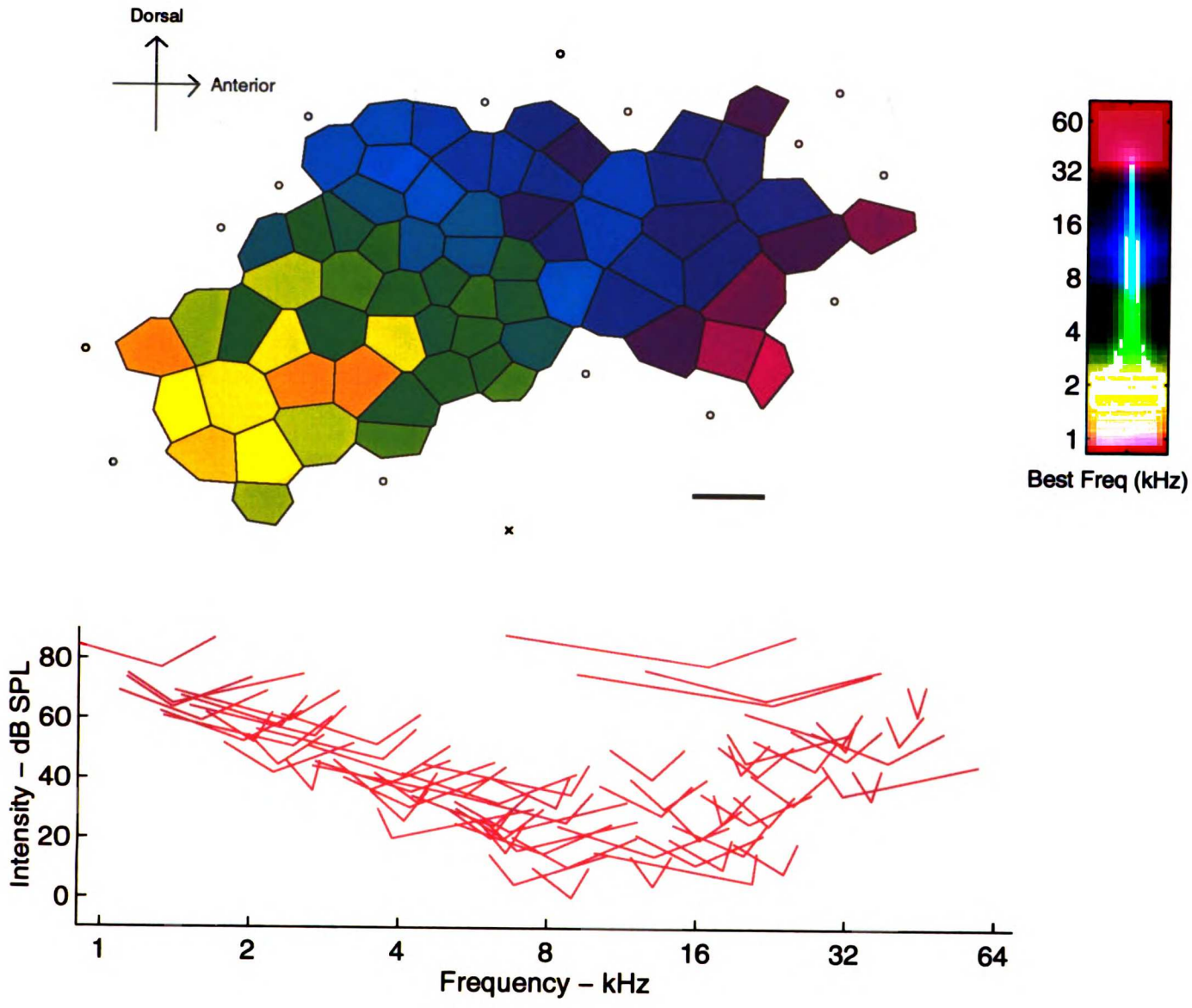

Dorsal
Anterior
60
32
16
8
4
2
1
Best Freq (kHz)
Intensity – dB SPL
80
60
40
20
0
1
2
4
8
16
32
64
Frequency – kHz

FIGURE 5-2

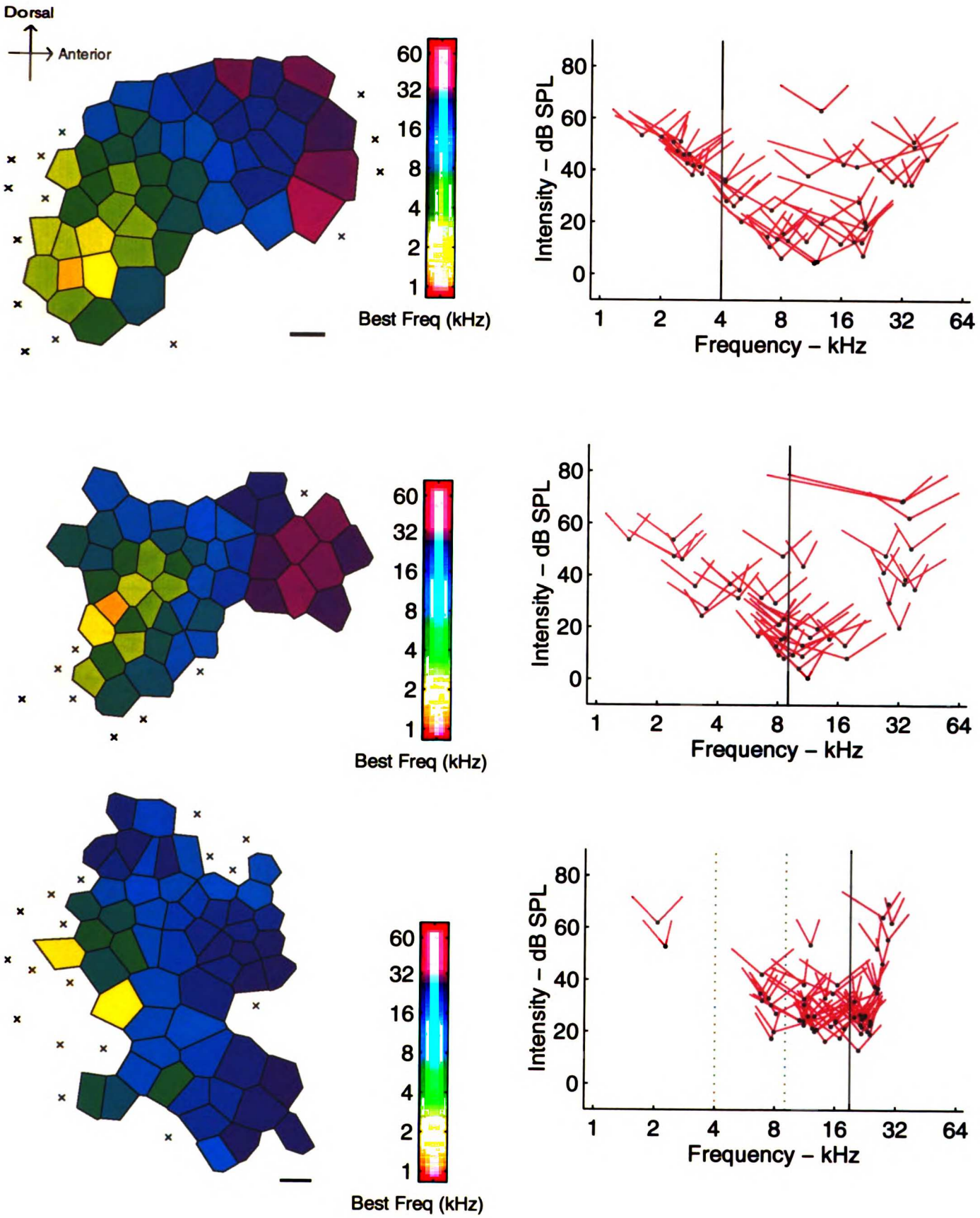

Dorsal
Anterior
60
32
16
8
4
2
1
Best Freq (kHz)
Intensity – dB SPL
80
60
40
20
0
1
2
4
8
16
32
64
Frequency – kHz

FIGURE 5-3

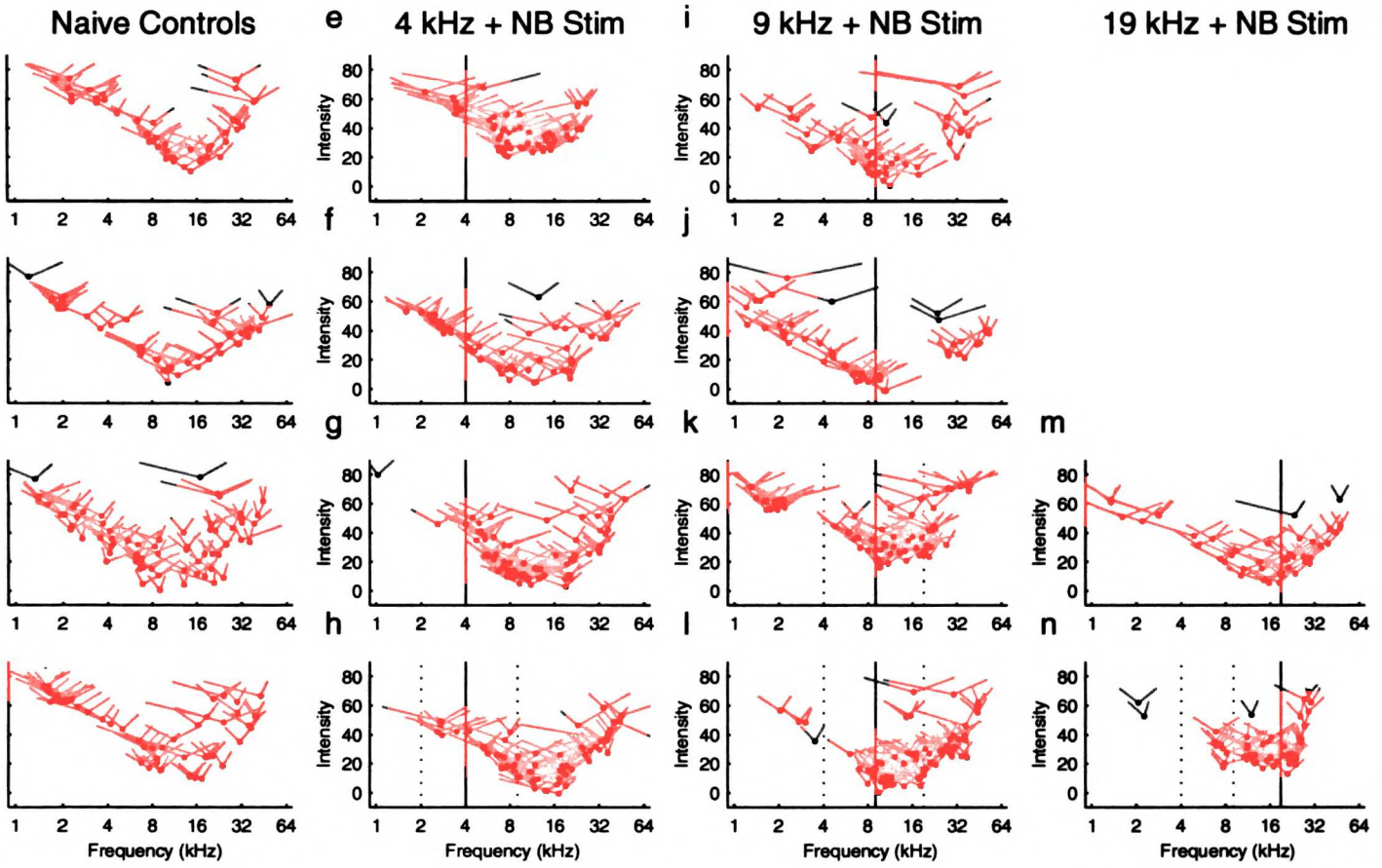

Naive Controls
e
4 kHz + NB Stim
i
9 kHz + NB Stim
19 kHz + NB Stim
f
j
g
k
m
h
l
n
Intensity
Frequency (kHz)
1 2 4 8 16 32 64
0 20 40 60 80

FIGURE 5-4

Bandwidth @ Threshold + 10dB (octave)
1.6
1.4
1.2
1
0.8
0.6
Two Frequencies
One Frequency (+ 2 unpaired)
Control
One Frequency
5 Hz Multi
7.5 Hz Multi
15 Hz Multi
15 Hz 9 kHz

FIGURE 5-5

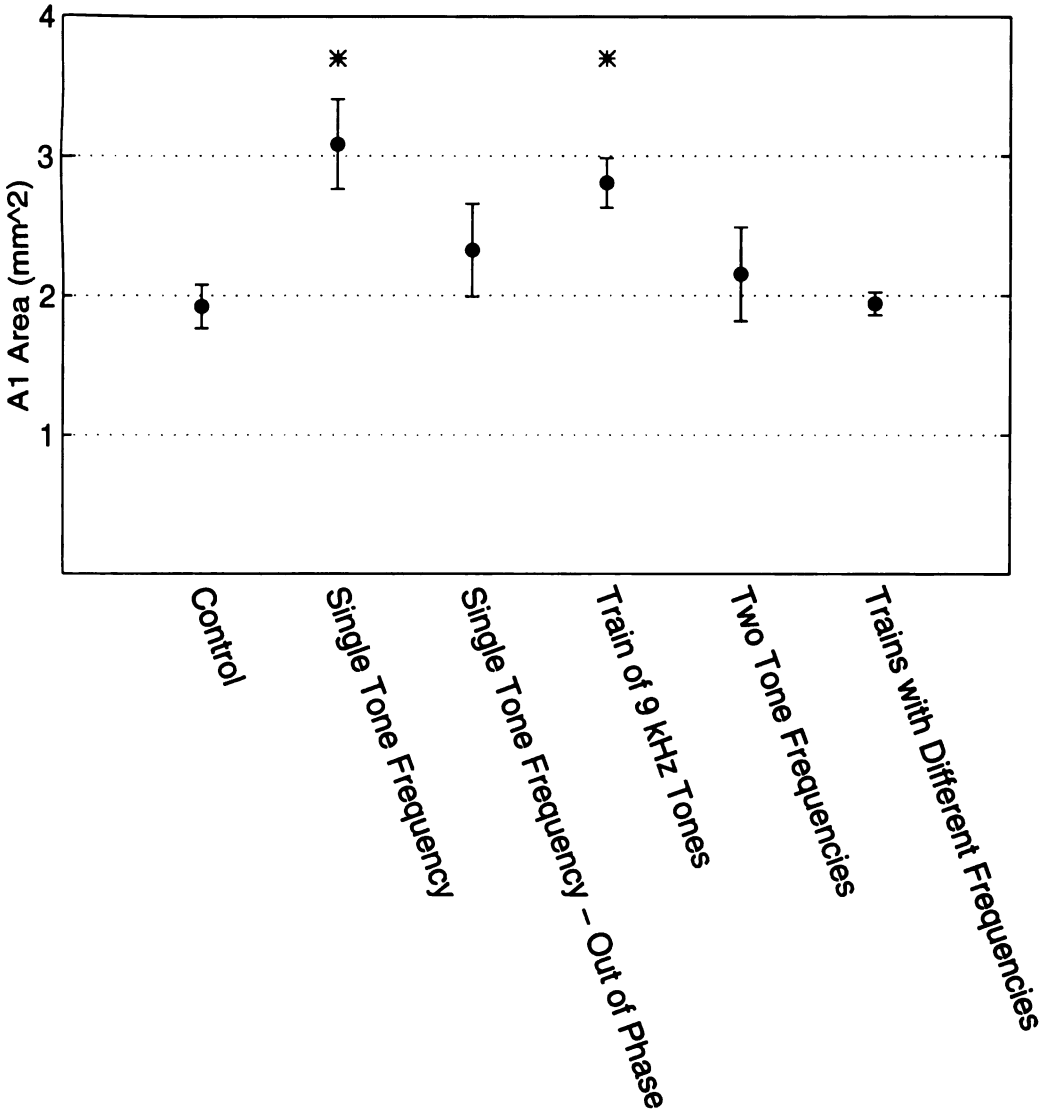

A1 Area (mm^2)
4
3
2
1
Control
Single Tone Frequency
Single Tone Frequency – Out of Phase
Train of 9 kHz Tones
Two Tone Frequencies
Trains with Different Frequencies

FIGURE 5-6

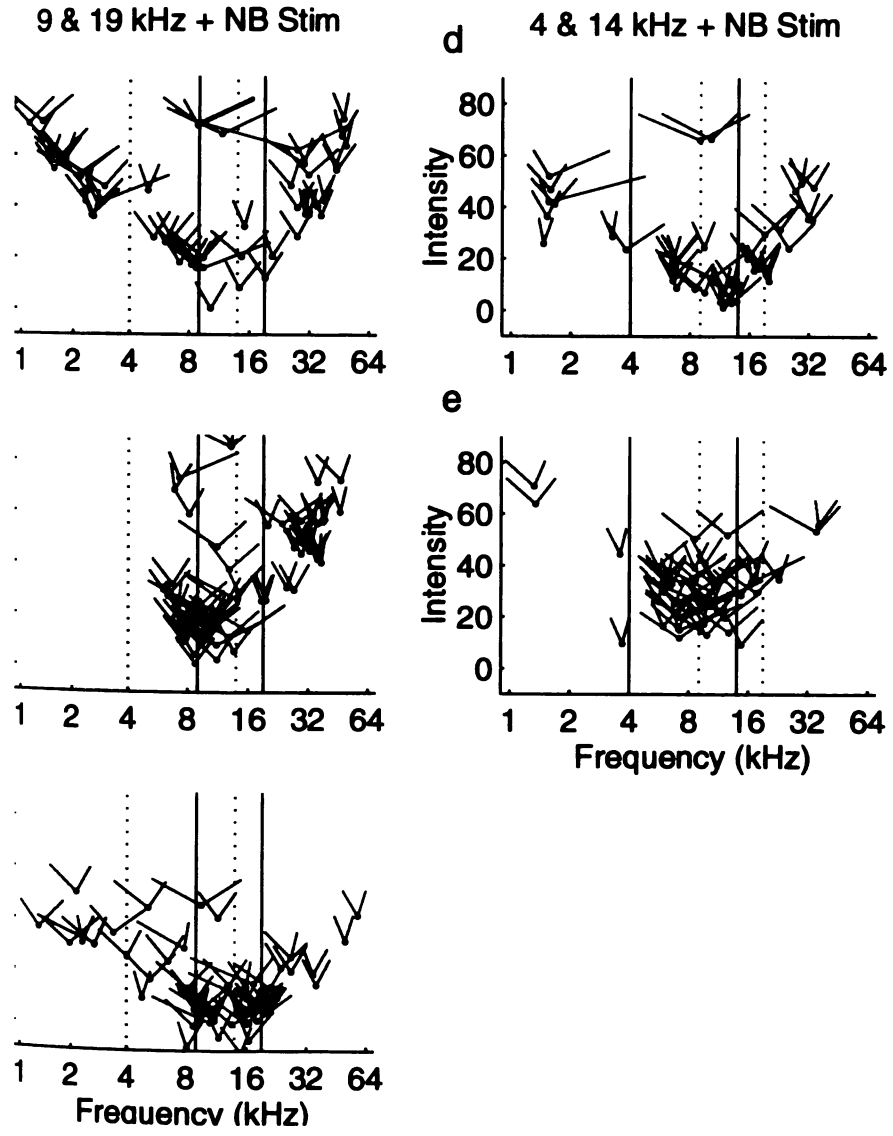

9 & 19 kHz + NB Stim
d
4 & 14 kHz + NB Stim
Intensity
80
60
40
20
0
1 2 4 8 16 32 64
e
Intensity
80
60
40
20
0
1 2 4 8 16 32 64
Frequency (kHz)
1 2 4 8 16 32 64
Frequency (kHz)

FIGURE 5-7

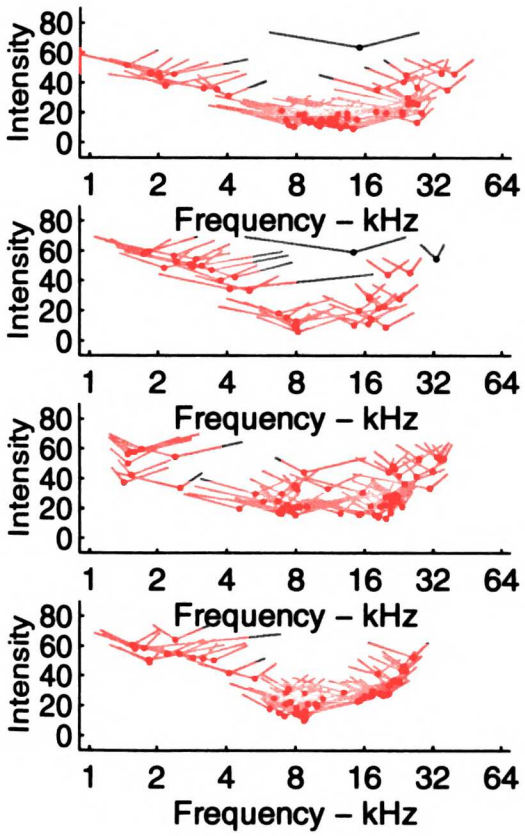


FIGURE 5-8

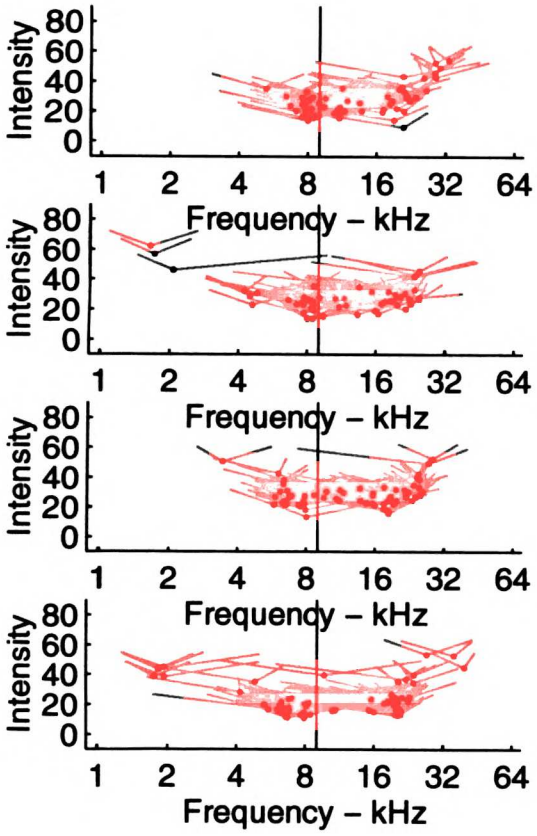


FIGURE 5-9

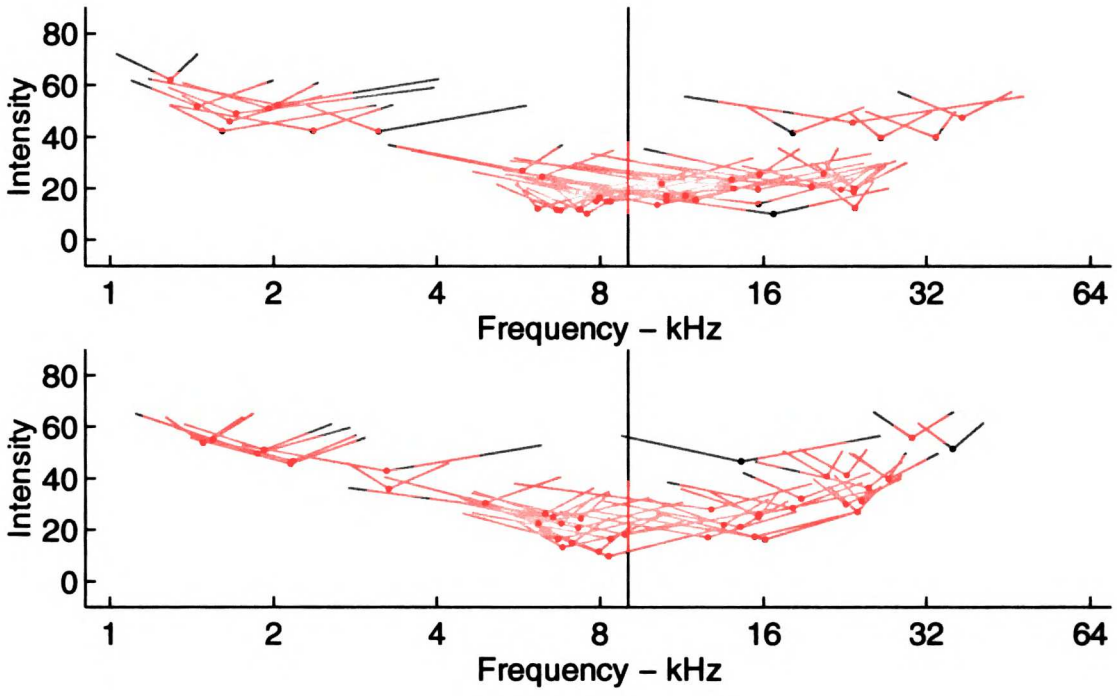

Intensity
80
60
40
20
0
1
2
4
8
16
32
64
Frequency – kHz
Intensity
80
60
40
20
0
1
2
4
8
16
32
64
Frequency – kHz

FIGURE 5-10

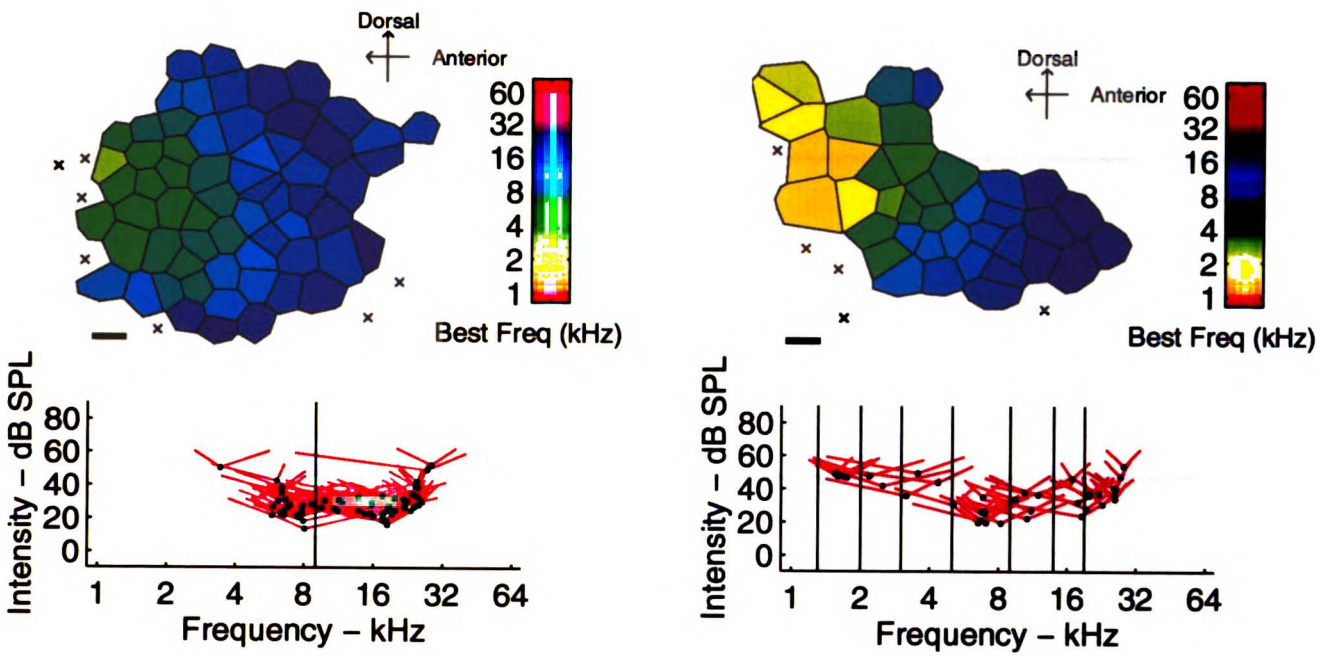

Dorsal
Anterior
60
32
16
8
4
2
1
Best Freq (kHz)
Intensity – dB SPL
80
60
40
20
0
1
2
4
8
16
32
64
Frequency – kHz
Dorsal
Anterior
60
32
16
8
4
2
1
Best Freq (kHz)
Intensity – dB SPL
80
60
40
20
0
1
2
4
8
16
32
64
Frequency – kHz

FIGURE 5-11

CHAPTER SIX:

Summary and Conclusions:

Principles of Cortical Self-Organization

The mammalian brain is a sophisticated self-organizing system that learns by transforming experiences into changes in circuitry that improve the animal's functional capacities, and ultimately its chances of survival. The same principles that guide day to day learning also allow the cortex to compensate for damage either to peripheral sensory structures or within the central nervous system. Although studies of long-term potentiation and depression have demonstrated that plasticity mechanisms are dependent on correlation-based rules, we still do not understand the principles that govern how sensory experience alters the distributed responses of thousands of cortical neurons in a behaviorally useful manner.

Experiments by Recanzone, Merzenich and colleagues have established that attention is required for sensory input to drive reorganization of cortical maps (Recanzone et al., 1992d; Recanzone et al., 1993; Recanzone et al., 1992e). This result and others suggest that attention gates cortical plasticity mechanisms, allowing correlation-based rules to operate preferentially on stimuli which are relevant to the animal (Singer, 1986; Ahissar et al., 1992). It has been proposed that the brain uses ascending neuromodulatory projections, such as the central cholinergic system, to differentiate important stimuli from among the tens of thousands of behaviorally irrelevant stimuli encountered each day (Weinberger, 1993; Ahissar and Ahissar, 1994).

The cholinergic input to the cortex arises from nucleus basalis (NB) neurons located in the basal forebrain (Mesulam et al., 1983). To explore the role of NB in long-term cortical plasticity, I developed a simple and robust plasticity paradigm using electrical activation of the basal forebrain and confirmed that activity of cholinergic NB neurons

paired with sensory stimuli is sufficient to generate enduring reorganizations of cortical circuitry.

Additionally, I used this model to investigate the principles of self-organization that specify how connection strengths and network dynamics are modified to result in representational plasticity that is likely to be behaviorally useful. NB stimulation was used to activate cortical plasticity mechanisms. Auditory cortex was chosen for these studies because the ease of auditory stimulus generation facilitated exploration of the rules that guide plasticity in response to stimuli located within a continuous multi-dimensional feature space.

A chronic stimulating electrode was used to pair activation of NB with the presentation of an auditory stimulus several hundred times daily. Stimulation was delivered to adult rats that were awake and unrestrained. After four weeks of such pairing, a detailed map of the response properties of primary auditory cortex neurons was reconstructed from up to one hundred microelectrode penetrations. The reorganizations that resulted were among the largest ever recorded in primary sensory cortex. Importantly, the plasticity observed was specific to the stimulus paired with NB stimulation. For example, when 9 kHz tones were paired, the region of the A1 map representing this frequency was expanded as neurons that previously responded to other frequencies shifted their responses toward 9 kHz. In contrast, stimuli presented without NB activation did not result in cortical reorganization. These results confirm the hypothesis that NB functions to demark significant stimuli allowing cortical plasticity mechanisms to operate specifically on important events.

The first principle of cortical plasticity I investigated relates changes in receptive field size to specific qualities of the stimulus paired with acetylcholine release. Recanzone and colleagues showed that cortical receptive field size decreased after monkeys practiced a task requiring fine discrimination of location on the receptor surface (cochlea or skin), and increased following training on a task requiring detection of changes in stimulus modulation rate. In my simplified preparation, this differential plasticity was mimicked, without behavioral training, by changing the statistics of the sensory input paired with NB stimulation. Receptive field sizes were increased when amplitude modulated stimuli were paired with acetylcholine release and decreased when different tone frequencies were paired. By pairing several different types of stimuli, I showed that receptive fields were altered as a continuous function of spatial variability and temporal modulation of stimuli. These results suggest that simple rules operate in the cortex to generate useful changes in circuitry based on the statistics of sensory stimuli marked by NB activity.

I also used chronic NB stimulation to examine representational plasticity of time-varying stimulus features. The maximum following rate of cortical neurons were significantly increased after repeated pairing of NB activation with stimuli modulated at 15 Hz, and significantly decreased after pairing with 5 Hz stimuli. Interestingly, my first attempt to generate temporal plasticity, by pairing 9 kHz tones modulated at 15 Hz, did not increase the cortical following rate, despite a large reorganization of the cortical map of frequency. In contrast, when the tone frequency was randomized while maintaining the 15 Hz modulation rate, dramatic plasticity of A1 temporal responses

occurred without any map reorganization. Thus, variability of one feature can profoundly impact plasticity of another.

Determining which features of a stimulus are behaviorally important is a difficult problem that has been largely ignored in the plasticity literature. In tasks involving simple tonal stimuli, it seems obvious that experience with 9 kHz should increase the 9 kHz region of the map, but how does the cortex know that tone frequency is important and not duration, intensity, modulation rate, bandwidth, or any other stimulus feature? Simultaneously adjusting the cortical tuning for every stimulus feature to match the novel stimulus would not only be inefficient, but would create representations that did not generalize well. As there is little evidence that primary sensory cortex has access to specific information about task goals, it is reasonable to hypothesize that the cortex uses information contained in the input itself to make an educated guess about how to improve performance (Figure 6-1).

Variability shapes behavioral generalization functions in humans and animals (Lively et al., 1993; Bower, 1994; Nygaard et al., 1995; Rescorla and Furrow, 1977). My results demonstrate that input variability can serve as an important cue for the cortex about which feature(s) of the stimulus contain information. This result further substantiates the hypothesis that NB activity marks important stimuli and allows simple cortical rules to improve the representation of features likely to be useful based on the statistics of input. Chronic NB stimulation will provide a powerful new tool to probe the principles that guide cortical reorganization. Future experiments are needed to explore the rules that guide representational plasticity of other simple features, including duration, intensity, bandwidth, FM direction and rate.

It is important to realize that these rules operate on the internal (neural) representation of the sensory stimulus. For this reason, it is probably inaccurate to suggest that they operate as a simple look-up table (i.e. if input is modulated, decrease frequency selectivity). Cortical plasticity is a dynamic and progressive process. As connections strengths and network time constants are altered, the input too is altered, allowing the history of the animal to influence the implementation of changes in cortical circuitry (Figure 6-2). It is possible that the variability of spatial and temporal reorganization observed across individuals in this series reflects this process.

In real-world situations learning rarely occurs on repeated identical stimuli, such as were used in my experiments. In addition to naturally-occurring variability, animals are generally able to manipulate the environment and significantly alter their sensory input. It is likely that such manipulations are quite important for shaping cortical responses to behaviorally important stimuli (Figure 6-3). For example, as an animal becomes familiar with one aspect (or feature) of a sensory object, it may focus on others (either via real-world manipulations or by selective attention). Given the dynamic processes that underlie learning and plasticity in the mammalian cortex, progressive variation of the sensory input will likely be required to approximate naturalistic learning using the chronic stimulation paradigm developed in this thesis.

NB stimulation will also provide a new approach to clarifying the functional principles relating cortical plasticity to behavioral performance. To date it has not been possible to definitively demonstrate that cortical plasticity is sufficient to improve performance. Many studies have correlated cortical plasticity with behavior, but could not establish causality. Because this model allows plasticity to be generated

independently of behavior, it will be possible to generate cortical reorganizations and then quantify the consequences on behavioral performance. Finally, NB stimulation will be a powerful tool in studies of the role plasticity plays in the genesis and remeditation of CNS pathology.

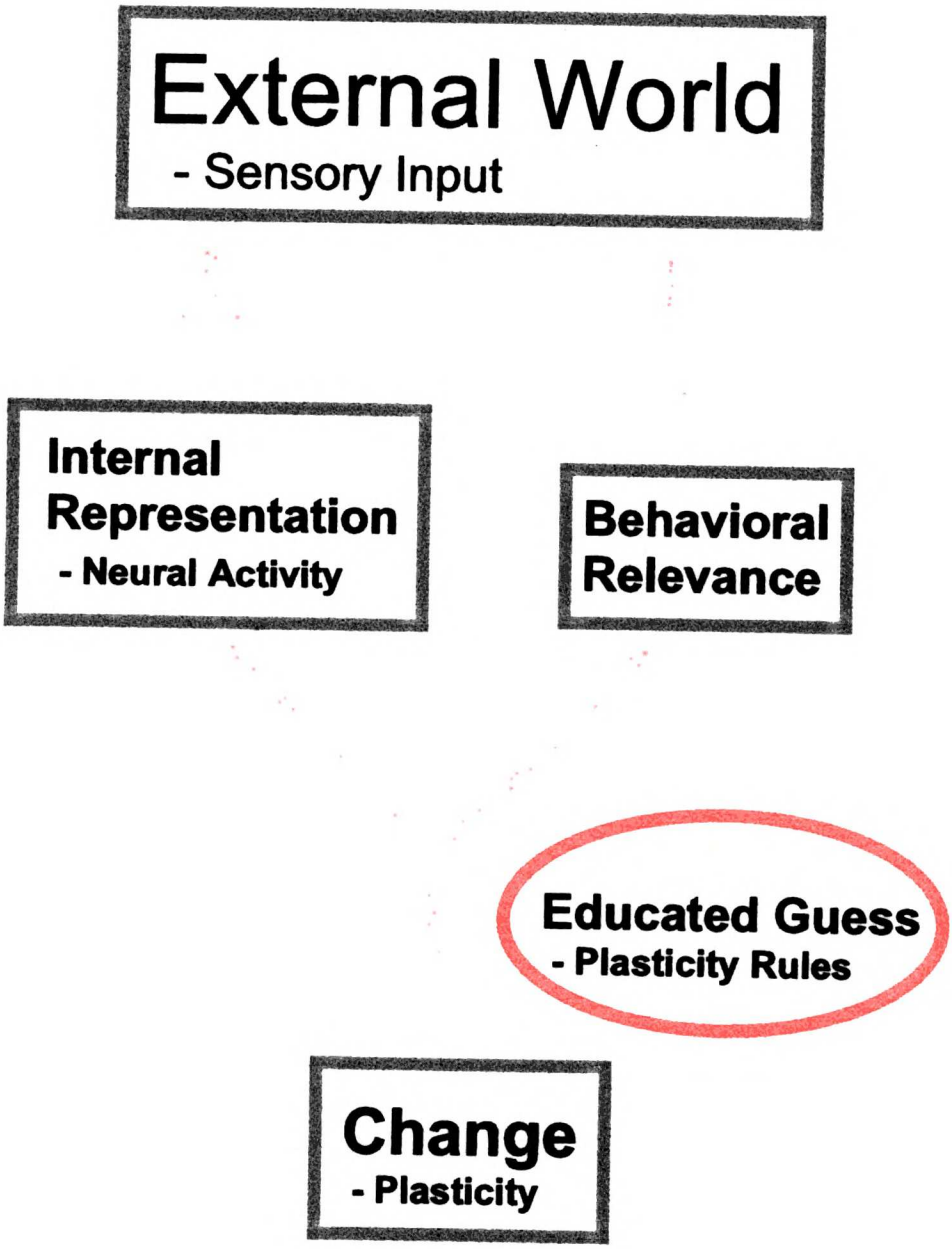
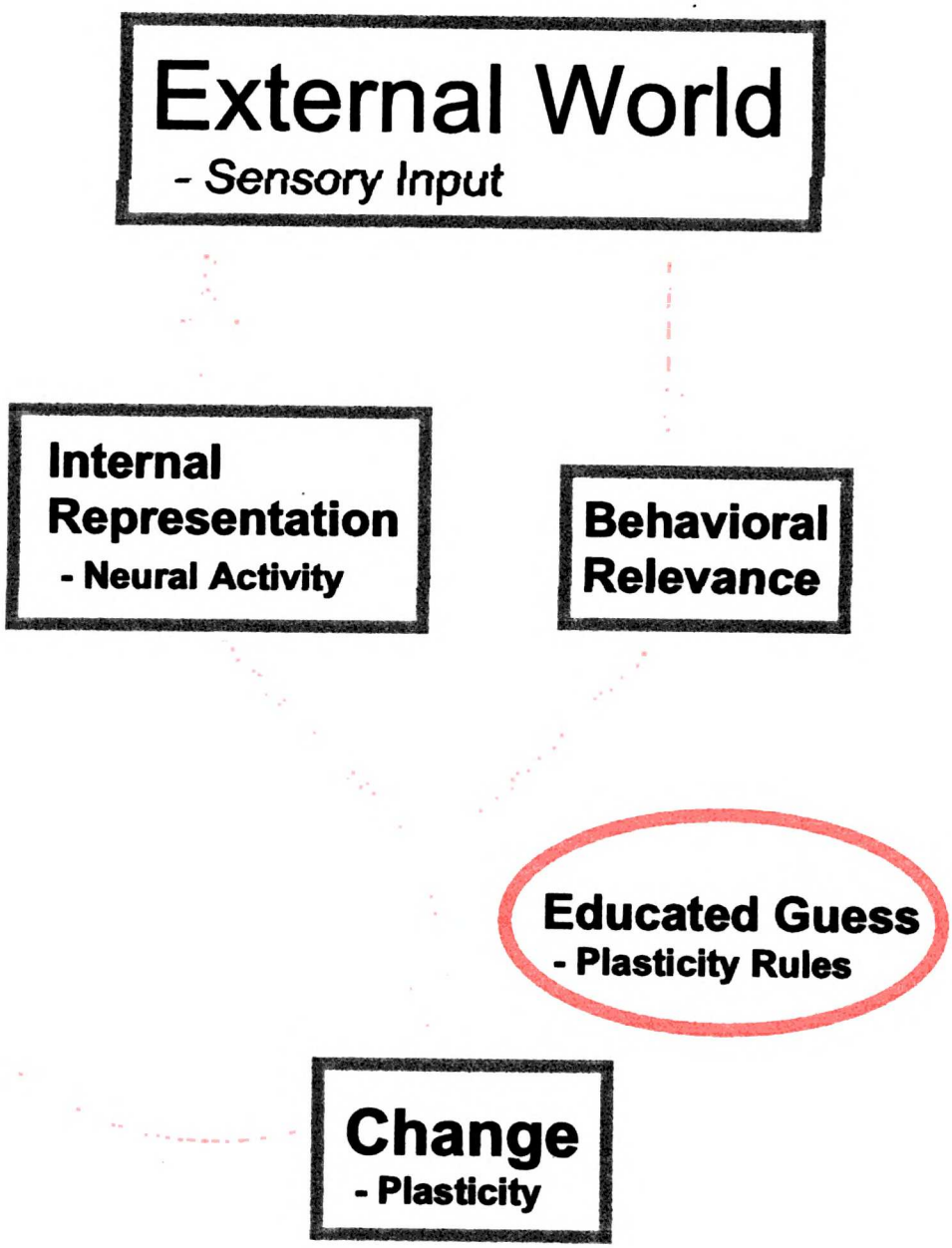

External World
- Sensory Input
Internal Representation
- Neural Activity
Behavioral Relevance
Educated Guess
- Plasticity Rules
Change
- Plasticity

FIGURE 6-1


External World
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- Neural Activity
Behavioral Relevance
Educated Guess
- Plasticity Rules
Change
- Plasticity

FIGURE 6-2

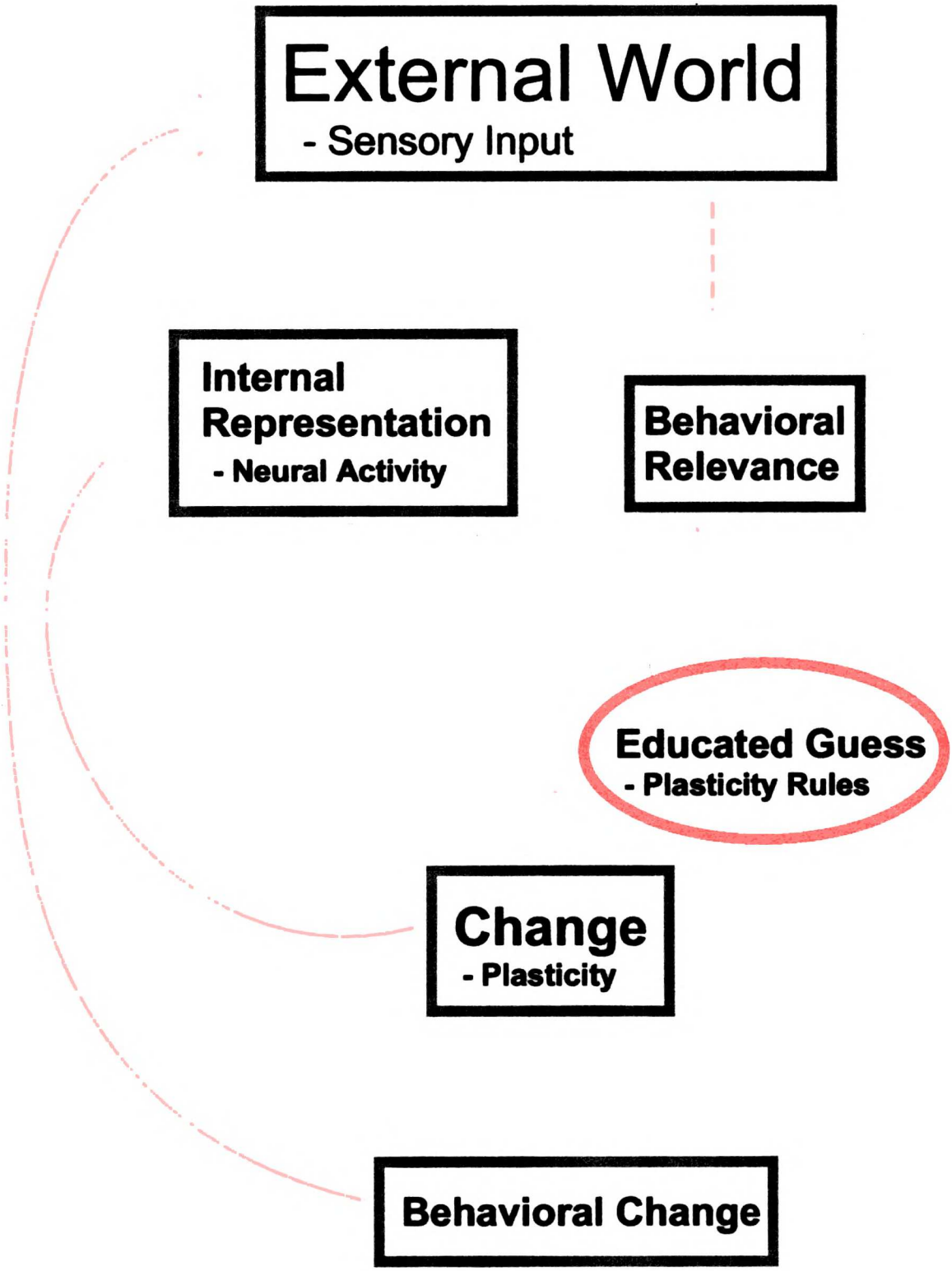
External World
- Sensory Input
Internal Representation
- Neural Activity
Behavioral Relevance
Educated Guess
- Plasticity Rules
Change
- Plasticity
Behavioral Change

FIGURE 6-3

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