ENVIRONMENTAL AND TRAINING-INDUCED PLASTICITY IN PRIMARY AUDITORY CORTEX

by

NAVZER D. ENGINEER, M.B.B.S., M.S.

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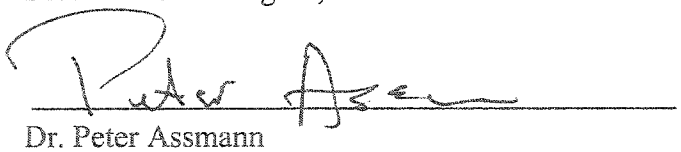
APPROVED BY SUPERVISORY COMMITTEE

Dr. Michael P. Kilgard, Chair



Dr. Peter Assmann

Dr Laurence J. Cauller

Dr. Stephen Lomber

DEDICATION

Dedicated to my parents

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Navzer D. Engineer, Ph.D.

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Supervising Professor: Michael P. Kilgard, Ph.D

Previous studies have demonstrated that a rich, stimulating environment results in both anatomic and physiologic changes in the cerebral cortex. In addition to generalized sensory experience, training on perceptual learning tasks also alters neurophysiologic responses in sensory and motor cortex. The effects of both enrichment and training on responses from auditory cortex neurons are examined in this dissertation. Although there is a vast literature spanning almost three decades on the anatomic and morphologic effects of enrichment, comparatively fewer studies have focused on the neurophysiologic consequences of enrichment. For example, neurophysiologic effects of enrichment have been demonstrated in visual (Beaulieu and Cynader 1990a, b) and somatosensory (Coq and Xerri 1998) cortex. In these studies, enrichment sharpened orientation tuning in visual cortex and increased the area of forepaw representation in somatosensory cortex. The effects of enrichment on the processing of auditory cortex neurons are not known. In the first part of my dissertation, the effects of enrichment on the auditory cortex are documented. Changes in spectral and temporal responses from primary auditory cortex (A1) neurons of enriched rats were compared to responses from rats in standard conditions using multi-unit recordings. Enrichment dramatically enhanced cortical responses

across A1, increased frequency selectivity and sensitivity and altered temporal processing without affecting cortical map reorganization (Engineer et al. 2004). The next study was designed to document the effects of auditory discrimination training on responses from auditory cortex neurons. Both animals and humans get better at discrimination tasks with practice. More recently, these studies have been combined with electrophysiological recordings (unit recording, evoked potentials, fMRI) to probe task specific effects on cortical responses. The second part of this dissertation documents the neurophysiologic consequences of auditory sequence learning on rat A1 neurons.

The results of these and earlier studies indicate that exposure to a rich, stimulating environments or training on perceptual learning tasks can significantly alter sensory information processing of cortical neurons. Although the exact consequences of plasticity on cortical development or recovery from injury are not clear, numerous studies suggest that environmental enrichment and/or behavioral training may be useful remediation strategies in promoting recovery from neurological disability.

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CHAPTER 1
INTRODUCTION

Cortical maps and receptive fields are dynamic (Diamond et al. 1999). The initial emergence of primitive maps during development depends on molecular cues (Goodman and Shatz 1993) and spontaneous activity (Meister et al. 1991). Further refinement of cortical maps and receptive fields is activity-dependent and modulated by sensory experience (Katz and Shatz 1996). Although refinement of cortical maps and receptive fields are typical features of developmental plasticity, they are not restricted to developing neural circuits. Plastic changes in *adult* sensory cortex can result from peripheral and central lesions and from modifications induced by environmental enrichment or behavioral training. Many of the early studies on cortical plasticity were studied by inducing lesions of the sensory receptor surface. For example, when a specific region of the cochlea was destroyed, the deprived cortex increased the representation of the functional neighboring cortical areas (Robertson and Irvine 1989). Soon, it was established that environmental enrichment and behavioral training could also result in topographic reorganization and receptive field alterations in primary sensory cortex. Exposure to enriched environments stimulates dendritic growth, increases the number of synapses, dendritic branching and stimulates neurogenesis in adult cortex. Many of these anatomic and morphologic changes reflect changes in the underlying functional architecture of cortical circuits such as changes in synaptic efficacy. Focused behavioral training on tactile,

visual and auditory tasks also alters responses, temporal processing and sensory maps in cortical networks. For instance, the region of the cortex engaged in a tactile discrimination task shows increased representation of the trained digit and narrow receptive fields after weeks of training (Recanzone et al. 1992a).

During early post-natal development the cortex is sensitive to passive stimulus exposure while adult cortex is more resilient to passive exposure. Exposure to a variety of acoustic environments during development alters the tonotopic map and frequency tuning in primary auditory cortex (A1) neurons. Experiments with young rats (during the critical period for auditory cortex maturation) demonstrated that environmental exposure to sounds during a critical period can result in map and receptive field plasticity that persist into adulthood (Zhang et al. 2002, 2001). Moreover, the same study also demonstrated that adult cortical maps are resilient to passive acoustic exposure. These results demonstrated that appropriate acoustic input patterns during development are important for normal maturation of auditory cortical circuitry and that the same passive manipulations do not alter cortical neurons in adults. The role of attentive versus passive sound exposure in adult cortex will also be addressed in this dissertation.

The body of this dissertation is divided into two parts. The first part (Chapter 3) documents the effects of generalized enrichment of responses from auditory cortex neurons and compares them to responses from standard housed rats. The results from this experiment have been published in the *Journal of Neurophysiology* (Engineer et al. 2004). The latter half (Chapter 4) describes the effects of sequence discrimination training on responses from A1 neurons.

CHAPTER 2
RATIONALE FOR RESEARCH

Enrichment-induced plasticity (Chapter 3)

Aim #1: *To document the effects of enriched environments on responses of primary auditory cortex neurons.*

Over the last three decades, numerous studies have shown that rearing animals in enriched conditions alters brain anatomy and neuron morphology. Some of these alterations include increased dendritic length and branching, increase synaptic density, neurogenesis of hippocampal neurons. The neurophysiologic consequences of enriched environments have been documented in visual and somatosensory cortex including narrow receptive field and cortical map reorganization (Beaulieu and Cynader 1990a, b; Coq and Xerri 1998). The effects of generalized enrichment on auditory cortex neurons have not been studied. In the auditory cortex of young rats, cortical topography and receptive field development can be disrupted by abnormal sensory input (e.g. pulsed noise bursts) and these changes persist into adulthood. These developmental studies highlight the importance of patterned input during normal development and that any environmental manipulation or insult during cortical maturation results in abnormal cortical tonotopy, disrupted receptive fields and altered temporal processing. These studies also demonstrated that in contrast to developing cortex, adult auditory cortex is resilient to these manipulations. This led to support for the existence of a 'critical period' for auditory cortex maturation and appears to be about 4 weeks after birth. We asked the question if generalized environmental enrichment could alter neurophysiologic

responses of auditory cortex neurons well beyond their critical period for auditory cortex maturation.

Adult rats were reared in enriched and standard conditions at 4 weeks of age for 2 months after which extracellular recording techniques were used to document changes in cortical maps, receptive fields and temporal response characteristics from primary auditory cortex neurons. Our hypothesis was the enrichment would increase response strength of cortical neurons compared to rats reared in standard conditions. Based on earlier studies in visual and somatosensory cortex, enrichment will narrow receptive fields of A1 neurons. This decrease in receptive field size would not be specific for a frequency region of the A1 map because rats would be exposed to a variety of simple and complex sounds that would span the frequency spectrum of the rat hearing range. Finally, enrichment was expected to increase the cortical processing rate of auditory neurons compared to standard housed rats.

Training-induced plasticity (Chapter 4)

More than a decade ago, studies by Weinberger, Recanzone and others demonstrated that the cortical topography, receptive fields and temporal processing can be modified after animals train on a perceptual learning task (Recanzone et al. 1992a; Recanzone et al. 1993; Weinberger 1993). Practice on tactile and auditory frequency discrimination tasks improved performance with practice (Recanzone et al. 1992a; Recanzone et al. 1993). Training restricted to one section of the finger or to one region of the acoustic spectrum resulted in a 3-4 fold increase in the cortical representation of the trained area, narrow receptive field and altered temporal responses (Recanzone et al. 1992a; Recanzone et al. 1993). Neurons that responded to the trained digit showed increased coherence with the trained stimulus. Some, but not all of these plasticity effects were correlated with improved behavioral performance. From these experiments, it

became apparent that cortical reorganization and temporal processing can be induced simply by training an animal on a perceptual learning task. Although studies have reported the neural consequences of perceptual learning in the primary visual cortex of animals (Crist et al. 2001; Ghose et al. 2002; Schoups et al. 2001), relatively fewer studies have looked at the psychophysical and neurophysiologic consequences of complex sound discrimination training in primary auditory cortex.

The second motivational factor for this study was previous work that employed an "unnatural" form of inducing cortical plasticity, namely cholinergic nucleus basalis stimulation. Cholinergic projections from the nucleus basalis (NB) to the cortex have been implicated in learning and memory. Lesions of the cholinergic NB have been shown to prevent cortical reorganization (Baskerville et al. 1997). In this paradigm, pairing a tone with nucleus basalis stimulation resulted in a specific reorganization of the paired tone representation (Kilgard and Merzenich 1998). It was later demonstrated that the specific spatio-temporal parameters of the paired acoustic stimulus were critical for driving cortical changes (Kilgard et al. 2001). This form of unnatural stimulation induced cortical plasticity without attention or reinforcement in the natural (behavioral) sense. When a tone-tone-noise sequence was paired with NB stimulation, neurons in A1 became combination-sensitive to the sequence elements (Kilgard and Merzenich 2002). The discrimination experiments in the present study were designed to test the hypothesis that perceptual learning on auditory tasks could also drive cortical changes in a task-dependent manner.

Aim #2: *To document the effects of easy to difficult sequence discrimination training on responses of primary auditory cortex neurons.*

The temporal pattern of acoustic input is critical for processing complex sounds including speech. In primary auditory cortex neurons of many species, the response of certain sound components can be either facilitated or suppressed by preceding stimuli (Brosch and Schreiner 2000; Brosch et al. 1999). The effects of sequence discrimination training on responses of cortical neurons are not known. In this series of experiments, we were interested in two aspects of auditory perceptual learning: *sequence learning* and *task difficulty*.

In many specialized species, sounds (e.g. vocalizations) that have ecological value will drive auditory neurons only if presented in a certain order. Neurons exhibit a selective response to the behaviorally relevant sequence and a degraded or absent response to individual components of the sequence or if the sequence order is changed. In rats, learning to discriminate a sound sequence from other sequences could increase the behavioral relevance of that sound and consequently improve cortical processing.

Task difficulty plays an important role in determining the nature of the neural response. Many studies have documented the effects of task difficulty on responses of visual cortex neurons. For example, neurons responded differently in a difficult task compared to an easy task (Ahissar and Hochstein 1997; Spitzer et al. 1988; Spitzer and Richmond 1991). In the present study, rats were trained to discriminate a sound sequence from other sound sequences. In some cases, the task was easy (frequency discrimination) whereas in others it was very difficult (reverse order discrimination).

Hypothesis: On easy tasks, rats will learn to discriminate sound sequences and their performance will improve over the course of training. The reverse order discrimination is more difficult and rats may not learn the task. Frequency discrimination training may result in an increase in the area of representation of the CS+ sequence and show narrow receptive fields as seen in the

Recanzone study with monkeys. More recently however, contrasting results obtained in cats trained on a frequency discrimination task (Brown et al. 2004) makes it difficult to predict the outcome of the present study. Species differences, task differences and/or experimental design could contribute to these differences. Training will increase the response strength of A1 neurons and decrease latency responses. Neurons may become combination-sensitive to the CS+ sequence if rats are able to learn the sequence and show no combination-sensitivity if they fail to learn the task or if simply exposed to the sequence.

CHAPTER 3

ENRICHMENT-INDUCED PLASTICITY IN THE AUDITORY CORTEX

Introduction

Environmental enrichment results in morphologic, molecular and physiologic changes in sensory and motor cortices of young and adult animals (van Praag et al. 2000). Rats raised in enriched conditions exhibited increases in cortical thickness, gene expression, acetylcholinesterase levels, oligodendrocyte to neuron ratio, dendritic branching, and number of synapses per neuron compared to animals raised in standard conditions (Bennett et al. 1966; Diamond et al. 1966; Diamond et al. 1972; Globus et al. 1973; Greenough and Volkmar 1973; Greenough et al. 1973; Rampon et al. 2000; Sirevaag and Greenough 1987; Staiger et al. 2002; Volkmar and Greenough 1972).

Neurophysiologic responses can also be altered by experience. Cats raised in enriched conditions had sharper orientation tuning in primary visual cortex and were able to resolve higher spatial frequencies compared to cats raised in standard conditions (Beaulieu and Cynader 1990a). Enrichment narrowed receptive fields and sharpened the topographic organization of adult rat primary somatosensory cortex (Coq and Xerri 1998). Experience-dependent remodeling of receptive fields and topographic organization of auditory cortex has also been observed in behaviorally trained animals (Ahissar and Ahissar 1994; Edeline 1999; Weinberger and Bakin 1998). Practice on frequency discrimination improved behavioral performance, narrowed receptive fields, and expanded the region of A1 responding to the trained frequency (Recanzone

et al. 1993). The effects of more generalized enrichment have not been reported in auditory cortex.

In the present study, our aims were to document the consequences of environmental enrichment on response properties of auditory cortex neurons. Extracellular recordings from anesthetized rats show significant changes in response strength, receptive field characteristics and temporal response properties.

Methods

Environmental Conditions

Sixteen female Sprague-Dawley rats were used in this study. Rats were randomly assigned to either the enriched environment (n=8) or the standard condition (n=8). The enriched rats were raised 4 per cage (in two sessions), while rats in the standard condition were raised 2 per cage (Figure 1A). Rats in the enriched environment (Figure 1B) were housed together in a single large cage in a separate room from the main rat colony at the University of Texas at Dallas. All protocols and recording procedures conformed to the Ethical Treatment of Animals (NIH) and were approved by the University Committee on Animal Research at the University of Texas at Dallas.

The enriched environment consisted of a large cage (45 L x 76 W x 90 H cm) of four levels connected by ramps. Touch plates at the bottom of two ramps triggered different tones (2100 or 4000 Hz) when the rat stepped on the plates. In addition, chains, wind chimes, or bells were hung across the entrance of each ramp so that a unique sound was elicited when rats passed from one level to the next. A motion detector emitted an electronic chime each time a rat crossed the infrared beam in front of the water source. An exercise wheel emitted a tone (3000 Hz Piezo Speaker) and activated a small green light emitting diode with each rotation. Each movement-

triggered sound had unique spectral and temporal characteristics that provided behaviorally meaningful information about the location and activity of other rats in the cage.
The power spectrum of these sounds spanned the entire hearing range of the rat (1-45 kHz). All sound intensities were less than 75 dB SPL.

A CD player presented randomly selected sounds every 2 to 60 seconds. These sounds included simple tones, amplitude modulated and frequency modulated tones, noise bursts and other complex sounds (rat vocalizations, classical music, rustling leaves, etc.). Seven of the seventy-four sounds activated a pellet dispenser (Med Associates) that delivered a sugar pellet to encourage attention to the sounds. The rewarded tracks included modulated tones with different carrier frequencies and frequency modulated sweeps. The sound sources added to the enriched environment were provided 24 hours a day. After one month, a vasectomized male rat was introduced into the enrichment cage to encourage more natural social interactions appropriate for these ages, since it is known that rats reach sexual maturation by 8-12 weeks of age.

The acoustic environment of the standard condition consisted of vocalizations from 20-30 other rats housed in the same room, and sounds resulting from daily room traffic, feeding and cleaning, which were also heard by the enriched group. However, sounds in the enriched condition were more diverse and provided more behaviorally relevant information than the sounds in the standard condition. Rats in both conditions were on a reverse 12-hour light/dark cycle. As a result, both groups heard the sounds of room traffic while they were most active. For both housing conditions, constant temperature and humidity were maintained. Food and water were provided ad libium for all rats. All the rats used in this study were housed with their mothers and littermates until weaning at four weeks of age. Acute microelectrode mapping was performed after eight weeks in each environment. Although acute experiments using

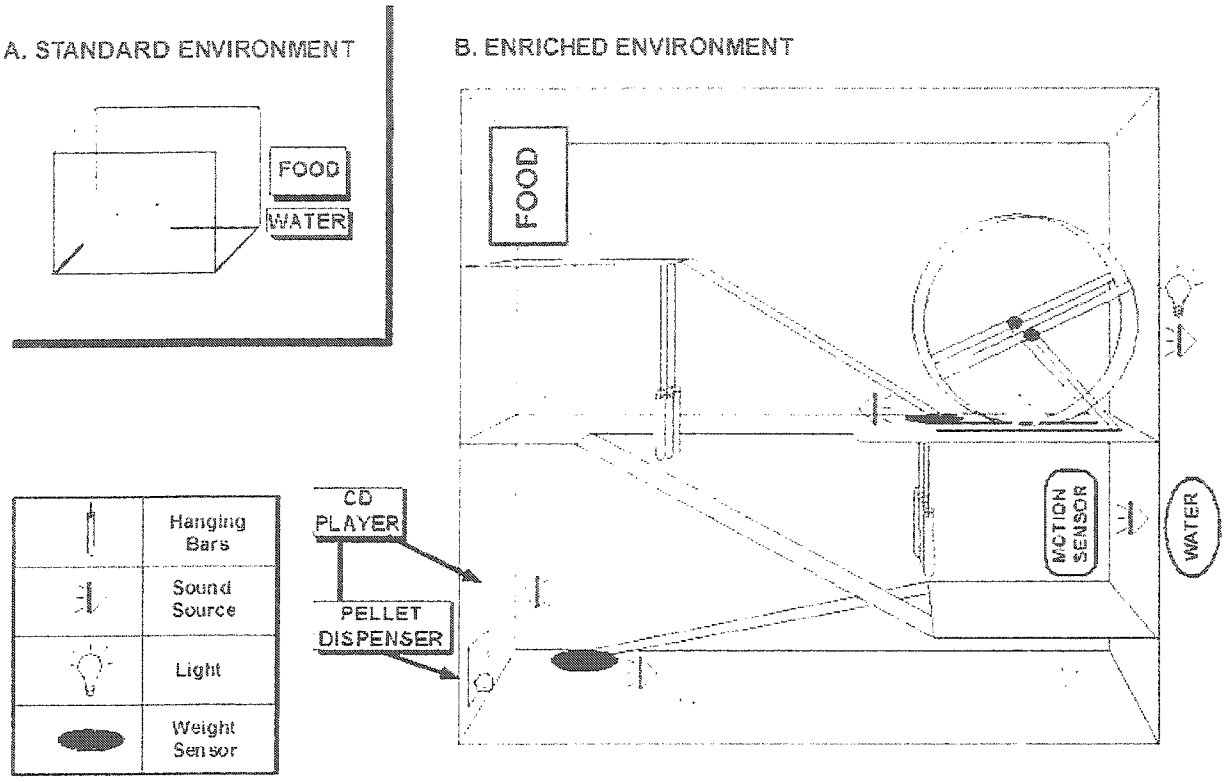


Figure 1. Schematic of standard and enriched housing conditions. A) The standard condition consisted of 1 or 2 rats housed in hanging cages within a rat colony room. B) The enriched condition consisted of 4-8 rats housed in a rich environment with devices that generated different sounds when rats crossed a motion detector path, stepped on weight sensors, or passed through hanging bars. In addition, each rotation of the running wheel triggered a brief tone and light flash, and a CD player played 74 sounds, including tones, noise bursts, musical sequences and other complex sounds, in random order. Some of these sounds were associated with delivery of a sugar reward.

enriched and standard housed rats were interspersed, some experimenters were not blind to the identity (enriched or standard) of the rats because of the unkempt state of the fur that typically identified the enriched rats. Therefore, the possibility of unintentional bias cannot be excluded.

Acute Surgery

Surgical anesthesia was induced with sodium pentobarbital (50 mg/kg, i.p.). A state of areflexia was maintained throughout the surgery and recording phases with supplemental doses of dilute pentobarbital (8 mg/ml, i.p.). The interval between supplements varied depending on the anesthetic state of the rat but was typically every 1-1.5 hr. Anesthesia depth was evaluated by heart rate, breathing rate, toe-pinch responses and corneal reflexes. These indicators were indistinguishable between the two groups. Circulatory function was monitored with EKG and pulse oximetry. Fluid balance was maintained with a 1:1 mixture of 5% Dextrose and Ringer's Lactate (~0.5 ml/hour). Body temperature was maintained at 37° C. The trachea was cannulated to minimize breathing sounds and ensure adequate ventilation. Humidified air was delivered to the open end of the cannula. After the cisterna magnum was drained to minimize cerebral edema, the right auditory cortex was exposed and the dura resected. The cortex was maintained under a layer of viscous silicon oil to prevent desiccation during the 24-30 hr experiment and a detailed map of auditory cortex was generated from 50-100 microelectrode penetrations. The sampling density and depth of recordings made in enriched and standard housed rats were indistinguishable. Parylene coated tungsten microelectrodes (FHC) were lowered 550 μm below the pial surface (layer 4/5) of the right auditory cortex. Spikes from a small cluster of neurons were collected at each penetration site. Penetration locations were referenced using cortical vasculature as landmarks.

Stimulus Presentation and Data Analysis

Auditory stimuli were delivered from the left side of the rat via a calibrated speaker in a shielded, double-walled sound attenuating chamber and were generated using Brainware (Tucker-Davis Technologies). Auditory frequency tuning curves were determined at each site by presenting 81 logarithmically spaced frequencies from 1 to 32 kHz at 16 intensities from 0 to 75 dB (1296 total stimuli). The tones were randomly interleaved and separated by 500 ms. Tone and noise burst repetition rate transfer functions (RRTF) were also derived at each site by randomly interleaving 12 repetitions of 14 different repetition rates (3-20 Hz) for tones and 4 repetition rates (5, 10, 15 and 20 Hz) for noise bursts. A 2 second silent period separated each train. The frequency of the RRTF tones was selected to generate the strongest response at each recording site. The RRTF stimuli were presented at 70 dB SPL. All stimuli were 25 ms long with 3 ms rise and fall time. An example of an RRTF from a single site is shown in Figure 4B. The RRTF to tones and noise burst was also quantified using the vector strength and Rayleigh statistic measure (Liang et al, 2002). Vector Strength (VS) quantifies the degree of synchronization between action potentials and repeated tones pips. A value of one indicates perfect synchronization and zero indicates no synchronization. The Rayleigh statistic combines the degree of synchronization with the number of spikes.

Action potentials were recorded simultaneously from two Parylene coated tungsten microelectrodes (2 MΩ). The neural signals were filtered (0.3-15 kHz) and amplified (10,000X). Action potential waveforms were recorded whenever a set threshold was exceeded.

Tuning curve parameters were defined by an experienced blind observer using custom software that randomized the order of data from each recording site across both groups. Best frequency (BF), bandwidth measures (BW), response threshold, spontaneous rate, and latency

measurements for each penetration were recorded (Figure 3). The CF is the frequency that evokes a reliable response at the lowest intensity (response threshold). Frequency bandwidth is the range of frequencies that each site responds to at 10, 20, 30 and 40 dB above threshold. First spike latency is the time from stimulus onset to the earliest reliable neural response. The end of response was defined as the time after tone onset when the poststimulus time histogram (PSTH) created by summing the responses to all of the tones within each site's tuning curve returned to baseline. The borders of A1 were defined based on continuous topography of CF and short response latency. Sites with high thresholds, long latencies, broad tuning and discontinuities in CF topography were considered non-A1 (Kilgard, et al. 2001; Doron et al. 2002). Criteria for identifying non-A1 sites were subjective and were applied blindly by well trained blind observers.

Cortical maps were reconstructed using the Voronoi tessellation procedure. The percent of A1 responding is the sum of the areas of all of the Voronoi tessellations that responded to the particular frequency and intensity combination, divided by the total area of A1 (Bao et al. 2003a; Bao et al. 2003b; Kilgard and Merzenich 1998, 1999; Read et al. 2001). The tessellation procedure generates polygons from a set of non-uniformly spaced points so that every point in the polygon was nearer to the sampled point than to any other. This allowed area information to be estimated from a number of discretely sampled recording sites by assigning each point on the cortical surface the qualities of the closest sampled point. The area measures generate reliable estimates of the percent of primary auditory cortex that is responsive to a given frequency-intensity combination.

Results

Neurophysiologic responses were recorded from rats housed either in standard laboratory conditions or in an enriched environment. Action potentials were recorded from small groups of A1 neurons at more than 800 sites in sixteen rats. Although rats in the enriched environment were exposed to a variety of broadband and narrowband stimuli, environmental plasticity was quantified by recording A1 responses to simple tones and white noise bursts. Comparisons between responses from rats housed in enriched (n=8 rats; 462 sites) and standard (n=8 rats; 358 sites) conditions indicated that environment substantially altered response strength, receptive field size, intensity threshold and spontaneous rate. Enrichment also altered temporal properties of rat A1 neurons including response latency and tone and noise burst modulation transfer functions.

Map and receptive field properties

Neurophysiologic responses were recorded from rats housed either in standard laboratory conditions or in an enriched environment. Action potentials were recorded from small groups of A1 neurons at more than 800 sites in 16 rats. Although rats in the enriched environment were exposed to a variety of broadband and narrowband stimuli, environmental plasticity was quantified by recording A1 responses to simple tones and white noise bursts. Comparisons between responses from rats housed in enriched (n=8 rats; 462 sites) and standard (n=8 rats; 358 sites) conditions indicated that environment substantially altered response strength, receptive field size, intensity threshold, spontaneous rate, and response latency. Enrichment did not significantly alter the A1 map of tone frequency. In both groups, best frequency was highly correlated with anterior-posterior position (r^2=0.83 ±0.02 and r^2=0.82 ± 0.03 for enriched and standard). The average change in best frequency as a function of anterior-

posterior distance was not significantly different between the 2 groups (-0.31± 0.01 and -0.27± 0.02 for enriched and standard, in octaves/mm). The total size of A1 was also unaffected by enrichment (1.03± 0.17 (enriched) 0.80± 0.15 mm^2 (standard), $p > 0.05$). No overrepresentation of any frequency-intensity combination (including the 2.1, 3 or 4 kHz frequencies used in the enriched condition) was observed (see methods).

Table 1. Response properties (mean and standard error) of primary auditory cortex neurons recorded from rats housed in enriched and standard conditions. Student's *t*-tests were used to determine statistical significance. ± refers to the standard error of the mean.

Response Parameter	Enriched N=462 A1 sites	Standard N=358 A1 sites	P Value
Response Strength (spikes/tone)	1.61 ± 0.04	1.24 ± 0.06	< 0.00001
Response Strength (spikes/noise)	1.44 ± 0.04	1.04 ± 0.06	< 0.00001
Neural Threshold (dB)	17.19 ± 0.47	19.88 ± 0.61	< 0.001
RF Bandwidth (BW40)	2.01 ± 0.04	2.16 ± 0.05	< 0.05
Peak Latency (ms)	19.18± 0.18	18.32 ± 0.25	< 0.01
End of Peak Latency (ms)	36.09 ± 0.39	34.41 ± 0.40	< 0.01
Spontaneous (spikes / 20 msec)	0.114 ± 0.004	0.094 ± 0.006	< 0.01

The number of action potentials in response to tones or noises increased by one-third in enriched rats compared to rats housed in the standard condition (Table 1 and Figure 2C, D).

A1 neurons in enriched rats were nearly 3 dB more sensitive to tones and were more selective for tone frequency than neurons in the standard group (Table 1 and Figure 2).

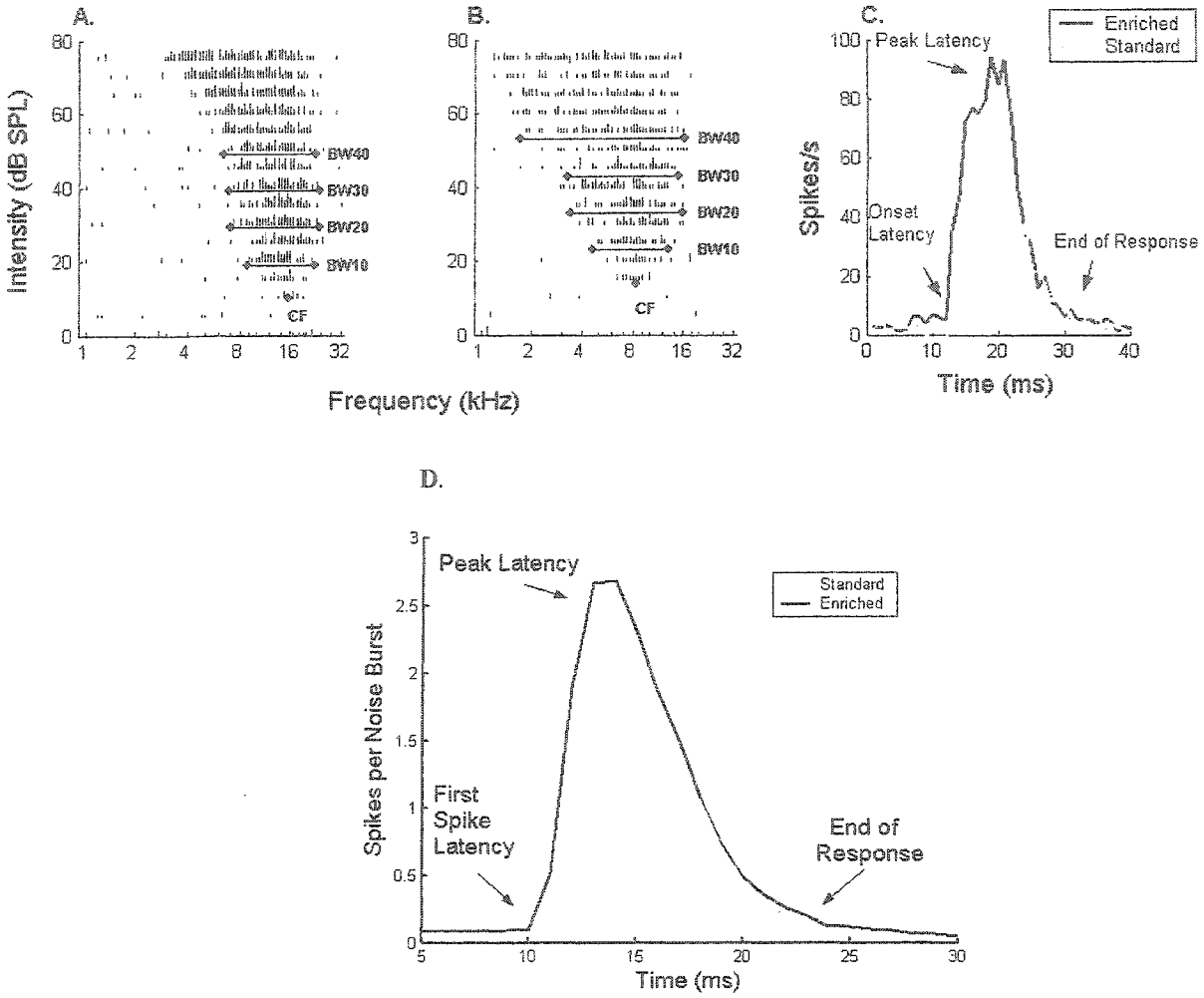


Figure 2. Tone responses from an enriched rat (A) show more spikes per tone, narrower tuning and lower thresholds compared to tone responses from a standard rat (B). C) Post-stimulus time histograms (PSTH) for these two sites illustrate the greater response strength after enrichment. Binwidth is 1 ms. D) Mean noise burst PSTH's for (N (enriched) = 462, N (standard) = 358). Enriched rats exhibit a higher firing rate, late peak and end of response latency. The gray shaded regions represent the standard errors of the mean for each group. Binwidth is 1 ms.

Bandwidth at 10-40 dB above threshold was narrower for enriched rats, but only reached statistical significance at 40 dB above threshold. End of response latency was later as a result of the greater number of spikes evoked by each sound (Table 1 and Figure 2D). The average signal to noise ratio increased by 11% due to the greater rise in driven rate over spontaneous rate. Spontaneous activity in enriched rats was 21% higher. Each of these changes were observed in all regions of the frequency map.

Modulation Rate Transfer Functions

Enrichment significantly affected the following rate of cortical neurons. Figure 3A is a representative example of a spike raster plot from a single site. Each point represents a spike. The lower panel of this figure shows response to tones (red) whereas the upper panel shows response to noise burst trains (green). Decrease in the number of spikes can be seen at faster rates. This is reflected in the RRTF shown in Figure 3B. Figure 4A shows the mean normalized repetition rate transfer function from enriched and standard rats. At slower rates (< 8 Hz), enriched rats showed more spikes/tone compared to naïve rats. At faster rates however, enriched rats responded with fewer spikes/tone. When vector strength and the Rayleigh statistic was used to quantify the degree of synchronization between the tone trains and action potentials, similar results were obtained (Fig. 4B & C). These results indicate that compared to standard housed controls, enriched rats exhibited *increased* response strength and synchronization at slow rates, but exhibited *decreased* response strength and synchronization at fast rates. The cortical following rate for noise burst trains increased for slow rates in enriched but failed to reach significance at faster rates. Best rate (rate at which maximum spikes per tone are obtained) for enriched rats was significantly decreased compared to naïve rats ($6.7 \pm .1$ vs. $7.8 \pm .2$, $p > 0.00001$).

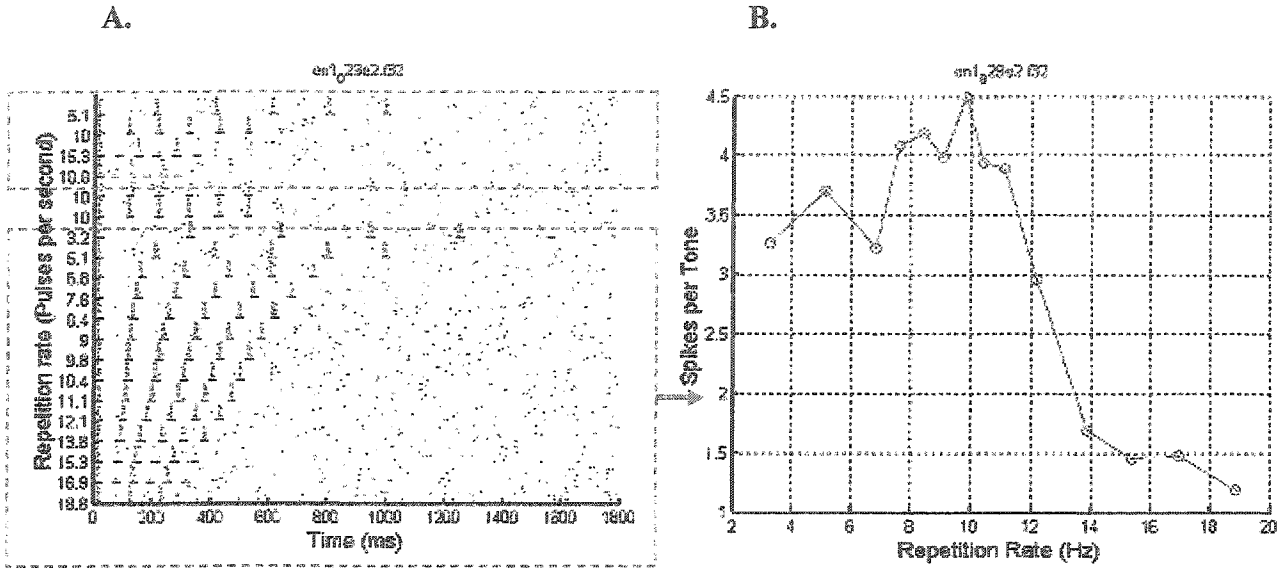


Figure 3. A) Dot-raster at a single site from an enriched rat. Tone and noise burst repetition rate transfer functions (RRTF) were derived at each site by randomly interleaving 12 repetitions of 14 different repetition rates (3-20 Hz) for tones (red box) and 4 repetition rates (5, 10, 15 and 20 Hz) for noise bursts (green box). In addition, a 5 kHz and 12 kHz tone train (10 pulses per second) was also presented at each site. Each dot represents a spike. The frequency of the RRTF tones was selected to generate the strongest response at each recording site. The RRTF stimuli were presented at 70 dB SPL. All stimuli were 25 ms long with 3 ms rise and fall time. B) Repetition rate transfer function at a single site in an enriched rat. Figure shows mean spikes per tone for each of the rates that were presented. Mean spikes per tone decreased at faster rates. The best rate was also calculated at each site. Best rate is the rate that gives the maximum spikes/tone, in this case 10 Hz.

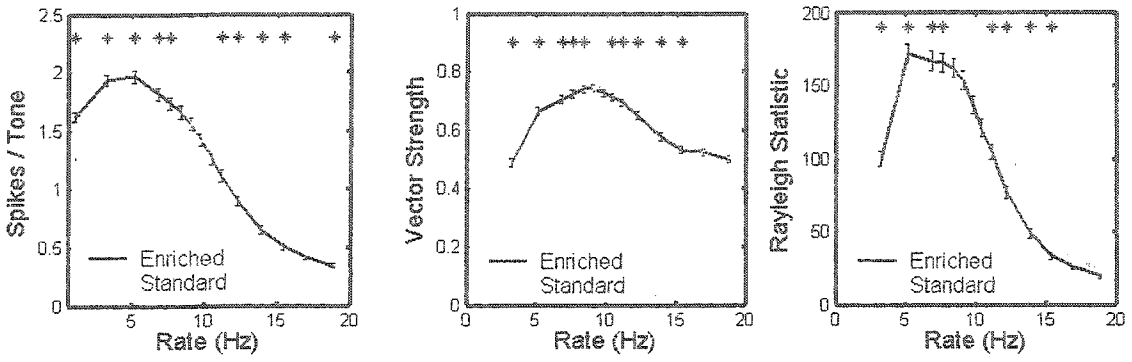


Figure 4. Repetition rate transfer functions (RRTF) for tones in enriched (solid black) and standard (dashed grey) rats. RRTF's were derived at each site by randomly interleaving 12 repetitions of 14 different repetition rates (3-20 Hz) as shown on the x-axis. The tone frequency was set to the best frequency for each site. A) Spikes per tone as a function of repetition rate. Enriched rats responded with more spikes to tones presented at slow rates (<8Hz) and to isolated tones than standard housed rats, while the reverse was true for rates above 11 Hz. B) Vector Strength (VS) quantifies the degree of synchronization between action potentials and repeated tones pips. A value of one indicates perfect synchronization and zero indicates no synchronization. C) The Rayleigh statistic combines the degree of synchronization with the number of spikes. At slower modulation rates, RS was larger in enriched rats compared to standard rats. At faster rates, however, many neurons were not as well synchronized. These results indicate that compared to standard housed controls, enriched rats exhibited *increased* response strength and synchronization at slow rates, but exhibited *decreased* response strength and synchronization at fast rates.

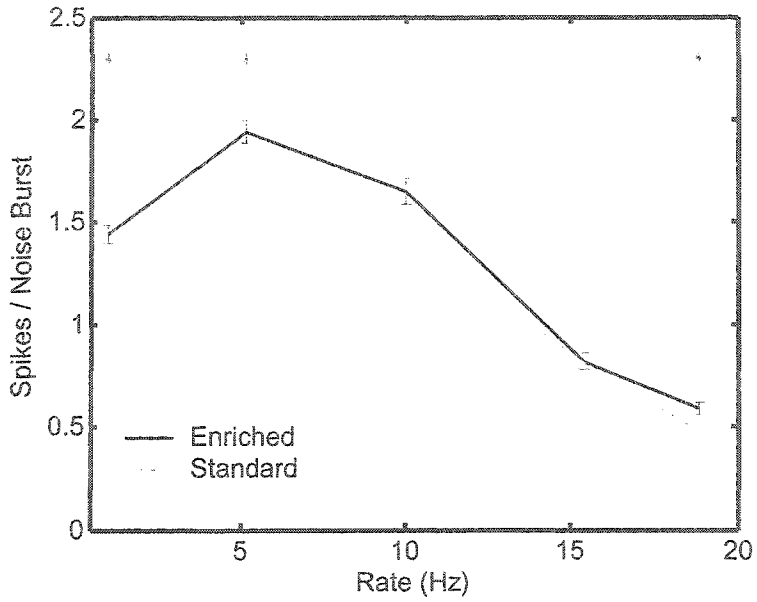


Figure 5. Spikes per for noise bursts as a function of repetition rate. Noise burst RRTF's were derived at each site by randomly interleaving 12 repetitions of 4 repetition rates (5, 10, 15 and 20 Hz). For the slow rates (5-10 Hz), greater spikes/tone was observed for enriched rats compared to standard housed rats.

Figure 5 is the mean normalized RRTF for noise burst trains. For noise bursts too, greater increase in response strength was observed at rates <10 Hz.

Discussion

This study was designed to evaluate the neurophysiologic consequences of environmental enrichment on auditory cortex neurons. Microelectrode mapping provided the greatest spatial precision in documenting cortical plasticity. This technique made it possible to document improvements in A1 response strength, latency, sensitivity, and frequency selectivity. Changes in cellular, synaptic or network properties may contribute to the experience-dependent modifications induced by environmental conditions (Gilbert 1998; Katz and Shatz 1996). Acute

sampling from A1 neurons in enriched rats revealed that increased response strength was accompanied by decreases in onset latency, response threshold and receptive field size. Smaller receptive fields were also observed in visual and somatosensory cortex after enrichment (Beaulieu and Cynader 1990a, b; Coq and Xerri 1998).

The persistence of these effects under general anesthesia is consistent with the earlier conclusions that structural changes contribute to environmental plasticity. Reduced inhibition could explain the increased excitability, decreased threshold, and onset latency of A1 neurons in enriched rats. Enriched cats had 25% fewer GABAergic synapses in visual cortex than standard housed cats (Beaulieu and Colonnier 1987). Application of the GABA antagonist bicuculline increased response sensitivity, spontaneous activity, and maximum discharge rate in visual, somatosensory, and auditory cortex (Dykes et al. 1984; Eysel et al. 1998; Wang et al. 2000; Wang et al. 2002). Although GABAergic blockade mimics several of the effects of enrichment, bicuculline causes receptive fields to increase in size, not decrease. While it appears that decreased inhibition influences physiologic properties in enriched cortex, additional mechanisms are likely to contribute to environmental plasticity. Differences in other modulatory neurotransmitter levels may also affect response properties in enriched cortex. Enrichment increases levels of cortical norepinephrine, dopamine, acetylcholine, and acetylcholinesterase (Feenstra et al. 1995; Giovannini et al. 2001; Naka et al. 2002; Park et al. 1992). Interestingly, iontophoretic application of norepinephrine decreases receptive field size in auditory cortex neurons and acetylcholine decreases thresholds (Manunta and Edeline 1997; Metherate et al. 1990). These results indicate that changes in multiple neurotransmitter systems could explain most of our physiological findings; however, structural changes are likely to contribute as well.

Our findings that enrichment-induced strengthening develops over many days and is maintained under general anesthesia support earlier evidence of neuroanatomical changes induced by environmental enrichment. Previous studies have shown that multiple factors influence the degree of plasticity generated by environmental enrichment. These include physical activity, enrichment duration, social experience, behavioral relevance of sensory events, and age. For example, simple wheel running has been shown to increase cell proliferation and neurogenesis in the adult mouse dentate gyrus (van Praag et al. 2000). Even a few hours of daily enrichment increased brain weight, acetylcholinesterase staining, and RNA/DNA ratios (Ferchmin and Bennett 1975; Will et al. 1977). While passive sensory enrichment failed to alter brain weight in rats, social interactions significantly increased brain weight (Ferchmin and Bennett 1975). Studies in primates have shown that focused attention is required if sensory inputs are to stimulate cortical plasticity (Ahissar and Ahissar 1994; Recanzone et al. 1993). These results indicate that both social interactions and attention contribute to the expression of cortical plasticity. The enriched environment used in this study was designed to expose rats to a wide variety of behaviorally meaningful sensory inputs. While background sounds were present in both environments, in the standard environment these sounds had little behavioral relevance and were less diverse than the sounds in the enriched environment. The enriched housing condition was also designed to increase the behavioral relevance by providing greater social interactions than the standard environment. However, this study cannot determine whether social experience, behavioral relevance of sensory events, attention, physical activity, or enrichment duration were important factors in altering cortical responses.

Exposure to behaviorally relevant sounds that are spectrally restricted (i.e. tones) can alter A1 topography, receptive field size and latency (Recanzone et al. 1993; Weinberger and

Bakin 1998). These changes are typically restricted to the region of the cortical map activated by these sounds. Although some tonal stimuli were part of the environmental enrichment, the plasticity effects documented in this study were not frequency-specific and were found across A1. Sensory sensitization due to random foot shock also strengthens responses across A1 (Bakin and Weinberger 1990). However, these changes develop and fade much more quickly than the effects observed in this study and would likely not persist under the general anesthesia used in some of our experiments. Despite these differences, it remains likely that many of the same mechanisms involved in fear conditioning and perceptual learning are also involved in environmental plasticity.

Cellular mechanisms and paired pulse depression

Neurons in the visual, auditory and somatosensory cortex fail to respond at very high stimulation rates (Creutzfeldt et al. 1980; Hawken et al. 1996; Kilgard and Merzenich 1999; Simons 1985). This temporal low-pass behavior observed in cortex is not evident in subcortical structures including thalamic nuclei that respond vigorously to stimuli presented at fast rates. The cellular mechanisms responsible for the suppressed responses of cortical neurons at faster rates are not clear and could result from synaptic interactions that depend on the previous firing history of the neuron. A presynaptic mechanism that may be responsible for the suppressed responses is paired-pulse depression where an increase in response strength of the first pulse results in suppression of the subsequent pulse. The degree of facilitation or depression of the postsynaptic response depends on the fraction of vesicles released from the presynaptic neuron. In depressing synapses, this fraction is high whereas in facilitating synapses it is low. The release fraction depends on a number of factors such as Ca+2 channel density and Ca+2 buffers in the presynaptic neuron.

More specifically, in vitro recordings from layer II/III pyramidal neurons of primary auditory cortex revealed two distinct populations of neurons: high probability connections (HPC) and low probability connections (LPC) (Atzori et al. 2001). HPC neurons were associated with depressing synapses whereas in LPC neurons the second pulse (of the postsynaptic cell) showed either facilitation or depression. These experiments also showed that the concentration of extracellular $Ca+^{2}$ strongly influences the release probability of HPC neurons but not LPC neurons. Elevated $Ca+^{2}$ concentrations increases the probability of release and results in depression of the second pulse resulting in a decrease in the paired-pulse ratio (P_2/P_1). Similar depression was also observed between hippocampal pyramidal neurons with elevated $Ca+^{2}$ levels (Debanne et al. 1996).

Post natal studies vs. adult enrichment

Earlier studies have shown that primary sensory cortex development can be disrupted by abnormal sensory input in very young animals (Weliky and Katz 1997; Zhang et al. 2002). Rats exposed to pulsed noise before four weeks of age exhibited disruption of tonotopicity and receptive field plasticity (Zhang et al. 2002). Pulsed noise exposure after four weeks of age resulted in no significant plasticity. These results indicate that some forms, particularly deleterious forms, of auditory cortex plasticity are limited to the first four weeks of post-natal development. This may be due to protective safeguards innate in the central nervous system. However, our finding that environmental enrichment can cause plasticity in primary auditory cortex indicates that cortical responses can be substantially altered even in animals well beyond these early sensitive periods. This finding extends the observation that highly focused behavioral training can alter cortical responses in adults by demonstrating that even generalized environmental enrichment can significantly enhance responses in primary auditory cortex. Since

the present study was not designed to determine how specific factors influence environmental plasticity, additional studies will be needed to evaluate the potentially interacting factors responsible for the profound physiological changes observed in this study.

CHAPTER 4

PERCEPTUAL LEARNING OF SOUND SEQUENCES IN THE AUDITORY CORTEX

Introduction

Temporal processing of auditory information is critical for processing complex sounds including speech. In primary auditory cortex neurons of many species, sounds presented as part of a sequence show facilitation or adaptation to the following elements. This response enhancement or attenuation depends on the spectral and temporal separation of sounds. For example, context dependent facilitation was maximal when temporal separation of tones was approximately 100 ms and separated by 1 octave (Brosch and Schreiner 2000; Brosch et al. 1999). Adaptation to successive elements of sound sequences (forward masking) has been demonstrated with pure tones (Abeles and Goldstein 1972; Brosch and Schreiner 1997; Calford and Semple 1995; Horikawa et al. 1997; Phillips et al. 1989), click trains (Eggermont 1991), amplitude and frequency modulated sounds (Eggermont 1994; Schreiner and Langer 1986; Schreiner and Langner 1988) and natural calls (Creutzfeldt et al. 1980; Glass and Wollberg 1983).

In many specialized species, sounds that have ecological value drive auditory neurons only if presented in a particular order. In the bat, for example, many auditory neurons will only respond to a specific combination of the pulse-echo pair compared to individual components of the pair (Suga et al. 1978). Similar context-dependent facilitation has also been documented in auditory

neurons of the song-bird (Margoliash 1983) and cat (McKenna et al. 1989). Thus, auditory neurons in higher brain regions can exhibit a selective response to certain sequences and a degraded or absent response to individual components of the sequence or if the sequence order is changed.

The ability to discriminate patterns in sounds is an important property of the central auditory system. Auditory cortex lesions can disrupt complex discriminations in both animals (Diamond and Neff, 1957; Harrington and Heffner, 2000; Heffner, 1986; Ohl and Scheich H, 1999) and humans (Kaga et al. 1997; Tramo et al. 2002). In contrast, the discrimination of simple tones is preserved which suggests that the auditory cortex may play an important role in processing complex spectro-temporal transitions including animal vocalization and speech.

Perceptual learning on auditory, visual or tactile discrimination tasks alters cortical responses in a task-specific manner (Edeline 1999). Training can result in reorganization of cortical maps, receptive field plasticity and altered temporal responses that are specific to the trained stimulus. For example, long-term operant training on a tactile and auditory discrimination task can substantially increase the cortical representation of the trained frequency, narrow receptive fields and sharpen temporal responses (Recanzone et al. 1992a; Recanzone et al. 1992b; Recanzone et al. 1993). The effects of long term training on sound sequence discrimination have not been studied. In rats, learning to discriminate a sound sequence from other sequences increases the behavioral relevance of the acoustic pattern and consequently improve cortical processing. In this study, rats were trained on a variety of sequence discrimination tasks that ranged from an easy to difficult task.

Methods

Thirty-five female Sprague-Dawley rats were used in this study. All protocols and recording procedures conformed to the Ethical Treatment of Animals (NIH) and were approved by the Institutional Animal Care and Use Committee at the University of Texas at Dallas.

General description of operant chamber

Rats were trained in an operant chamber placed inside a sound-attenuated booth affixed with a light source. A second light source was placed just above a lever inside the cage. Lever press triggered the delivery of a sugar pellet from a dispenser into a receptacle inside the cage. Sounds were delivered via a calibrated speaker (Optimums Bullet-horn Tweeter- 40-1221) mounted ~10 cm away from the rat's left ear. Acoustic stimuli were presented at 50 dB SPL. The booth was placed in a closed room, and the rat's behavior was monitored on a video screen. Lever presses and light status were monitored and recorded using custom-made programs (MATLAB, Mathworks) and TDT (Tucker-Davis) hardware and software. Rats trained two sessions per day (each session lasted ~1-hr) five days a week for a period of 2-3 months. They were food deprived ~20-hrs prior to each training session. Constant temperature and humidity were maintained in the rat colony room. Rats were maintained on a reverse 12-hour light/dark cycle.

Sequence Discrimination Training

Rats were trained on a variety of sequence discrimination tasks that varied from easy to difficult. The specifics of each of these tasks are detailed below but in all cases, training involved two phases; an initial shaping phase followed by discrimination training. During the shaping phase, rats learned to make appropriate contact with the lever in order to receive a food reward. Both light and the appropriate sound sequence (CS+) were used during shaping. After shaping

and before the onset of discrimination training, rats were trained to *detect* the target (CS+) sequence for ~1 week. Once they mastered this task, they attempted to *discriminate* the CS+ sound from one or more CS- sounds as a go/no-go task.. CS+ and CS- sequences were randomly interleaved and presented every 7-10 seconds. Every session also included "silent" trials during which no sounds were presented and served as catch trials. Including these silent trials prevented the rats from adopting a timing strategy and brought them under good stimulus control. If the rat stopped performing the task (missed 5 consecutive CS+ sounds without any lever presses) during a session, they would receive a time-out with house light off until the reinitiated the task by pressing the lever. Such a break in the task generally only occurred when the rats reached satiety and fell asleep or while the rats were drinking from the water bottle.

A schematic of the discrimination task is shown in Figure 6. Rats were rewarded when they pressed the lever within 3 seconds of hearing the CS+ sound (hit). A miss was recorded if they failed to respond to this sound. Pressing the lever any other time resulted in a time out (lights off for 5-6 seconds). If they continued to press the lever during this time, the presentation of the next sound was delayed. On CS- sound trials, pressing the lever within 3 seconds from sound onset resulted in a time out (false alarm) whereas avoiding it resulted in a correct rejection. All sounds in the sequence were 25 ms long with 3 ms ramps and had a stimulus onset asynchrony (SOA) of 100 ms between each of the sound elements. Reaction time was documented for lever presses to both the CS+ and CS- sound.

Rats were trained on sequence discrimination tasks that ranged from an easy frequency discrimination task to a more difficult discrimination task (Fig. 7 A-D). For the frequency discrimination task (Fig. 7A), rats (n=8 rats) attempted to discriminate a Low-Low-Low (LLL) sequence (CS+) from a High-High-High (HHH) sequence (CS-). The frequencies of the low and

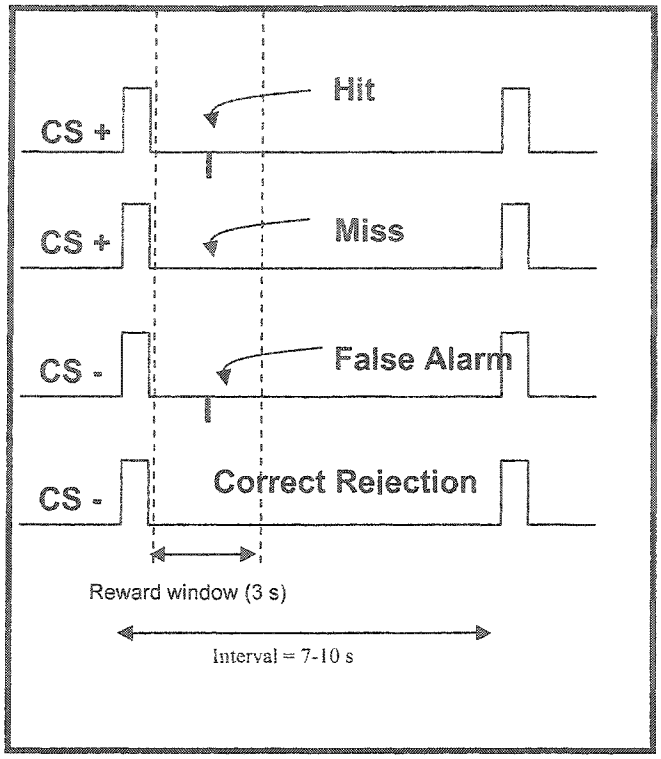


Figure 6. Schematic of the Go/no-go task for sequence discrimination. The CS- and CS+ sequences were randomly interleaved and presented every 7-10 seconds. Hit window was defined 300 ms after sound onset unto 3 seconds later. Rats were rewarded if they pressed the lever within this time window to the CS+ sequence. If lever press occurred 300 ms before the hit window onset, or 3 seconds later, rats received a time-out. During the time-out, booth lights were switched off for 5-6 seconds. If rats continued to press the lever after lights came back on and before the presentation of the next sound, they received a time-out again and presentation of the next sound was delayed by about 40 ms. A miss was recorded if they failed to respond to the CS+ sound. For the CS- sound, lever press during this window also resulted in a time-out (false alarm) whereas avoiding it resulted in a correct rejection.

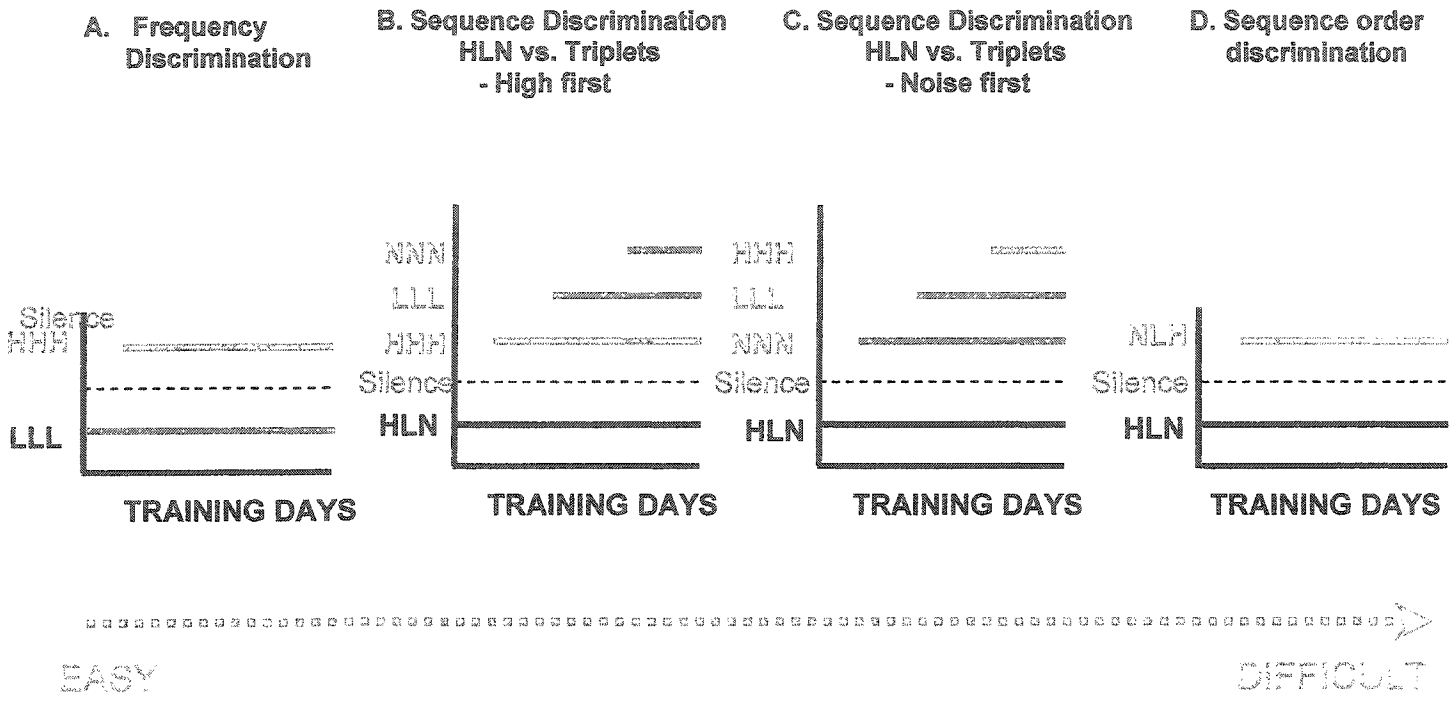


Figure 7. Auditory sequence discrimination tasks. Panels A-D show sequence discrimination tasks that ranged from easy to difficult. In all tasks, silence (catch) trials were used (dashed line) to prevent the rat from adopting a timing strategy and brought them under good stimulus control. In all tasks, rats were shaped with the CS+ sound (not shown) in order to make appropriate contact with the lever. After shaping, rats were switched to a go/no-go task wherein the CS+ sound and silence trials were randomly interleaved. The CS – sounds were introduced later, once they learned the CS+ detection task. A) Frequency discrimination. Rats attempted to discriminate the LLL sequence from the HHH sequence. B & C) HLN sequence discrimination. Rats attempted to discriminate the HLN sequence from three CS- triplet sequences (HHH, LLL and NNN). The only difference between the 2 tasks was the order of presentation of the CS – sounds. In the High-first task, HHH was the first CS- triplet whereas in the Noise-first task, NNN was the first CS- triplet. The approximate number of days spent on each triplet was the same for both groups. D) Reverse order discrimination. Rats attempted to discriminate a HLN sequence from its reverse order NLH triplet.

high tone were 5 kHz and 12 kHz respectively. For the next two tasks (Fig. 7B & C), rats attempted to discriminate a High-Low-Noise (HLN) sequence (CS+) from triplet CS- sequences (High-High-High; Low-Low-Low; Noise-Noise-Noise). The two groups differed only in the order of presentation of the CS- sounds. In one group (n=4 rats), HHH was the first CS- (the High-first group), while the other group (n=7 rats) had NNN as the first CS- sound (Noise-first group). For the final task, rats (n=5 rats) attempted to discriminate the HLN sequence from its reverse Noise-Low-High (NLH) sequence (Fig. 7D).

Exposure to sound sequences

A group of rats (n=4) was simply exposed to the sound sequences HLN, NNN, LLL and HHH and served as a control. These sounds were randomly interleaved with the delivery of food. On average, rats heard approximately the same number of sounds and received approximately the same reward amount as that of the trained groups. They were exposed to the sequence sounds for a period of ~ 2 months. Although this group was food deprived and motivated just like the trained group, no association was made between stimulus and reward. The trained and exposure groups were compared to naïve rats (n=7).

Acute Surgery

Surgical anesthesia was induced with sodium pentobarbital (50 mg/kg, i.p.). Supplemental doses of dilute pentobarbital (8 mg/ml, i.p.) induced a state of areflexia throughout the surgery and recording phases. The interval between supplements varied depending on the anesthetic state of the rat but was typically every 1-1.5 hr. Anesthesia depth was evaluated by heart rate, breathing rate, toe-pinch responses and corneal reflexes. These indicators were indistinguishable between the two groups. Circulatory function was monitored with EKG and pulse oximetry. Body temperature was maintained at 37° C. Fluid balance was maintained with a 1:1 mixture of 5% Dextrose and Ringer's Lactate (~0.5 ml/hour). The

trachea was cannulated to minimize breathing sounds and ensure adequate ventilation. Humidified air was delivered to the open end of the cannula. After the cisterna magnum was drained to minimize cerebral edema, the right auditory cortex was exposed and the dura resected. The cortex was maintained under a layer of viscous silicon oil to prevent desiccation during the 24-30 hr experiment and a detailed map of auditory cortex was generated from 50-100 microelectrode penetrations. The sampling density and depth of recordings made in trained, sound exposed and naïve rats were indistinguishable. Parylene coated tungsten microelectrodes (FHC) were lowered 550 μm below the pial surface (layer 4/5) of the right auditory cortex. Spikes from a small cluster of neurons were collected at each penetration site. Penetration locations were referenced using cortical vasculature as landmarks.

Behavior data analysis

For all trained rats, hit rate (number of hits/total number of CS+ trials) and false alarm rate (number of false alarms/total number of CS- trials for a particular sequence) were determined at the end of each training session. Behavioral performance was measured with standard signal detection theory criteria (Green and Swets, 1966). The d' measure allows one to measure the sensitivity of the rats performance regardless of the criterion used. The value of d' is calculated from the hit rate and false alarm rate for each session by using the MATLAB function norminv (inverse of the normal cumulative distribution function (cdf) :

$$d\text{- prime} = \text{norminv}(1-\text{false}) - \text{norminv}(1-\text{hit})$$

where false is the false alarm rate and hit is the hit rate for each session. This function essentially computes the z-score of the noise (N) distribution (1-false) and signal + noise (SN) distribution (1-hit) and then computes their difference (i.e. $Z_N - Z_{S+N}$). Thus, the value of d' is simply the

number of z-score units between the mean of the SN and S distributions. Paired *t*-test was used to compare early and late *d* primes for each sound sequence.

Stimulus presentation and neurophysiologic data analysis

Stimuli were generated using SigGen and Brainware. Frequency-intensity-tuning curve were derived at each site. Auditory frequency tuning curves were determined at each site by presenting 41 logarithmically spaced frequencies from 1 to 32 kHz at 16 intensities from 0 to 75 dB SPL (656 total stimuli). The tones were randomly interleaved and separated by 1300 ms. Response latency of A1 neurons from all rats was quantified from tuning curve data and from sequence data. In the first case, minimum latency was defined as the time from stimulus onset to the earliest consistent response for any of 15 intensities of the three frequencies that were nearest the CF (45 stimuli). The end of response latency was defined as the time after tone onset when the PSTH (peristimulus time histogram) created by summing the responses to all of the tones within each site's tuning curve returned to baseline. All analysis was done using Matlab 6.5. In all cases, error bars reflect standard error of the mean.

Neural responses to the stimuli presented during behavior training and variations of the paired sequence were also included as part of the stimulus set (See Appendix B). Twenty repetitions of each of the sequence elements presented alone and in the following combinations were randomly interleaved: HLN (50, 100, 200 ms), NLH (100 ms), LHN (100 ms), LN (100 ms), HN (200ms), LLL (100 ms), HHH (100 ms) and NNN (100 ms). All stimuli were 50 dB SPL 25-ms long with 3-ms amplitude ramps and presented every 3 seconds. Neural responses were evaluated by documenting the number of spikes to a sequence element when preceded by other elements of the sequence compared with the response to the same element in isolation. Context-dependent facilitation or suppression was quantified by comparing the mean

facilitation/suppression index for all sites from experimental and control rats. The index is 100 times the logarithm base-two of the ratio of the number of spikes in response to a stimulus element in the context of a sequence and the number of spikes in response to the same element in isolation.

Results

Behavioral performance was recorded over the course of training. After 2-3 months of training, rats were anesthetized and underwent multi-unit microelectrode mapping of the primary auditory cortex. Auditory cortices of sequence exposed and naïve rats were also mapped and their A1 responses were documented and compared to the trained rats.

Behavior

Frequency discrimination

Rats learned to discriminate the HHH CS- sequence from the target LLL sequence (Fig. 8A & B). *d* primes improved over the course of training. Figure 8C shows the mean *d* primes for all rats binned every 2 sessions. On average, rats heard the LLL (CS+) sound for ~ 60-120 days. The HHH (CS-) sequence was introduced after the rats were good at detecting the LLL sequence. *d* primes for the HHH sequence increased over the training period which indicated an increase in discrimination sensitivity (d'>1.5). When compared to first versus the last day of training (Fig. 8D), paired *t*-test analysis revealed a significant improvement (Silence: 0.2 ± 0.1 vs. $2.3 \pm .28$, $p < 0.0001$; HLN vs. HHH: 0.2 ± 0.08 vs. 1.8 ± 0.31, $p < 0.0001$).

High-Low-Noise sequence discrimination

Rats attempted to discriminate the target HLN sequence from CS- triplet sequences (HHH, LLL and NNN). After reaching good stimulus control for the HLN CS+, one set of rats

was presented first with the HHH CS- sound (High-first group) followed by LLL and NNN, and the other group was presented first with the NNN sequence (Noise-first group) followed by LLL and HHH. The only difference between these two groups was the order of addition of new CS- sounds (Fig. 7).

In the last phase of training, rats in both groups were attempting exactly the same task (i.e. to discriminate the same CS- sequences from the HLN sequence). The duration of presentation of an individual CS- sound was on average similar in both groups. For example, in the High-first group, the HHH sequence was presented for ~ 28.2 days and in the Noise-first group, the same sequence was presented for ~30 days. Although in the latter group, rats were already hearing the LLL and NNN sequences by the time the HHH sequence was introduced, rats attempted to discriminate all the sequences towards the end of training. Similarly, for the High first group, rats were already attempting to reject the HHH and LLL sequence by the time NNN was added later. For both groups, on average, rats were listening for all the CS- sequences during the last 4-4 ½ weeks of training.

The High-first group was able to discriminate all the triplet sequences from the HLN sequence (Fig. 9A & C). Although there was some variability in the time it took for rats to discriminate the HHH from HLN sequence (from 5-25 days), on average, rats were able to discriminate the first CS- sound (HHH) within 25 days. When the LLL sound was introduced next, they were already good at discriminating this sequence from HLN in the presence of the HHH sequence (Fig. 9C). Finally, they learned to discriminate the NNN sequence from the HLN sequence (in presence of the other two CS- sounds).

Paired *t*-test analysis showed that *d* primes achieved during the last session were significantly greater than the first session for the HHH and NNN sequence (HHH: 0.07 ± 0.1 vs.

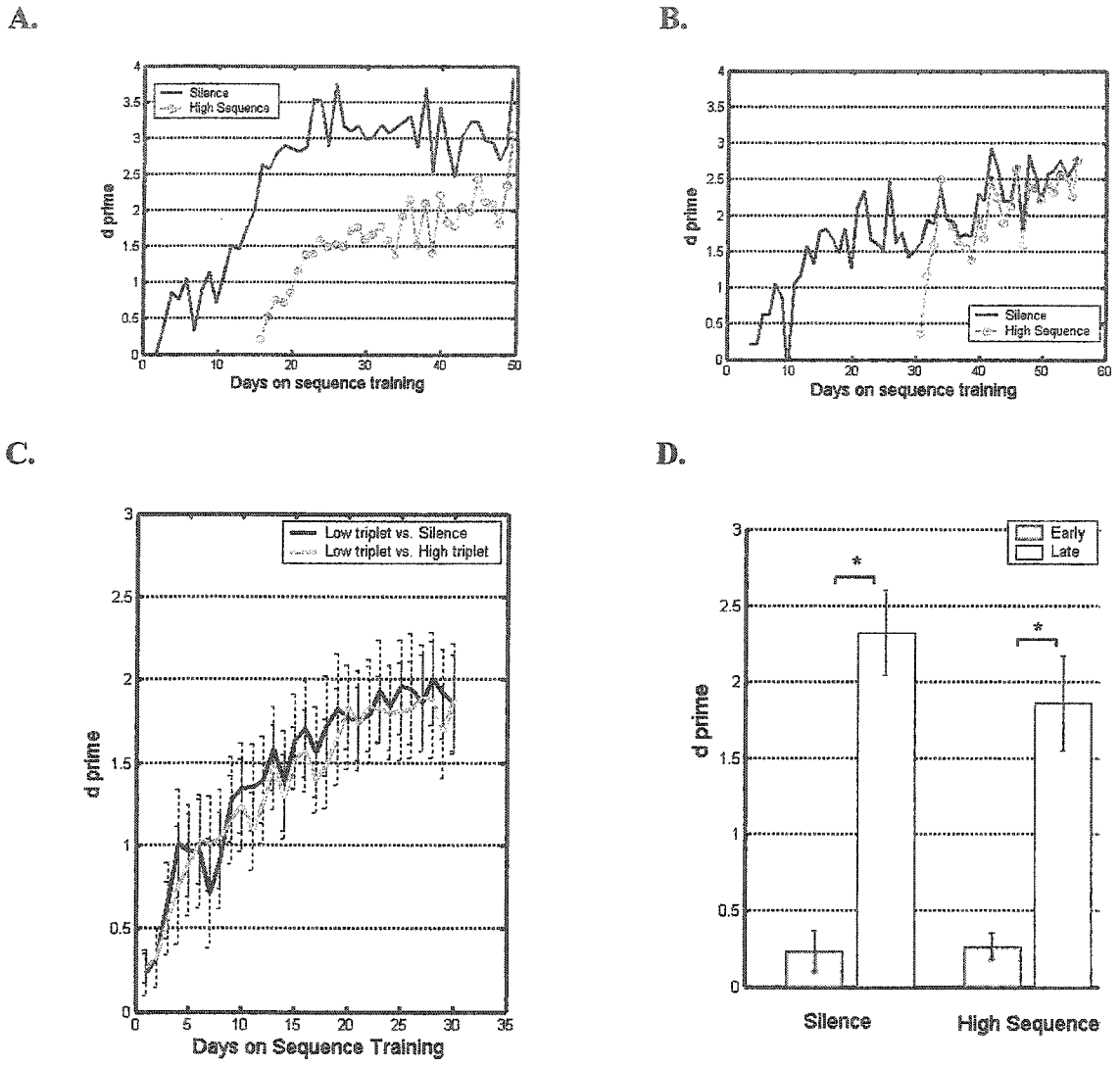


Figure 8. Frequency discrimination training. Rats attempted to discriminate a LLL (CS+) sequence from the HHH (CS-) sequence. Improvements in performance are measured with *d* prime (see methods) A-B) Discrimination performance in 2 rats. The black line is the LLL detection task (versus silence). During silence trials (catch trials), no sounds were presented, and brought rats under good stimulus control. Performance on the HHH sequence (unfilled circles) improved with practice. This sequence was added after rats were able to detect the LLL sequence. C) Data averaged across all rats (n=8). Green line represents the improvements for the HHH discrimination performance. Data is shown only for the first 30 days. The HHH sequence has been offset to the start of the silence trials and represents the total number of days on that sequence. D) Early and late d-primes for silence and HHH discrimination. Early (grey) *d* primes represent the first day of training and late (white) *d* primes represent the last day of training for silence and the HHH sequence. Rats were able to discriminate the LLL sequence from the HHH sequence on the last day of training (0.2 ± 0.08 vs. 1.8 ± 0.31, $p < 0.0001$).

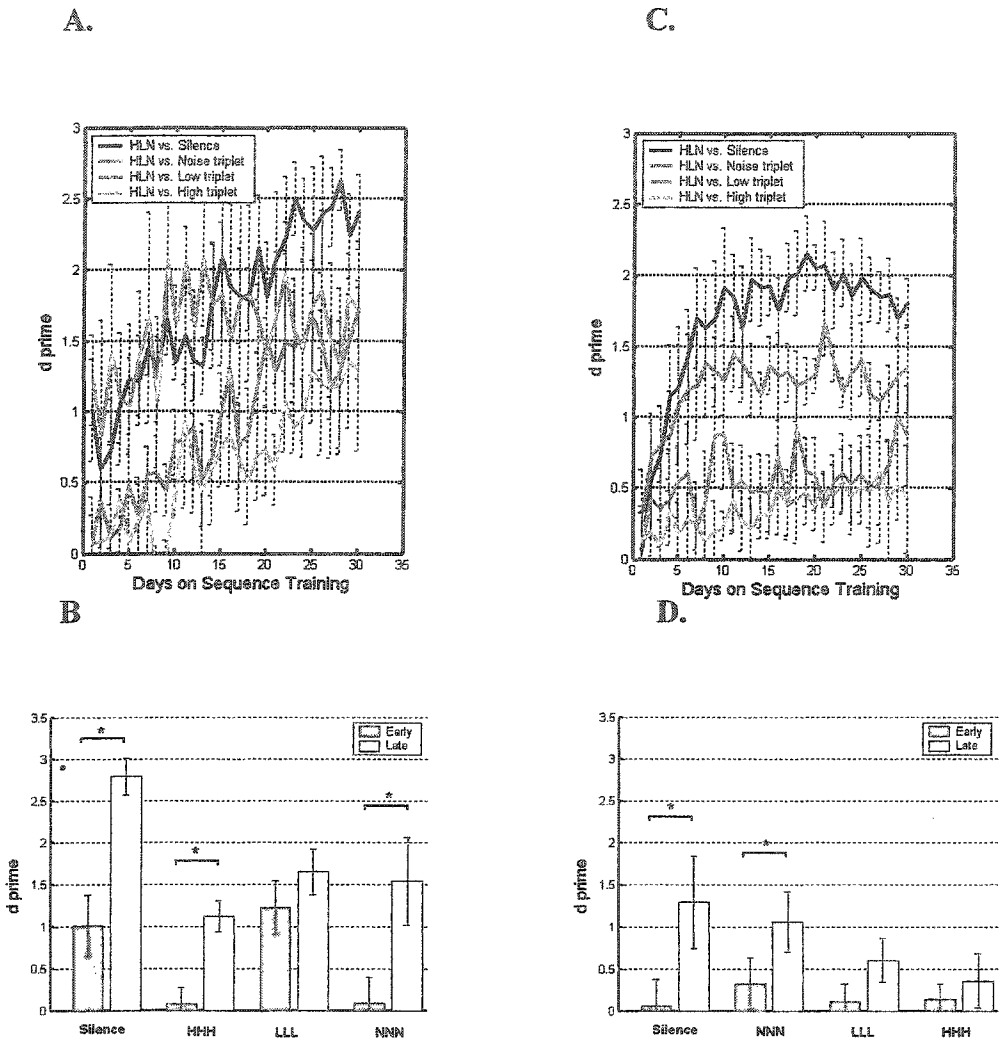


Figure 9. HLN vs. triplet discrimination. Improvements in performance are measured with the *d* prime measure (see methods). In panel A & C, average *d* primes are shown for every 2 sessions (bins). A & B) High-first group. Rats attempted to discriminate the HLN sequence from HHH (green), LLL (red) and NNN (blue) sequence (in that order). The sequences have been offset to the beginning of the axes to represent the total number of days on a sequence and not the day that these sequences were added. Data is shown for the first 30 days. Black line depicts detection of the HLN sequence compared to silence trials. During these silence trials, no sounds were presented to the rat, and served as catch trials and brought rats under good stimulus control. Panel B shows early (grey) vs. late (white) *d* primes. Rats were able to discriminate the HLN sequence from all CS- sequences (paired *t*-test). C & D) Noise-first group. Rats attempted to discriminate the HLN sequence from NNN, LLL and HHH sequence in that order. Data shown for first 30 days. Panel D) shows early (grey) vs. late (white) *d* primes. In this group, rats were unable to discriminate the tone sequences from HLN when preceded by the noise sequence. They were however, able to discriminate the noise sequence from HLN.

1.11 ± 0.1, $p < 0.01$; NNN: 0.08 ± 0.3 vs. 1.54 ± 0.5, $p < 0.01$). Rats in the High-first group were already good at discriminating the LLL sequence from the HLN sequence on the first day of exposure and did not improve significantly (paired *t*-test, $p > 0.05$). When the order of CS- presentation was reversed, rats were able to discriminate the first triplet (NNN) from the HLN sequence within ~11 days but had difficulty discriminating the two tone sequences LLL and HHH that were added later (Fig. 9B).

In contrast, the High-first group, rats had no difficulty discriminating all the CS- sequences if HHH was the first CS- sound. Since the amount of time that the Noise-first group heard the HHH sequence was comparable to the amount of time for the same sequence in the previous group (~30 days-HHH group vs. ~28.2 sessions-NNN group), the likely reason for the inability to discriminate the HHH sequence was the presence of the NNN and LLL sequence.

Rats in the noise-first group learned to discriminate only the NNN sequence (early dprime vs. late dprime: 0.3 ± 0.3 vs. 1.05 ± 0.3, $p < 0.05$) whereas they were unable to correctly reject the HHH and LLL tone sequences (Fig. 9D). In summary, it appears that even though the sound stimuli are exactly the same in both these groups, discrimination performance depends on the order of presentation of the CS- sounds.

Reverse order discrimination

In the reverse order task, rats attempted to discriminate the HLN sequence from its reverse order NLH sequence. This proved to be difficult and rats were unable to do the reverse discrimination. Figure 10A & B shows data from individual rats that were unable to discriminate the two sequences, although performance on the HLN sequence was good. On average, rats achieved poor *d* primes (Fig. 10C) even after weeks of training on this task. When compared to

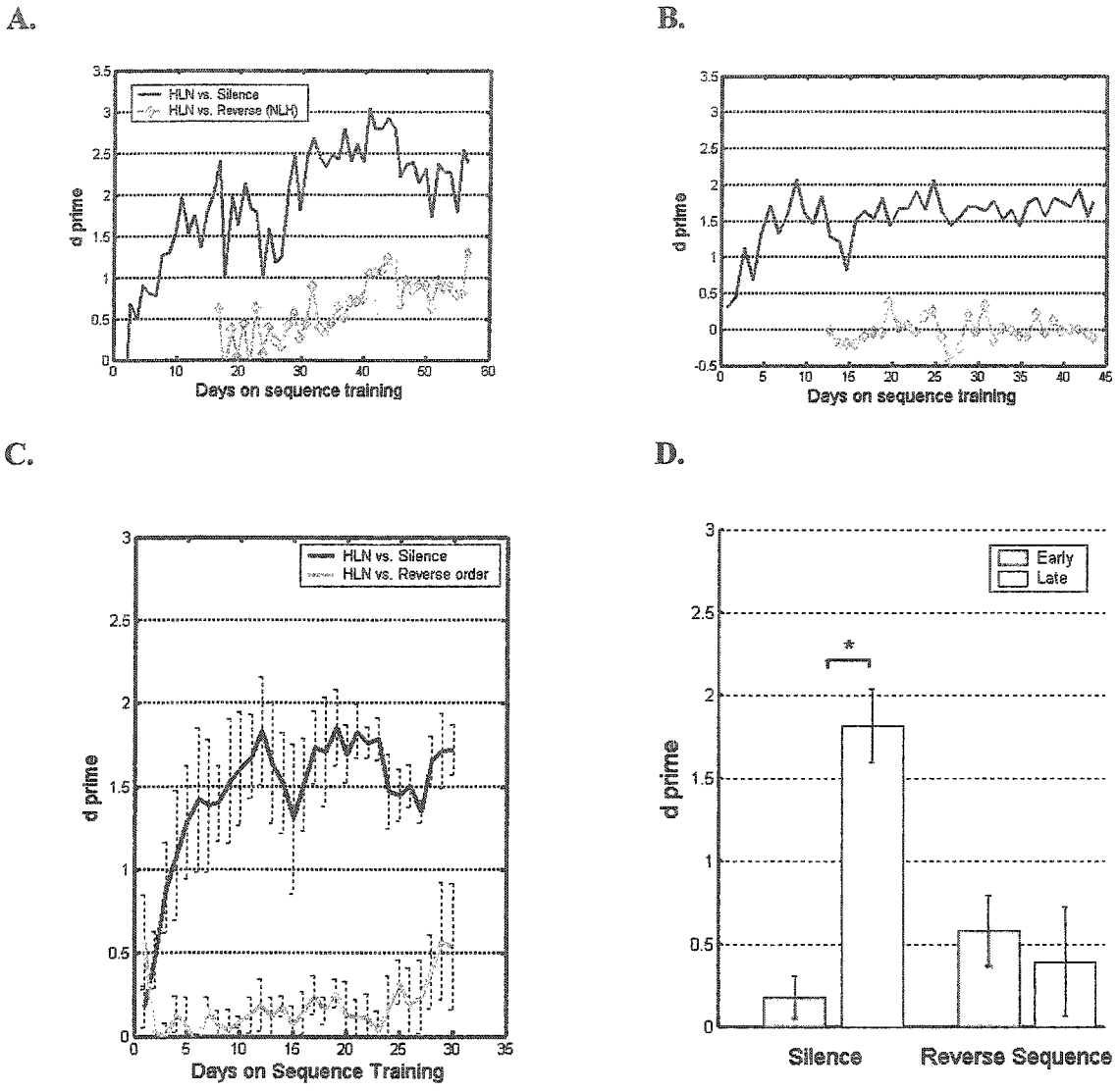


Figure 10. Reverse order discrimination. Performance is measured with the *d* primes (see methods) A-B) Discrimination performance in 2 rats. The black line shows detection of the HLN sequence compared to silence trials. During these silence trials, no sounds were presented to the rat, and this served as catch trials and brought rats under good stimulus control. NLH discrimination is represented by unfilled diamonds. Poor *d* prime for the NLH sequence can be seen for both rats.

C-D) Data averaged across all rats. The teal line represents the NLH performance. Data is shown only for the first 30 days. The NLH sequence has been offset to the beginning of the x-axes and represents the total number of days on that sequence. Panel D) shows that rats were unable to discriminate the HLN from the NLH sequence (paired *t*-test, $p > 0.05$).

first versus the last day of training (Fig. 10D), paired *t*-test analysis did not show a significant improvement of *d* primes for the reverse sequence (0.57 vs. 0.39, n.s.).

Reaction time

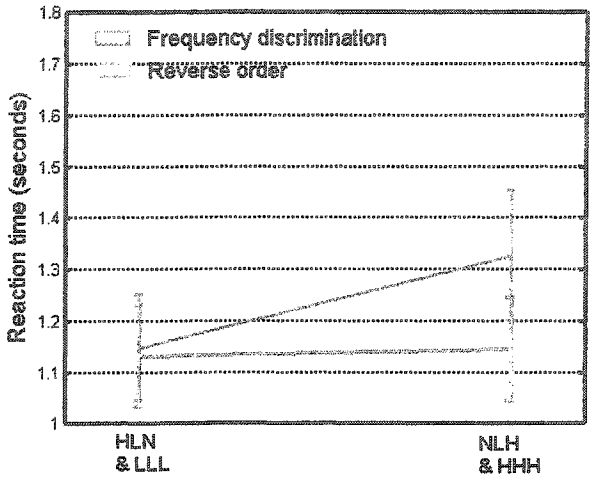
Reaction times for lever presses were documented with each hit or false alarm. Mode was used as the measure of central tendency rather than mean or median because this value represented the most likely reaction time for each sequence in a single session. Reaction times were measured for the CS+ and CS- sounds for the duration of the hit window. Lever presses beyond 3 seconds were also recorded but excluded from reaction time analysis. The reaction time data is shown for the last 10 sessions in each group.

After frequency discrimination training, no significant differences in reaction time were observed for the CS+ or CS- sequence (paired *t*-test, $p > 0.05$) (Fig. 11A). This was probably because rats treated the CS- sequence as the CS+ sequence. The reverse order, high first and noise first groups also did not show any significant changes in reaction time between the CS+ and CS- sequences (Fig. 11A & B). However, reaction times for the CS+ sequences were different across groups (Figure 12). Reaction times for the Noise-first was greater compared to the frequency discrimination task (1.50 ± 0.1 vs. 1.15 ± 0.1, $p < 0.05$, one-tailed *t*-test). No significant difference was observed in the CS+ reaction times of the High-first and Noise-first group (1.35 ± 0.1 vs. 1.50 ± 0.1, $p > 0.05$, tw0-tailed *t*-test).

Summary of behavior results

Rats were trained on a variety of sequence discrimination tasks that ranged from easy to difficult. A summary of behavior performance is shown in Figure 13. Frequency discrimination was the easiest task whereas reverse order discrimination was the hardest. When this same

A.



B.

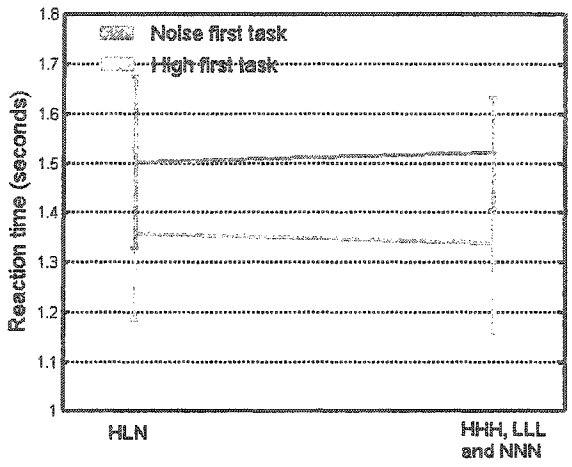


Figure 11. Reaction time data (mode) after discrimination training. Panel A. Reaction times for LLL and HHH are not significantly different after frequency discrimination training (magenta) (LLL vs. HHH, 1.14 ± 0.1 vs. 1.32 ± 0.1, $p > 0.05$). After reverse order training (teal), reaction times for HLN and NLH are also not significantly different (HLN vs. NLH, 1.13 ± 0.09 vs. 1.14 ± 0.1, $p > 0.05$). Panel B Reaction time date for the Noise-first (red) and High-first (green) groups. The reaction time for CS- sequences is the average reaction times for NNN, LLL and HHH sequences since all rats in both these groups were attempting to discriminate the three triples during the 5 days (10 sessions). There is no significant difference between reaction times for the CS+ and CS- sequences in both groups. Error bars represent standard error of the mean (s.e.m).

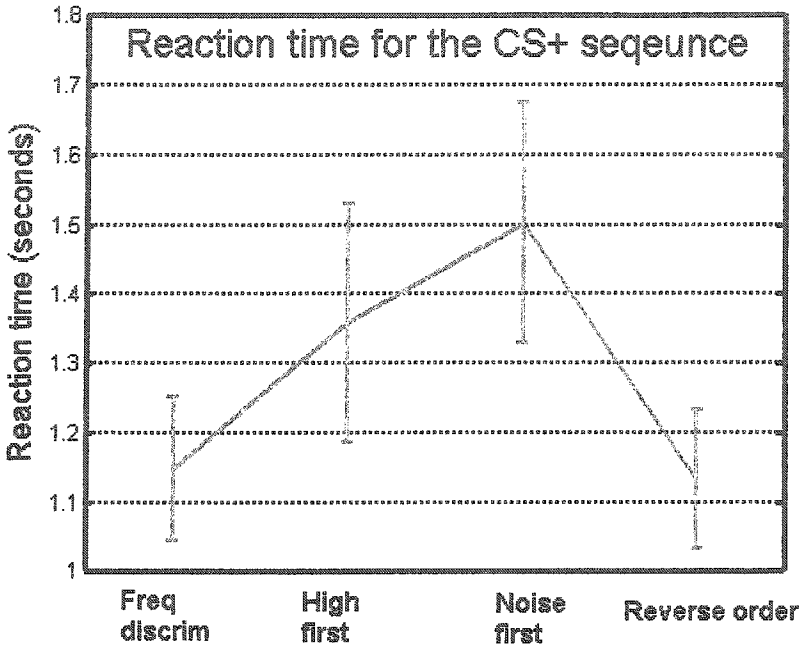


Figure 12. Reaction time data (mode) for the CS+ sequences. Compared to the frequency discrimination group, the noise-first group shows an increase in reaction time (one-tailed *t*-test, $p < 0.05$). There were no significant differences between the other groups.

sequence (HLN) served as the CS+ sequence with triplet CS- tones and noise burst, it produced different results. If HHH was the first triplet, rats were able to discriminate all the triplet sequences. Changing the order of presentation of these triplet sequences (NNN first) prevented subsequent tone sequence learning in presence of noise. Previous results combining behavioral training with electrophysiology have demonstrated that long term training on discrimination tasks alters cortical topographic organization, receptive field and temporal response properties of auditory cortex neurons. Our next goal was to document the responses from primary auditory cortices of these rats at the end of training. The results of the acute electrophysiology from these rats (and naïve and sequence exposed rats) are discussed next.

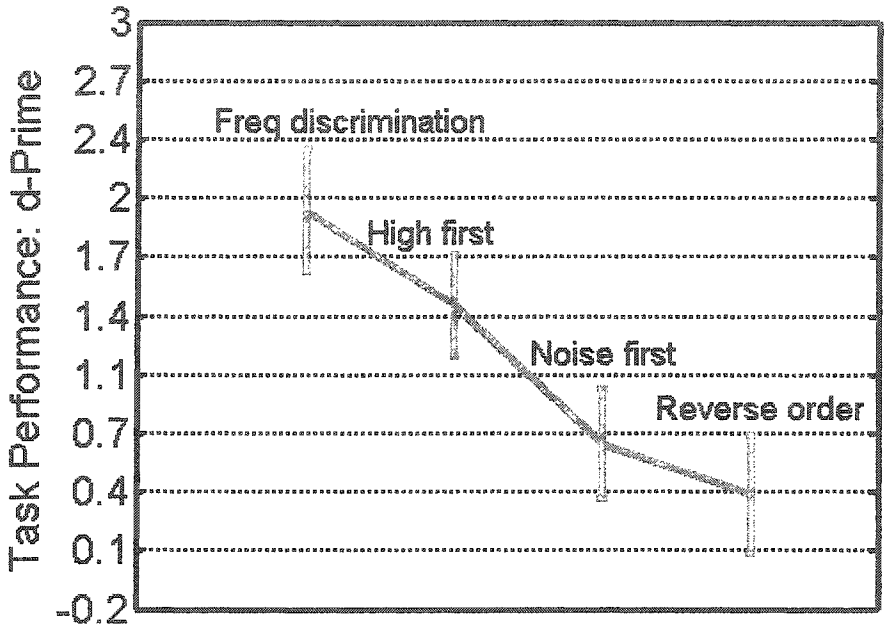


Figure 13. Summary of behavior performance. Frequency discrimination was the easiest task with the largest *d* prime. The most difficult task was the reverse order discrimination. Performance on the high first and noise first task was intermediate between the other two tasks. *d* primes represent the average *d* prime for all the CS- sequences in the high first and noise first task. Error bars represent standard error of the mean (s.e.m).

Electrophysiology

Action potentials were recorded from small groups of A1 neurons from ~2000 A1 sites in 35 rats. Long term sequence training generated different forms of plasticity in A1 depending on the nature of the task. Sequence discrimination training substantially altered response strength, frequency selectivity, latency and the degree of suppression to the following elements of the sequence.

Table 2. Number of rats and A1 sites from control and experimental groups.

Experimental groups	# Rats	# A1 Sites
A) Controls	7	329
B) Sequence Exposure	4	263
C) Frequency Discrimination	8	444
D) High-first	4	189
E) Noise-first	7	433
F) Reverse (HLN vs. NLH)	5	329
Totals	**35**	**~2000**

Strength of evoked response

The strength of evoked response to tones in the receptive field of A1 neurons increased after training on the sequence discrimination tasks (Fig. 14). Compared to control rats, the greatest increase in response strength of A1 neurons was observed in the High-first and Noise-first group (~1.5 ± .04 spikes/tone vs. 1.2 ± .03 spikes/tone, $p < 0.00001$). This represented ~30% increase in the number of spikes compared to control rats. When rats were simply exposed to these same sequences, the increase was only 10% (1.4 ± .04 vs. 1.2 ± .03 spikes/tone, $p < 0.05$).

In the easy task (frequency discrimination) and difficult tasks (reverse order discrimination), response strength increased was only 10-15% compared to controls This difference was even more when they were compared to the High-first and Noise-first group. Sequence exposure also increased response strength but only by ~10% compared to controls. In addition to the observed increase in overall response strength of A1 neurons in trained and sound exposed rats, we also quantified responses to the sequence elements that rats were trained on. In the next section, responses to the individual elements of the sequence (High, Low or Noise) will be documented first followed by responses to the second and third elements of the sequence.

Response to individual elements of the sequence

For the frequency discrimination group, responses to the low (5 kHz) and high (12 kHz) tone in specific regions of the A1 frequency map were quantified in order to document frequency specific effects on response strength. Responses were quantified by binning the frequencies half an octave on either side of 5 kHz (3.5 - 7.1 kHz) or 12 kHz (8.5 – 16.9 kHz). In the low frequency region, there was a greater increase in response to the 5 kHz tone in trained rats compared to controls ($1.5 \pm .08$ vs. $1.2 \pm .06$ spikes/tone, $p < 0.01$). Similarly, in the high frequency region, response to the 12 kHz tone increased compared to controls. Interestingly, response to the low tone in the high frequency region increased significantly by more than 40% compared to controls. In contrast, response to the high tones in the low frequency region of trained rats was similar to controls (Fig. 15).

For the intermediate tasks, response to the individual high, low and noise elements increased compared to control rats (Fig. 16 A-C). The sound exposure group also showed an increase in response strength to the first elements compared to controls. Smaller increases in

response strength were also observed for the reverse order discrimination group and the frequency discrimination group (data not shown).

Response to sequences after training

Suppression or facilitation to the following elements was quantified by using a suppression/facilitation index. The index is 100 times the logarithm base-two of the ratio of the number of spikes in response to a stimulus element in the context of a sequence and the number of spikes in response to the same element in isolation. For example, an index of 100 signifies twice the number of spikes to the following element compared to the number of spikes in isolation whereas -100 denote half as many spikes. Sites that show no facilitation or suppression have an index of zero. In addition to quantifying responses to the triplet elements that were presented during the training, responses to other variations of the triplets were also quantified (see methods).

After training on the frequency discrimination task, response to the second element of both the low and high triplet was suppressed compared to the first element (- 18 and - 20 respectively). However, this suppression was less when compared to control rats (Low: - 18 vs. - 43, $p < 0.05$; High: - 20 vs. - 56, $p < 0.00001$). The third element of both tones also showed less suppression when compared to controls (Appendix A). Suppression also resulted when the low tone preceded either the high tone (Low-High-Noise) or the noise burst (Low-Noise). This suppression of the following elements of the sequence was most likely a consequence of the increased response strength to the first sequence element. Thus, frequency discrimination training resulted in less suppression to the second element of both the CS+ and CS - sequence compared to control rats (Fig. 17A & B).

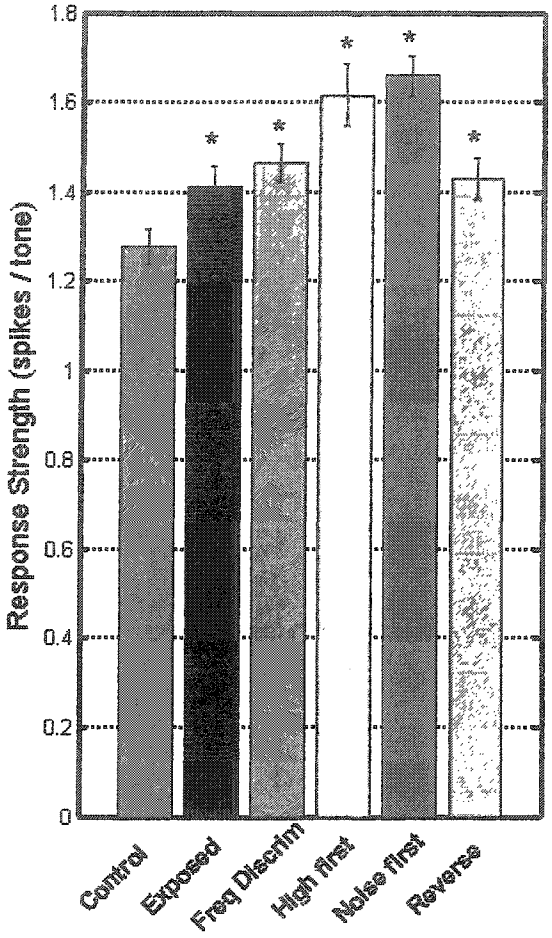


Figure 14. Response strength of A1 neurons after training and sound exposure. Response strength of A1 neurons increased across all trained groups compared to controls. Greatest increase in strength was observed in rats that discriminated the HLN sequence from the three triplet sequences. There was no significant difference in response strength between these two training groups. Response strength also increased for the sound exposure group by ~ 10% compared to controls. Similar increases were observed in the frequency discrimination and reverse discrimination groups. (*) compared to controls.

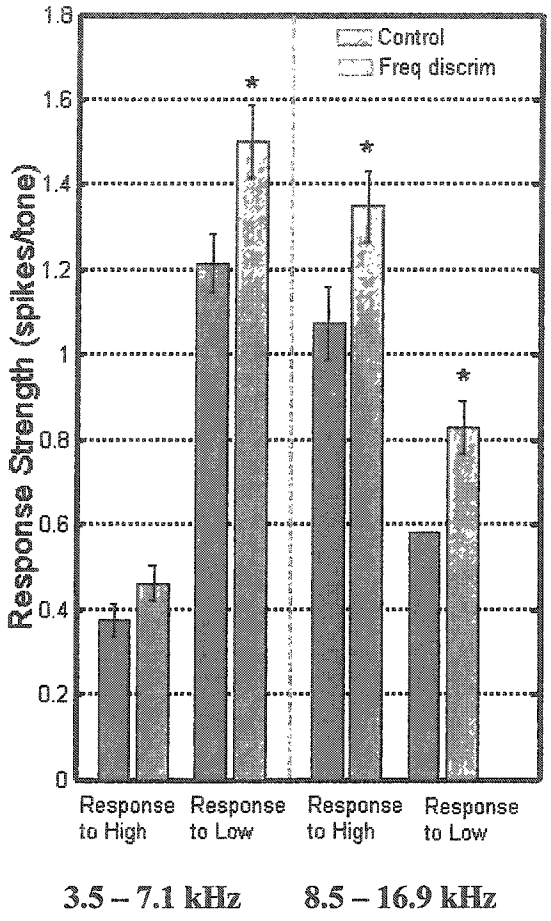


Figure 15. Response to Low and High tone in different frequency bins. Dashed line divides the figure into a low frequency region (3.5 – 7.1 kHz) and a high frequency region (8.5 – 16.9 kHz). Both ranges are within half an octave from 5 kHz and 12 kHz. In trained rats (magenta), response to the low tone increased significantly in both the low (23%) and high (42%) frequency region compared to control (blue) rats.

For rats that trained to discriminate the HLN sequence from the three triplet sequences, simply changing the order of presentation of the CS – sounds resulted in interesting and contrasting results. The High-first group learned to discriminate all the triplet sequences from HLN by the last day of training. In contrast, the Noise-first group was able to discriminate just the noise triplet and unable to discriminate the tone sequences that were added subsequently. For both groups, the strength of evoked response to the high tone, low tone and noise burst was substantially increased (Figure 16 A-C). The groups differed in their response to the following elements of the CS- triplet sequences. As can be seen from Figure 16, the second element of the tone and noise triplets were suppressed for the Noise-first group. In contrast, the High-first group did not show this suppression. When responses were further quantified with facilitation index measures, A1 neurons of the High first rats showed less suppression to the second and third element of the triplet sequences compared to control rats. This was not the case for Noise-first group in which A1 neurons showed greater suppression to the second and third elements (Fig. 17A & B and Table 3). Simply switching the order of presentation of the CS- sounds determined the degree of suppression. Only the group that learned all the CS- sequences showed less suppression to the CS- sounds compared to controls. Response to the HLN sequence was increased for both these trained groups compared to controls (data not shown). Interestingly, the sound exposure group (exposed to the same sequences as the trained groups) also showed an increase in the evoked response strength of A1 neurons to the HLN sequence compared to controls. Suppression to the second and third elements of the CS – sequence was also observed for this group. For the difficult reverse order discrimination task, the low element of HLN (CS+) as well as the low element of NLH (CS-) showed significant suppression compared to controls (HLN: - 35 vs. 3, $p< 0.00001$; NLH: - 85 vs. - 12, $p< 0.00001$).

	Second Element				Third Element			
	Control	High First	Noise First	Sound Exposure	Control	High First	Noise First	Sound Exposure
HHH	-56	-33	-71	**-102*** **	-35	-31	-34	**-65** **
LLL	-43	-46	**-81*** **	**-80** **	-31	-33	-26	**-53***
NNN	-7	2	**-40**** **	**-71**** **	15	17	**-10**** **	**-18**** **

Table 3. Suppression index measures for four groups of rats. The index is 100 times the logarithm base-two of the ratio of the number of spikes in response to a stimulus element in the context of a sequence and the number of spikes in response to the same element in isolation.
* Student *t*-test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.00001$

This suppression also generalized for the second element of other sequences that were not part of the training (LLL, HHH, NNN).

Figure 17A & B shows a summary of the suppression results for all groups for the triplet tone sequences. The only groups that showed less suppression compared to control rats were the frequency discrimination and High-first group. When the task was not learned (reverse order discrimination), or only partially learned because of prior interference from other sequences, or when simply exposed to sequences, A1 neurons showed suppressed to following elements of the sequence.

Receptive field plasticity

Bandwidth was measured at 10, 20, 30 and 40 dB above threshold. Exposing rats to sound sequences resulted in expansion of bandwidth tuning (2.05 vs. 1.73 octaves @ 20 dB above threshold, $p < 0.00001$) by almost 20%. In contrast, training on the same sequences (High-first and Noise-first groups) resulted in a slight decrease in bandwidth (10% for noise-first) to no change (high-first) in bandwidth. Similarly, frequency discrimination training did not result in

any bandwidth plasticity. The reverse group on the other hand showed a slight increase in bandwidth plasticity. The mean bandwidth measures for all groups are shown in Figure 18.

For the frequency discrimination group, we also documented bandwidth in different frequency bins to document any frequency specific effects. Significant increases in bandwidth tuning were observed only at 30 and 40 dB above threshold. At these thresholds, the high frequency region around 12 kHz showed a greater increase in bandwidth (~23% increase) compared to the low frequency region around 12 kHz (~7% increase). These results are similar to those obtain by Brown et al after frequency discrimination training in cats (Brown et al, 2004). In that study, increased bandwidth tuning was observed not at the training frequency but at frequencies immediately after it. In contrast, frequency discrimination training in monkeys decreased receptive field size (Recanzone et al, 1993). These differences are discussed later.

Response latency

Response latency of A1 neurons from all rats was quantified from tuning curve data and sequence data (see methods). Frequency discrimination training decreased onset latency by ~ 1 ms (15.8 vs. 14.9 ms, $p < 0.001$). Similar decreases in latency were seen in the auditory cortex of cats trained on a frequency discrimination task (Brown et al 2004). In contrast, increase in latency was observed in monkeys after training on a frequency discrimination task (Recanzone et al 1993). Factors such as species differences, operant conditioning paradigm and experimental design methodologies (e.g. adaptive tracking) could have contributed to these differences. Both sound exposure and training on the difficult task increased onset latency by ~1-1.2 ms. Onset latency measures are shown in Table 4. Only the sound exposure group showed an increase in both peak and end of peak latency compared to controls. Trained rats did not show any significant changes.

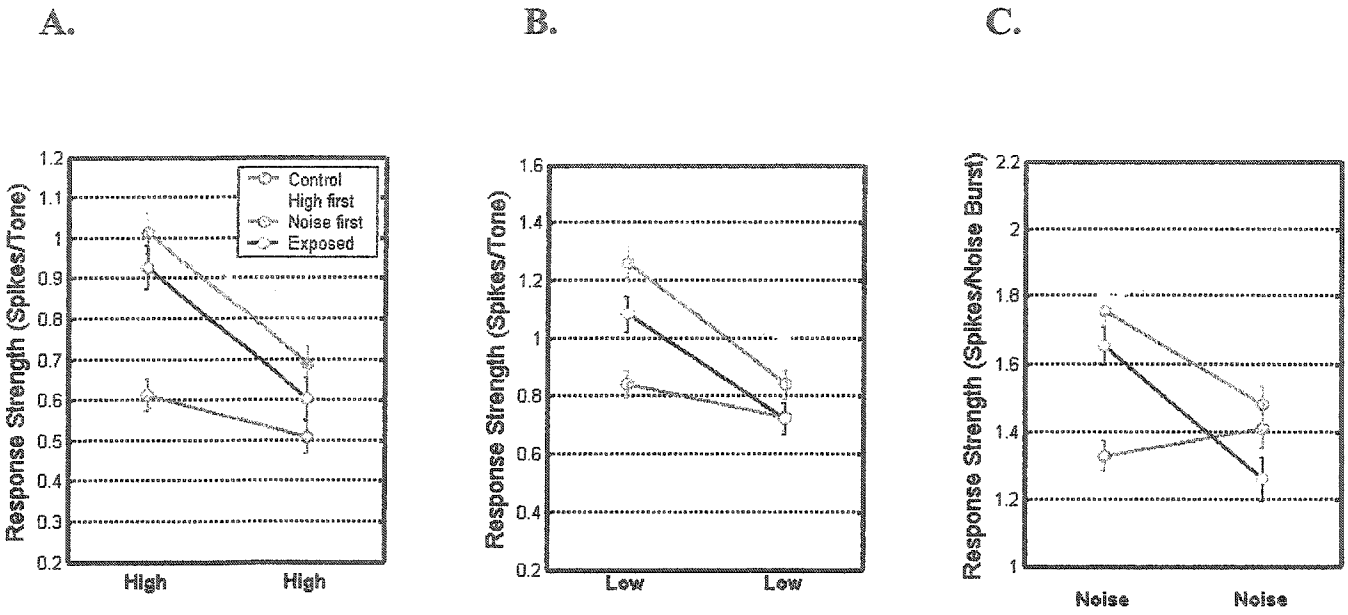


Figure 16. Response to the first and second element of the triplet CS – sequences in naïve, exposed, high first and noise first groups. In panels A-C, there is an increased response to the first element of the High, Low or Noise of trained and exposed rats compared to controls (blue). In the sound exposed (black) and noise-first (red) rats, the second element of the sequence is suppressed. This suppression is greater than the suppression to the second element of control rats. In contrast, the high-first group (green) shows less suppression to the second element of the sequence compared to controls. This can be easily appreciated in panel C for the noise sequence. This group showed less suppression even though response to the first element of the sequence was enhanced.

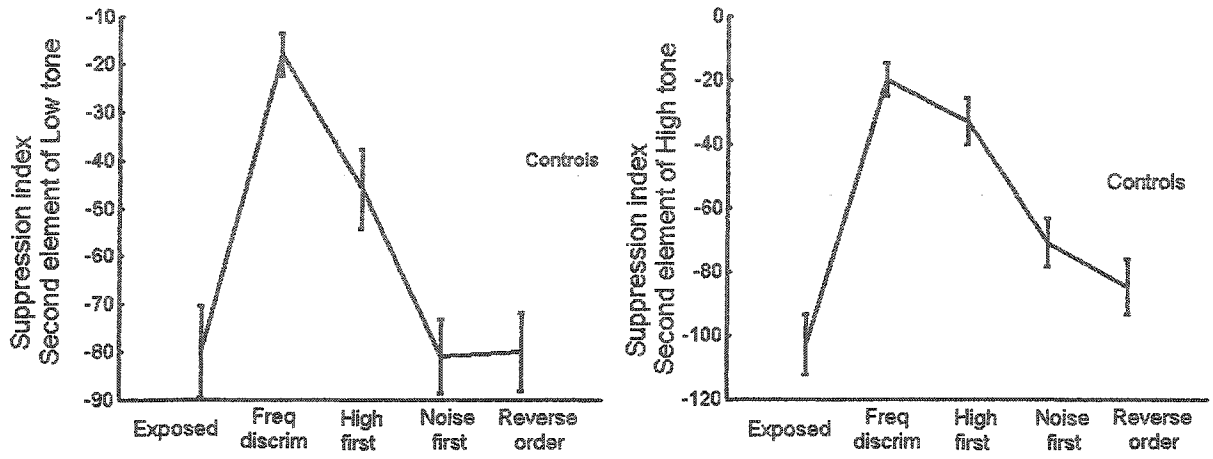


Figure 17. Summary figure of suppression indices in naïve, trained and sound exposed rats compared to controls. Green dotted line is the standard error of the mean for controls. The index is 100 times the logarithm base-two of the ratio of the number of spikes in response to a stimulus element in the context of a sequence and the number of spikes in response to the same element in isolation. For example, an index of 100 signifies twice the number of spikes to the following element compared to the number of spikes in isolation whereas -100 denote half as many spikes. Sites that show no facilitation or suppression have an index of zero.

Data is shown for the second element of the high (Panel A) and low (Panel B) sequence. The sound exposed group and the reverse order group showed maximal suppression to the tones compared to control rats. Similar suppression was also observed for the noise sequence (data not shown). In contrast, the frequency discrimination group (easy task) and the high-first group showed the least amount of suppression or no suppression compared to controls. Error bars indicate standard error of the mean (s.e.m).

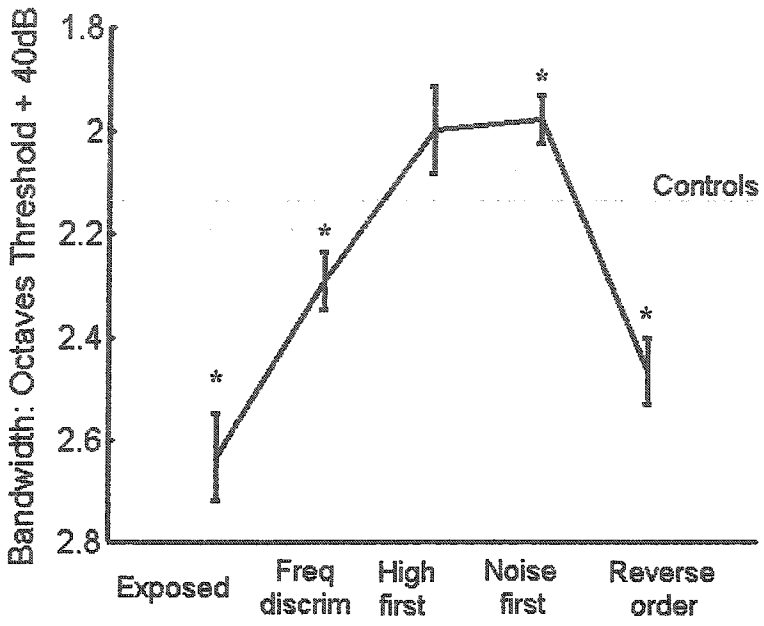


Figure 18. Bandwidth plasticity of A1 neurons from trained and sound exposure rats compared to controls (green horizontal line). Green dotted line is the standard error of the mean for controls. Compared to controls, receptive field size increased in rats that were either exposed to the sound sequences or in rats that did not learn the task (20% and 10% increase respectively). The only group that showed a small but significant decrease in bandwidth tuning was the noise-first group (~10% decrease). Bandwidth for the frequency discrimination and the high-first group did not differ from controls. Error bars indicate standard error of the mean (s.e.m). (*) indicate significant difference from controls.

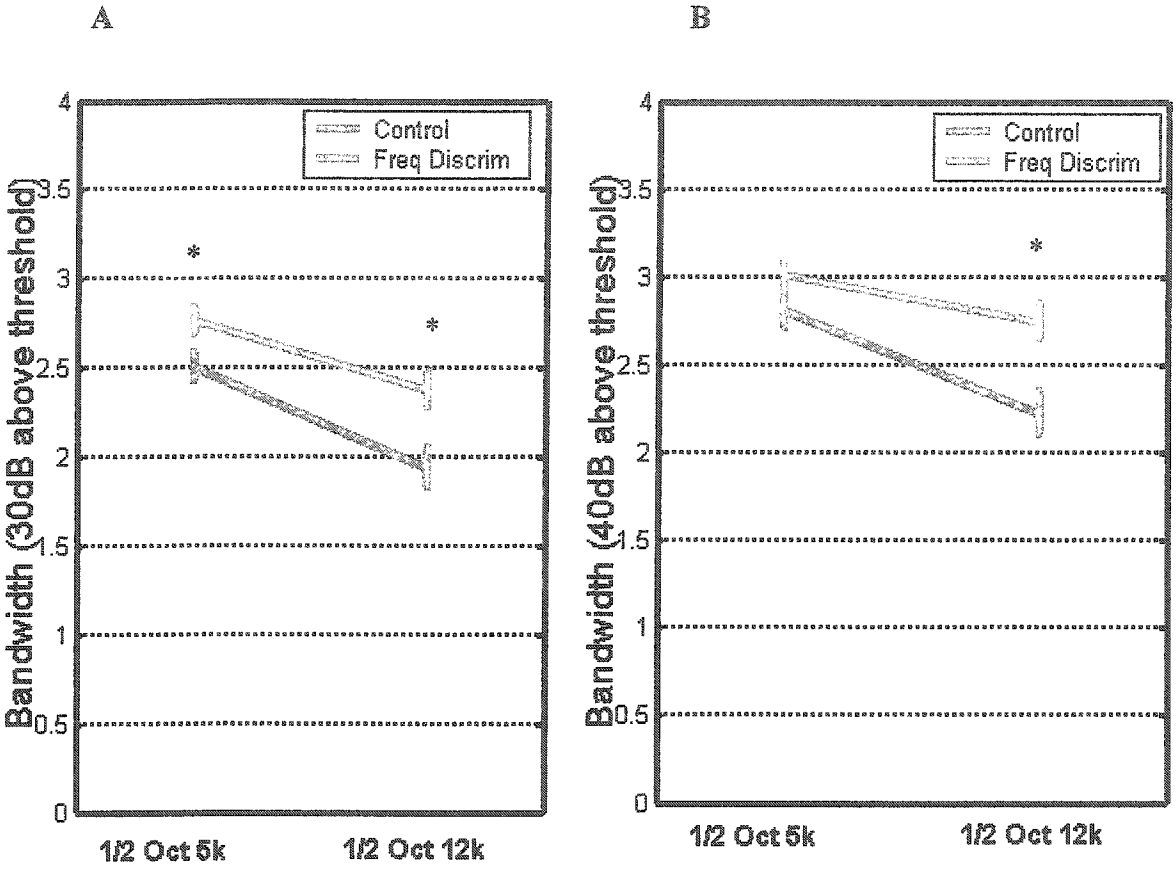


Figure 19. Bandwidth tuning half an octave from 5 kHz and half an octave from 12 kHz after frequency discrimination training. Both Panel A & B show bandwidths at 30 and 40 dB above threshold. Increase in bandwidth tuning after frequency discrimination training (magenta) compared to controls (blue). This increase was greater in the high compared to low frequency region. Error bars indicate standard error of the mean (s.e.m). (*) indicate significant difference from controls.

Onset, peak and end of peak latencies from the sequence data were quantified for tones and noise bursts in isolation and also for the second and third elements. A number of interesting and significant latency differences emerged. Data for onset latency is shown in Figure 20.

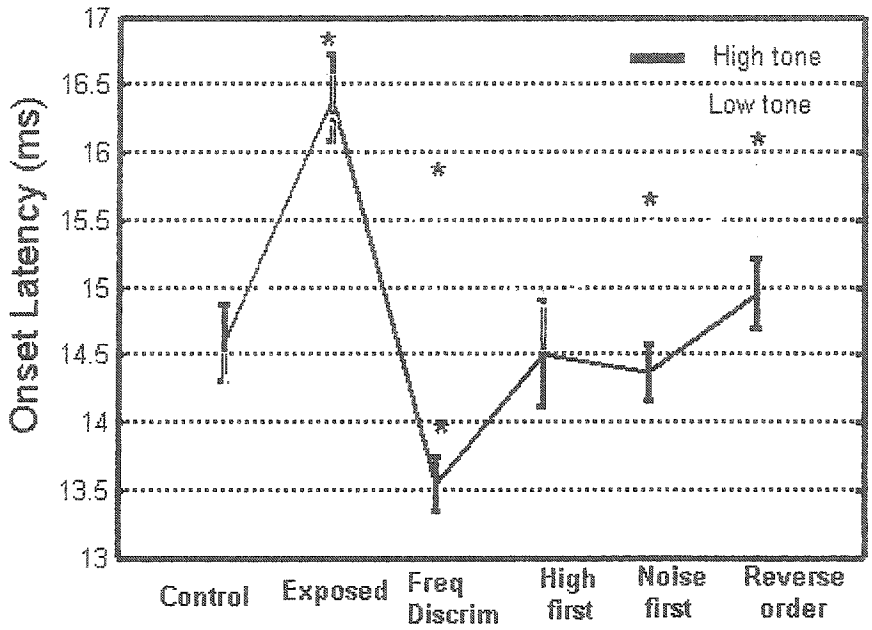


Figure 20. Onset latency for trained and exposed rats to the high (black) and low (grey) tones in isolation. (*) represents significant differences in onset latency compared to controls. The sound exposure group shows an increase in onset latency for both high and low tones. Frequency discrimination training resulted in a decrease in onset latency for the high tone and increased onset latency for the low tone. Even though the high-first and noise-first group were listening to exactly the same sequences by the end of training, significant latency differences between the two groups emerged simply by changing the order in which they were trained.

Differences in peak and end of peak latency measures will be documented in the text. After sound exposure, onset latency for both the high as well as the low tone increased compared to controls (~2 ms increase, $p < 0.00001$). This increase was also observed for the noise burst. Peak and end of peak latency for tones and noise bursts also increased in exposed rats compared to controls. An interesting dissociation between the onset latency for low and high tones was observed after frequency discrimination training. Counter intuitively, onset latency for the CS- tone (HHH) decreased ($p < 0.01$) whereas for the low tone it increased ($p < 0.05$). One explanation for this difference could be the greater increase in response strength to the low tone in both the high and low frequency regions of the A1 map resulting in a longer latency. This difference was also reflected in the peak latency.

Rats trained to discriminate the HLN sequence from the CS- triplet sequences showed latency differences even though they were listening for the same CS- sequences (except that the order of presentation was different). No latency changes were apparent for the High-first group whereas for the Noise-first group, latency for the low tone increased (Fig. 17). Even within the same trained group, paired *t*-test analysis revealed shorter onset latencies for the high tone compared to the low tone. Significant differences in latencies were also observed for the following elements of the sequence (Fig. 21A-C). For the NNN sequence for example, in both the frequency discrimination group as well as the high-first group, onset latency decreased by ~ 1.8 ms compared to controls whereas onset latency was much delayed in the sound exposure group by ~ 3 ms compared to controls ($p < 0.00001$). Interestingly, this same pattern (Fig. 21C) was also observed for the HLN, LLL, HHH and NLH sequences (Appendix C).

For both the trained and sound exposure groups, we did not observe any significant correlation between improvements in behavioral performance and responses from A1 responses.

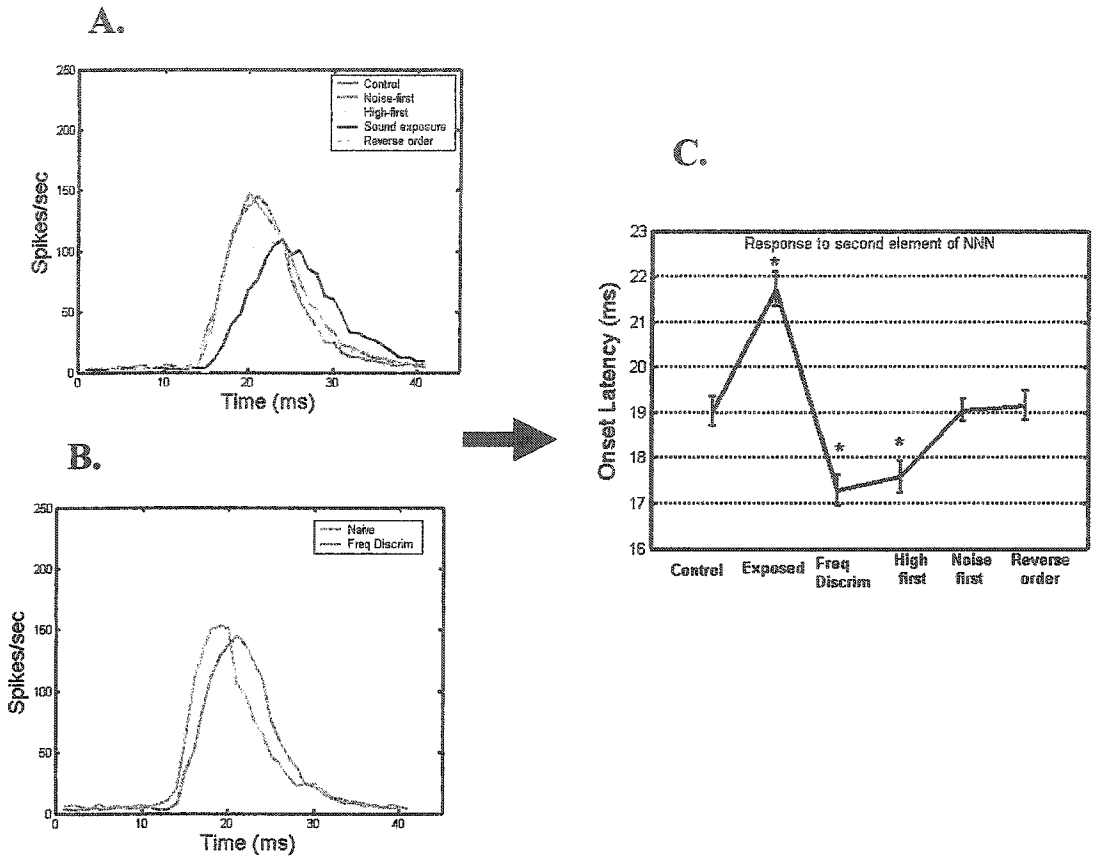


Figure 21. Responses to the second element of the noise burst. A & B) Population PSTH of trained and sound exposure groups. Response window is 150-190 ms after tone or noise burst onset (onset of the second element). The axes in the figure has been offset (i.e. 0 ms actually represents 150 ms). A) Decrease in onset latency for the high-first group and increased onset latency for the sound exposure group. B) Population PSTH for the frequency discrimination group shows decreased onset latency compared to controls. Shaded areas represent standard error of the mean C) Mean onset latencies obtained from the population PSTH.

Experiment	Behavior (d prime)	Response Strength	Receptive field plasticity	Latency (ms)	Suppression
Sound Exposure	---	+ 10%	+ 20%	+ 1.3	**More Suppression**
Freq Discrimination	1.8	0	0	- 0.9	**Less Suppression**
HLN vs. HHH, LLL, NNN	1.4	+ 26%	0	0	**Less Suppression**
HLN vs. NNN, LLL, HHH	0.6	+ 30%	-12%	- 0.6	**More Suppression**
Reverse Discrimination	0.3	+ 11%	+ 10%	+ 1.1	**More Suppression**

Table 4. Summary of behavioral performance and neurophysiologic responses for tasks that ranged from easy to difficult. Neurophysiologic responses are compared to naïve controls. The d' measures is the average d' on the last day of training (average of two sessions). The d' values for the HLN vs. 3 CS- triplet sequences is the average d' of all the CS- sequences. Response strength is measured in spikes/tone. Receptive field plasticity is bandwidth tuning at 20 dB above threshold. Suppression reflects the suppression to the second element of CS+ and CS- sequences. Frequency discrimination was the easiest task with the best performance whereas sequence order discrimination was the most difficult task with poor *d* primes. Response strength was greatest for rats that discriminated three CS- sequences from the CS+ (HLN) sequence.

Overall, rats that were exposed to the sound sequences showed only a small increase in response strength, greatest increase in bandwidth tuning, longer latencies and more suppression of the second and third elements of the tone and noise triplet sequences. Interestingly, rats that were unable to do the discrimination task (reverse discrimination) also showed more suppression, longer latencies, greater bandwidth and a small increase in response strength. Although rats trained on the high-first and noise-first task showed the largest increase in response strength, the noise-first group showed greater suppression compared to the high-first group. Zero represents no change.

Table 4 is a summary table of behavioral performance and responses from A1 neurons in trained and sound exposed rats. The results are compared to control rats. Frequency discrimination resulted in the best *d* primes whereas sequence order discrimination which was a more difficult task had poor *d* primes. Training on the noise-first and high-first task resulted in the greatest increase in response strength. The major difference between these two groups was the amount of suppression to the following elements. Simply adding the NNN sequence as the first CS- resulted in greater suppression in this group. A short summary is also presented in the legend of Table 4.

Discussion

Rats were trained on a variety of discrimination tasks that ranged from easy (frequency discrimination) to difficult (reverse order discrimination). In the frequency discrimination task, rats were able to discriminate the LLL from HHH sequence. Three out of nine rats trained for ~4-5 months compared to other rats that trained for ~2-3 months. Responses were not significantly different for the early or late trained groups. In both cases, rats were able to discriminate the high sequence from the low sequence within ~ 1 month of adding the high sequence. In the late-training group, rats continued to maintain high d' scores through several weeks of training. In both the Brown and Recanzone studies, animals were also able to achieve high *d* primes (~3-3.5). The frequency discrimination task was different from previous discrimination studies, with respect to acoustic stimuli and the nature of the behavioral task. While tones used in this study were part of a sequence, previous studies used single tones. The Brown study (Brown et al, 2004) was a limited-hold task in which cats initiated each trial resulting in a series of tones of a single frequency. At the offset of this series of tones, a tone with a different frequency was presented. Release of a foot pedal upon hearing this tone was

associated with reward. The frequency of the second sound was constantly adjusted throughout each session to achieve best performance (adaptive tracking). The Recanzone study in monkeys also used adaptive tracking in a limited hold paradigm similar to the Brown study (Recanzone et al 1993). The task differed slightly, in that, tone pip pairs were presented with each trial and monkeys had to discriminate whether the tone pip pairs were same or different. Our study employed randomly interleaved CS+ (5 kHz) and CS- (12 kHz) frequencies in a go/no-go task instead of limited-hold adaptive tracking. Although frequency discrimination was the common goal in all these studies, plasticity effects varied considerably as will be discussed in the next section.

Two groups of rats attempted to discriminate the HLN sequence from tone and noise burst triplets. The only difference between the two groups was the order of presentation of the CS- sequences. When HHH was the first CS- sequence, followed by LLL and then NNN, rats were able to discriminate all triplet sequences from HLN. Interestingly, if NNN was the first CS- triplet, rats were unable to discriminate the tone triplets that were added later. It appears that prior training with the noise sequence interfered with subsequent tone discrimination. For this noise-first group, performance on the high triplet was slightly worse compared to the low triplet on the last day of training due to the shorter time spent on the high sequence (~30 days on high compared to ~50 days on low). The high-first group spent almost exactly the same number of days on the high triplet (~28 days) but this was in the absence of the other triplets. Moreover, rats in the high-first group were already good at discriminating the LLL sequence from the HLN sequence when it was added subsequently. Rats were unable to discriminate the HLN sequence from its reverse NLH sequence. They were unable to do so even after several days of HLN detection training. They performed well on the detection task, just like the other groups, and

although the hit rate for the CS+ remained high throughout, the false alarm rate for the CS- sound also increased. They trained for ~ the same number of days as rats in the other group and even after ~ 2-2 ½ months on this task, they were unable to discriminate the two sequences.

Changes in the response strength of A1 neurons were also observed across all groups (Table 4). After frequency discrimination, A1 response strength increased by about 15% compared to controls. In both the cat and monkey studies, response strength was not quantified (Recanzone et. al, 1993, Brown et. al, 2004). Greatest increase in response strength was observed in the more difficult tasks, namely the HLN vs. three triplet sequence discrimination (~30% increase). Although rats in the noise-first group were unable to discriminate all the CS- sequences from the HLN sequence, the increased response strength was not different from the high-first group. When the task was made too difficult (reverse discrimination), response strength increased by only 10% compared to controls. Both training and sequence exposure altered the degree and direction of bandwidth tuning. Exposing rats to sound sequences increased receptive field size by almost 20%. Interestingly, the reverse order group that was unable to discriminate sequences also showed an increase in receptive field size. A small decrease in receptive field size was observed only for the noise-first group. Bandwidth tuning for the high-first and frequency discrimination training was not significantly different from controls.

Frequency discrimination training increased response strength and receptive field size in the 5-12 kHz region of A1 neurons. This receptive field plasticity was apparent only at bandwidths 30 and 40 dB above threshold. The cortical topographic organization of A1 was unaltered after frequency discrimination. Two previous studies in monkeys and cats examined plastic changes in A1 following frequency discrimination training. In monkeys, frequency discrimination resulted in an expansion of the region of cortex specific to the trained frequency and narrowed

receptive fields (Recanzone et al. 1993). In the cat study however, no change in cortical topography was observed, and there was a slight increase in bandwidth tuning an octave greater than the trained region (Brown et al. 2004). This study was more in accordance with our findings. Interestingly, responses from primary visual cortex neurons also showed no changes in cortical topography (Crist et al. 2001; Ghose et al. 2002; Schoups et al. 2001). Similar findings were also observed after basal forebrain stimulation was paired with a sound of a single frequency. Receptive field expansion occurred in neurons with CF's upto 2 octaves away from the paired frequency (Kilgard et al, 2001).

The average onset latency of A1 neurons was significantly affected by sound exposure and training. Sequence exposure significantly increased onset latency of the 5 and 12 kHz tones as well as the noise burst by almost 2-3 ms compared to the trained rats. The frequency discrimination group shows dissociation in onset latency for the high and low tones. Counter intuitively, average onset latency for the CS- tone (12 kHz) decreased whereas it increased for the CS+ tone (5 kHz) compared to controls. Frequency discrimination training in owl monkeys led to an increase in response latency to the trained frequency (Recanzone et al, 1993) whereas in cats, latency in the trained frequency decreased (Brown et al, 2004). For the HLN vs. three triplet tasks, response latency was not significantly different for controls. Like the exposure group, rats that were unable to learn the task showed an increase in the average response latency.

There is no doubt that training alters receptive field size, cortical map reorganization, temporal processing and correlated activity of neurons. Only some of these plasticity effects correlated with improvements in behavioral performance. In the Recanzone study, there was no correlation between improvements in performance and receptive field size. On the other hand, behavioral performance was strongly correlated with the temporal coherence of the responses

(Recanzone et al, 1992). It is possible that the plasticity effects that do not correlate with improvements in behavioral performance may simply be inevitable consequences of some other underlying change such as synaptic strengthening, spike-timing and changes in yet unknown cellular and network properties of cortical neurons. Another possibility is that plasticity effects in non-primary regions of cortex could correlate with behavioral performance. This could not be confirmed since our study only documented changes in primary auditory cortex neurons.

Sequence processing and suppression effects

Two-tone interactions have been previously studied in naïve cortex of cats, monkeys and bats. In these studies, the effects of preceding tones on a subsequent tone of a sequence were studied. Neural activity of the subsequent tone was either inhibited or facilitated depending on the intensity, spectral and temporal separation of tones (Brosch and Schreiner 1997, Brosch and Schreiner 1999, Brosch and Schreiner 2000). Inhibition of the subsequent tone was maximal when the preceding tone was centered on the neurons excitatory receptive field or if the intensity of the masker increased. Inhibition decreased when the SOA was increased (Brosch and Schreiner 1997).

On the other hand, response facilitation was maximal when the spectral separation was ~ 1 octave and the tones were separated by about 100 ms. However, if the two tones had the same frequency, suppression was observed (Calford and Semple 1995, Brosch and Schreiner 1997). In cats, ~90% of A1 neurons responded more strongly to tones as part of a sequence that in isolation (Brosch and Schreiner 2000) whereas in monkeys, ~ 67% showed facilitation (Brosch and Schreiner 1999). In the present study, most A1 neurons from naïve rats showed response suppression when there the two tones were the same. Facilitation of the second tone was absent even when the two tones of the sequence had different frequencies (e.g. H-L) in naïve or trained

rats. This study differs from the previous studies by Brosch and Schreiner in a number of ways including species differences, the nature and separation of probe and maskers, range of different stimuli presented, intensity of the tones, effects of anesthesia and technical considerations (e.g. free-field sound presentation).

In almost all groups, response to the second (and sometimes the third) element of the sequences was suppressed compared to the first element especially when the first and second element were the same. Compared to naïve rats, the degree of suppression after training was of greater or lesser magnitude depending on the training condition. Only the frequency discrimination group and the high-first group showed less suppression compared to controls. On the other hand, the noise-first group, reverse group and the sequence exposure group showed the greatest suppression. This suppression was apparent both for the CS+ and CS- sounds. In some cases, suppression also generalized to other sequences such as the NLH sequence, even though rats were not trained on this sequence. The probable cause of suppression after training was an increase in response strength of the first element of the sequence. For the groups that showed suppression to the CS- and CS+ sequences, maximal suppression resulted when the spectral content of the sequence elements was the same (Table 3).

The different latency changes could result from cellular mechanisms such as paired-pulse depression in which the enhanced response to the first pulse delays recovery resulting in a decreased number of discharges and a longer time to spike for the second pulse. This will be elaborated more in the section on cellular mechanisms. In general, it appears that both response enhancement and response suppression are involved in the temporal processing of sound sequences in both specialized and non-specialized mammals and that training significantly alters temporal processing of rat auditory neurons.

Task difficulty and sequence learning

Studies in visual cortex have shown that task difficulty plays a role in modulating cortical plasticity. Different forms of plasticity result depending on whether rats are trained on an easy or difficult task. In visual cortex V4 neurons, responses were different when monkeys were trained on an easy or difficult orientation or color discrimination matching to sample task (Spitzer et al 1988). They showed that increasing the effort required to perform the task resulted in plasticity effects that were different compared to the easy task. Neurons responded more strongly in the difficult task compared to easy task (increased by 18%). Narrow receptive fields were also observed in the difficult task (20% decrease in bandwidth) compared to the easy task.

In a follow-up paper, the same group asked the question whether it was really task difficulty that mattered (Urbach and Spitzer 1995). Although performance scores and neural responses differed depending on whether the task was easy or hard, it is possible that the differences could arise depending on the attentional effort by the rat. Thus, not only is the nature of the discriminating stimuli (the resolution between the 2 stimuli) a critical factor, but also the subject's internal response. If the subject pays more attention to the difficult task, there will be reduced errors on that task, whereas if the subject pays less or same attention as the easy task, then errors will increase.

In the present study, auditory discrimination tasks ranged from easy to difficult. The easy frequency discrimination task did not change response strength of A1 neurons compared to controls. For the tasks that were more difficult (Noise-first and High-first), increase in A1 response strength was the greatest even though the average performance on the last day of training was less compared to the easy frequency discrimination task. The HLN vs. three triplet tasks may require greater attentional effort compared to the frequency discrimination task. It is

however possible that these differences in response strength could result from the nature of the task itself wherein the CS+ stimulus was different between the two tasks. In the reverse order discrimination task however, the CS+ stimulus was the same but rats were unable to learn this difficult task. In this case, response strength increased by only 10% and receptive fields of A1 neurons increased. Challenging rats with a difficult task, so much so that they are unable to learn it, does not appear to generate useful plasticity.

Sound exposure and perceptual learning

Exposing rats to sound sequences increased response strength by only 10% compared to a 30% increase after training. Paying attention to the sounds, rather than simply being exposed to them may be important for causing significant increases in response strength. Sound exposed rats were also hungry and motivated just like the trained rats. Although there was almost no temporal association between the sounds presented and delivery of food, heightened arousal and food anticipation may have been sufficient to trigger plastic changes. These factors could have contributed to the slight increase in response strength. Again, it is not clear whether these changes are specific for A1 neurons and it is quite possible that non-primary auditory neurons and even non auditory areas could generate similar the plasticity observed in A1. In the Recanzone study, rats that were exposed to sound frequencies while performing a tactile discrimination task failed to show plastic changes in the auditory cortex (Recanzone et al, 1992b). In our study, sequence exposure was associated with the delivery of random delivery of food. This kind of exposure resulted in a slight increase in response strength, increased bandwidth tuning, longer latencies and suppression of sequences in A1 neurons. It would be interesting to know if similar plastic effects would occur after sequence exposure alone (without anticipation for food).

Some studies have also shown that prior exposure to sounds improves subsequent discrimination performance. Rats that were exposed to two amplitude modulated sounds (S+ and S-) for 48 hrs resulted in enhancement of discrimination between these two sounds. Furthermore, they showed that bilateral application of APV (an NMDA blocker) blocked this enhancement (Sakai et al. 1999). The latter result suggests that LTP may be responsible for these changes. Interestingly, behavioral relevance is not necessary to induce LTP in auditory cortex. Tetanic white matter stimulation (Kudoh and Shibuki 1994) was sufficient to induce LTP in rat auditory cortex. In another sound exposure experiment, rats were exposed to two sounds (S+) associated with reward and (S-) not associated with reward. Sound discrimination tested 2 weeks later showed better discrimination performance for the same S+ sound rather than if the S- was rewarded in the discrimination test (Watanabe et al. 2001). This result demonstrates 1) the specificity of discrimination stimuli and 2) that it is long lasting (~ 2 weeks) whereas in the Sakai study, simple exposure to sound stimuli and subsequent training were maintained only for 3 days. Prior association of a stimulus with reward appears to encourage rats to pay attention to the S+ and S- sounds used in the subsequent discrimination training. In the present study, reward was not paired with sounds but was randomly interleaved and we did not test for subsequent discrimination ability. It is interesting to speculate whether this group of rats would show subsequent improvement in discrimination performance, since none of the sounds presented during exposure were associated with reward.

Role of neuromodulators

Many studies have shown that the central cholinergic system (projections from the basal forebrain to cortex) is important for modulating plasticity. Cholinergic lesions are known to block learning and plasticity (Baskerville et al. 1997). Pairing of electrical stimulation of the

basal forebrain with sensory stimuli generates different forms of cortical plasticity (Bakin and Weinberger 1990; Edeline et al. 1994; Kilgard and Merzenich 1998a; Kilgard and Merzenich 1998b; Kilgard et al. 2001). However, acetylcholine is not the only neurotransmitter that is known to gate cortical plasticity. Cortical responses can also be modulated by the activity of dopaminergic ventral tegmental (VTA) neurons. For example, dopamine release has been observed during auditory learning (Stark and Scheich 1997) and is known to modulate LTP (Gurden et al. 2000; Otmakhova and Lisman 1998). In addition, similar to basal forebrain pairing, pairing electrical stimulation of VTA neurons with a tone increases the representation and selectivity of that tone in A1 (Bao et al. 2001). More recently, noradrenergic neurons have also shown to modulate the frequency tuning of auditory cortex neurons (Manunta and Edeline 1997).

For most of the training tasks in the present study, the CS+ stimulus (HLN) was the same stimulus that was paired with basal forebrain stimulation in the Kilgard and Merzenich study (Kilgard and Merzenich 2002). After repeated basal forebrain pairing with the three element sequence, 25% more A1 neurons showed facilitation to the low tone when preceded by the high tone, and more than 60% of A1 sites showed facilitation for the noise burst when preceded by the two tones. In addition, increase in population discharge synchrony was also observed after sequence pairing with basal forebrain stimulation. Background triplet sequences LLL, HHH, NNN and NLH sequences were also presented randomly but were not paired with basal forebrain stimulation.

Training on the HLN (CS+) sequence showed no facilitation to the low or noise element in our study. In fact, the second and third element was either suppressed (noise-first group) or did not change (high-first group). It seems that behavioral training, unlike basal forebrain stimulation

results in a different form of plasticity. Neurons in A1 did not become order-selective after training on the HLN sequence. A most likely explanation for the different results is that basal forebrain stimulation is not a "natural" form of learning. Although Ach release is known to modulate attentional processes, it is possible that phasic release of Ach occurred only for the paired stimulus in the NB stimulation paradigm. During natural learning, however, rats were not only motivated but were also "paying attention" not to hit to the CS- sounds. In other words, they were paying attention to both the CS+ and CS- sounds. Attention to both the behaviorally relevant and irrelevant stimuli could contribute to the different forms of plasticity generated by training. An important difference in A1 responses was that behavioral training resulted in increased response strength to the high low and noise sequences (in isolation) whereas no increase in response strength was observed in the basal forebrain study. The increased response strength to the first element resulted in suppression of the subsequent element of the sequence.

Perceptual learning in other systems

Perceptual learning can substantially alter neural responses in somatosensory, visual and auditory cortices (for review see Edeline 1999). Perceptual learning in the visual system is highly specific to stimulus parameters used during training which suggest the involvement of early stages of sensory processing (Crist et al. 2001; Fahle 1997; Karni and Sagi 1991). Even though specific changes in receptive field dynamics have been observed in the early stages of visual processing after training, the magnitude of these effects are relatively small compared to responses higher cortical areas such as V2 and V4. For example, Crist showed that training on a bisection discrimination task did not change basic receptive field properties (Crist et al. 2001). Monkeys training on an orientation discrimination task showed only modest changes orientation tuning in V1 (Ghose and Maunsell 2002; Schoups et al. 2001). More recently, however, Gilbert

and colleagues showed that training monkeys on a shape discrimination task resulted in responses specific to the shape attributes (Li et al, 2004). It appears that at least in primary visual cortex, contextual influences can modulate plasticity in V1 (Crist et al. 2001; Gilbert et al. 2001).

These observations contrast with those observed for the somatosensory and auditory system in which maps and receptive fields undergo substantial reorganization after extensive discrimination training (Recanzone et al. 1992; Recanzone et al. 1993). Even here, there are differences in response plasticity. For example, in monkeys, auditory discrimination training resulted in extensive tonotopic map reorganization and narrow frequency tuning whereas in cats there was no change in map organization and only a modest bandwidth effect (Brown et al. 2004; Recanzone et al. 1993).

Cellular mechanisms

Cellular, molecular and network level rules operate to bring about plasticity effects in cortex. The pattern of sensory input can alter cortical responses in the scale of milliseconds. One mechanism that has stood the test of time is Hebbian plasticity (Hebb 1949): synapses that are co-activated are strengthened. This so called "rule" has undergone numerous modifications over the last couple of years and now incorporates novel mechanisms including synaptic weakening, anti-Hebbian mechanisms and spike timing-dependent plasticity (STDP). The processes of long-term potentiation (LTP) and long-term depression (LTD) are the consequences of these learning rules that operate on the time scale of millisecond and ultimately contribute to network level changes.

Fregnac showed that receptive field properties of visual cortex neurons can be modified by electrically stimulating the neurons while showing the animal two different stimuli only one of which was associated with electrical co-stimulation (similar to classical conditioning). This

resulted in shifting of the neurons receptive field towards to stimulus that had the co-stimulation (Fregnac et al. 1992; Shulz and Fregnac 1992). Weinberger and colleagues would demonstrate a few years later that neurons in adult guinea pigs can also shift the peaks of their tuning curves by pairing auditory tones with electrical stimulation of single neurons in primary auditory cortex (Cruikshank and Weinberger 1996). These experiments in visual and auditory cortex showed that the co-stimulation was sufficient to induce receptive field plasticity without the need for behavioral relevance.

Recanzone demonstrated that behavioral training on tactile discrimination tasks increases the map representation of the digit and narrows receptive fields. How could these changes relate to the co-activation protocols mentioned earlier? The answer would lie in the elegant experiments conducted by Ahissar and colleagues who showed that Hebbian co-activation is affected by behavioral context (Ahissar et al. 1992). Increased synaptic strength (as measured by the cross-correlation between 2 primary auditory cortex neurons recorded simultaneously) resulted only when the monkey was engaged in a frequency discrimination task. Network-level alterations such as map plasticity, receptive field plasticity and changes in temporal processing that occur as a result of perceptual learning are strongly dependent on cellular learning rules that operate on a timescale of hundreds of milliseconds.

Potential concerns and problems

TIME COURSE OF PLASTICITY. Within a single group of trained rats there was some variability as to when the sequences were introduced. Some rats were able to learn sequences more quickly than others. This was most apparent in the HLN vs. the three triplet task, which were difficult tasks and the time point at which the subsequent sequence was added could not be exactly the

same for all rats. In the end however, all rats in both group were attempting to discriminate all the three CS- sequences from the HLN sequence.

The second point of concern was the end point at which the auditory cortex was mapped. On average, extra-cellular recordings from auditory cortex were performed once the rats performance reached good performance scores (*d* primes). Since this study was done under anesthesia, plasticity effects could be observed at only one point in time. It is not known when the plasticity effects would start to appear. For the HLN vs. three triplet discrimination tasks, the CS- sequences were added one after another. We do not know what influence the additional CS- sounds had on cortical responses that may already have generated some kind of plasticity. Future experiments employing evoked potential recordings and/or chronic multi-channel recordings from the auditory cortex of awake rats should be able to answer some of these questions.

EFFECTS OF ANESTHESIA. All the studies in this dissertation were done under barbiturate anesthesia. A potential concern in these studies is that anesthesia affects cortical responses. This is reflected in the driven discharge rate, spontaneous activity, frequency tuning and temporal processing of cortical neurons. In somatosensory cortex, enlargements in receptive fields and latency changes were observed under anesthesia but not in the awake state (Simons et al. 1992). In the visual system, anesthetics abolished the rhythmic discharges of thalamic and cortical neurons (Albrecht and Davidowa 1989). Bandwidth tuning of auditory cortex neurons was changed after both barbiturate and ketamine anesthesia (Zurita et al. 1994). Thus, anesthesia appears to have significant influences on the responses of cortical and subcortical neurons. In our studies, barbiturate anesthesia was used. These drugs enhance GABA-mediated chloride currents and cause inhibition of cortical responses. One of the effects seen with barbiturates on auditory cortex neurons is to increase the sharpness of tuning which may result from increased inhibitory

side-band activity (Gaese and Ostwald 2001). Although anesthesia does affect cortical responses to some degree, these effects are reduced because we recorded cortical responses under identical conditions of anesthesia in both experimental and control rats.

INVOLVEMENT OF NON-PRIMARY AREAS. Recordings in this study were obtained from primary auditory cortex of anesthetized rats. Studies have shown that cortical fields surrounding A1 respond to more complex spectro-temporal transitions in sounds including speech. Although we recorded from auditory cortex neurons in other fields in some of our rats, the sample obtained from each field was too small to make any meaningful comparisons. Due to time constraints of the experiments, we were unable to get a reasonably large number of recordings from all the fields.

CHAPTER 5

CONCLUSIONS

The cerebral cortex is dynamic and sensitive to manipulations of the environment both during their critical period and in adulthood. In adults, enrichment increased response strength, narrowed receptive fields and altered temporal processing of auditory cortex neurons. The persistence of these effects under general anesthesia is consistent with the earlier conclusions that structural changes contribute to environmental plasticity. Perceptual learning on auditory discrimination tasks also generated plasticity effects in primary auditory cortex. Rats trained on a range of easy to difficult discrimination tasks which in turn resulted in different forms of plasticity. Simply exposing rats to sound sequences broadened frequency tuning, increased latency and resulted in paired-pulse depression. Training rats on exactly these same sequences (High-first) resulted in shorter latencies, less suppression and greater increase in response strength.

During development, appropriate spectro-temporal transitions of acoustic input are important for normal maturation of auditory cortex circuitry. Experiments with young animals (before the critical period for auditory cortex maturation) demonstrated that exposure to sounds during a critical period disrupted tonotopic maps and broadened receptive fields. These plasticity effects persisted into adulthood (Zhang et al. 2002, 2001). Similar effects were also observed in the IC neurons of mice (Chiu et al. 2003). Rats that were exposed to a 9 kHz from birth to one month showed a decrease in response threshold, increase in spontaneous activity and clustering at the 9 kHz region when mapped at 15 months of age (adults). The Zhang study also demonstrated that

adult cortical maps are resilient to passive acoustic exposure. Exposing rats to an enriched environment however altered A1 responses in ways that were different compared to simple exposure to tones or noise bursts. Enrichment increased response strength, lowered threshold and narrowed receptive fields in adult rats without altering the tonotopic map organization of A1 (Engineer et al, 2004). There are a number of reasons why these plasticity effects are in some ways similar to the previous studies and in some ways different. First, enrichment is different from simple sound exposure. Second, some responses may be more resilient than others in adults. Third, a number of other factors in the enriched environment could have resulted in these changes.

Deprivation of the appropriate pattern of input during a critical period of development can result in aberrant plasticity. An interesting experiment in rats looked at the relationship between whisker deprivation and subsequent exploratory behavior. There was an increase in the area of representation of the principal whisker when all the neighboring whiskers were removed. If the animal was then given the opportunity for exploratory behavior, there was a contraction of the area of representation (Polley et al. 1999). Thus, it appears that novel or enriched environments can dramatically alter or reverse cortical dynamics when switched from one environment to another. Although not part of this dissertation, the time course of enrichment effects has also been documented (Engineer et al, 2004). Enriched housing improved cortical processing within just 2 weeks. These beneficial effects were lost when rats were switched back to the standard housing condition. These experiments show that rapid remodeling of cortical responses can occur even in adults.

Task difficulty, neuromodulation and clinical relevance

It is well known in psychology literature that animals and humans learn differently depending on the degree of motivation. Learning appears to be least when the animal has either low motivation (and hence boredom) or if the animal is highly motivated. There appears to be an intermediate zone where learning is maximal when the motivation is just right. This results in an inverted U-shaped function as seen in Figure 22A. Could a similar U-shaped function explain task difficulty and its role in differential plasticity effects? Is it possible that if the task was too easy or too difficult, the plastic changes in cortex would be different from those obtained in a task that was somewhat intermediate (Figure 22B)? In our experiments, the high first group, a task that was not too difficult or not too easy generated the most optimal plasticity whereas the difficult reverse order discrimination did not show improvements in responses compared to controls.

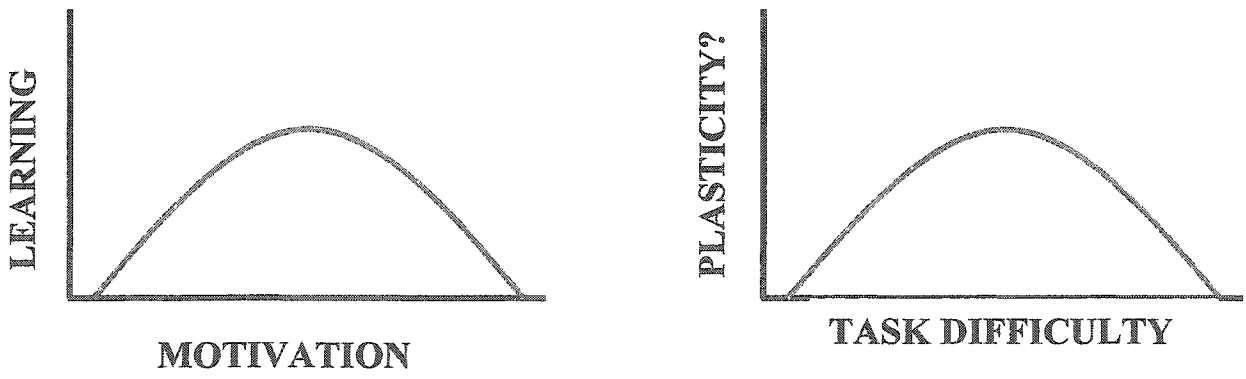


Figure 22 A. Learning and motivation U-shaped function. Learning is minimal when subjects are either too motivated or under motivated. B. Task difficulty and plasticity function. It is possible that there is an intermediate zone where one gets optimal plasticity when the task is not too easy or not too difficult.

In these experiments, we did not study attentional mechanisms that may have played a part in inducing plastic changes nor did we study the influence of neuromodulators such as acetylcholine, dopamine, norepinephrine, etc. Micro-dialysis studies in rats show that Ach efflux in the cortex is maximal when rats perform a sustained attention task and only slight Ach efflux when simply pressing a lever. It is also possible that difficult tasks induce stress and raise blood cortisol levels. It would be interesting to correlate these changes in cortisol level to task difficulty, with difficult tasks showing the greatest increase in cortisol levels and easy tasks showing no increase.

Although it is interesting to study how the auditory cortex responses to sound sequences after training, this study could also be of clinical importance especially in the field of neuro-rehabilitation, for example, aphasia therapy for stroke patients, computer based training protocols for dyslexia and speech learning impaired children. Some of the current protocols employed by speech language pathologists, for example involve intensive exercises for aphasic patients. Making the task very difficult may in fact lead to anxiety and poor performance. Could it also be possible that this in turn would lead to only modest plasticity effects? This experiments described here are only a first step at helping to understand how task difficulty influences cortical plasticity but future experiments with patients undergoing neuro-rehabilitation (and concurrent evoked potential recording, fMRI) will need to be done to better understand the role of task difficulty in improving behavioral training protocols in humans.

Clinical relevance

Rich and stimulating environments can significantly improve the sensory information processing of cortical neurons. Although the exact consequences of plasticity on cortical development or recovery from injury are not clear, numerous studies suggest that environmental

enrichment may be useful in promoting recovery from neurological disability. The decreased responsiveness of auditory cortex in congenitally deaf cats can be reversed by behaviorally meaningful electrical activation of the cochlea (Klinke et al. 1999). Plasticity has also been observed in deaf patients following cochlear implantation and likely contributes to improvements in sensory and language function (Sharma et al. 2002a; Sharma et al. 2002b, c). Exposure to an enriched environment significantly improved cortical circuitry in animal models of traumatic brain injury and may improve functional recovery in humans (Jones and Schallert 1994; Nudo and Friel 1999; Risedal et al. 2002). Collectively, these studies suggest that neural plasticity mechanisms underlie much of the functional improvements resulting from rehabilitation.

The ability to discriminate modulations in sounds is an important property of the central auditory system. Studies in humans have shown that discrimination training on acoustic tasks alters responses of auditory cortex neurons as revealed by MEG, fMRI and evoked potential studies (Cansino and Williamson 1997; Jancke et al. 2001; Menning et al. 2000). The perception of sequential sounds is impaired in individuals with language disorders. For example, language impaired children showed a deficit in the detection of altered time intervals for a four-tone sequence but not two-tone sequence (Kujala et al. 2000). Deficits in the perception of rapid sequences of sound have been implicated in central auditory processing disorders (Kraus et al. 1996).

Treatments designed to ameliorate non-linguistic perceptual deficits have benefited a large number of language-impaired children (Merzenich et al. 1996; Nagarajan et al. 1998; Tallal et al. 1996). Understanding how sensory experience in the form of auditory discrimination training alters physiologic responses in auditory cortex will aid in the development of training strategies to alleviate central auditory processing disorders. In addition, determining what

features of the acoustic stimulus are important in processing auditory information will provide new insights into plasticity mechanisms that the brain uses to process acoustic information.

Remaining issues and future directions

Although the entire focus of this dissertation was on plasticity effects in cortex, one cannot rule out changes taking place in subcortical structures. Indeed, reorganization that is observed at the cortical level is also present within subcortical structures (Florence and Kaas, 1995; Faggin, Nicolelis, 1997). It might appear that cortical plasticity may simply reflect changes in subcortical structures. Unfortunately, this simple scenario is not true because there are nearly ten times as many fibers projecting back from the cortex to thalamus. Top-down cortico-thalmaic feedback influences representation in the lower neuraxes and these cortico-fugal connections contribute to thalamic plasticity in a very specific manner (Yan and Suga, 1998; Ergenzinger et al, 1998). In fact, experiments have shown that the cortex may actually be necessary to induce thalamic plasticity (Krupa et al. 1999). Future experiments employing simultaneous recording from both thalamus and cortex and deactivating (e.g. cooling) cortical tissue will be helpful in understanding the dynamics of this complex circuitry.

Responses from A1 neurons were recorded at one point in time. It is not known when the plasticity effects would begin to appear. Future experiments employing evoked potential recordings and/or chronic multi-channel recordings from the auditory cortex of awake rats should be able to shed light on this important issue. Auditory responses from awake rats could be obtained while rats train or after every training session. Motion artifacts and implant injuries during training are potential concerns. It would be interesting to know if plasticity effects depend on the nature of the operant procedure. The present tasks employed a go/no-go paradigm. Other experiments have made use of limited hold experiments and adaptive tracking. An

experiment in which rats train on exactly the same stimulus sets but different operant procedures would be interesting.

Discrimination training on sequences did not generate combination-sensitive neurons in auditory cortex. We employed a variety of different tasks including two recent experiments that are an extension to this study. One was a simple HLN detection task and the other task was a HLN vs. tone (H, L, and N) discrimination task. Preliminary results show that these tasks too failed to generate order-sensitive neurons. It appears that behavioral training is different from the basal forebrain stimulation paradigm in which A1 neurons became order sensitive. During behavioral training, rats are hungry, motivated and are attending not only to the CS+ sound to get rewarded, but are attending to avoid the CS- sound. This more naturalistic training could recruit limbic circuits, arousal and attentional systems and a variety of neurotransmitters that may not be part of the basal forebrain training even though it is know that stimulation of this nucleus increases the behavioral relevance of the paired sound.

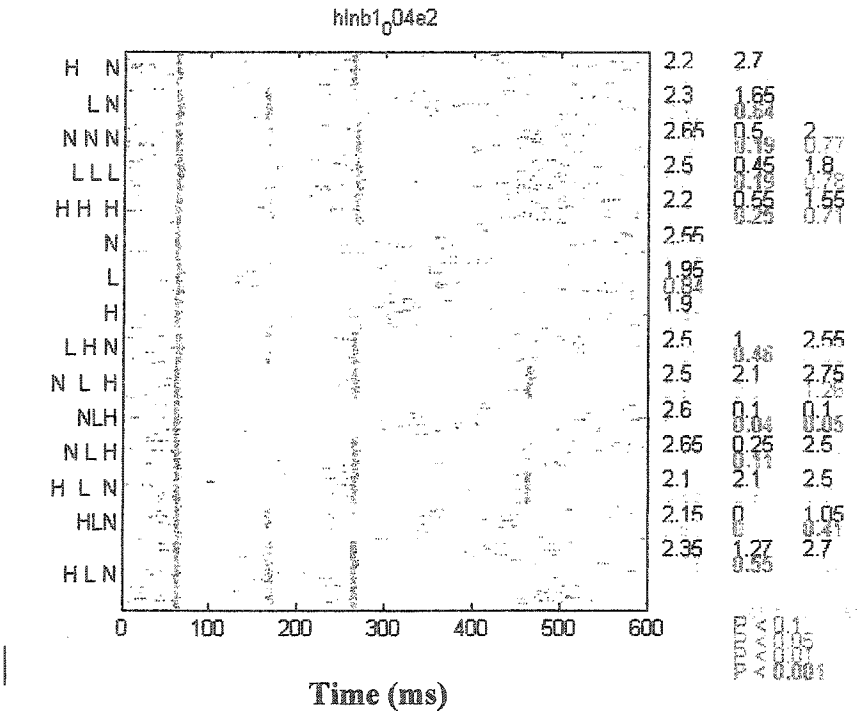
Patterns of both activity independent and experience dependent neural activity drive the refinement of precise cortical circuits in the young and adult brain. Temporal processing of auditory information in the range of tens to hundreds of milliseconds is important not only for simple discriminations (e.g. tones, intervals, and durations) but also for more complex acoustic processing such as language and music. This processing of acoustic information relies on the spatio-temporal firing pattern of trains of action potentials. How the cortex processes sequence and integrates temporal information over time is important is an important issue for computational neuroscientists. Incorporating such realistic properties of neurons into computational models may allow us to 'predict' how network-level rules operate to generate useful plasticity.

APPENDIX A

Group IV (Frequency Discrimination) N = 9 behavior rats (n=292 A1 sites) N = 7 naïve rats (n =221 A1 sites)		Suppression Index			
		Second Sequence Element		Third Sequence Element	
		Naïve	Trained	Naïve	Trained
Paired Sequence	H L N – 100 ms	3	1	0	11 (p<.05)
Tones Reversed	L H N – 100 ms	-31	-2 (p<.001)	14	8
Repeat Low	L L L – 100 ms	-43	-18 (p<.05)	-31	-10 (p<.01)
Repeat High	H H H – 100 ms	-56	-20 (p<.00001)	-35	-9 (p<.001)
Repeat Noise	N N N - 100 ms	-7	-14	15	9
Sequence Reversed	N L H – 100 ms	-12	0	25	18
Stretched	H L N – 200 ms	13	13	15	12
Degraded Sequence	L N – 100 ms	-14	-2 (p<.05)	-	
	H N – 200 ms	5	4	-	
HLN-Fast	H L N – 50 ms	-128	-150	-76	-62

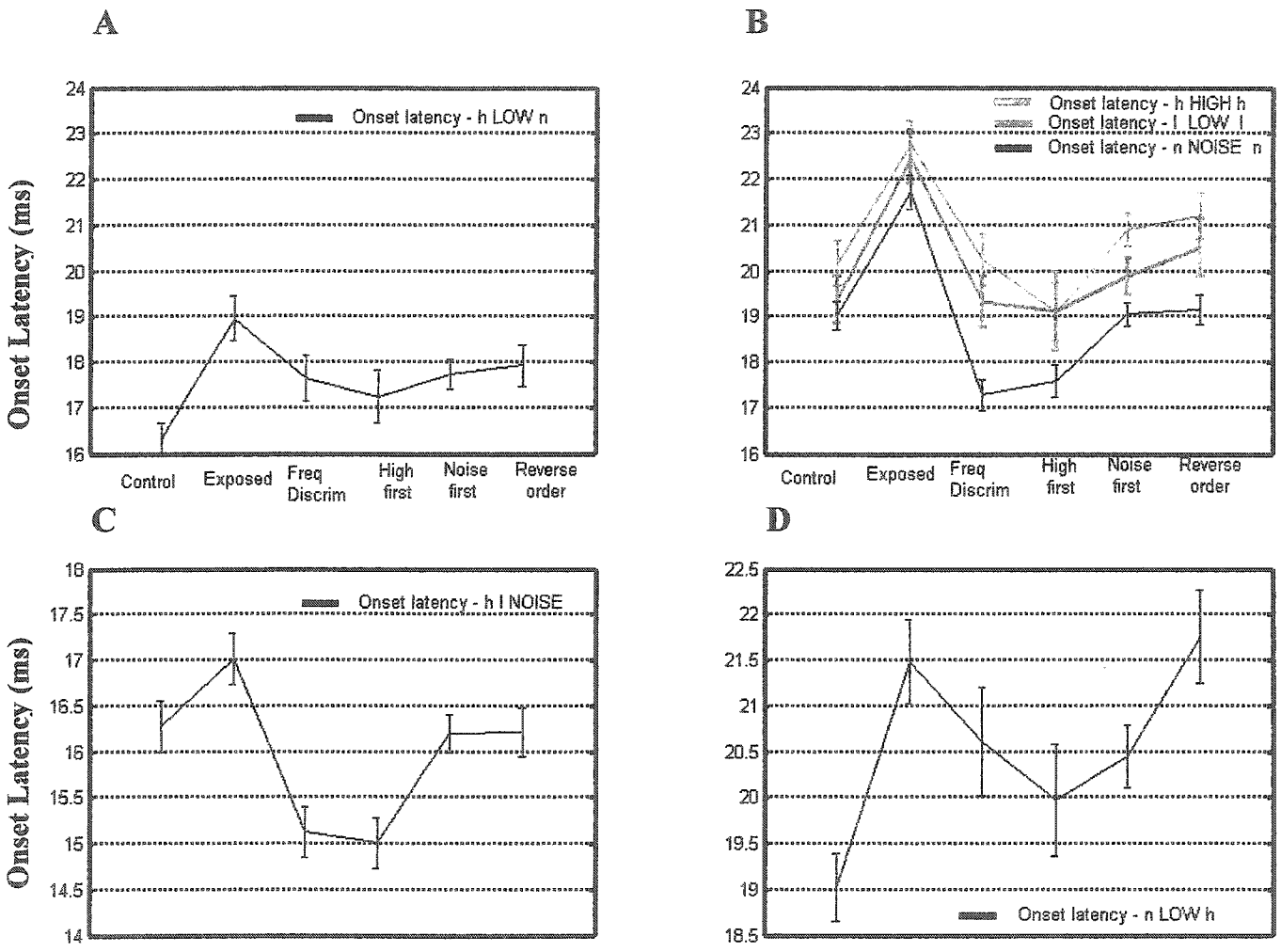
Suppression indices after frequency discrimination training. Suppression or facilitation to the following elements was quantified by using a suppression/facilitation index. The index is 100 times the logarithm base-two of the ratio of the number of spikes in response to a stimulus element in the context of a sequence and the number of spikes in response to the same element in isolation. For example, an index of 100 signifies twice the number of spikes to the following element compared to the number of spikes in isolation whereas -100 denote half as many spikes. Sites that show no facilitation or suppression have an index of zero.
Yellow highlighted area: The LLL (CS+) and HHH (CS-) sequences show significantly less suppression compared to controls.

APPENDIX B



Dot raster from trained rat (noise-first group) showing response suppression to the following elements of the sequence. The different sequence combinations are shown on the y-axis. Time is shown on the y-axes (ms). Each red dot represents on spike.

APPENDIX C



Mean onset latency for the second or third element of sequences. A) Mean onset latency for the second element of HLN B) Mean onset latency for the second element of HHH, LLL and NNN C) Mean onset latency for the noise element of HLN D) Mean onset latency for the second element of NLH. In all panels, sound exposure resulted in a significantly increased onset latency compared to controls and in most cases, when compared to trained rats.

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VITA

Navzer D. Engineer was born in Bombay, India, on November 18, 1971, the son of Navaz and Dara Engineer. After completing his elementary education at Bombay Scottish High School in 1988, he enrolled in Jai Hind College, Bombay and received the Bachelor of Science degree in Biology. He received the M.B.B.S degree after finishing medical school at BVMC Medical College, India in 1996, following which he did a residency in the Department of Cardiology and Neurology at P.D. Hinduja National Hospital, Bombay, India. After completing his work there, he entered the University of Texas at Dallas where he received the Master of Science degree in Cognition and Neuroscience in 2001. He entered the Doctoral program in Cognition and Neuroscience thereafter under the supervision of Dr. Michael P. Kilgard.