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Differences In Brainstem Level Encoding Of Am And Fm Signals In A Rat Model For Dyslexia

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DIFFERENCES IN BRAINSTEM LEVEL ENCODING OF AM AND FM SIGNALS IN A RAT MODEL FOR DYSLEXIA

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by

Stephen Anthony Chabot

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To Gabrielle, Rosie, the little one on the way, and the one we lost. To Mom, Dad, brothers and sisters, nieces and nephews. Thank you for all of your support through my education.

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Chabot, Stephen Anthony. M.S., Purdue University, December 2014 Differences in Brainstem Level Encoding of AM and FM Signals in a Rat Model for Dyslexia. Major Professor: Edward Bartlett.

Developmental dyslexia is a language learning disorder that affects a significant portion of the population. Many subjects who suffer from dyslexia show auditory processing deficits as children, some of which persist into adulthood, which impair their ability to learn one or more aspects of language. There is evidence that some types of training can correct the auditory processing deficits and bring the subject to a normal reading or speaking proficiency for their age group. One common rodent model for dyslexia is the cortical freeze lesion model. This model induces a lesion in the rat cortex similar to the microgyria present in many dyslexic brains, as well as changes to the thalamus similar to those seen in human dyslexics. The goal of this study was to determine what effect, if any, did the cortical lesions have on brainstem level responses to simple sinusoidal amplitude or frequency modulated stimuli. The study showed reduced response strengths of lesioned rats to AM signals below a 1000 Hz amplitude modulation rate. In comparing recordings done under anesthetic to recordings done under sedation, the recordings under sedation showed no significant differences between sham and dyslexic rats under a 256 Hz modulation frequency, while the rats recorded under anesthetic showed differences down to 64 Hz modulation frequency. This would suggest

that the cortex influences the brainstem responses on both a permanent and transient level, as the changes in the responses are evident even when cortical activity is at a minimal level to the effect of anesthetics.

CHAPTER 1. BACKGROUND

1.1 Peripheral Auditory System

The following section was written using information from Principles of Neural Science (Kandel, Schwartz, & Jessell, 2000). The auditory system is the sensory system responsible for the sensation of sound. Sound is defined as a mechanical wave which is an oscillation of pressure within a medium, and composed of frequencies within the range of hearing. A sensory system is a part of the nervous system responsible for processing sensory information. Every sensory system is composed of several basic parts: sensory receptors, neural pathways, and parts of the brain involved in sensory perception. Sensory receptors convert the relevant stimuli into neural signals which are then transmitted and modified by the neural pathways to the part of the cerebral cortex responsible for the sensation of that particular modality.

The auditory system is divided into two main parts: the peripheral auditory system and the central auditory system. The peripheral auditory system (figure 1.1) is responsible for transducing sound waves into neural signals which can then be processed by the central auditory system. The peripheral auditory system consists of three main parts: the outer, middle, and inner ear.

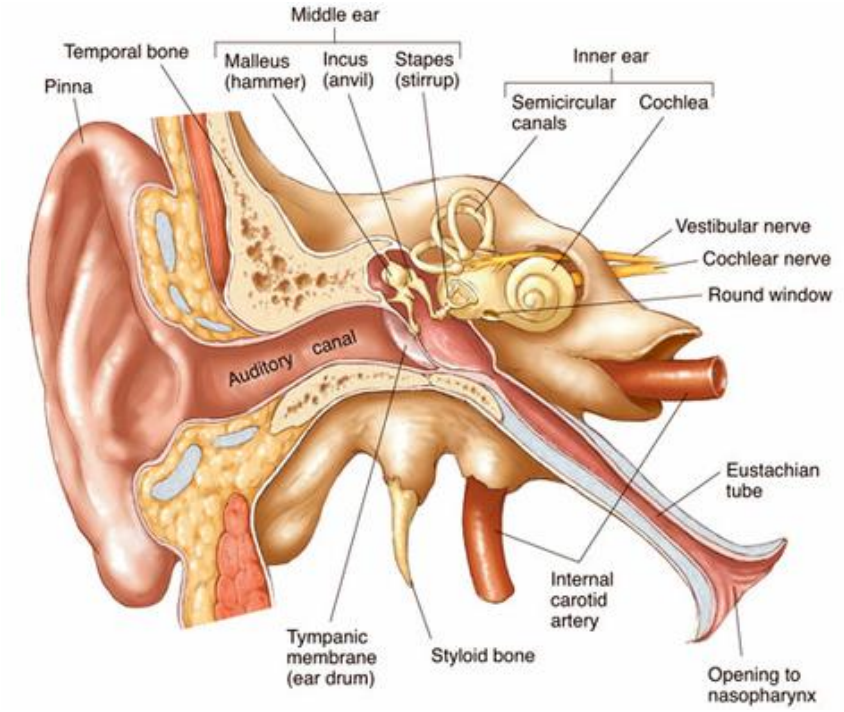

Figure 1.1 Peripheral Auditory System ("Peripheral Auditory system image")

The outer ear consists of the pinna, the external auditory meatus (ear canal), and ends with the tympanum (eardrum). The ear canal starts at the auricle and ends at the tympanum, or eardrum, a thin diaphragm about 9 mm in diameter. The middle ear is an air-filled pouch extending from the pharynx, to which it is connected by the Eustachian tube. The middle ear contains a linkage of three small bones, the malleus, incus, and stapes (or hammer, anvil, and stirrup). The base of the malleus is connected to the eardrum, and its other end is connected by a ligament to the incus, which also has a ligamentous connection to the stapes. The terminal end of the stapes inserts into the oval window, and opening in the bony covering of the cochlea.

The inner ear consists of the cochlea and the semicircular canals. The semicircular canals, while utilizing a mechanism similar to that of the cochlea, are not involved in hearing, but are involved in sensation of rotary movements and positions. The cochlea is

structure in which sound waves are converted into neural signals for processing by the nervous system. The interior of the cochlea contains three fluid-filled tubes wound around a bony core. In a cross-section of the cochlea, the uppermost fluid-filled compartment is the scala vestibuli. At the base of this chamber lies the oval window, which is sealed by the footplate of the stapes. The lowermost of the chambers is the scala tympani, which also has a basal aperture, the round window, which is sealed by an elastic membrane. The scala media, or cochlear duct, separates the other two compartments along most of their length. The scala vestibule and scala tympani communicate at the helicotrema, an absence of the scala media at the apex of the cochlea. The scala media is bounded by a pair of membranes: the Reissner's membrane, which divides the scala media from the scala vestibule, and the basilar membrane, which forms the partition between the scala media and the scala tympani. The basilar membrane is a complex structure, and the location of the organ of Corti, and is where signal transduction occurs. The organ of Corti consists of approximately 16,000 hair cells which are innervated by approximately 30,000 nerve cells of the 8th cranial nerve. The hair cells are divided into two groups, the outer hair cells and the inner hair cells.

1.2 Central Auditory System

Following transduction into nerve signals in the cochlea, the signal is transmitted through the eighth cranial nerve into the central auditory pathway. The major nuclei in the pathway are illustrated in figure 2.2. The auditory nerve (cranial nerve XIII) terminates first in the cochlear nuclear complex. From here, projections terminate in the ipsilateral superior olivary complex and after terminating in the trapezoid body, they

project to the contralateral superior olivary complex, as well as the contralateral lateral lemniscus through two separate pathways: the dorsal acoustic stria and the intermediate acoustic stria. The superior olivary complexes both project up to the ipsilateral lateral lemniscus. Information is passed both laterally in the lateral lemnisci to the opposite side, as well as projecting higher in the pathway to the inferior colliculus, which is the last part of the pathway that resides in the brainstem. After the inferior colliculus, the next stop is the medial geniculate nucleus in the thalamus, which then terminates in the auditory cortex. (Kandel et al., 2000)

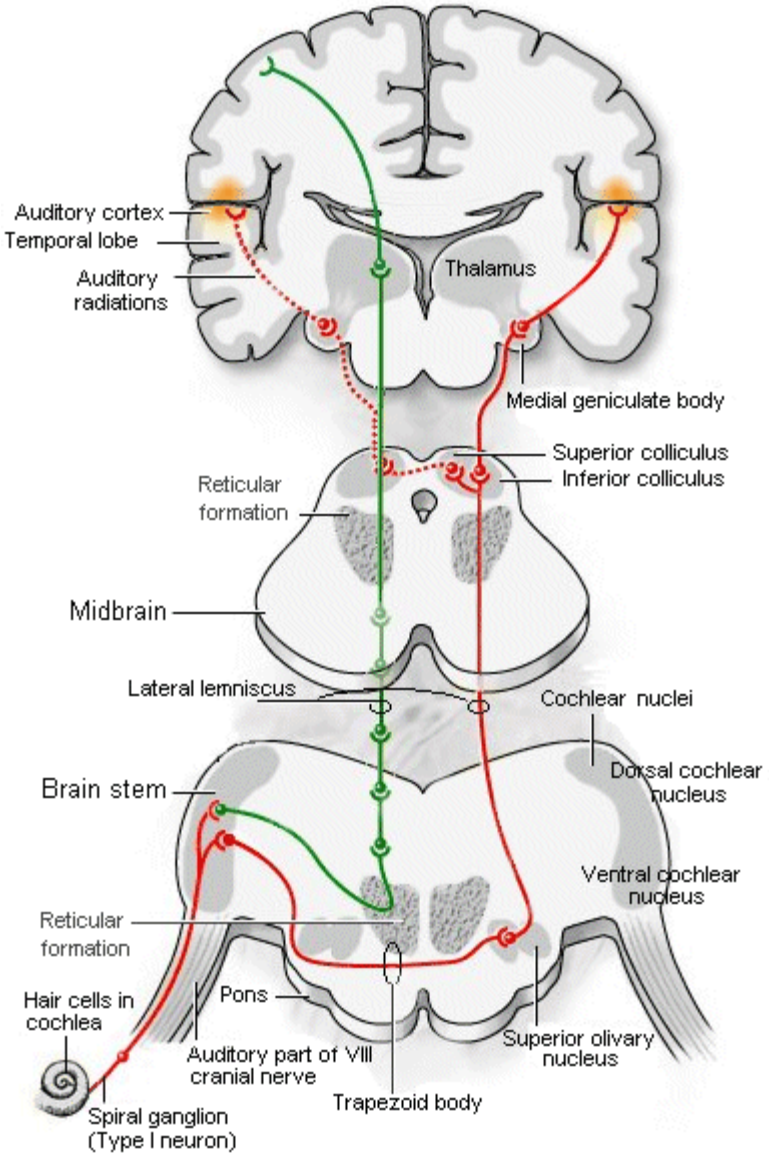

Figure 1.2 Central Auditory pathways("Central Auditory Pathways")

The role of the inferior colliculus (IC), medial geniculate body (MGB), and auditory cortex are of primary interest in this project. The following information is taken primary again from Principles of Neural Science (Kandel et al., 2000). The IC consists of two primary components, the dorsal part and the central nucleus. The dorsal part of the IC receives both auditory and somatosensory inputs, and the function of this region

remains uncertain. The central nucleus lies beneath the dorsal layers, with the cell bodies arranged in layers. The cell bodies within each layer exhibit similar characteristic frequencies, so the tonotopic map in this layer extends orthogonally to the layers. The IC contains many neurons sensitive to interaural timing or intensity differences, which would suggest involvement in sound localization. The MGB is constitutes the thalamic relay of the auditory system. The MGB consists of three major subdivisions, of which the principal (ventral or lateral) nucleus is best understood. Neurons from the central nucleus of the IC project to the principal nucleus through the brachium of the IC. The remaining components of the MGB receive both somatosensory and visual inputs in addition to the auditory projections. The tonotopic map is maintained in the MGB, but here the neurons specific to a frequency are arranged in one layer, so the nucleus consists of a stack of neuronal laminae that represent successive stimulus frequencies. The ascending auditory pathway terminates in the cerebral cortex, with several distinct auditory areas. The primary auditory cortex contains aa tonotopic representation of the various frequencies. The cortex is responsive to stimulation originating in either ear, but not to the same extent, but is instead divided into two zones of different types. In half of these strips, known as summation columns, the neurons are excited by stimulation coming from either ear, though the contralateral input is typically stronger. The alternating bands, known as suppression columns, contain neurons that are excited by ipsilateral inputs, but inhibited by contralateral inputs. Because the summation and suppression columns extend at right angles to the tonotopic mapping, the overall map of the cortex consists of columns responsive to all of the different audible frequencies. The primary auditory area is

surrounded by several distinct regions which help in the processing of particular types of auditory information.

Information does not only flow upwards in the auditory system, but backwards as well. All sensory systems have extensive descending projections, and the size of these projections is in many cases greater than that of the ascending pathways, but we still have a poor understanding of role that is played by these connections (Bajo & King, 2013). The auditory cortex has confirmed descending connections to auditory and non-auditory thalamus, midbrain, and medullary regions, meaning that auditory corticofugal influence reaches sites both immediately presynaptic to the cortex, sites remote from the cortex, as in periolivary regions that may have a centrifugal role, and to the cochlear nucleus, which could influence processing early in the central auditory pathway (Winer, 2006). Targets outside the central auditory pathway include the striatum (possible premotor functions), the amygdala and central gray (prospective limbic and motivational roles), and the pontine nuclei (for precerebellar control) (Winer, 2006).

1.3 Measurement Techniques

An Auditory Evoked Response (AER) is a measurement of electrical activity within the auditory system that is produced or stimulated by sounds presented to the subject. The AER has some similarities to electroencephalography (EEG), but the measurement technique is able to measure the response to a specific stimulus instead of measuring brain activity as a whole. The AER consists of electrical activity from the auditory structures that is recorded from electrodes placed at specific locations on the head. The signal picked up by the electrode is typically passed through a pre-amplifier,

filters, and an Analog/Digital Converter before being stored on a computer. There is some variation in the type of electrode used, depending on the type of recording being done and the species that is being recorded from. The typical electrodes used for recording in humans is a flat disc that is taped directly onto the skin, while the typical electrode used in rodent studies is a needle electrode placed under the scalp. There are several limitations associated with using surface electrodes to measure electrical activity. The first of these is that the electrode will pick up all electrical signals generated in its vicinity, so determining what specific population of neurons is generating the signal of interest can be challenging in some circumstances, and impossible in others. However, the electrode has excellent temporal sensitivity due to collecting real time electrical data. A contrast to these tradeoffs would be the fMRI type of measurement, which can give good spatial resolution of activity, but poor temporal resolution. Another difficulty in using surface electrodes to measure neural activity is the problem of extracting the signal of interest from background brain activity. The activity of interest is of an extremely small voltage at the surface, on the order of microvolts, due to the voltage field decreasing proportionately to the distance between the structure generating the field and the electrode that is measuring it. To extract the signal of interest from the background activity of the brain, two processes are necessary. The first process is amplification of the signal before any analysis takes place. The second process is signal averaging, which averages hundreds of recorded responses to the same stimulus to average out all of the background electrical activity, which is assumed to be of a random nature. After the signal has been amplified and averaged, filtering is also needed to compensate for baseline drift and high frequency noise.

The following information was obtained primarily from Handbook of Auditory Evoked Responses (Hall, 2007). While the general procedure for recording an AER is outlined above, there are several different kinds of AER commonly used in clinical and research settings. One of the most common types of AER used is the Auditory Brainstem Response (ABR). The ABR measures the response to transient stimuli, typically a short click (around .1 millisecond duration), or a short tone burst stimulus is used. The ABR waveform gives distinct peaks, which are typically numbered with roman numerals for analysis purposes. In clinical application, the peaks of the ABR can give information on anatomical structures within the auditory system. Generally speaking, the anatomic origins of the different parts of the ABR waveform are better known for earlier parts of the ABR, and less precise information is given by the later waveforms. Clinical knowledge of the origins of the peaks of the ABR has come chiefly from lesion studies in the auditory system. Because lesion studies cannot be done in humans, other than on subjects with pre-existing lesions, the data concerning the origin of the peaks on human subjects is limited. Animal model studies, while useful, do not necessarily correlate because of how the signal is measured. In a small animal like a rat, a surface electrode will be much closer to a corresponding generator site than it may be in a human, and due to the effects of distance on an electrical measurement, the relative contributions of nuclei in the auditory pathway can vary significantly from model to human subject. Also confounding the matter, especially in the later peaks of the ABR, is the fact that multiple generator sites can contribute to the same wave component, and conversely the same anatomic structure can contribute to multiple components of the ABR. The anatomic origin of wave I component of the ABR has been clearly defined as the far-field

representation of the compound action potential of the distal portion of the eighth nerve, that is, the activity of the eighth nerve as it leaves the cochlea and enters the internal auditory canal. The reason that this can be confidently stated is based on evidence from direct recordings from the eighth nerve potentials, and also from supporting evidence from ECochG studies. Wave II also originates from the eighth nerve, but from the proximal end rather than distal end. Evidence for this is supported by the relationship between the latencies of waves I and II and the relatively slow conduction time of the auditory nerve. The origin is further supported by evidence of a persistent presence of wave II in brain death, as well as interoperative recordings directly from the root entry zone of the nerve. Due to the size differences, the wave II component in small animals corresponds to wave III in humans. In small animals, there is no ABR component corresponding to the wave II component in humans.

CHAPTER 2. DYSLEXIA

2.1 Human Dyslexia

Developmental dyslexia is a learning disability affecting around 5-10% of children and adults (Shaywitz, 1996). It is characterized by severe reading and spelling difficulties that are resistant to usual teaching methods and remedial efforts (GersonsWolfensberger & Ruijssenaars, 1997). Dyslexia has a high rate of co-occurrence with specific language impairment, which is characterized by difficulty in assimilating a language as a child (Flax et al., 2003). Both disorders are linked to the presence of polymicrogyria in the cortex of the patient (Guerreiro et al., 2002). Post-mortem analysis in human dyslexic subjects has shown hemispherical differences in dyslexic patients, with the left dyslexic MGN typically having a greater percentage of small neurons compared to the right dyslexic MGN, and no significant difference existing between right dyslexic and right control MGNs, or between right and left control MGNs (Galaburda, Menard, & Rosen, 1994).

Dyslexia in adults is associated with a reduced ability to phase lock to slow amplitude modulation (<10-Hz) (Hamalainen, Rupp, Soltesz, Szucs, & Goswami, 2012). One study (Witton, Stein, Stoodley, Rosner, & Talcott, 2002), associated only deficits at 20-Hz AM and 2-Hz FM as having a significant correlation to pseudo-word reading

accuracy, suggesting that although the brain changes which cause dyslexia may cause deficits across a range of frequency sensitivities, not all of the frequencies would have an effect on written or spoken language acquisition. Another study using fMRI showed differences in MGB activity of adults with dyslexia when attending to phonemes compared to other speech features (Diaz, Hintz, Kiebel, & von Kriegstein, 2012). AMFR studies in dyslexic adults have shown a reduced AMFR amplitude with a normal click ABR response (McAnally & Stein, 1997). An analysis of speech ABR in dyslexics showed that up to 40% of dyslexia patients have abnormally long latencies in the later speech ABR peaks while maintaining normal click ABR responses (Banai, Abrams, & Kraus, 2007). One question that is of interest in the study of dyslexia is whether the deficits in processing start in the brainstem encoding of the sound, or if the deficits of the brainstem encoding are a result of a deficit higher up in the brain. Recent studies into the effect of speech and music experience on brainstem encoding of complex stimuli, such as differences in pitch coding by native Mandarin speakers (Krishnan, Gandour, & Bidelman, 2010) or music experience (Bidelman, Gandour, & Krishnan, 2011) suggest that a top down model can be used to explain some of the brainstem changes in dyslexics. One possible explanation for the effect of experience and learning on brainstem encoding is the reverse hierarchy theory (Ahissar & Hochstein, 2004), which postulates that conscious perception is based on the highest possible representation along the perceptual hierarchy, and that repeated exposure to a stimulus causes the higher levels to influence the ways that lower levels encode the stimuli. Some of the more recent work in treating dyslexia in younger subjects has taken advantage of the cortical plasticity in the young brain using either phonological discrimination training (Collet et al., 2012)or

neurofeedback training (Nazari, Mosanezhad, Hashemi, & Jahan, 2012) in order to reduce the symptoms of dyslexia, often bringing the subjects up to a normal speaking or reading level.

2.2 Rat Dyslexic Model

One common rat model for dyslexia is the cortical freeze lesion model, which utilizes freeze lesion induced on postnatal day 1 (P1) in the primary somatosensory cortex which are similar in morphology to polymicrogyria in human dyslexics (Dvorak & Feit, 1977). This model also showed a similar difference in MGN morphology to that observed in human dyslexic patients, with lesioned subjects having a greater percentage of small cells and fewer large cells in the MGN compared to sham littermates (Peiffer, Rosen, & Fitch, 2002).

In the induced microgyria rodent model, there are rapid auditory processing deficits in adult rats with induced lesions similar to those seen in human dyslexics (Peiffer, McClure, Threlkeld, Rosen, & Fitch, 2004; Peiffer et al., 2002). Lesioned rats show deficits in discrimination of two-tone stimuli with a duration of 64 msec or less, showing a deficit in rapid auditory processing (Clark, Rosen, Tallal, & Fitch, 2000), as well as lowered ability to detect short silent gaps in a cued startle experiment (Threlkeld, McClure, Rosen, & Fitch, 2006). Experiments using enriched auditory environments to reduce the effects of cortical injury gave mixed results, with one study (Peiffer et al., 2002) showing little to no effect of acoustic experience on coding outcomes, and another more recent study (Threlkeld, Hill, Rosen, & Fitch, 2009) showing some improvement on adult subject, but better results when acoustic experience started at a young age.

2.3 Experiment Aims

It is known that there are projections from the auditory cortex to the inferior colliculus (Bajo, Nodal, Moore, & King, 2010; Peterson & Schofield, 2007; Winer, 2005), which are thought to play a role in modulation of stimulus-specific adaptation (SSA), but are not thought to be essential to its formation (Anderson & Malmierca, 2013). Stimulus specific adaptation involves either the strengthening or inhibition of a neuronal response to a sustained stimulus, and can happen both in the short term and long term. If SSA in the IC is affected by the cortex only in the short term, then there would be differences in the IC level responses to a sustained stimuli in test cases involving inactivation or disruption of normal cortical activity vs cases where the cortex was left to function normally. In the case of long term SSA, the corticofugal projections would affect the development of the lower parts of the brainstem if the focal lesions disrupted them. To explore this possibility, we are comparing the ABR and FFR responses of rats with focal lesions to those of rats who have undergone a sham operation. If the corticofugal projections affect the lower levels of brainstem development, there should be significant differences in response amplitude between the sham and lesioned subjects, both in awake sedated recordings and in anesthetized recordings. If the responses affected are the cortical responses, then the differences should either not be present or should be greatly reduced when cortical activity is reduced in anesthetized recordings. We would also like to explore the effects of Chinese tone exposure on pitch coding in the brainstem, and see if it is enhanced through repeated exposure. This would further strengthen the corticofugal hypothesis of the development of the lower auditory system.

2.4 Materials and Methods

2.4.1 Subjects

24 young (age P21 to P30, weighing ~ 50-100g) male Long-Evans rats obtained from Harlan were used in this study. The animals were housed in the animal care facility for the period of the study in relatively quiet, standard laboratory housing conditions, on a 12-hour on, 12 hour off reversed day/night cycle. The protocol used was approved by the Purdue animal care and use committee (PACUC 06-105). Only male subjects were used in this study, due to previous reports of gender differences for auditory processing and loss of MGB neurons (Higgins, Escabi, Rosen, Galaburda, & Read, 2008; Peiffer, Rosen, & Fitch, 2004).

2.4.2 Induction of Focal Cortical Lesions

Pregnant Long-Evans rats were obtained from Harlan. On postnatal day 1 (P1), the pups were pseudo-randomly assigned to receive either a bilateral freezing injury or a sham operation. Subjects were anesthetized via induced hypothermia, and a midline incision was made in the skin covering the skull. Somatosensory lesions were made with a cooled (-70°C) probe placed on the skull at bregma, approximately 2 mm lateral to the sagittal suture for 5s. For the sham surgery, the same surgical procedure was carried out, but the probe was maintained at room temperature (Higgins et al., 2008). The induced subjects were marked in the footpad with an injection of ink, and the sham induced animals were not marked.

2.4.3 Experimental Setup

The experimental setup was similar to that described in (Parthasarathy, Cunningham, & Bartlett, 2010). Experiments were performed in a 9'x9' double walled acoustic chamber (Industrial Acoustics Corporation). There were two groups of animals recorded from; for the first group, all recordings were done under isoflurane anesthesia and for the second group, the recordings were done using the sedative Dexdomitor. For both groups, the animals were initially anesthetized using isoflurane anesthesia. Induction was performed in a separate induction chamber at 4% concentration of isoflurane. The animals were then transferred to a manifold and maintained under anesthesia at 1.5-2% isoflurane. For the subjects recorded under anesthesia, the animals were maintained under anesthesia using the manifold as described. For the subjects recorded using the sedative Dexdomitor, they were maintained under anesthesia using the manifold while the leads were attached. Afterwards, they were removed from the manifold and given an intramuscular injection of 0.2mg/kg Dexdomitor into the rear leg, and recording started 15 minutes after removal from the isoflurane manifold to allow the effects of isoflurane to wear off. The animals were placed on a water circulated warming blanket (Gaymar) set to 37°C for the duration of the experiment. The pump was placed outside the chamber to minimize electrical and acoustic interference. Stimuli were presented free field using a speaker to the right ear of the animal, at a distance of 115cm from the ear. The sound level of the speaker was calibrated using a Bruel Kjaer microphone and SigCal software (Tucker Davis Technologies). The channel 1 electrode was place on the forehead of the subject along the midline from Cz to Fz, the channel 2 electrode was placed perpendicular to the channel 1 electrode between the midline of the animal's ears, the

negative electrode was placed along the mastoid beneath the animal's right ear, and the ground electrode was placed in the nape of the neck. Impedances were tested using the head-stage (RA4LI, Tucker Davis Technologies or TDT) and were always < 1 kΩ. Control recordings were created using electrodes placed in a potato instead of an animal to ensure there was no stimulus bleed-through, and ensure that the data collected represented physiological data.

Sounds were generated using SigGenRP (TDT). Stimulus presentation and acquisition were done using BioSig (TDT). Waveforms were converted to signals through a multi-channel processor (RX6, TDT) and presented using a Bowers and Wilkins DM601 speaker. ABRs and FFRs were recorded using sub-dermal needle electrodes (Ambu) connected to a low impedance headstage (RA4LI, TD) and amplified (RA4PA preamplifier, TDT). Digitized waveforms were recorded using a multichannel recording system (RX-7-2, TDT, and RZ-5, TDT).

2.4.4 Stimulus and Recording Descriptions

Auditory brainstem responses (ABRs) were recorded using rectangular click stimuli of alternating polarity, 0.1 ms long, presented at 26.6 clicks/s. The acquisition window was 20 msec and each ABR was averaged over 1500 repetitions. The intensity of the clicks started at 5dB and was increased in 10dB increments to obtain the click threshold,

Sinusoidally amplitude modulated tones were 200 msec long with a 5 msec cosine squared ramp ant onset and offset and played at a rate of 3.1/s. All stimuli were played at 30 dB above click ABR threshold. The acquisition window was 300 msec long, and each

AMFR was an average of 200 repetitions. The carrier frequency for the stimuli was 8 kHz. To test the change in tracking ability with modulation depth, the tone was modulated starting at 7 different modulation depths at each frequency ranging from 0dB depth to -30dB depth. To obtain the tMTF, the modulation depth was maintained at 0dB depth and the modulation frequency was varied from 32 to 4096 Hz in half octave steps.

Sinusoidally frequency modulated tones were 200 msec long with a 5 msec cosine squared ramp at onset and offset and played at a rate of 3.1/s. The carrier frequency was 8 kHz, and modulation frequencies varied in 7 increments from 40 (0.5%) to 4000 Hz (50%) in approximately octave steps. These stimuli were also presented at 30dB above click ABR threshold.

2.4.5 Histology

After electrophysiology data was collected on the animals, they were euthanized using an intraperitoneal injection of Beuthanasia (200mg/kg), then transcardially perfused with phosphate buffered saline and 4% paraformaldehyde. After perfusion, the subject was decapitated and the brain removed from the skull, and left in 4% paraformaldehyde overnight. The next morning, it was removed from the paraformaldehyde solution and placed in a 30% sucrose solution for cryoprotection. After the cryoprotection step, the brain was wrapped in foil and placed in a -80°C freezer to await further processing.

Tissue was sectioned into 40µm coronal sections using a cryotome (Thermo Scientific). Alternating sections were reserved for Nissl staining procedure (Paul, Beltz, & Berger-Sweeney, 2008) and for Calbindin antibody staining. Antibody staining was done in individual wells using a primary antibody against Calbindin(Sigma –Aldrich,

rabbit anti-Calbindin, 1:500 dilution), with a secondary biotinylated antibody (Sigma-Aldrich, goat anti-rabbit, 1:200 dilution) followed by DAB visualization. After staining the sections were mounted onto slides and examined for the presence of necrotic lesions. The subjects showing evidence of lesions were considered dyslexic for the purposes of data analysis, and if no lesions were present the subjects were considered sham (Figures 1 and 2). The rats with ink injected into the rear footpads were known to have received the freezing probe, but it is not known if the lesions result until the tissue is processed.

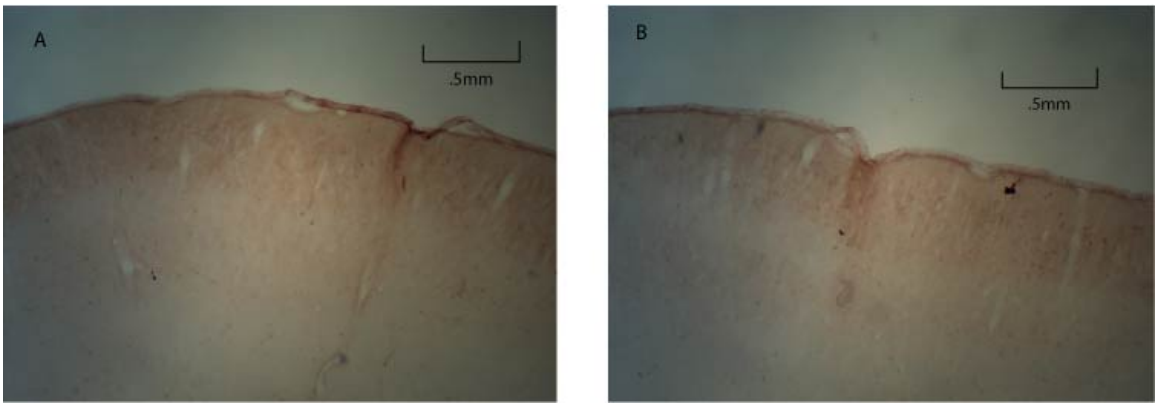

Figure 2.1 Normal cortex structure

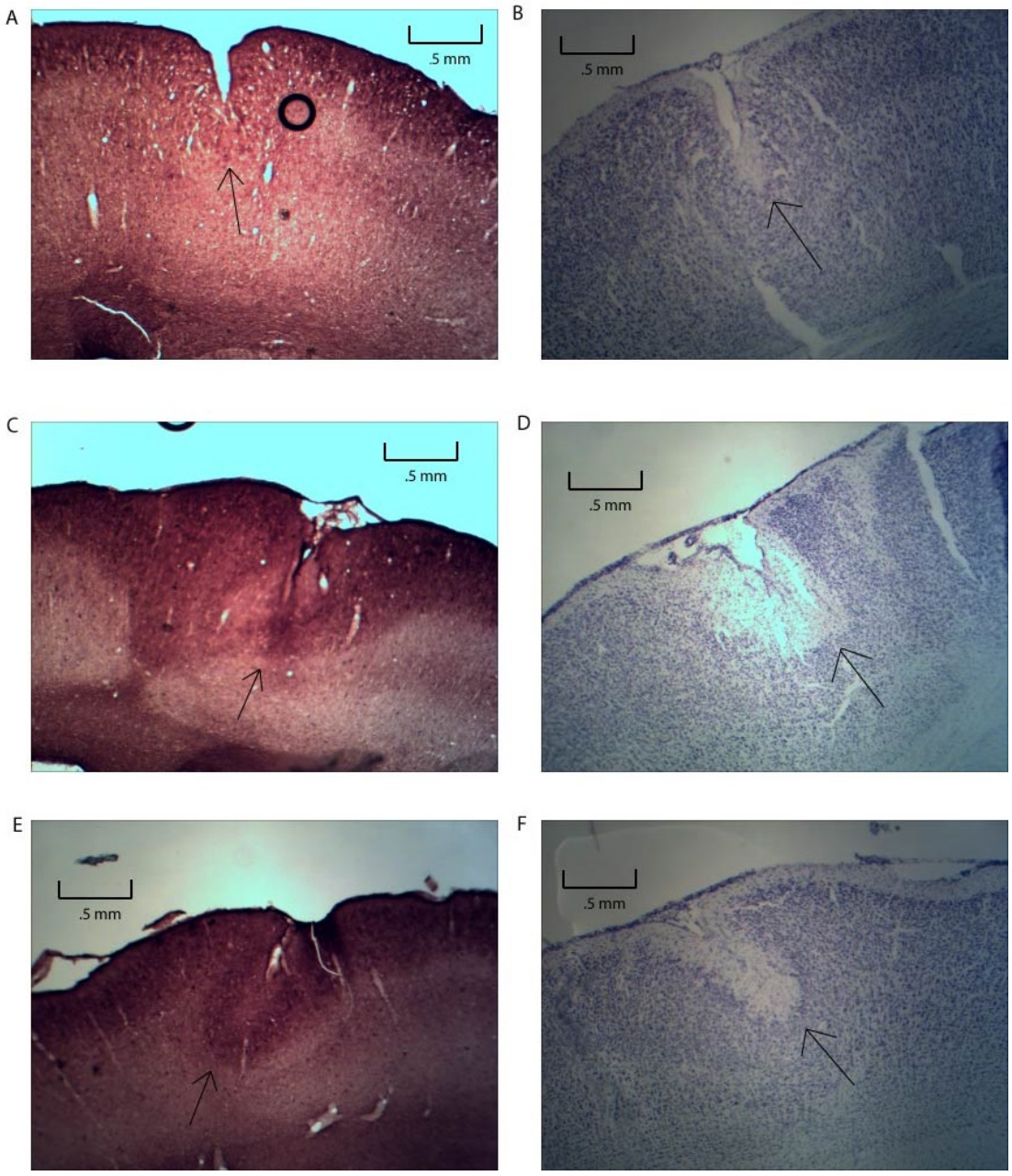

Figure 2.2 Microgyria identified through Calbindian and Nissl stain techniques

2.4.6 Data Analysis

The unfiltered click stimuli ABR stimuli were analyzed at 75dB (typically about 30 dB above detection threshold). The amplitude and latency of wave I were recorded, as

well as the amplitude and latency differences of the wave III-V complex. All other data was bandpass filtered using an 8-pole Butterworth filter from 80-3kHz, with the exception of 32 and 64 Hz stimuli, which were filtered with a high-pass corner frequency of 30 Hz instead of 80 Hz. The response power at modulation frequency was calculated using the Welch power spectral density method using 8192 discrete Fourier transform points and plotted using Matlab.

The amplitude and latency values of the ABR data were tested for statistical significance using the ranksum function in Matlab, and all other statistical analysis was done using the SAS 9.3 software package. The tMTF responses were analyzed using a 2-way ANOVA (condition vs. modulation frequency), and the SAM depth and SFM depth recordings were tested using a repeated measures ANOVA (condition vs. modulation frequency vs. modulation depth). A value of $p<.05$ was the standard for statistical significance.

2.5 Results

2.5.1 Introduction

This study compared the response strengths of two different groups of age-matched microgyric and sham rats. The first group was recorded using the anesthetic isoflurane, and the second group was recorded using the sedative Dexdomitor. The recordings were performed when the subjects were between the ages of postnatal day 20 (P20) and postnatal day 30 (P30). The weight of the subjects was between 100-150 grams. Microgyric lesions were confirmed in subjects after histological processing (see figure 2.1 for examples. Arrows point to the lesions). Examples of normal brain sections are shown in figure 2.2.

2.5.2 tMTF Comparison

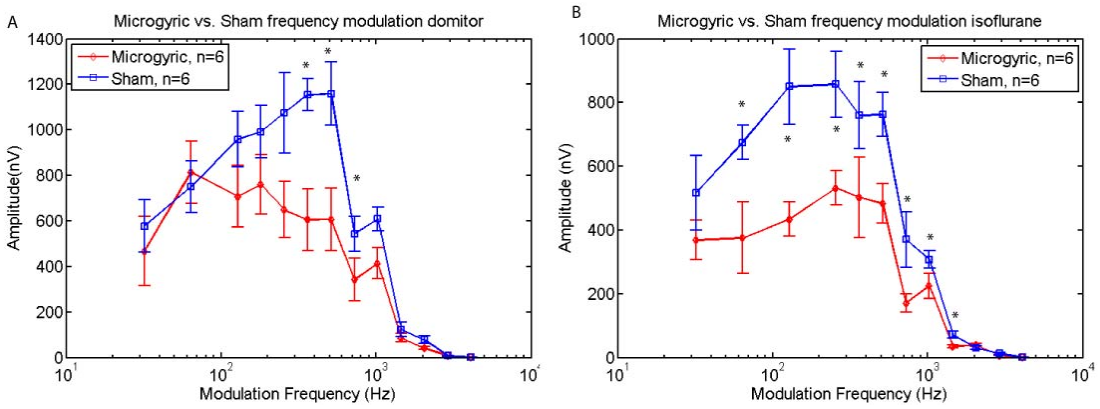

Figure 2.3 tMTF comparison. Tone modulated transfer functions of microgyric vs sham animals, contrasting between sedative (domitor) and anesthetic (isoflurane). Error bars show standard error and (*) signifies statistically different values ($p<0.05$).

Table 2.1 tMTF comparison Domitor

Domitor	Microgyric	Sham		
Modulation frequency(Hz)	Mean(nV)	Stdev(nV)	Mean(nV)	Stdev(nV)
32	466.81	152.31	578.01	113.86
64	814	136.9	751	114.34
128	708.32	134.26	957.56	121.86
181	759.51	131.01	992.24	113.95
256	649.36	124	1074.08	176.77
362	604.96	134.22	1154.12	69.62
512	607.63	137.32	1159.14	138.82
724	342.07	92.57	542.98	75.27
1024	413.18	68.1	608.41	52.93
1448	86.15	19.23	123.27	31.91
2048	40.85	7.27	77.46	16.98
2896	7.55	0.86	7.54	1.53
4096	0.11	0.02	0.09	0.01

Table 2.2 tMTF Comparison Isoflurane

Isofluorane	Microgyric	Sham		
Modulation frequency(Hz)	Mean(nV)	Stdev(nV)	Mean(nV)	Stdev(nV)
32	368.35	61.33	516.59	116.98
64	375.8	112.43	675.12	53.36
128	434.3	54.19	849.65	117.13
181				
256	532.14	53.27	857.52	103.45
362	502.79	126.34	759.89	105.16
512	483.17	62.71	761.8	69.4
724	170.59	27.82	370.45	86.99
1024	225.26	39.22	307.81	27.71
1448	35.28	4.62	71.99	10.18
2048	39.36	5.93	30.45	5.38
2896	7.15	0.64	11.97	2.84
4096	0.1	0.01	0.11	0.01

The comparison of the responses to a sinusoidal amplitude modulated carrier wave at multiple modulation frequencies gives an indicator of how well the subject can phase lock to the envelope frequency. Figure 2.1 shows the response of microgyric and sham animals to an 8-kHz carrier wave modulated from a range of 32-Hz to 4096-Hz. The results showed that the sham animals recorded using Dexdomitor had significantly greater response strength between 362-Hz and 724-Hz. In the group recorded from under isoflurane anesthesia, the sham animals had significantly greater response strength when modulation frequencies were between 64-Hz and 1024-Hz.

Some differences were expected between the tMTFs of the rats recorded under isoflurane and those recorded under dexdomitor because of the inactivation of the cortex caused by the anesthetic. The corticocollicular projection are known to have a continuous effect on subcortical processing (Bajo & King, 2013), and the differences between the responses under dexdomitor and isoflurane are likely due to the presence and absence of cortical feedback during the recording. The presence of differences between the microgyric and sham animals under both recording conditions suggests both brainstem level deficits as well as cortical deficits in the microgyric animals. Comparing the range of frequencies that the deficits are present suggest that the cortical feedback is able to help compensate for the brainstem level deficiencies at both the lower and higher frequencies, but not at the mid-range frequencies. The lack of significant differences above the 1024 Hz frequency corresponds with an overall decrease in the amplitude of the responses. The ability to phase lock to a stimulus decreases at higher frequencies, so if the differences between microgyric and sham animals are due to difficulty in phase locking, the differences would be expected to disappear when the frequencies reach the range where phase locking can no longer occur.

2.5.3 Sinusoidal Amplitude Modulated Depth Response

The response to sinusoidal amplitude modulated signals of varying depths gives some complementary information to the information obtained by recording from a wide range of frequencies. At 0dB (100%) modulation depth, it is the same signal as that used for recording the range of frequencies. By observing the differences in the responses to an amplitude modulated depth stimulus, it can give an indicator of how well the auditory

system can utilize envelope information. In comparing the results of the responses in figures 2.2 and 2.3 to those in figure 2.1, there are some inconsistencies in results. In figure 2.2, there is statistical significance at the 256-Hz modulation frequency at the three highest modulation depths, but there is no corresponding significant difference at the 100% modulation depth at 256-Hz modulation in figure 2.1. In figure 2.3, there is no significant difference at any depth for the 256-Hz modulation frequency and 1024-Hz modulation frequency despite there being significant differences at both frequencies in figure 2.1. The significant differences in figure 2.2 suggests that the sham animals encode the modulation envelope more strongly at the three highest depths, which likely corresponds to a lower threshold of depth detection. The responses in figure 2.3 show a similar trend at the 64-Hz modulation frequency.

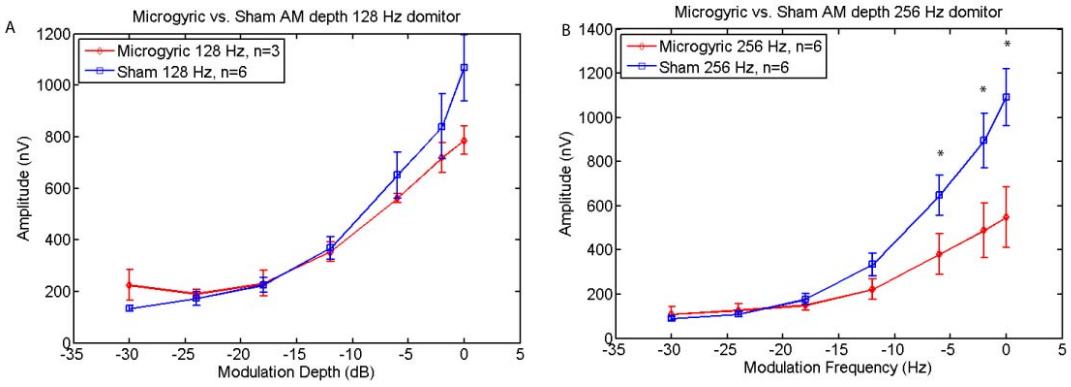

Figure 2.4 SAM Depth Response Domitor. The results of amplitude modulated tones at different depths. Animals sedated with Dexdomitor. (*) signifies statistical significance ($p<0.05$). Error bars show standard error.

Table 2.3 AM depth domitor

	Microgyric		Sham	
Domitor	Mod. Freq.: 128 Hz		Mod. Freq.: 128 Hz	
Modulation Depth (dB)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)
0	786.73	53.94	1067.38	128.49
-2	719.08	57.92	840.04	125.82
-6	561.97	16.93	651.61	88.97
-12	353.66	38.86	368.51	44.18
-18	231.67	49.84	225.18	27.21
-24	192.28	14.54	172.82	27.97
-30	225.48	59.26	134.36	12.14
	Microgyric		Sham	
	Mod. Freq.: 256 Hz		Mod. Freq.: 256 Hz	
	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)
0	548.52	136.66	1091.34	129.99
-2	489.72	123.41	895.15	123.68
-6	380.71	92.64	647.37	90.91
-12	222.81	45.49	334.49	51.61
-18	151.17	23.99	177.7	24.87
-24	128.01	29.59	110.34	11.55
-30	109.64	33.33	89.42	9.62

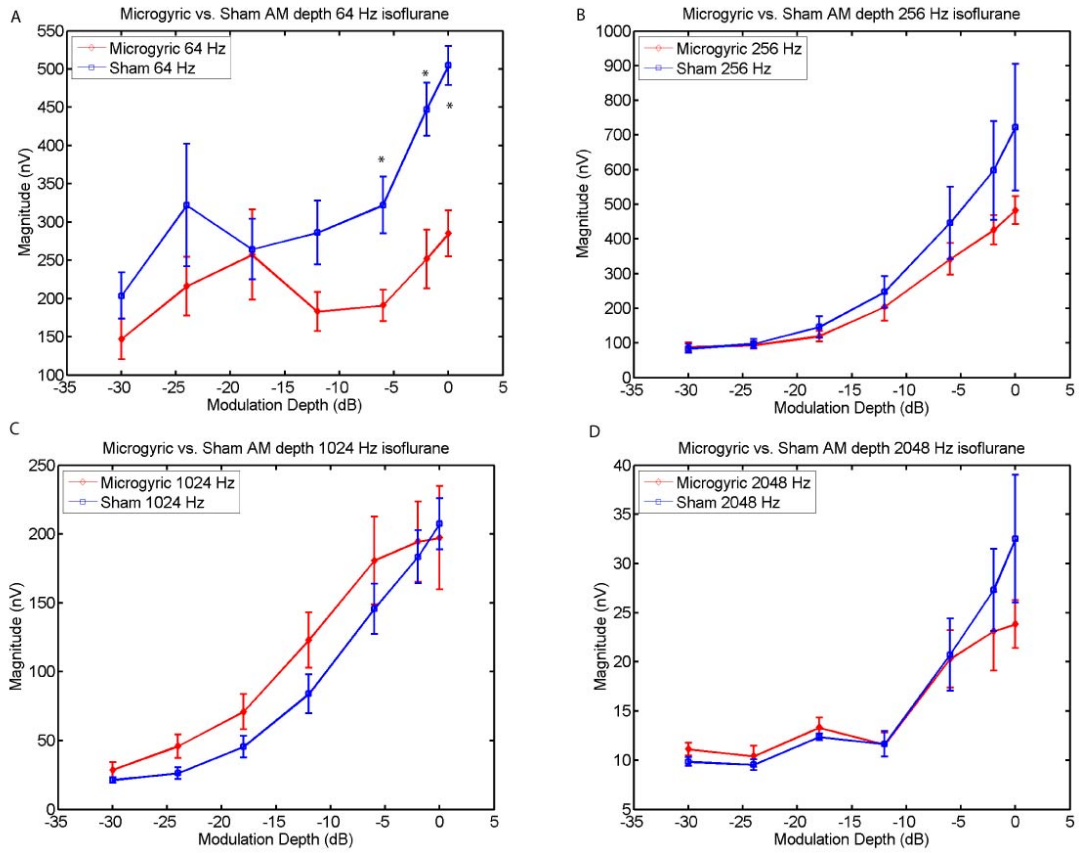

Figure 2.5 SAMDepth Response Isofluorane. Sinusoidal amplitude modulated tones with varying depths. Error bars Showing standard error, and (*) shows statistically significant differences ($p<0.05$). Recording performed using isoflurane anesthetic.

Table 2.4 AM depth Isoflurane

	Microgyric		Sham		Microgyric		Sham	
Isofluorane	Mod. Freq.: 64 Hz		Mod. Freq.: 64 Hz		Mod. Freq.: 256 Hz		Mod. Freq.: 256 Hz	
Modulation Depth (dB)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)
0	284.91	30.02	504.63	25.41	722.45	40.13	722.45	182.29
-2	251.82	38.32	447.28	34.58	597.96	41.82	597.96	142.5
-6	190.87	20.33	322.06	37.1	446.37	45.53	446.37	104.11
-12	183.09	25.44	286.13	41.72	247.21	39.99	247.21	45.92
-18	257.4	59	264.43	39.76	146.16	15.26	146.16	30.41
-24	216.08	38.38	322.2	79.86	98.09	9.75	98.09	13.29
-30	147.24	26.45	203.61	30.51	84.37	12.66	84.37	12.57
	Mod. Freq.: 1024 Hz		Mod. Freq.: 1024 Hz		Mod. Freq.: 2048 Hz		Mod. Freq.: 2048 Hz	
	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)
0	197.26	37.47	207.42	18.65	23.83	2.44	32.51	6.49
-2	194.44	29.29	183.47	19.31	23.13	4.01	27.31	4.17
-6	180.66	31.89	145.7	18.31	20.31	2.93	20.72	3.68
-12	122.98	20.24	83.91	14.25	11.59	1.22	11.64	1.29
-18	70.91	12.83	45.51	7.7	13.33	1.01	12.37	0.33
-24	45.91	8.62	26.27	4.2	10.4	1.07	9.54	0.56
-30	28.64	5.77	21.33	1.96	11.13	0.65	9.87	0.47

2.5.4 Sinusoidal Frequency Modulated Depth Response

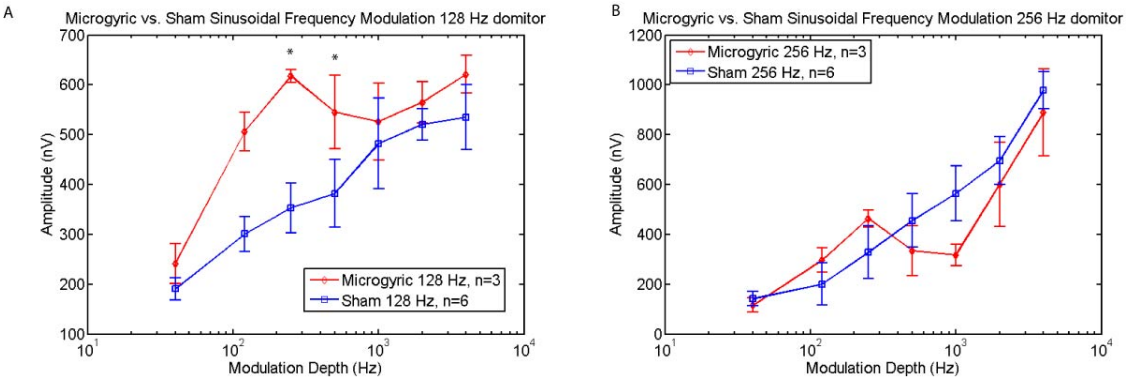

Figure 2.6 Microgyric vs. sham sinusoidal frequency modulation under Dexdomitor. Responses of sinusoidal frequency modulated tones at varying modulation depths under sedative. Error bars show standard error. Statistical significance ($p<0.05$) denoted by (*).

Table 2.5 Sinusoidal Frequency Modulation Domitor

Domitor	Microgyric		Sham		Microgyric		Sham	
	Mod. Freq.: 128 Hz		Mod. Freq.: 128 Hz		Mod. Freq.: 256 Hz		Mod. Freq.: 256 Hz	
Modulation Depth (Hz)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)
40	241.14	40.13	190.34	22	114.78	28.74	141.12	29.32
120	505.74	38.9	300.6	34.54	296.02	48.19	200.29	84.82
250	617.47	13.22	352.83	50.52	462.31	33.41	327.1	105.46
500	545	73.95	382.01	67.93	333.68	98.88	454.22	107.12
1000	526.29	77.42	482.33	91.6	316.89	42.58	563.69	110.37
2000	564.78	41.59	520.65	31.28	600.73	168.7	695.82	94.97
4000	620.77	38.02	535.27	64.85	888.38	174.73	977.8	73.35

The response to sinusoidal frequency modulated signals can give an indication of how well the auditory system tracks frequency cues in auditory stimuli. In the recording done using domitor, the response to the sinusoidal frequency modulation showed significant differences at modulation depths of 250-Hz and 500-Hz at the modulation frequency of 128-Hz. In the recordings done using isoflurane, there was a significant difference at 500-Hz modulation depth at the 256-Hz modulation frequency. In both cases, the microgyric animals had a higher amplitude response than the sham animals. Due to the low number of subjects used in the SFM recordings, it is difficult to establish significant differences between the groups. The difference in perception thresholds of frequency modulation in humans could contribute to difficulties in discriminating between complex phonemes in speech, especially in cases where the speech stimulus is degraded (e.g. a noisy environment).

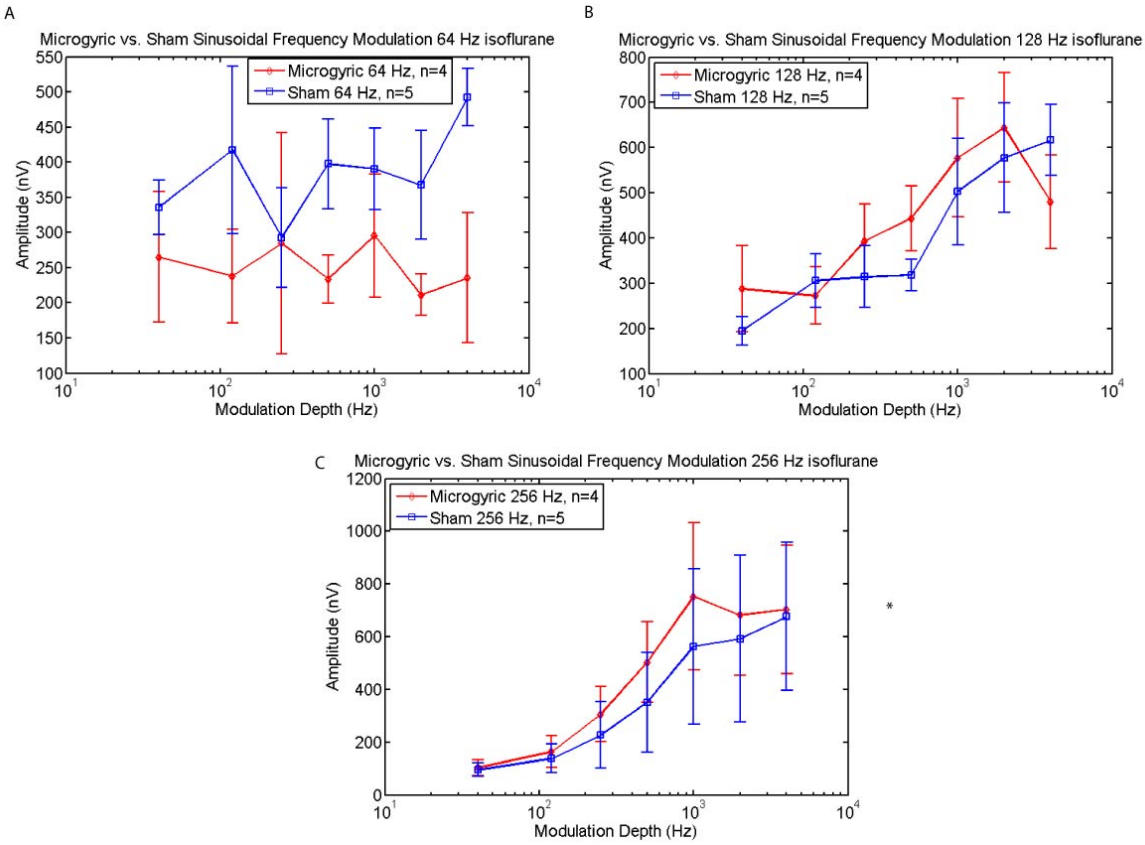

Figure 2.7 Microgyric vs. sham sinusoidal frequency modulation under isoflurane. Response to sinusoidal frequency modulated tones of varying modulation depths. Error bars show standard error. Statistical significance ($p<0.05$) denoted by (*).

Table 2.6 Sinusoidal Frequency Modulation Isoflurane

Isofluorane	Microgyric		Sham		Microgyric		Sham	
	Mod. Freq.: 64 Hz		Mod. Freq.: 64 Hz		Mod. Freq.: 128 Hz		Mod. Freq.: 128 Hz	
Modulation Depth (Hz)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)
40	265.28	92.51	335.9	38.32	287.83	94.69	194.44	32.48
120	238.17	66.28	417.51	119.12	272.34	63.72	305.6	59.16
250	284.79	157.07	292.68	70.52	393.5	81.29	314.28	68.53
500	233.94	34.08	397.59	64.08	443.75	71.92	318.03	34.68
1000	295.79	87.73	390.71	58.21	577.52	131.55	503.22	117.61
2000	211.35	29.43	367.65	77.15	644.43	120.67	577.41	120.9
4000	235.67	92.04	492.48	40.61	479.68	103.95	616.9	78.25
	Mod. Freq.: 256 Hz		Mod. Freq.: 256 Hz					
	Mean (nV)	Stdev(nV)	Mean (nV)	Stdev(nV)				
40	103.92	29.76	95.67	25.75				
120	163.66	60.35	138.15	54.05				
250	306.2	104.51	227.3	125.99				
500	503.71	154.17	351.07	188.76				
1000	752.8	279.5	563.24	295.61				
2000	682.27	227.6	592.29	317.16				
4000	704.11	243.57	676.3	280.75				

2.5.5 ABR Analysis

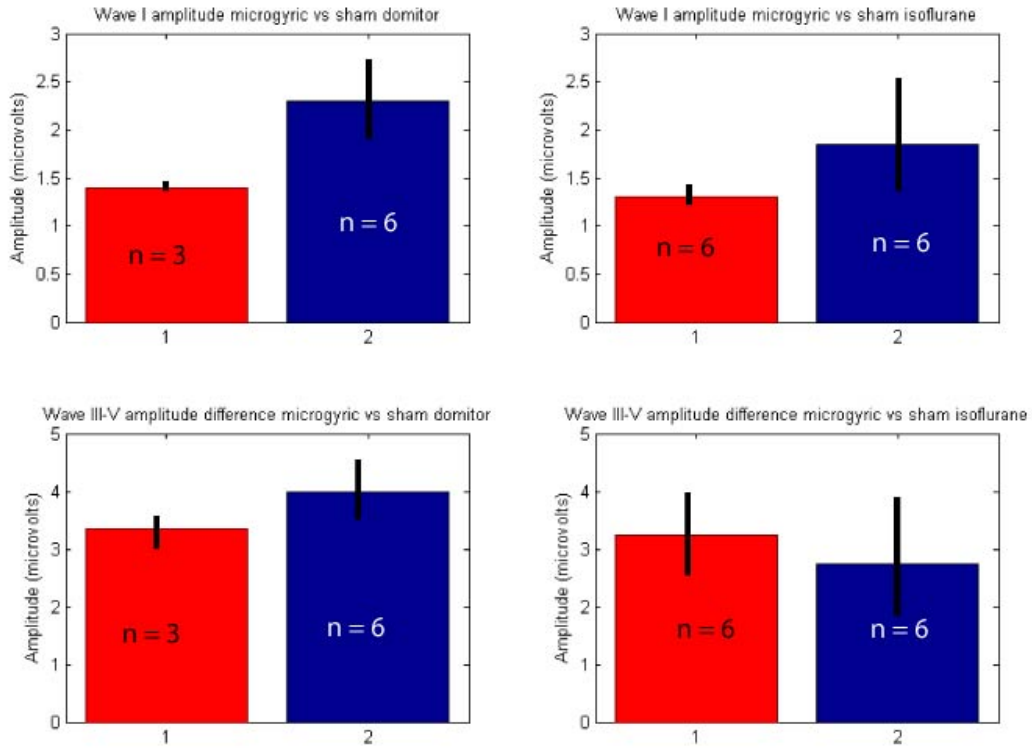

Figure 2.8 ABR amplitude comparison in microgyric and sham animals. Microgyric (red) vs sham (blue) ABR amplitudes. There were no significant differences between the groups using a ranksum test in Matlab.

The ABR analysis shows the response of the auditory brainstem to click stimuli. Changes in the early peaks of the click ABR are generally not associated with dyslexia (McAnally & Stein, 1997), while increases in latency in peak 5 and beyond in a speech ABR is common in human dyslexic patients (Banai et al., 2007). This study showed no significant differences in the amplitudes of the peaks between microgyric and sham animals, and in the case of domitor recordings, no significant differences were seen in

latencies. The isoflurane group showed a significant difference between microgyric and sham for the peak III latency. Because the latency shifts of the ABR peaks can increase under higher concentrations of isoflurane (Santarelli, Carraro, et al., 2003), it is not certain if the difference is due to the microgyria, or a result of variability in the isoflurane concentration during recording.

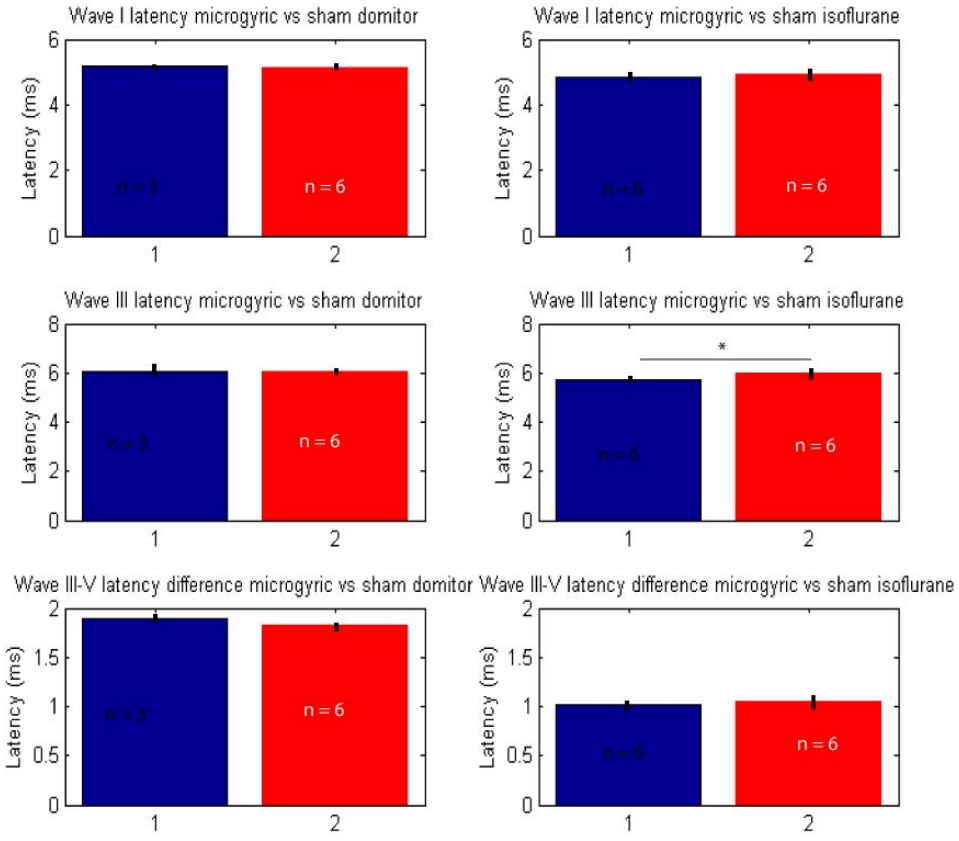

Figure 2-7 ABR latency comparison in microgyric and sham animals

2.6 Discussion

This study compared the responses of microgyric and sham animals recorded in two separate groups. The first group was recorded from using the anesthetic isoflurane, and the second group was recorded from using the sedative Dexdomitor. We expected that within each group, there would be significant differences in response amplitudes between the microgyric and sham animals, and we expected the significant differences to vary between the two groups to reflect the level of cortical activity during the recording.

The tMTF responses and the AM depth responses both show that there are significant differences in the response to amplitude modulated stimuli between dyslexic and sham animals. Which frequencies are affected differs according to whether the recording is done using an anesthetic (in this case isoflurane) or a sedative (Dexdomitor). The lower amplitude responses in microgyric subjects are similar to those seen in language impaired human subjects (Wible, Nicol, & Kraus, 2005; Witton et al., 2002).

Studies on the effects of isoflurane on rat steady state responses (SSRs) and mid latency responses (MLRs) show reduced SSR amplitude and an increased MLR latency with no effect on amplitude (Santarelli, Carraro, et al., 2003). From analyzing the ABR and MLR responses of animals under isoflurane, the latencies of ABR waves I-IV, as well as the MLR waves were increased, without the amplitudes being affected (Santarelli, Arslan, et al., 2003). In the cat, *in vivo* cortical responses had greatly reduced sensitivity and poor temporal response properties (Cheung et al., 2001). Sedation instead of anesthetic would leave the cortical responses intact, so comparing the responses of the animals recorded using dexdomitor and those recorded using isoflurane can help to separate the effects of the microgyria on the brainstem as compared to the effect on the

entire auditory system. The tMTF shows that the band of frequencies where there is significant difference is much greater using isoflurane anesthesia than when dexdomitor sedation is used. If you assume that the differences are to the (relative) activity or inactivity of the cortex, it would mean (a) that the microgyria do have an effect on the long-term responses to stimuli in the brain stem and (b) that cortical activity can help to compensate for the deficits in brainstem encoding. The existence of projections from the cortex to the inferior colliculus, superior olive, and cochlear nucleus are well established (Bajo & King, 2013; Peterson & Schofield, 2007; Schofield & Coomes, 2005). It is thought that these projection play a role in learning induced plasticity and stimulus specific plasticity in the inferior colliculus (Anderson & Malmierca, 2013; Bajo et al., 2010). Learning induced plasticity would result in long-term adaptation of the lower structures to specific stimuli, and the changes would be evident both in the presence and absence of cortical activity. The stimulus specific adaptation would enhance the response to a sound being played in real time, and this activity would be absent if cortical activity were reduced. The effect of the P0 induced cortical microgyria appears to be present and significant, but the reason for the effect is not clearly understood. The lesions are induced in the somatosensory cortex, and not in either primary or secondary auditory cortex. If the auditory cortex were lesioned and malformed, understanding a disruption of the normal cortical feedback to the brainstem is pretty straightforward. The question remains whether there are also projections from the somatosensory cortex back to the auditory brainstem, if there is a significant amount of auditory processing being done in the somatosensory cortex, or if the mere physical proximity of the auditory cortex to the freeze-lesion in the somatosensory cortex is enough to disrupt normal activity in the

descending pathways. The changes in the area immediately surrounding the lesion was characterized in a study which showed enhanced activity in cortical layer II/III following a theta burst stimulation in layer VI (Peters et al., 2004). A similar study showed an area of hyperexcitability in the focal area surrounding a freeze lesion showing activity similar to epileptiform activity similar to that in human polymicrogyria (Jacobs, Gutnick, & Prince, 1996). Both studies showed that the region of abnormal cortical activity extended from 1-3 millimeters from the site of the lesion. Another effect of the freeze lesion was an inability for long term potentiation in layer IV of the surrounding cortex (Peters et al., 2004) which could inhibit the ability of the cortex to adapt in response to the stimuli it is presented with. Due to the proximity of the somatosensory cortex to the auditory cortex, the changes in corticocollicular feedback are likely due to the proximity of the lesion to the auditory cortex and the disruption of the normal function of neighboring cortical structures by the presence of the lesion.

The development of the central auditory system has been shown to be experience-dependent, and can be helped or hindered by the acoustic environment in which the animal (or person) is raised (Sanes & Bao, 2009; Sanes & Woolley, 2011). Learning dependent processes depend primarily on the long term potentiation of the synapses involved, which is one of the mechanisms disrupted by an adjacent freeze lesion. Induction of microgyria on P0 would reduce the feedback from the cortex into the brainstem through both learning dependent mechanisms as well as disruption of normal short term function of the cortex, in which case both the learning induced plasticity and stimulus specific activity would be impaired. This would suggest that the difference between microgyric animals recorded from under anesthetic and sedative would be less

than those of sham animals recorded under anesthetic and sedative, if the induction of microgyria does in fact interfere with the corticocollicular projections. This appears to be the case from a visual comparison of the responses, but because two separate groups of animals were used in this study rather than record from the same animals using both an anesthetic and a sedative, the comparison is not as clear as could be.

The responses to the sinusoidal frequency modulated stimuli showed some statistical significance, with the microgyric subjects showing an enhanced response compared to the sham in the case of 3.2% and 6.4% modulation depths at 128-Hz modulation frequency under domitor, and 6.2% modulation depth at 256-Hz modulation frequency under isoflurane. Given a larger sample size, it is likely that more of the stimuli would have a significantly different response, as some of the cases were approaching significance. Interestingly, the significance occurs in the direction opposite to that of amplitude modulation, with the microgyric rats showing greater response amplitude as compared to the shams. This could suggest that the detection of frequency modulated tones occurs through a mechanism distinct from that of detecting amplitude modulated tones, and the mechanism is affected differently by the presence of cortical lesions.

2.7 Future Directions

The effect of microgyric lesions showed some interesting results, but a better comparison could be made if the isoflurane and domitor recordings were done on the same animals. Expanding the size of the study would give clearer statistics, especially in the case of the sinusoidal frequency modulated data. The possibility of using early

acoustic experience to attempt to overcome the deficits from the induced lesion has been done for some types of acoustic stimuli (Threlkeld et al., 2009). A study has not yet been done to see if the AM and FM tone deficits would respond in a similar manner. Another direction the study could go would be to use auditory discrimination tasks to determine the psychophysical performance of lesioned and non-lesioned animals, and see if the discrimination task has any effects on the differences in evoked responses between the groups.

CHAPTER 3. EXPOSURE AND ASSOCIATION EXPERIMENTS

3.1 Introduction

This experiment was an initial study to explore the enhancement of pitch coding in the brain stem of rats either exposed to a specific Chinese tone for an hour daily, or given a treat when a specific tone was played and given no reward when a second tone was played. Native speakers of tonal languages show enhanced pitch tracking capabilities compared to non-native speakers of tonal languages (Krishnan et al., 2010). This experiment was an attempt to replicate the results of the human study in a rodent model in order to have an established animal model for tonal language experience. For the rat to work as an established animal model for this phenomenon, the rats would need to show a similar experience-dependent enhancement in their pitch tracking ability through the course of the experiment as was seen in the human studies. The animal model could then be further studied to explore the mechanism behind the enhancement in pitch tracking that was seen in the original study.

3.2 Materials and Methods

3.2.1 Subjects

For the exposure and association experiments, 2 adult male Long-Evans rats (weight >350g) obtained from Harlan were used, housed in standard laboratory housing.

3.2.2 Exposure and Association

For the exposure experiment, the rats were brought up from the colony daily and placed in their cages in the recording booth. A target sound (standardized Mandarin tone 2) was played continuously for an hour at a sound level of 30dB above click ABR threshold, after which the rat was returned to the colony. For the associative training experiment, the rat was placed in a training cage in the recording booth, and either a target sound (Mandarin tone 2 in speech) or non-target (Mandarin tone 4 in speech) was presented. If the target sound was presented, the door was opened and the rat was given a food reward (Fruit Loops), and if the non-target sound was presented, the door was opened and no reward was given. Recording was performed in the same manner as for sinusoidal amplitude modulated tones. The animals were recorded from monthly for the duration of the experiment using both the sounds used in training and one tone not used in training to determine if any changes were specific to a tone, or if pitch tracking in general was improved.

3.2.3 Stimulus and Recording Descriptions

The Chinese tones used in the exposure and associative training experiments were artificially generated using a Matlab program. They were of a standard length of 250 ms

and presented at a rate of 3.1/s. The tones used were tone 1 (flat), tone 2 (rise), and tone 4 (dip). For recording purposes, these tones were presented at 30 dB above click ABR threshold. The Chinese tones used in the study are shown in figures 3.1 – 3.3.

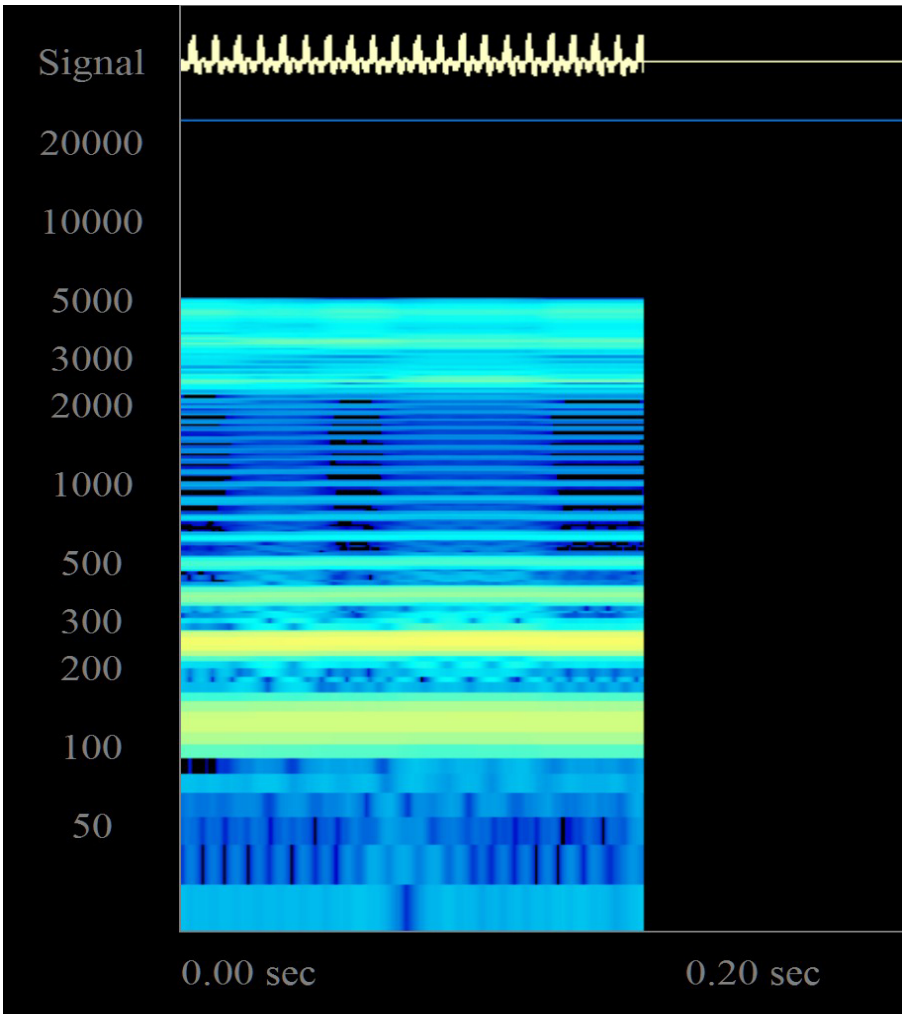

Figure 3.1 Chinese Tone 1 Natural Time Waveform and Spectrogram

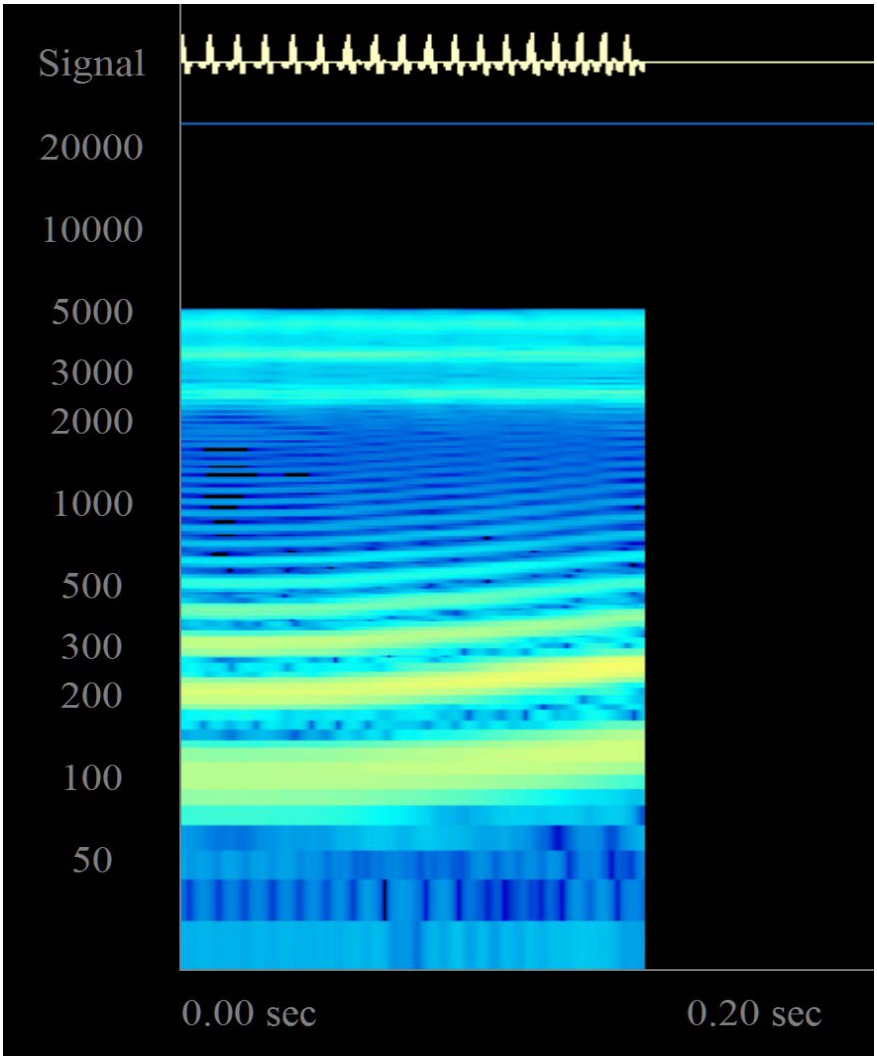

Figure 3.2 Chinese Tone 2 Natural Time Waveform and Spectrogram

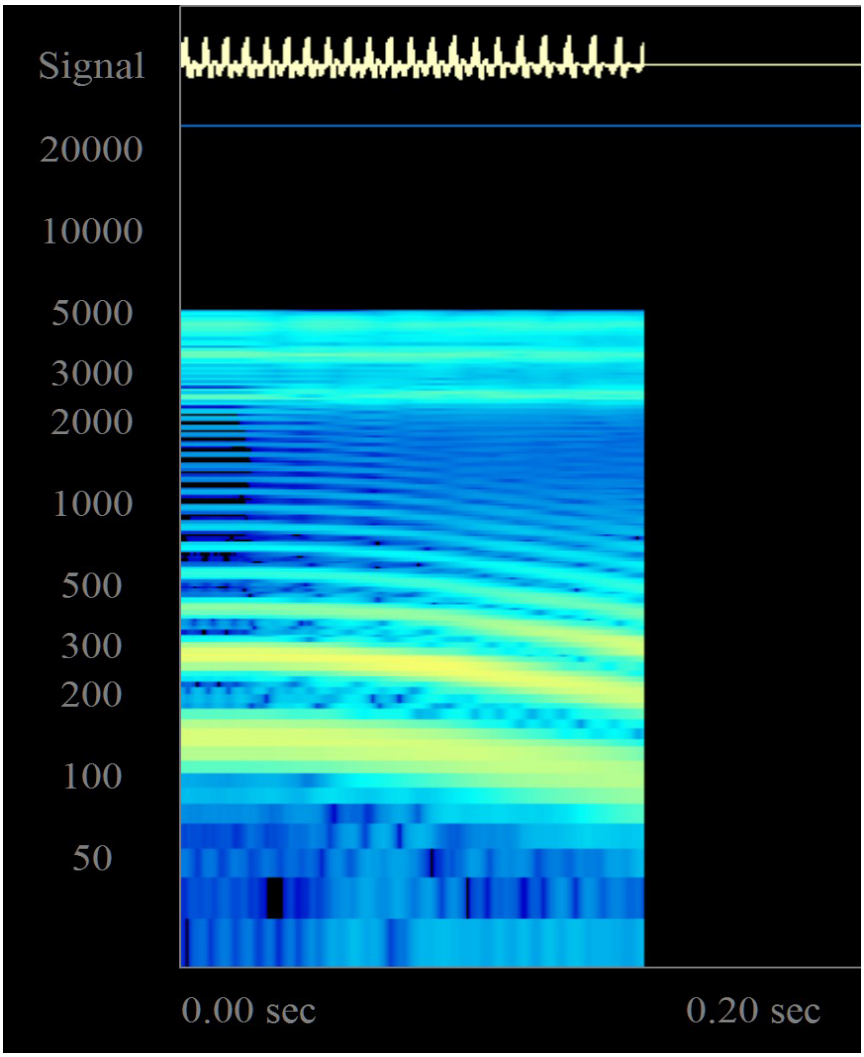

Figure 3.3 Chinese Tone 4 Natural Time Waveform and Spectrogram

3.2.4 Data Analysis

The tone pitch strengths were calculated using software from the Krishnan lab (department of Speech Language Hearing Sciences, Purdue University). The program took as input the stimulus file and multiple response files. It first cross-correlated the stimulus with the response to obtain the time lag between the two. It then divided the stimulus and response into 6 50-msec sections and calculated the autocorrelation function

(ACF) for each section. The pitch strength is defined as the highest non-zero peak of the ACF, which would be a measure of how well the dominant frequency is represented in the recording. The study was only preliminary, and no statistical analysis was done.

3.3 Results

3.3.1 Exposure Results

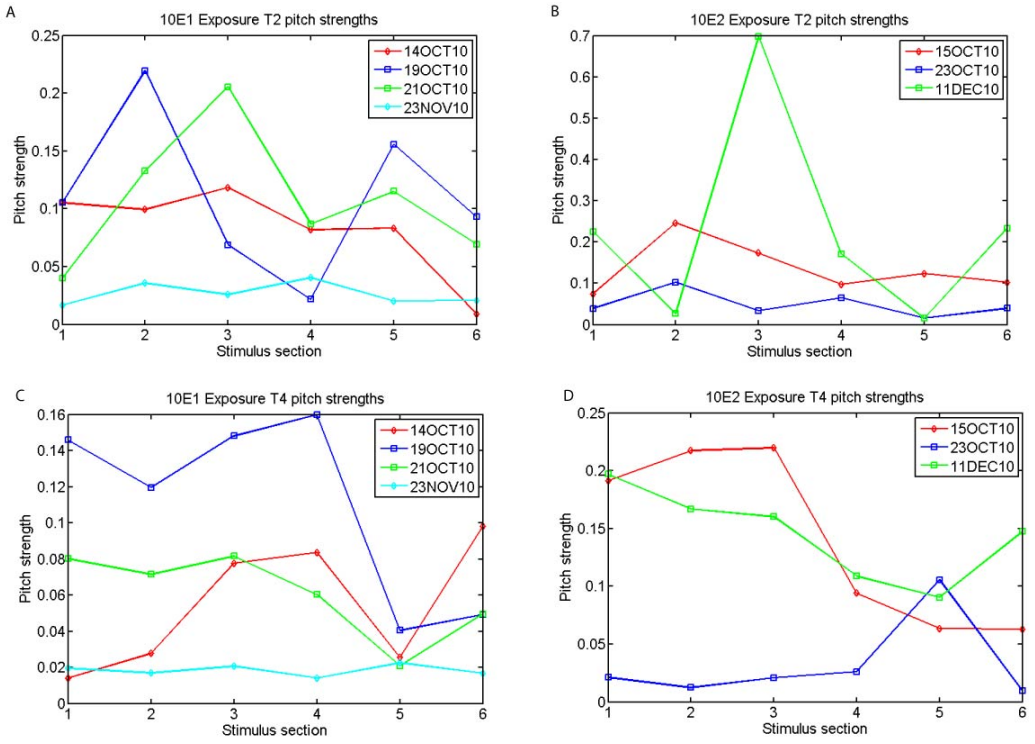

Figure 3.4 Pitch strengths of animals exposed to Chinese tone 2. Figures showing pitch strengths of two different animals over time. Pitch strength is defined as the first peak of the autocorrelation function.

The results of pitch strength analysis for the 6 sections of Chinese tones 2 and 4 are shown in figure 3.8. Pitch strength is defined as the peak of autocorrelation function corresponding with the fundamental frequency of the stimulus file. There was no clear trend in the data showing higher or lower pitch strengths developing over time.

3.3.2 Associative Training Results

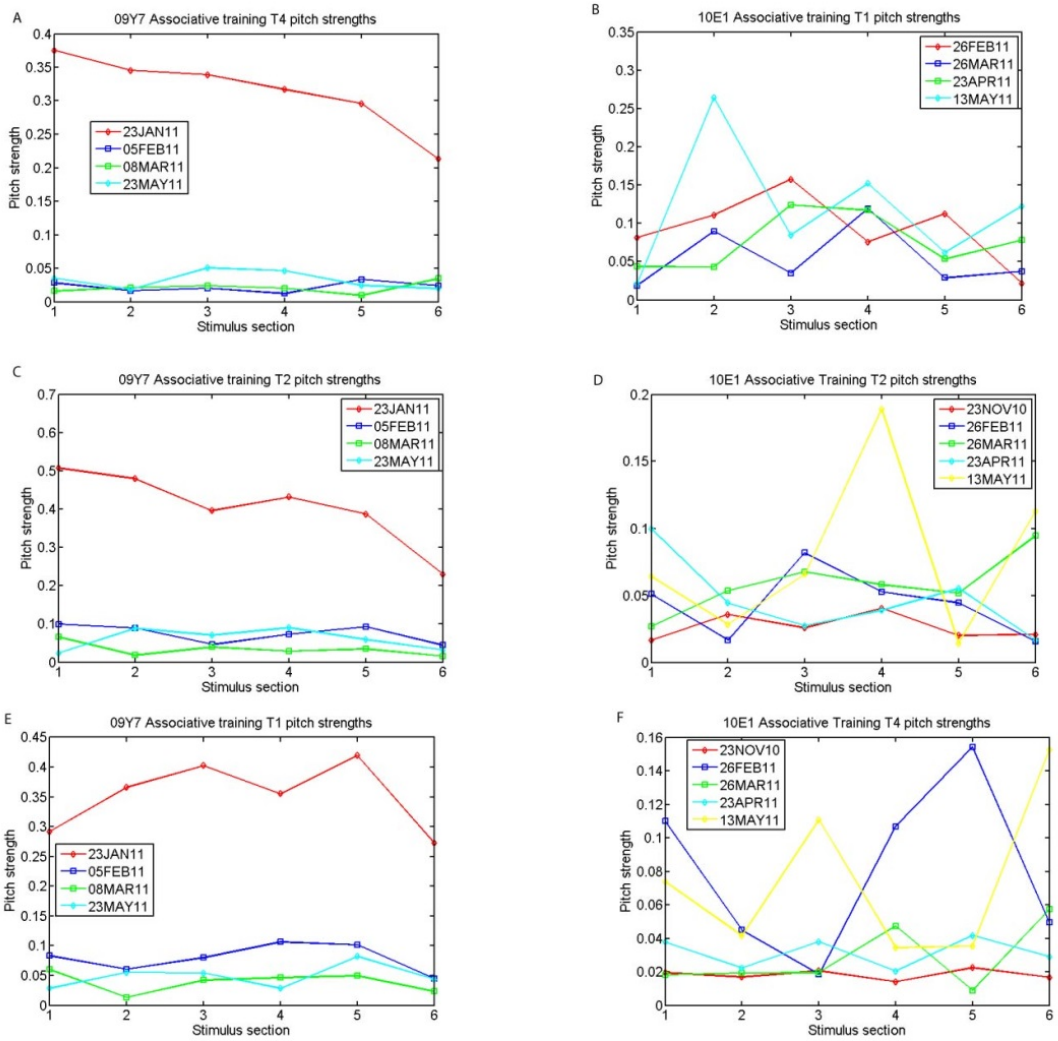

Figure 3.5 Pitch strengths of animals during and after associative training. Figures showing change in pitch strength of animals over time. Pitch Strength is defined as the first peak of the autocorrelation function.

The results of calculated pitch strengths in animals given a reward after exposure to Chinese tone 2 are shown in figure 3.9. Again there is no clear trend in the data (pitch strength calculated the same as in figure 3.8). Animal 09Y7 appears to have higher pitch strength in the initial recording, but the recording had a high frequency noise element which inflated the numbers. The lack of any clear results are likely due to using adult

animals in the experiment, as the results of early exposure in another study show a positive correlation(Threlkeld et al., 2009).

3.4 Discussion

For the animals in the exposure and associative training experiments, there was no clear trend in pitch strength over the length of the experiment. This was a preliminary study to determine an animal model to study the effects of tonal language experience on enhanced pitch coding in the brain stem in humans (Krishnan et al., 2010). A similar study (Threlkeld et al., 2009) shows that early acoustic exposure does ameliorate the deficits of microgyric lesions. Because we were using adult rats in our study, any benefit would likely have been limited. This is similar to the studies done in humans on training and music experience (Collet et al., 2012) which show that the greatest benefits are realized when the training begins early in life. The results would suggest that the subjects require earlier exposure for us to see any positive results, and that a more active paradigm (behavioral training) may be necessary.

3.5 Future Directions

Due to the previous studies on the effect of age on plasticity, the experiment could be done using very young rats to see if exposure or associative training would give any meaningful results. Using an active training paradigm involving response levers to indicate which tone was presented could possibly give stronger results, as the rat would have to actively discriminate between the two different tones instead of just passively listen to them. If positive results were obtained (higher pitch strengths in the trained rats),

combining the tone discrimination task with the variation of microgyric animals could be a way to help determine on what pathways the enhancement of pitch encoding is dependent.

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