CHANGES CAUSED BY CORTICAL DAMAGE AND EVENT PAIRED VAGUS NERVE STIMULATION

by

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For being my motivation and my better half,

this work is dedicated to Amy.

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by

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DISSERTATION

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PREFACE

This dissertation was produced in accordance with guidelines which permit the inclusion as part of the dissertation the text of an original paper or papers submitted for publication. The dissertation must still conform to all other requirements explained in the "Guide for the Preparation of Master's Theses and Doctoral Dissertations at The University of Texas at Dallas." It must include a comprehensive abstract, a full introduction and literature review and a final overall conclusion. Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported.

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

January, 2011

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I would like to thank my family for supporting me as I have journeyed down this road. My parents have given me curiosity and a desire to help people that has helped me to select a career, and they have provided me with the basic tools to pursue it. To Amy, I thank you for more than I could possibly ever write about in this brief section. I could write another dissertation about your patience, desire to help, and want for more for me and for us (it would probably be a lot longer paper than the current one). And to Luke, thank you for the necessary breaks and comic relief. Being a parent is the greatest experiment I have ever started.

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January, 2011

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Despite great progress in neuroscience over the last forty years, the consequences of brain injury are difficult to predict and to treat. Basic research is needed to clarify these deficits in a controlled manner to allow clinicians to more effectively shape rehabilitative methods to improve recovery. For example, auditory cortex damage results in impaired discriminations of complex sounds, such as animal vocalizations, but a recent study has shown the discrimination of comparatively complex speech sounds is unimpaired (Floody et al. 2010, 260-268). This result seems to suggest that auditory cortex does not play a role in speech discrimination. We confirmed with an operant training method that whole word discrimination is unimpaired following bilateral auditory cortex lesions, but the discrimination of the rapidly occurring acoustic transitions at the beginning of speech sounds is impaired for at least one-month post lesion (Chapter 2). These results support the hypothesis that auditory cortex is required to accurately discriminate the subtle differences between similar consonant and vowel sounds.

Methods that enhance cortical reorganization beyond training alone may increase functional recovery after cortical injury. Pairing vagus nerve stimulation (VNS) with a tone enhances the representation of that tone in the auditory cortex (Engineer et al. 2011, 101-104). Additionally, tone-paired VNS can treat tinnitus, a disorder of the auditory system. Many of the ways for directing cortical change in sensory systems also direct change in the motor system. We paired VNS with specific movements to test if similar enhancements would occur for the paired movement in the motor cortex as seen in the auditory cortex with tone-paired VNS (Chapter 3). There was a larger cortical area associated with those movements paired with VNS and smaller areas representing movements not paired with VNS. Rats that received identical motor training without VNS pairing did not exhibit motor cortex map reorganizations. These results suggest that pairing VNS with specific events may act as a general method for increasing cortical representation of those events. VNS-movement pairing could provide a new approach for increasing functional recovery of movements following brain damage.

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CHAPTER 1

INTRODUCTION

Neuroscience research ultimately aims to improve the quality of life in humans. I became interested in neuroscience in large part because of this noble endeavor. Although not all neuroscience research is obviously tied to this goal, improving our understanding of the how the brain interacts within itself and in relation to the body will lead to advances in our daily lives. The intent of this dissertation was to provide research that could one day be used to further advance the quality of life in human beings.

Cortical damage to the sensory and motor cortices results in deficits that impair the injured person's daily life. Observed deficits depend on the area of the cortex damaged. In the motor cortex, the location of the damage in the somatotopic organization determines which part of the body has impaired voluntary movements. Damage in the visual cortex results in corresponding blind spots in a patient's visual field. The locations of the blind spots are directly related to where the damage occurs in the retinotopic map of the visual cortex. Despite also having a predictable representative organization, damaging the auditory cortex does not result in clearly predictable deficits in hearing.

Damaging the auditory cortex does not result in deafness and only impairs the perception of certain types of sounds. Animals with bilateral auditory cortex lesions are still able to detect and discriminate different frequency tones from each other (Ohl et al. 1999, 347-362; Kudoh et al. 2006, 1761-1766). Animals with auditory cortex lesions can also still discriminate different

intensities of the same frequency tone (Rosenzweig 1946, 127-136). Discrimination of most complex sounds, such as frequency patterns (Diamond, Goldberg, and Neff 1962, 223-225), vowel-like stimuli (Whitfield 1980, 644-647), and animal vocalizations (Heffner and Heffner 1986, 683-701; Heffner and Heffner 1989, 275-285; Harrington, Heffner, and Heffner 2001, 1217-1221), is impaired following auditory cortex lesions. However, our recent study demonstrated unimpaired speech discrimination following bilateral auditory cortex lesions in rats (Floody et al. 2010, 260-268).

Speech is considered a complex sound and, based on the previous literature, should be impaired following auditory cortex lesions. Rats received bilateral auditory cortex lesions and completed a series of speech discrimination tests using acoustic startle response (Floody et al. 2010, 261-264). The lesioned rats did not demonstrate impaired discriminations, seemingly contradicting the previous research that suggested the auditory cortex is necessary for processing complex sound. We determined two possible alternative explanations for the lack of an impairment following the lesions. The previous studies demonstrating impaired complex sound discrimination used operant training methods while our study used the acoustic startle response. Therefore, we tested a new set of animals on speech discriminations before and after bilateral auditory cortex lesions using an operant training method. A second possible explanation was based on a neurophysiological study from our lab demonstrating that the auditory cortex processes the initial portion of a speech sound in a way more behaviorally relevant than the processing of the entire speech sound (Engineer et al. 2008, 603-608). If auditory cortex specializes in processing the initial part of a speech sound, then whole speech sounds may

provide extra cues that the rest of the auditory system is using to form discriminations. Chapter 2 describes the experiments and results of testing these two hypotheses.

After learning how auditory cortex lesions effected speech discrimination, I wanted to develop a model that would help patients recover their lost abilities following brain damage. At the time, a study in our lab was demonstrating the use of vagus nerve stimulation (VNS) paired with a tone to cause enhanced representations of the paired tone in the auditory cortex (Engineer et al. 2011, 101-104). Additionally, this method of VNS-tone pairing was being used to treat tinnitus in rats by pairing multiple tones around the phantom frequency to cause a selective decrease of the overrepresentation. This renormalization of the tonotopic organization in the auditory cortex eliminated the perception of the phantom noise for at least several months. Having seen the benefit of event paired VNS in the auditory cortex, we decided to test if this method could be used in the motor cortex to generate similar plasticity in healthy rats. If VNS paired with movements could enhance cortical representations of the paired movements, then it may offer a new method for treating movement disorders related to abnormal motor representations. Chapter 3 describes the experiments and results of pairing VNS with specific movements to enhance their representations in the motor cortex.

This dissertation consists of four chapters. Chapter 2 examines the role of the auditory cortex in speech discrimination. Chapter 3 examines if cortical changes can be generated from performing a motor task paired with vagus nerve stimulation. The findings and implications from Chapters 2 and 3 are discussed in Chapter 4.

CHAPTER 2

DISCRIMINATION OF BRIEF SPEECH SOUNDS IS IMPAIRED IN RATS WITH AUDITORY CORTEX LESIONS

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2.2 ASTRACT

Auditory cortex (AC) lesions impair complex sound discrimination. However, a recent study demonstrated spared performance on an acoustic startle response test of speech discrimination following AC lesions (Floody et al. 2010, 260-268). The current study reports the effects of AC lesions on two operant speech discrimination tasks. AC lesions caused a modest and quickly recovered impairment in the ability of rats to discriminate consonant-vowel-consonant speech sounds. This result seems to suggest that AC does not play a role in speech discrimination. However, the speech sounds used in both studies differed in many acoustic dimensions and an adaptive change in discrimination strategy could allow the rats to use an acoustic difference that does not require an intact AC to discriminate. Based on our earlier observation that the first 40 ms of the spatiotemporal activity patterns elicited by speech sounds best correlate with behavioral discriminations of these sounds (Engineer et al. 2008, 603-608), we predicted that eliminating additional cues by truncating speech sounds to the first 40 ms would render the stimuli indistinguishable to a rat with AC lesions. Although the initial discrimination of truncated sounds took longer to learn, the final performance paralleled rats using full-length consonant-vowel-consonant sounds. After 20 days of testing, half of the rats using speech onsets received bilateral AC lesions. Lesions severely impaired speech onset discrimination for at least one-month post lesion. These results support the hypothesis that

auditory cortex is required to accurately discriminate the subtle differences between similar consonant and vowel sounds.

2.3 INTRODUCTION

Bilateral auditory cortex lesions cause long-lasting impairments in the discrimination of complex sounds. Lesioned animals are impaired in the discrimination of frequency patterns (Diamond, Goldberg, and Neff 1962, 223-225), vowel-like stimuli (Whitfield 1980, 644-647), and animal vocalizations (Heffner and Heffner 1989, 275-285; Heffner and Heffner 1986, 683-701; Harrington, Heffner, and Heffner 2001, 1217-1221), with little recovery even a month later. Japanese macaques are unable to discriminate between coo vocalizations even years after receiving bilateral auditory cortex lesions (Harrington, Heffner, and Heffner 2001, 1217-1221). Rats' auditory cortex neurons encode speech sounds in a manner that is highly correlated with the rats' ability to discriminate these sounds (Engineer et al. 2008, 603-608). These results suggest that the auditory cortex serves a critical role in the discrimination of complex sounds.

However, a recent study reported that bilateral auditory cortex lesions do not impair consonant discrimination by rats (Floody et al. 2010, 260-268). This result seems to conflict with earlier studies suggesting that the auditory cortex is necessary for discriminating complex stimuli. One possible explanation for the spared speech discrimination following auditory cortex lesions is that the rats were tested with pre-pulse inhibition in an acoustic startle reflex paradigm, while previous lesion studies reporting deficits for complex stimulus discriminations used operant training methods. An alternate explanation is that the rats were able to quickly adapt their strategy to use some cue present in the speech sounds that does not require the auditory

cortex to discriminate, such as amplitude (Rosenzweig 1946, 127-136; Raab and Ades 1946, 59-83; Oesterreich, Strominger, and Neff 1971, 251-270) or duration differences (Scharlock et al. 1965, 673). To test these potential explanations, we conducted two experiments. Experiment 1 tested whether auditory cortex lesions impair rat performance on an operant speech discrimination task. Experiment 2 tested whether auditory cortex lesions impair performance on an operant speech discrimination task in which the speech stimuli were truncated to reduce potential acoustic cues that could be used to accomplish the task.

2.4 METHODS

2.4.1 Experiment 1

General Procedure

Rats learned to perform a Go/No-Go procedure in three phases: shaping, detection, and discrimination testing. During the shaping phase, the rats learned to press a lever for a food pellet reward. In the detection phase, the rats learned to press the lever only after a presentation of the target sound, “dad”. Pressing the lever without the presentation of a target sound resulted in a brief period during which the rats did not receive sugar pellets and the lights were extinguished to serve as negative reinforcement. Once the rats consistently pressed the lever as a response to the target stimulus, the discrimination testing phase began. In discrimination testing, the rats had to press the lever to the target stimulus, “dad”, and ignore presentations of the distracting stimulus, “tad”. A past study using the identical stimuli showed discriminating the voiced “dad” from the unvoiced “tad” was a moderately difficult but reliably accomplished task (Engineer et al. 2008, 605). Discrimination testing occurred in quiet and in four levels of white noise (48 dB SPL, 60 dB SPL, 72 dB SPL, or 84 dB SPL) presented as blocks within a session. Addition of the background noise helped to avoid a ceiling effect in discrimination performance by creating multiple levels of difficulty. After learning the discrimination task, rats received bilateral aspiration lesions to the auditory cortex and continued to test on the same task for an additional fifteen days.

Subjects

This experiment used three female Sprague-Dawley rats averaging 363 g (standard deviation 21 g). Rats' activity increases during the dark cycle of the day; therefore, they were housed in a 12:12 hr reversed light cycle environment to increase activity during daylight hours. Rats were food deprived to not less than 85% of their normal body weight for the purpose of motivation. Rats had free access to water at all times except during testing. The University of Texas Institutional Animal Care and Use Committee approved all handling, housing, surgical procedures, and behavioral testing of the rats.

Apparatus

All training and testing sessions occurred in double-walled sound booths designed to reduce outside noise. Booths were 67 cm x 67 cm x 67 cm at the outer dimension with a 20 cm x 20 cm x 20 cm wire cage inside. A pellet dispenser provided 45 mg sugar pellets through a tube into the booth that attached to a pellet dish on the inside of the cage. The dispensing mechanism was attached to the outside the sound booth to reduce the noise associated with the delivery of a pellet. A lamp located above the pellet dish indicated the beginning of a trial and a lever located to the right of the pellet dish provided the rats a means to respond. Roughly 20 cm to the left of the rats' estimated head position was a Tucker-Davis Technologies FF1 free-field, flat spectrum speaker that played stimuli to the rats. A lamp located in the booth provided additional light during sessions. Programs created in MATLAB were used to run sessions and collect data via a Tucker-Davis Technologies RP2.1 real-time processor connected to an Hewlett Packard personal computer.

Stimuli

Two of the exact stimuli used by Engineer et al., (Engineer et al. 2008, 607) “dad” and “tad” were chosen for the discrimination task. These words differ only in the presence or absence of voicing in the initial consonant, and not the place of articulation of the initial consonant, vowel, or terminal consonant sound. An ACO Pacific microphone taking samples every 10 μs with 32-bit resolution recorded a native English-speaking female saying the words. The STRAIGHT vocoder program was used to shift the fundamental frequency and spectrum envelope of each speech sound higher in frequency by a factor of two to approximate the rats hearing range (Kawahara, Masuda-Katsuse, and de Cheveign´e 1999, 187-207; Kelly and Masterton 1977, 930-936). Amplitude levels from the most intense 100 ms of each sound were calibrated to 60 dB. The stimuli were presented randomly within blocks consisting of six target “dad” stimuli, six distracting “tad” stimuli, and one silent catch trial.

During behavior, a program created in MATLAB generated a white-noise background for each block of trials. The same microphone used in creating the speech stimuli was also used to calibrate noise levels at 48, 60, 72, and 84 dB SPL. Linear ramps using one-fifth steps occurred between noise levels to reduce stress to the rats by removing sudden amplitude increases. Pilot studies had revealed that the rats quickly habituate to the noise and demonstrate no behavioral aversions to even the loudest intensities. The first block of trials occurred in quiet. Afterwards, the silent background block was randomly interleaved with the four noise blocks throughout the remainder of the session to create five background noise conditions.

Behavioral testing procedure

Rats began by training twice daily for thirty minutes, five days a week on a go/no-go procedure. Initial training focused on shaping the rats to press the lever for a food reward. After being introduced to the cage, rats explored the area and received a sugar pellet reward whenever they approached the lever. Any time a pellet dispensed during the shaping phase, the target "dad" sound played over the speaker. Requirements for a reward were gradually restricted in regards to the rats' proximity to and actions involving the lever so the rat had to be close to the lever, then touching the lever, and finally pressing the lever to receive a sugar pellet. Rats generally started to press the lever for a sugar pellet within a single training session and trained to a criterion of two consecutive sessions of 100 sugar pellets earned by lever press within four one-half hour training sessions.

After reaching the criterion for learning to press the lever, training was increased to two sixty-minute sessions per day (five days per week), during which the rats learned to press the lever only in response to a presentation of the target "dad" sound. The target sound was no longer played in response to a lever press, but acted as the cue for the rats to press the lever. Silent trials were interleaved with presentations of the target sound as catch trials. Catch trials accounted for 25% of the trials, and ensured the rats were responding to the target sound and not randomly pressing the lever. Responses to the silent catch trials resulted in a time out, during which lamps in the booth were extinguished and the beginning of the next trial delayed. Rats did not receive any food during time out periods. Sessions started with either a target sound or a silent catch trial occurring every 10 seconds, allowing 8 seconds for the rats to respond, and with an 8-second timeout period for false alarms. As the rats' performance progressed, trial spacing

was eventually reduced from every 10 seconds to 8 seconds, the hit window reduced from 8 seconds to 3 seconds, and the timeouts reduced from 8 seconds to 6 seconds to allow for optimal training efficiency. Rats were unable to respond in less than 0.3 seconds from the beginning of a trial, therefore the hit window did not begin until after this period.

Rats demonstrated a reliable response to the target sound within 40 sessions by reaching a d-prime (d') value of 1.5 for 10 consecutive sessions, after which they advanced to discrimination testing. All the parameters remained the same in discrimination testing as they were in detection training, except for the addition of the distracting sound, "tad", and white noise. Stimuli were presented in blocks of thirteen trials, with each block occurring in one of five, randomly presented noise conditions (silence, 48 dB SPL, 60 dB SPL, 72 dB SPL, 84 dB SPL). The rats were previously used in experiments using other noise levels in unrelated experiments before entering the 98 pre-lesion testing sessions reported on here (one rat tested for ninety-six sessions). Evaluation of the rats' performance used percent correct, which averaged the percent of responses to the target sound and the percent of correct rejections for the non-target sound (excluding the catch trials).

Auditory cortical lesions

Bilateral lesions were administered to determine the role of AC in the operant discrimination of speech sounds varying in the initial consonant. All surgeries used sterilized instruments in a clean environment. After rats received an initial anesthetic dose of sodium pentobarbital (50 mg/kg, i.p.) they were placed in a Kopf Stereotaxic device. Doses of atropine (0.54 mg, s.c.), dexamethasone (2 mg, s.c.), cefotaxime sodium (20 mg, s.c.), and 10 ml of dextrose/Ringer's solution (s.c.) given to the rats before and during the surgery reduced

inflammation, prevented infection, and provided nourishment and fluids throughout the surgery and recovery. Bupivicane (1 ml, s.c.) injections into the scalp acted as a local anesthetic at the beginning and end of the surgeries to ensure the rats felt no pain. Body temperature was maintained at 37° C and the application of opthomalic ointment prevented corneal drying. The initial incision was made mid-line on top of the skull. After resecting the temporalis muscle, micro-rongeurs were used to remove a piece of the skull, as delineated by the space between the temporal ridge, coronal suture, and the lateral suture. The exposed area of the brain is known from neurophysiology to contain primary auditory cortex (A1) and most of the surrounding auditory fields. After removing the dura mater, an aspiration lesion was used to remove the cortex to the approximate level of the hippocampus as judged by visual inspection of the tissue and a rough depth estimate. Removal of the entire exposed auditory cortex ensured that A1 was lesioned. Bleeding stopped through natural clotting and the skull piece was replaced. The procedure was then replicated on the other side of the brain within the same surgical session. After the completion of both lesions and the replacement of both skull pieces, a loose stitch was made connecting the two temporalis muscles that helped to hold the skull pieces in place. The skin was sutured and a topical antibiotic ointment applied. Rats recovered for one week with free access to food before testing began again. Rats received amoxicillin (5 mg) and carprofen (1 mg) in tablet form for the three days immediately following the surgery.

2.4.2 Experiment 2

General Procedure

Behavioral methods for Experiment 2 closely approximated Experiment 1 with two changes: no background noise was delivered and a greater number of distracting stimuli were delivered. In Experiment 2, rats discriminated “dad” from “bad”, “sad”, “tad”, “dood”, “deed”, and “dud”. Half of the rats were tested on speech sounds that were limited to the first 40 ms of the speech sounds and the other half tested on the full-length speech sounds. After twenty days of discrimination testing, the rats that tested using the shortened stimuli received either bilateral auditory cortex lesions or sham lesions. The rats were allowed to recover for one week following the surgical procedure and then continued testing for an additional fifteen days

Subjects

Experiment 2 used twenty-four female Sprague-Dawley rats averaging 264 g (standard deviation 15 g). Housing conditions and food deprivation protocols were the same as Experiment 1.

Stimuli

Two lists of stimuli were created for comparing the effects of onset speech sound discrimination following lesioning, and onset speech discrimination compared to full-length speech sound discrimination before lesioning. Creation of both lists followed the same initial steps. A native English speaking female was recorded saying the consonant-vowel-consonant sounds “dad”, “bad”, “sad”, “tad”, “dud”, “deed”, and “dood”. Engineer et al., (Engineer et al. 2008, 607) used four of the same stimuli varying in the initial consonant place (“dad”, “bad”, “sad”, and “tad).Three additional stimuli varying in the vowel place introduced a greater range of

testing to the lists (“dud”, “deed”, and “dood”). The speaker and recording procedures were the same as Experiment 1. Following amplitude calibrations, one list remained at full-length and the second list was truncated to the first 40 ms following sound onset (see Figure 2.1). The duration of cortical activity that correlated with behavioral discrimination in an earlier study served as the

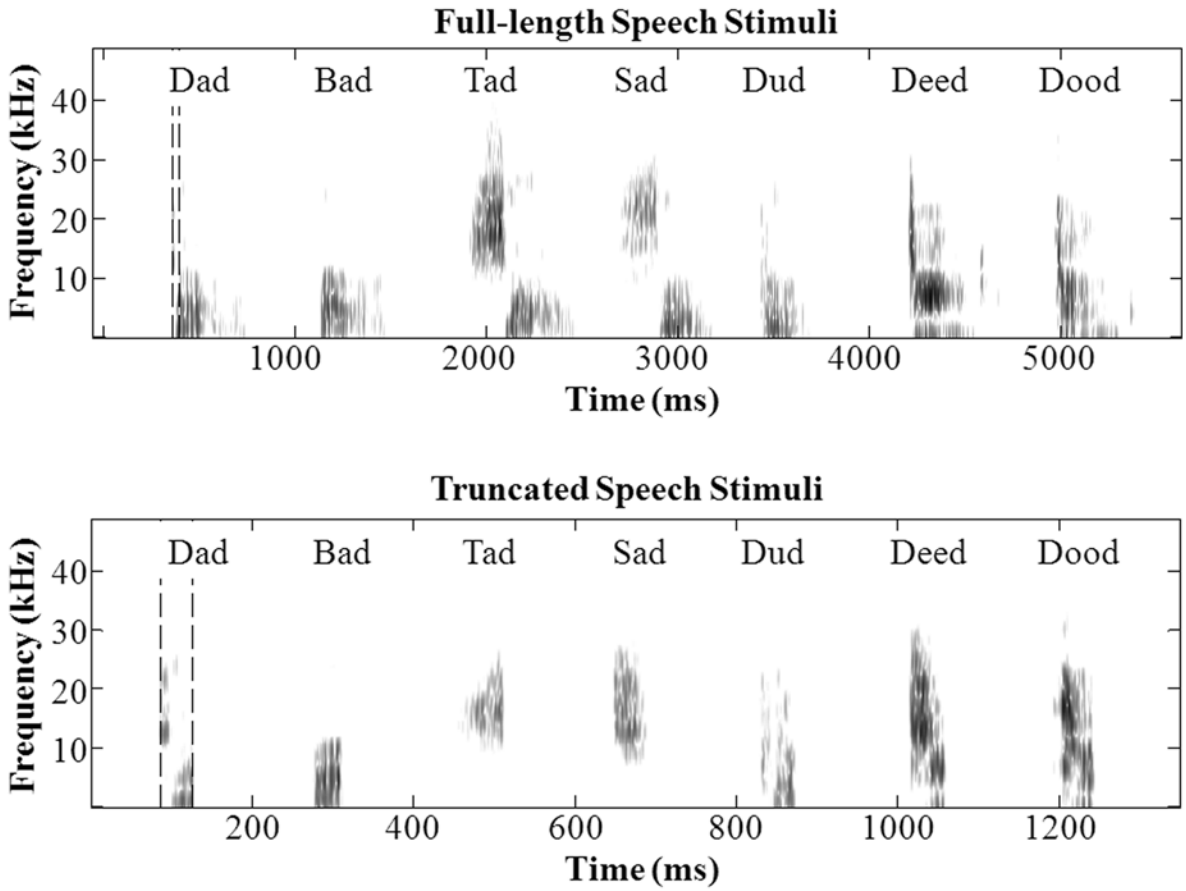


Figure 2.1
Spectrograms of the speech stimuli used. Spectrograms on the top represent the full-length stimuli and spectrograms on the bottom represent the same stimuli truncated to the first 40 ms of the sound. In Experiment 1, the full-length “dad” was discriminated from the full-length “tad” stimuli. In Experiment 2, the Full-length trained group discriminated the full-length “dad” from the full-length versions of “bad”, “tad”, “sad”, “dud”, “deed”, and “dood”. The onset trained group in Experiment 2 discriminated the first 40 ms of “dad” from the first 40 ms of “bad”, “tad”, “sad”, “dud”, “deed”, and “dood”. The space between the two dotted lines represents a 40 ms duration. The speech sound represented is listed above each spectrogram.

basis for the 40 ms duration (Engineer et al. 2008, 603-608). This duration is also similar to the duration limits suggested by psychophysical studies in humans and tests two of the consonants and two of the vowels used by Blumstein and Stevens (Blumstein and Stevens 1980, 649-651).

Behavioral testing procedure

Behavioral testing in Experiment 2 followed the same basic procedures and used the same apparatus as Experiment 1. Rats were trained using stimuli limited to the speech onsets, referred to as the "onset trained group", or the full-length versions of the sounds, referred to as the "full-length trained group". During the discrimination testing phase, rats heard full-length or truncated stimuli in blocks of thirteen, with the target version of "dad" presented six times, versions of the six distracting sounds "bad", "sad", "tad", "dud", "deed", and "dood", presented once, and one silent catch trial. Stimuli were presented in random order within each block. Unlike the first experiment, no noise was presented in the background. The full-length trained group tested for twenty sessions after which most rats demonstrated a plateau in their performance. Rats in the onset trained group tested for an additional twenty sessions to reach a consistent level of discrimination. Calculations for percent correct were the same as Experiment 1 using all the disctracting sounds in place of the single "tad" sound (excluding the catch trials). The same formula was used to calculate performance scores for individual contrasts, e.g. responses to "dad" compared to "sad".

After the last testing session, the onset trained group was randomly divided into two smaller groups of six. To test the effects of auditory cortex lesions on speech onset discrimination, one group received bilateral auditory cortex lesions focused on destroying A1, the lesion group, while the second group received sham surgeries, the sham group. Following a

week of recovery, all rats began testing again for an additional fifteen days using the 40 ms speech stimuli.

Auditory cortex lesions

Six rats from the onset trained group received bilateral auditory cortex lesions (Figure 2) while the other six received sham surgeries. All surgical procedures for lesioned rats were the same as Experiment 1. Previous research has indicated that penetrating the skull can result in neurochemical changes in the underlying brain regions (Adams, Schwarting, and Huston 1994, 947-952). Sham surgeries were similar to the lesion surgeries with the exception that the skull was not penetrated to avoid unwanted cortical damage.

2.4.3 Histological procedures

Following the last testing session, the rats from Experiments 1 and 2 were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Once the rats were unresponsive to toe pinch, they were transcardially perfused with 250 ml of 0.1 M PB solution with 0.02% heparin, followed by 500 ml of 4% formalin solution in 0.1 M PB. Brains were cryoprotected in a 30% sucrose solution for 2 days after removal from the skulls. Sections were taken at 40 µm intervals and stained with Cresyl Violet. Four sections used to define the lesion were spaced at 1 mm intervals and matched to reference sections in a widely used brain atlas for the rat (Paxinos and Watson 1998). Sections were then scanned into a computer, where a recreation of the lesion was created using Adobe Photoshop CS3 and the selected atlas slides. Several studies have suggested that the defined area for A1 in the cited atlas does not accurately represent A1 based on the neuronal response characteristics (Floody et al. 2010, 264; Doron, LeDoux, and Semple 2002, 345-360). The coordinates from Polley et al., (Polley et al. 2007, 3622) agree well with the

coordinates for A1 defined in an earlier lesion study (Floody et al. 2010, 264). These coordinates provided the approximate location of the neurophysiologically defined A1 used in this study.

2.5 RESULTS

2.5.1 Histology

Lesions bilaterally destroyed A1 and most of the surrounding auditory cortex in every rat (Figure 2.2). Five rats had their brains stained with Cresyl Violet for comparison. Methodological errors during sectioning prevented staining the sections from the remaining four rats. Visual inspection of the remaining four rats' brains revealed a similar pattern of destruction. The lesions typically extended bilaterally from roughly 1.6±0.2 mm to 8.2±0.2 mm caudal from bregma (mean ± standard error of the mean). The dorsal and ventral extent of the lesions was variable amongst the rats and occasionally included parts of the somatosensory cortex, parietal association areas, visual cortices, or temporal association cortex. Damage to the temporal association cortex may contribute to the observed behavioral deficits in complex sound processing, and requires a cautionary note when interpreting the results from this study (Kudoh et al. 2006, 1763-1765; Dewson, Pribram, and Lynch 1969, 579-591).

2.5.2 Experiment 1

The purpose of Experiment 1 was to evaluate the possibility that auditory cortex lesions would impair consonant discrimination when tested using operant procedures. Rats were rewarded for pressing a lever in response to the word "dad" and not the word "tad". Testing occurred in quiet and in four levels of background noise. The background noise was added to

increase the difficulty of the task to ensure that a negative result was not due to a ceiling effect. Rats were able to discriminate “dad” from “tad” in noise up to 72 dB SPL as accurately or better as they could in quiet (Figure 2.3; $t(11) \leq 0.28$, n. s.), but experienced a deficit in noise at 84 dB SPL (Quiet= 88±1%, 84 dB SPL= 67±3%, $t(11)= 4.8$, $P< 0.001$). Performance was still above chance when the background noise was 24 dB louder than the speech sounds (Chance= 50%;

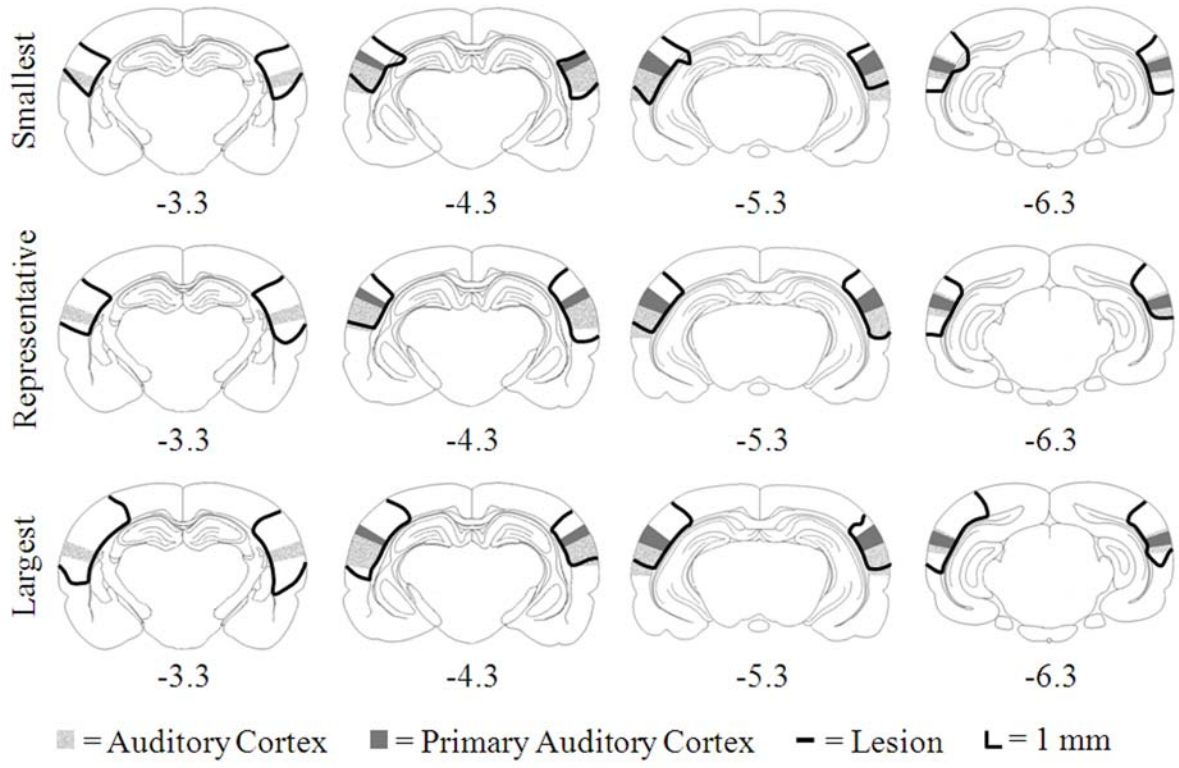


Figure 2.2
Estimated lesion locations on horizontal sections at -3.3, -4.3, -5.3, & -6.3 mm from bregma of a rat with a lesion representative of the groups in Experiment 1 and 2, and the two rats with the smallest and largest lesions. Five of the nine lesioned rats had their brains stained with Cresyl Violet for comparison. Visual inspection of the remaining four rats’ brains revealed a similar pattern of destruction. Stippled areas in light grey represent the location of auditory cortex and the dark grey shaded area represents primary auditory cortex determined using coordinates from Polley et al. (Polley et al. 2007, 3622). Areas outlined in a bold, black line represent traces of the destroyed cortex. Scale bars located in the bottom right hand corner of the figure represent 1 mm.

t(11)= 5.2, *P*< 0.001). The ability of rats to discriminate these sounds in high levels of noise is consistent with human performance (Miller and Nicely 1955, 338-352; Wang and Bilger 1973, 1248-1266; Phatak, Lovitt, and Allen 2008, 1220-1233).

Bilateral auditory cortex lesions failed to generate a long-term impairment in speech sound discrimination. Performance was well above chance on the first day of post-lesion testing, even when the background noise was 84 dB SPL (Performance in 84 dB SPL= 59± 2%, Chance=

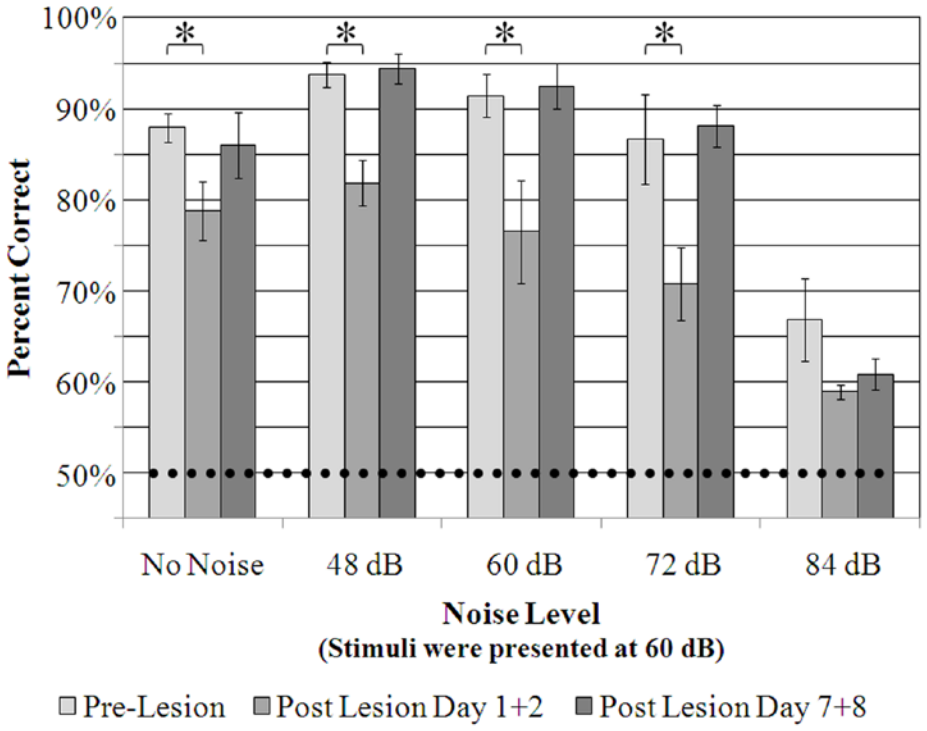


Figure 2.3
Percent correct discrimination of "dad" and "tad" by three rats in Experiment 1 before and after bilateral auditory cortex lesions. Discrimination scores are reported in quiet and in four levels of white noise across the last two days of pre-lesion, first two-days post-lesion, and days seven and eight post-lesion behavior. One rat used slightly different noise levels (no noise, 42 dB, 54 dB, and 66 dB) during the first session of retesting; therefore, only her performance in silence was included in the analysis of this session. The dotted line represents chance performance at 50%. Asterisks represent significant differences between pre-lesion and post-lesion scores, *P*< 0.05.

50%; $t(4)= 17.8$, $P< 0.001$). A small, but statistically significant impairment was observed over the first two days in all but the loudest noise level (In 84 dB SPL, $t(5)= 1.7$, n. s.; In all other levels, $t(5)\geq 2.4$, $P< 0.05$). By the eighth day of post-lesion testing, performance had recovered to pre-lesion levels ($t(5)= 1.19$, n. s.). The observation that discrimination performance recovered completely at every noise level argues against a ceiling effect that would obscure the impact of the lesion. These results support the conclusion of the startle reflex modification study that rats with auditory cortex lesions can accurately discriminate amongst consonant sounds (Floody et al. 2010, 260-268).

2.5.3 Experiment 2

Accurate speech sound discrimination following auditory cortex lesions suggests that the auditory cortex is not necessary for speech discriminations; however, it is possible that lesioned rats quickly adopt a new strategy that relies on the identification of cues in the auditory signal that are not affected by lesions of auditory cortex. Animals with bilateral auditory cortex lesions can discriminate tone frequencies (Whitfield 1980, 644-647; Harrington, Heffner, and Heffner 2001, 1217-1221; Kudoh et al. 2006, 1761-1766; Dewson, Pribram, and Lynch 1969, 579-591; Dewson 1964, 555-556; Evarts 1952, 443-448; Ohl et al. 1999, 347-362; Rybalko et al. 2006, 1614-1622; Goldberg and Neff 1961, 119-128; Butler, Diamond, and Neff 1957, 108-120), sound intensity (Rosenzweig 1946, 127-136; Raab and Ades 1946, 59-83; Oesterreich, Strominger, and Neff 1971, 251-270), sound duration (Scharlock, Neff, and Strominger 1965, 673-681), slow amplitude modulations (Cooke, Zhang, and Kelly 2007, 90-99), and click trains (Heffner 1978, 963-976; Cranford, Igarashi, and Stramler 1976, 69-81) as accurately as unlesioned animals. Since the “dad” and “tad” stimuli used in Experiment 1 varied in cues other

than voicing, including their first and second formant frequencies (846 and 2286 Hz vs. 957 and 1984 Hz, respectively), as well as the overall stimulus duration (414 ms vs. 465 ms), it is possible that the lesioned rats used these unrelated cues to perform the task. In Experiment 2, we tested rats on consonant discrimination tasks with speech sounds truncated to 40 ms to eliminate duration differences and make it more difficult to use differences occurring during the vowel component of the sounds.

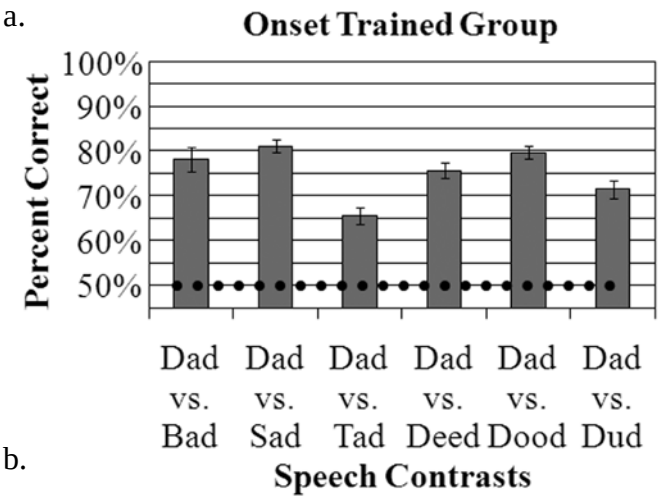
The sounds were truncated at 40 ms because 1) this interval is known to be sufficient for humans to accurately discriminate consonant sounds (Blumstein and Stevens 1980, 648-662; Bertoncini et al. 1987, 31-37) and 2) rat consonant discrimination using full-length speech sounds is best correlated with neural activity when only the first 40 ms of the A1 response is used (Engineer et al. 2008, 605-606). These results suggest that the auditory cortex activity within the 40 ms of speech onset is used to discriminate between consonant sounds. No prior study has reported speech discrimination by animals using such short stimuli. Animals can accurately discriminate consonant (Floody and Kilgard 2007, 1884-1887; Kuhl and Miller 1975, 69-72; Reed et al. 2003, 205-215) and vowel sounds (Kudoh et al. 2006, 1761-1766; Dewson 1964, 555-556; Baru and Shmigidina 1977, 197-204), and exhibit similar phonetic boundaries to humans in fricative durations (Reed et al. 2003, 205-215) and voice onset time (Kuhl and Miller 1975, 69-72). We predicted that, like humans, un-lesioned rats would also be able to discriminate speech onsets.

Rats were trained to discriminate the first 40 ms of the target speech sound, “dad”, from similarly truncated versions of distracting speech sounds (“tad”, “bad”, “sad”, “deed”, “dood”, and “dud”). Since discrimination was accurate at every noise level after lesioning in Experiment

1, noise was not used to increase task difficulty in Experiment 2. Instead, six stimuli varying from the target stimulus in either the consonant (“tad”, “bad”, “sad”) or vowel (“deed”, “dood”, and “dud”) were used to provide a range of easy and difficult discriminations. A group of rats tested on full-length versions of the stimuli served as a comparison group to determine if rats could discriminate truncated speech as accurately as full-length speech. Both groups performed the task correctly on 75±1% (onset trained) and 76±2% (full-length trained) of the trials over the last two days of testing. These levels of performance are well above chance (50%; $t(23) \geq 16.6$, $P < 0.001$), indicating that both groups accurately discriminated the target sound from the of performance of the full-length trained group was not significantly different from the performance of the onset trained group ($t(46) = 1.7$, n. s.). This comparable performance between the groups supports predictions made from neurophysiology (Engineer et al. 2008, 603-608) and human psychophysics (Blumstein and Stevens 1980, 648-662; Bertoncini et al. 1987, 31-37) that rats can discriminate speech onsets as accurately as they can discriminate full-length speech.

Performance on individual contrasts over the last two days of testing were examined to confirm that rats could accurately discriminate between both consonant and vowel sounds using only the speech onsets (Figure 2.4 a+b). Both onset and full-length trained groups consistently rejected speech sounds that differed from the target sound in the initial consonant or the following vowel. All six speech contrasts tested were well above chance ($t(23) \geq 9.1$, $P < 0.001$). For both groups, the most difficult consonant to discriminate from the target sound, “dad”, was “tad” (onset trained group= 66±2%, full-length trained group= 65±1% correct). The most difficult vowel to discriminate from the target sound, “dad”, was “dud” (onset trained group=

a.



b.

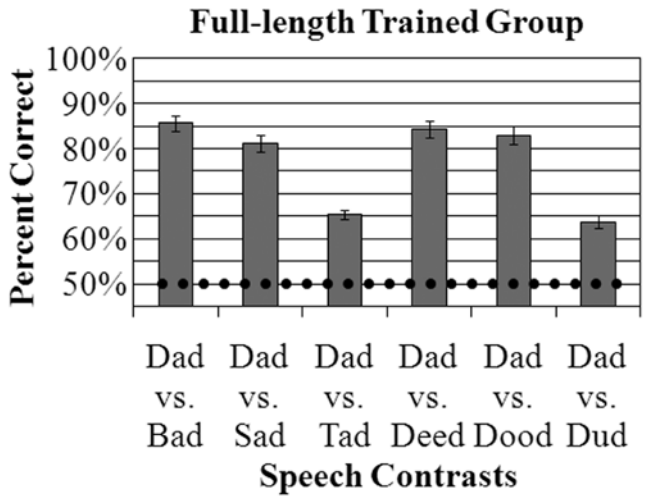


Figure 2.4
Comparison of the percent correct discriminations of "dad" with individual distracting stimuli over the last 2 days of testing in Experiment 2. a) Animals in the full-length trained group, n=12. b) Animals in the onset-trained group, n=12. The dotted lines represent chance performance at 50%. Error bars show the standard error of the mean. Both groups performed significantly above chance on every task ($t(23) \geq 9.1$, $P < 0.001$).

71±2%, full-length trained group= 64±1%). Thus, the overall pattern of consonant and vowel discrimination was similar whether the rats used the entire speech sound or only the initial 40 ms of the speech sound. These results indicate that the first 40 ms of the speech sounds tested contain sufficient information to explain the discrimination ability of rats trained on full-length speech sounds.

Rats in the onset trained group learned the task at a notably slower rate compared to the full-length trained group (Figure 2.5). The full-length trained group learned the task to a level of 68% correct in 1.8±0.3 days, while the onset trained group took 10.0±1.8 days to reach the same criterion ($t(12)= 4.7$, $P < 0.001$). The 68% criterion is the best single day performance of the rat with the worst performance of all the rats tested (i.e. allows all the rats in the study to reach the criteria). Full-length trained rats performed significantly better

than the onset trained rats each day during the first ten days of testing ($t(22) \geq 2.6$, $P < 0.01$). The slow rate of learning in the onset trained group indicates the truncated stimuli were more difficult to discriminate. These results show that the additional acoustic cues included in the full-length stimuli facilitates the learning of the speech discrimination task, but are not required to perform the task accurately.

To test the importance of auditory cortex in speech discriminations using the minimal acoustic information, six rats from the onset trained group received bilateral auditory cortex lesions and continued to test on speech onset discriminations after one week of recovery (Figure

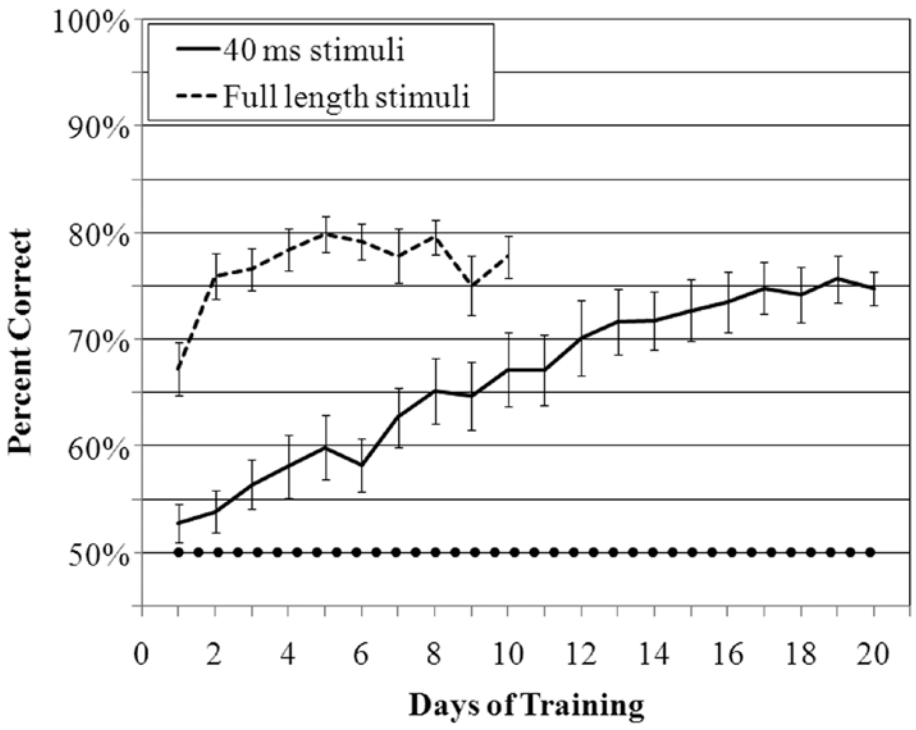


Figure 2.5
Timeline of the percent correct discrimination of "dad" from the distracting stimuli for the onset trained group (n=12) and the full-length stimulus trained group (n=12) in Experiment 2. Error bars show the standard error of the mean. The dotted line represents chance performance at 50%.

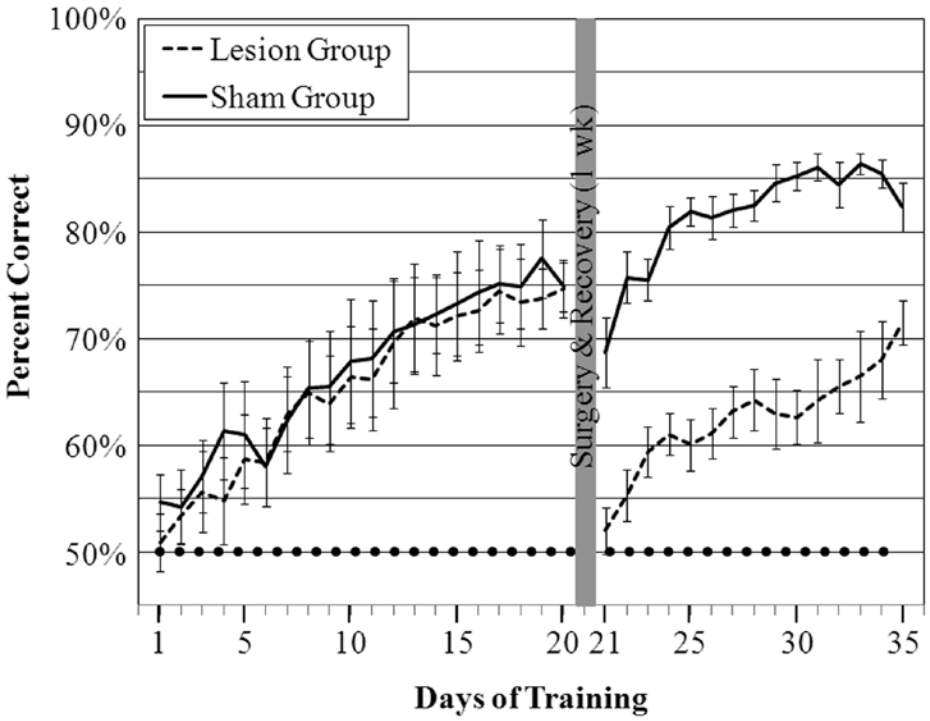


Figure 2.6
Timeline of the percent correct discrimination of "dad" from the distracting stimuli for the lesion group (n=6) and the sham group (n=6) from initial testing on the task through retesting after recovery from surgeries in Experiment 2. The grey bar represents the time for the surgery and one week of recovery. Error bars show the standard error of the mean. The dashed line represents chance performance at 50%.

2.6). Lesioned rats were able to reliably discriminate target speech sounds from silent catch trials, indicating that AC lesions did not deafen the rats or impair their ability to perform the operant task (Performance= 59± 3%, Chance= 50%; $t(11)= 3.0$, $P< 0.01$). Additionally, the unimpaired discrimination performance by the lesioned rats in Experiment 1 argues against non-auditory impairments from collateral damage to other cortical areas from the lesioning process. Rats in Experiments 1 and 2 received the same lesioning procedures and demonstrated similar extents of cortical damage. Despite their ability to hear the stimuli, the lesioned rats in Experiment 1 were impaired to chance levels of performance on the first day of continued testing

(Performance= 52± 2%, Chance= 50%; $t(5)= 0.92$, n. s.). The six remaining rats from the onset trained group underwent sham surgeries and continued testing with the onset stimuli to serve as an un-lesioned comparison group. These sham rats experienced only a slight impairment and their performance was well above chance on the first day of post-surgery testing (Performance= 69± 3%, Chance= 50%; $t(5)= 5.7$, $P< 0.001$). Sham rats demonstrate that the weeklong interruption in training following surgery may have caused a slight lapse in performance, but that the damage to the auditory cortex is responsible for the majority of the observed impairment in the lesion group. The lesion group performed the task at above chance levels as testing progressed, but remained significantly worse than the sham group on every day ($t(10)\leq -3.51$, $P< 0.01$). Auditory cortex lesions did not affect the whole word discrimination task in Experiment 1, but did impair speech onset discriminations in Experiment 2. These results are consistent with the hypothesis that rats use the first 40 ms of auditory cortex neural activity to discriminate between consonant sounds (Engineer et al. 2008, 603-608).

Performance of both the lesion and sham groups improved significantly from the twenty-first to the thirty-fifth day of testing ($t(5)\leq -3.3$, $P< 0.01$). Performance by the lesion group was approximately 19% lower than the sham group on every day of post lesion testing. If the rats were recovering a previous ability, we would expect to see an increased rate of improvement in the performance compared to the sham rats. Since the difference between the groups' scores remained consistent over the additional testing ($R^2= 0.07$, n. s.), we conclude that the deficit is stable for at least a month and that the improvement in both groups is due to ongoing learning.

Six distracting stimuli created a range of easy and difficult discriminations from the target speech sound to test if discriminating the subtle differences in speech onsets was impaired

following bilateral auditory cortex lesions (Figure 2.7). Lesion rats were significantly worse than the sham group on the average discriminations of the target sound from each of the distracting sounds over all post-surgery testing (Figure 6; Main effect of group, $F(1, 60)= 180.0$, $P< 0.001$); $t(10)\leq -2.47$, $P< 0.05$). Additionally, the degree of impairment from pre-lesion to post-lesion performance is similar for each of the contrasts tested (Average pre-lesion minus post-lesion performance= -12%; No main effect of task, $F(5, 30)= 0.89$, n. s.). With the exception of the "sad" contrast, the ranking of the highest to lowest scored contrast was the same between the

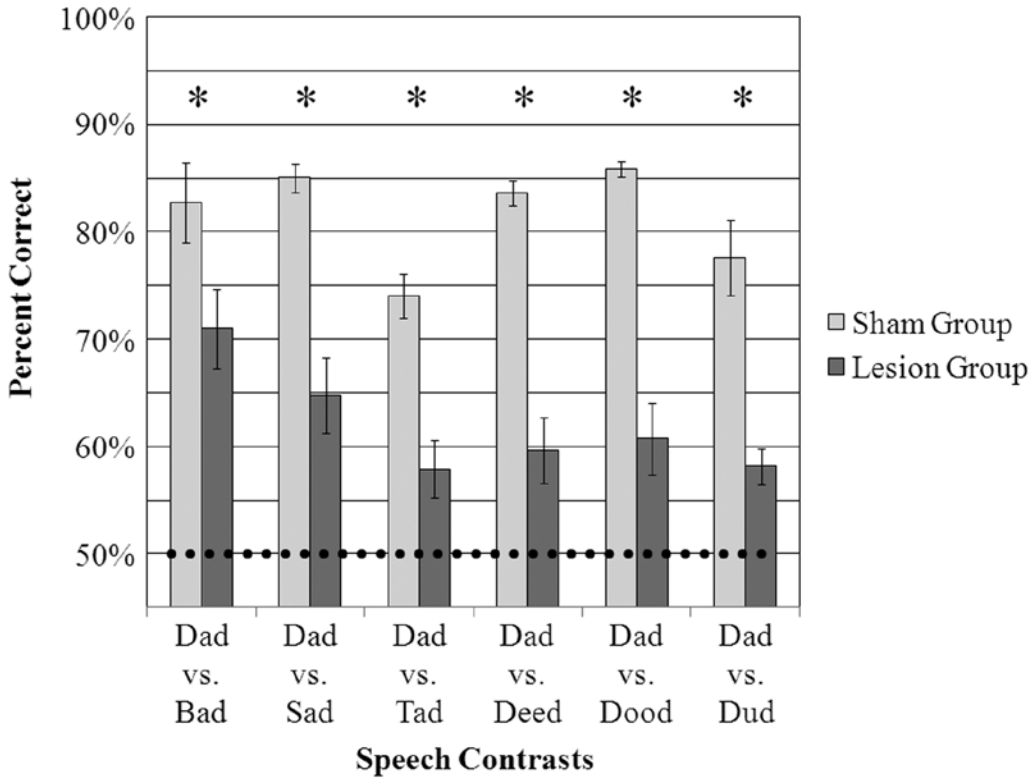


Figure 2.7
Percent correct across all post-surgery days for sham and lesion groups on "dad" discrimination from individual distracting stimuli in Experiment 2. Error bars represent the standard error of the mean. n= 6 in both groups. The dotted line represents chance performance at 50%. Asterisks represent significant differences between the lesion and the sham group, $P< 0.05$. Lesion group is significantly worse than the sham group on every speech contrast ($t(10)\leq -2.47$, $P< 0.05$).

lesion and the sham group (Figure 2.7; Interaction of group and individual contrast, $F(5, 60)= 2.0$, n. s.). This consistent pattern of impairment after bilateral auditory lesions suggests that the average impairment is not due to any one contrast, but is a general deficit across the consonant and vowel discriminations tested.

2.6 DISCUSSION

Operant speech discrimination ability was preserved after bilateral auditory cortex lesions unless the speech stimuli were limited to the first 40 ms. This result is in agreement with an earlier study finding spared discrimination of speech stimuli with an average duration of 150 ms (110-260 ms) tested using an acoustic reflex modification paradigm (Floody et al. 2010, 260-268). The longer stimuli contain several potential auditory cues, including stimulus duration, which could allow discrimination by rats with auditory cortex lesions. The sounds from Experiment 1 were shortened by 90% to reduce or eliminate these cues. Normal rats still accurately discriminated the truncated speech sounds. Auditory cortex lesions impaired the rats' discrimination ability of the truncated speech sounds. These results suggest that the auditory cortex plays an important role in distinguishing the rapidly occurring acoustic transitions at the beginning of speech sounds.

Similarities in the final performance of the onset and the full-length trained rats confirm earlier predictions that the entire speech sound is not necessary for accurate speech discrimination amongst consonants and vowels. Although human psychophysics supported this finding (Blumstein and Stevens 1980, 648-662; Bertoncini et al. 1987, 31-37), onset discrimination has not been previously reported in animals. Human performance was more accurate than rat performance, which might be expected since humans have thousands of hours experience with speech sounds in their native language. By comparison, the onset trained rats in

our study heard the speech for less than 80 hours total. Humans exhibit significantly worse discrimination of contrasts not used in their native language (Bradlow et al. 1997, 2299-22310; Goto 1971, 317-323; Sharma and Dorman 2000, 2697-2793; Winkler et al. 1999, 638-642). These psychophysical results suggest that humans and rats discriminate consonant and vowel sounds in a similar manner.

Our earlier study found that the differences in the first 40 ms of A1 neural activity better predict whole word discrimination in rats than the A1 responses to the whole sound (Engineer et al. 2008, 603-608). Predictions that these brief, spatiotemporal activity patterns in A1 are critical for onset discriminations were confirmed in Experiment 2 by the loss of discrimination ability following auditory cortex lesions. A recent fMRI study of native and non-native English speakers reported that (as in rats) the distinctiveness of the A1 activity patterns evoked by consonants was correlated with discrimination ability (Engineer et al. 2008, 603-608; Raizada et al. 2010, 1-12). Collectively, the results from previous studies and the current study suggest that humans and animals likely share similar neural processing mechanisms for encoding speech sounds up to the level of A1. Animal models have proven useful in elucidating the biological cause of some forms of communication disorders. For example, cortical microgyric lesions in the parietal cortex affect the temporal processing of speech sounds in rats in a manner that closely resembles the deficits seen in children with specific language impairment (Clark et al 2000, 828-839; Fitch et al. 1994, 260-270; Warrier et al. 2004, 431-441; Wright et al. 1997, 176-178). These results have contributed to the development of treatment models for specific language impairment in humans. Additionally, a genetic model in rats has contributed to explaining some of the underlying causes of dyslexia (Threlkeld et al. 2007, 508-514; Meng et al. 2005, 17053-

17058; Galaburda et al. 2006, 1213-1217). Understanding the contributions of the auditory system in discriminating speech sounds in rodent models may lead to the refinement of techniques for treating different sources of language impairments in humans.

CHAPTER 3

REPEATEDLY PAIRING VAGUS NERVE STIMULATION WITH A MOVEMENT REORGANIZES PRIMARY MOTOR CORTEX

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3.2 ABSTRACT

Although sensory and motor systems support distinctly different functions, both systems exhibit experience dependent cortical plasticity under similar conditions. If the mechanisms regulating cortical plasticity are common to sensory and motor cortices, then methods for generating plasticity in sensory cortex should also be effective in motor cortex. Repeatedly pairing a tone with a brief period of vagus nerve stimulation (VNS) increases the proportion of primary auditory cortex responding to the paired tone (Engineer et al. 2011, 101-104). In this study, we tested the hypothesis that repeatedly pairing VNS with a specific movement would result in an increase in the representation of that movement in primary motor cortex. To test this hypothesis, we paired VNS with movements of the lower or upper forelimb in two different groups of rats. Following VNS movement pairing for five days, intracranial microstimulation was used to quantify the organization of primary motor cortex. There was a larger cortical area associated with those movements paired with VNS and smaller areas representing movements not paired with VNS. Rats that received identical motor training without VNS pairing did not exhibit motor cortex map plasticity. These results suggest that pairing VNS with specific events may act as a general method for increasing cortical representations of those events. VNS-movement pairing could provide a new approach for treating disorders associated with abnormal movement representations.

3.3 INTRODUCTION

Although sensory and motor systems support distinctly different functions, both systems can exhibit topographic reorganization of the cortex following training or injury. Training on an auditory task significantly alters the trained tone representation in the auditory cortex (Bakin & Weinberger 1990, 271-286; Recanzone, Schreiner, and Merzenich 1993, 87-103). Somatosensory cortical representation of a digit was significantly increased as a result of operant conditioning (Recanzone et al. 1992, 1031-1056; Wang et al. 1995, 71-75; Byl, Merzenich, and Jenkins 1996, 508-520). Similar changes occur in the motor cortex following training with precise digit movements (Nudo et al. 1996, 785-807; Kleim, Barbay, & Nudo 1998, 3321-3325). Motivation and frequency of training influence the degree of cortical map plasticity (Kleim & Jones 2008, S225-S239; Recanzone et al. 1992, 1031-1056; Kleim et al. 2004, 628-633). Deprivation caused by peripheral injury changes the organization of sensory and motor cortices (Hubel, Wiesel, and LeVay 1977, 377-409; Merzenich et al. 1983, 33-55; Merzenich et al.1984, 591-605; Pons et al. 1991, 1857-1860; Yu et al. 2010, 1667-1676). For example, digit amputation or nerve transection causes receptive fields in the inactivated somatosensory cortex to shift to neighboring digits (Merzenich et al. 1983, 33-55; Merzenich et al.1984, 591-605). Likewise, transecting the facial nerve reduces the number of motor cortex neurons that elicit vibrissae movements while increasing the number eliciting forelimb movements (Sanes et al. 1988, 2003-2007). Targeted lesions to the sensory or motor cortex cause the surrounding healthy

cortical areas to take on some of the damaged area's lost functionality (Eysel et al. 1999, 153-164; Conner, Chiba, and Tuszynski 2005, 173-179). Collectively, these results suggest that the mechanisms regulating cortical plasticity are common to both sensory and motor cortices.

Our previous study demonstrated that repeatedly pairing vagus nerve stimulation (VNS) with a tone causes a greater representation of that tone in primary auditory cortex (Engineer et al. 2011, 101-104). This map expansion is specific to tones presented within a few hundred milliseconds of VNS. No previous study has reported the effects of pairing VNS with a specific movement on cortical plasticity. If the mechanisms regulating map plasticity in the auditory cortex are the same in the motor cortex, then VNS-paired with a movement should generate map plasticity specific to the paired movement. In this study, we paired VNS with a specific movement to test if this method could be used to direct specific plasticity in the motor cortex. Movement of the upper or lower forelimb was paired with VNS with the prediction that only the corresponding area of motor cortex would expand.

3.4 METHODS

General procedure

Rats were randomly assigned to receive a vagus nerve cuff electrode or a non-functional, sham vagus nerve cuff electrode. After recovery from the surgery implanting the cuff, rats were trained to perform one of two operant motor tasks using either their upper or lower forelimb in order to receive a sugar pellet reward. Tasks were learned in several stages to facilitate mastery of the required movement. The movements were paired with VNS during the final week of each motor task. Intracranial microstimulation (ICMS) was used to quantify the reorganization in the primary motor cortex the day after the last training session. An additional group of experimentally naïve rats that had not received motor training or VNS also underwent motor cortex ICMS.

Comparison of the motor maps from the naïve rats to those of the rats with sham cuffs allowed us to determine if training on the motor tasks without VNS generates cortical plasticity. Comparison of the motor maps from the rats with sham cuffs to the rats with functional cuffs allowed us to determine if pairing VNS with the movements enhances cortical plasticity.

Subjects

Thirty-three adult, female Sprague-Dawley rats (264± 44 g, mean ± s.d.) were used in this experiment. The rats were housed in a 12:12 hr reversed light cycle environment to increase their daytime activity levels and were food deprived to no less than 85% of their normal body

weight during training as motivation for the food pellet rewards. All handling, housing, surgical procedures, and behavioral training of the rats were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee.

Implantation surgery

Rats were implanted with a custom-built cuff electrode prior to training. Stimulating cuff electrodes were constructed as previously described (Engineer et al. 2011, S1). In brief, two Teflon coated multi-stranded platinum iridium (0.006") wires were connected to a 4 mm section of Micro-Renethane tubing (1.8 mm inner diameter). The wires were spaced 2 mm apart. An 8 mm region of the wires located inside the tubing was stripped of the insulation. A cut was made lengthwise along the tubing to allow the cuff to be wrapped around the nerve and then closed with silk threads. A second group of randomly chosen rats received similar cuffs, but with silk threads in place of the platinum iridium wires.

All the steps of the surgeries were the same regardless of the type of cuff implanted. Rats were anesthetized using ketamine hydrochloride (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) with supplemental doses provided as needed. After rats were no longer responsive to toe pinch, incision sites atop the head and along the left side of the neck were shaved and cleaned with betadine and 70% isopropyl alcohol. The application of opthomalic ointment to the eyes prevented corneal drying during the procedure and a heating pad maintained the rats' body temperature at 37° C. Doses of cefotaxime sodium (2 x 10 mg, s.c.) and a dextrose/Ringer's (10 x 1 ml total, s.c.) solution were given to the rats before and during the surgery to prevent infection and provide nourishment throughout the surgery and recovery. Bupivicaine (2 x 0.5 ml, s.c.) injected into the scalp and neck further ensured that the rats felt no discomfort during

surgical procedures. An initial incision and blunt dissection of the scalp exposed the lambda landmark on the skull. Four to five bone screws were manually drilled into the skull at points close to the lambdoid suture and over the cerebellum. After an acrylic mount holding a two-channel connector was attached to the anchor screws, an incision and blunt dissection of the muscles in the neck exposed the left cervical branch of the vagus nerve. Twelve rats received the platinum iridium bipolar cuff-electrodes (5-6 KOhm impedance) while another thirteen received the sham cuffs in which silk thread replaced the platinum iridium wires. The rats with silken threads looked identical to the rats with wired cuffs after the surgeries. Leads (or silk threads) were tunneled subcutaneously and attached to the two-channel connector atop the skull. All incisions were sutured and the exposed two-channel connector encapsulated in acrylic. A topical antibiotic cream was applied to both incision sites. Rats were provided with amoxicillin (5 mg) and carprofen (1 mg) in tablet form for three days following the surgeries and were given one week of recovery before training began. During the week of recovery, rats were habituated to having the stimulator cable connected to the two-channel connector on their heads.

Motor tasks

Rats were trained on either a wheel spin or lever press task. Training occurred in two daily sessions for five days each week. Both tasks involved quick movement of the forelimb in order to receive a sugar pellet reward. Rats initiated each trial, but a delay of at least two seconds was required between trials to allow the rats to eat the sugar pellet. The wheel spin task required the use of muscles located primarily in the lower forelimb, especially the wrist, while the lever press task required the use of the shoulder and the upper forelimb.

The initial shaping procedures were similar for both motor tasks. Rats were placed in a cage and allowed to freely explore the area. A tether was connected to the rats' heads to familiarize the animals with the feeling of the connection. Each time the rats approached the response device (i.e., the lever or wheel) they received a 45 mg sugar pellet dispensed into a pellet dish located within the cage. Restrictions were gradually placed on rewarding the rats' proximity to the response device until the rats had to be next to, then touching, and finally using the device to receive the reward. Shaping procedures were conducted manually by an experimenter. Rats typically took four 30 minute sessions to become familiarized to the response

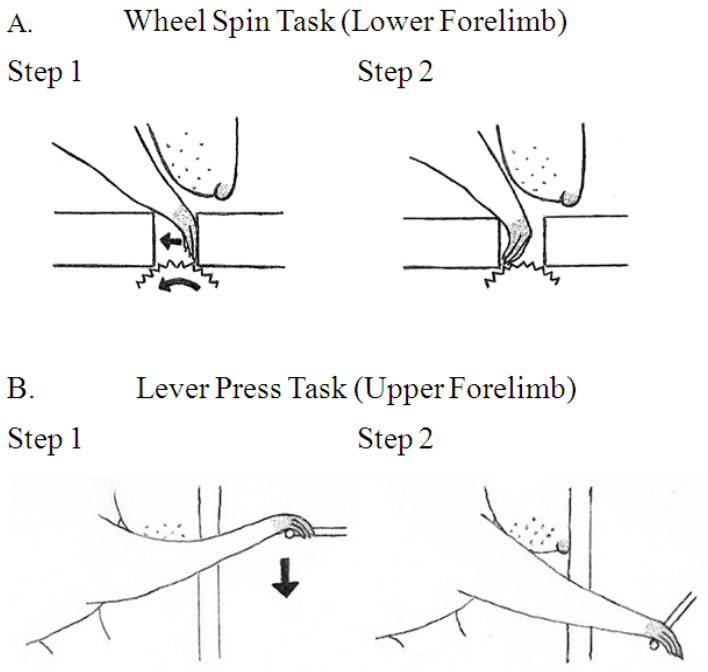

Figure 3.1
Sketches demonstrate the range of movements necessary to complete the motor tasks. A. The wheel spin task required the rat to spin a textured wheel towards themselves. Rats used movements of the wrist and digits to complete this task. Stimulation and reward occurred after the rat spun the wheel 145° within a 1 s period. B. The lever press task required the rat to depress a spring loaded lever twice within 0.5 seconds. The range of motion required to complete this task pivoted primarily around the shoulder joint. Stimulation and reward occurred after the second lever press.

device. After shaping, all training sessions were automated using custom-written MATLAB programs.

Wheel spin task

Rats that trained on the wheel spin task were required to spin a textured wheel below the floor of the training cage to receive a sugar pellet reward (Figure 3.1a). Training occurred in two 30 minute sessions daily. The acrylic training cage was 28 cm x 28 cm x 32 cm with a wheel placed just below an opening in the cage floor. The textured wheel was 32 mm in diameter and 9 mm thick. The 32 ridges providing the surface texture were 1.5 mm deep. Interchangeable acrylic pieces with rectangular holes 1 cm wide and ranging from 1.2 cm to 2.4 cm lengthwise were placed over the wheel to restrict access to the wheel. Reducing the size of the hole prevented broad movements of the forelimb and resulted in use of a flicking motion of the wrist and digits to spin the wheel. Rats learned the wheel spin task in five stages. Access to the wheel was progressively reduced at each stage. The size of the opening for each stage was as follows: 1) open access to the wheel, 2) 2.4 cm x 1 cm, 3) 1.9 cm x 1 cm, 4) 1.4 cm x 1 cm 5) 1.2 cm x 1 cm. Rats had to reach 1 cm through the floor to reach the wheel. Rats were rewarded for spinning the wheel 3° within a one-second period when each new stage began. Trials were initiated by the rats, but were spaced at least two seconds apart. After 35 successful spins of the wheel, the degree of rotation required for a reward increased to 30°, then 75°, and finally 145°. After 35 rewards at the highest rotational requirement, the rats advanced to the next stage of training (i.e. more restricted access to the wheel). Rats demonstrated a paw preference early in training and continued to use that paw for the remainder of the sessions (left paw, n=7).

Lever press task

Rats depressed a lever initially located inside the training cage to receive a sugar pellet reward. The training cage was a 20 cm x 20 cm x 20 cm wire cage with a Plexiglas wall opposite the door. All training sessions other than the shaping sessions were fifteen minutes long and occurred twice daily. After receiving 60 pellets in 2 sessions on their own in shaping, the rats learned to press the lever twice in a three second period for the same reward. Trials were initiated by the rats, but were spaced at least five seconds apart. The period between lever presses was reduced from three seconds to two seconds, then one second, and finally 500 ms, with fifteen successful trials as the criterion for advancing. After successfully pressing the lever twice within 500 ms forty-five times, the lever was gradually withdrawn out of the cage. The lever was initially located 4 cm inside the cage, then moved to 2 cm inside the cage, and then to 0.5 cm, 1.5 cm, and 2.0 cm outside of the cage. The criterion for advancing the lever was 15 successful double-lever presses for each position, except for 0.5 cm outside the cage which required 30 successful trials. Rats reached through a 1 cm x 8 cm window in the Plexiglas wall to reach the lever outside the cage (Figure 3.1b). The edge of the window was located 2 cm from the cage wall, while the lever was offset so that the middle of the lever lined up with the edge of the window furthest from the wall. This arrangement restricted the rats so that they could only comfortably press the lever with their right paw.

VNS-Movement pairing

During the final stage of the motor tasks, reaching through a 1.2 cm wide window and spinning the wheel 145° within a 1 second period or pressing the lever located 2 cm outside the cage twice within 500 ms triggered a food reward and VNS. Rats typically continued to spin the

wheel or press the lever beyond the required criterion, such that the movements were still occurring during VNS. VNS was always delivered as a train of 15 pulses at 30 Hz. Each 0.8 mA biphasic pulse was 100 μs in duration. The train of pulses was 500 ms in duration. VNS-movement pairing during the final stage of training continued for one week (i.e. 10 x 30 minute sessions for the wheel-spin task and 10 x 15 minute sessions for the lever-press task), delivering around 1,200 total stimulations. Previous research has shown that this form of VNS does not alter heart rate, blood oxygenation level, or ongoing behavior, suggesting that the stimulation is neither aversive nor rewarding to the animals (Engineer et al. 2011, S2).

Connections and stimulations from the external stimulator to the rats were identical between rats implanted with functional or sham VNS electrode cuffs. The sham cuffs with silk threads in place of platinum iridium leads did not carry an electrical charge when stimulated. This difference in the cuffs allowed experimenters to remain blind during training to stimulated and sham rats.

Intracranial microstimulation

The day after the last training session, the organization of primary motor cortex contralateral to the trained paw was defined using standard ICMS mapping procedures (Nudo, Jenkins, and Merzenich 1990, 464-467; Conner, Kulczycki, and Tuszynski 2010, 2740-2741). An additional eight rats (283 g, 19 g standard deviation) that did not train or receive VNS also underwent ICMS procedures to the left cortex to compare the effects of training on motor cortex organization. Rats were anesthetized initially with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), with supplemental doses provided as needed. After placing the rat in a stereotaxic frame with a digital readout, a craniotomy was performed to expose the motor cortex.

Parylene-coated tungsten electrodes (~0.7 MOhm impedance) were inserted to a depth of ~1800 µm (corresponding to cortical layers V-VI). Stimulation occurred following a grid with 500 µm spacing. ICMS was delivered one stimulation per second and a stimulation consisted of a 40 ms pulse train of 13, 200 µs monophasic cathodal pulses delivered at 350 Hz. Stimulation intensity was gradually increased (up to 200 µA) until a movement was observed. If no movement was observed at the maximal stimulation, then the site was deemed nonresponsive. The borders of primary motor cortex were defined based on unresponsive sites, except on the posterior border that was also defined by vibrissae movements' characteristic of the posterior-lateral vibrissae area.

Motor mapping procedures were conducted with two experimenters, both blind to the experimental condition of the rat. The first experimenter placed the electrode at random within the mapping grid and recorded the data for each site. Because the motor cortex is somatotopically organized, electrode placements close together often yield the same area of movement. The second experimenter was kept blind to the electrode placement to avoid biasing any just noticeable movements. The second experimenter delivered stimulations while observing which parts of the body moved in response. Movements were classified based on the part of the body that moved using the lowest stimulation current. The movements elicited by ICMS were small muscle twitches. Complex movements, such as those required to complete the trained tasks, were never observed. Movements of the vibrissae, face, eye, and neck were classified as "head". Movements of the shoulder, elbow, and upper forelimb, i.e. proximal forelimb, were classified as "upper forelimb". Movements of the wrist and digits were called "lower forelimb", and "hindlimb" included any movement in the hindlimb of the rat. Cortical area was calculated

by multiplying the number of sites eliciting a response by 0.25 mm^2 (0.5 mm x 0.5 mm). Four sites equal 1 mm^2.

3.5 RESULTS

Rats reached the last stage of the wheel spin task in 30±5 sessions and the lever press task in 12±1 sessions (mean ± standard error of the mean). The percent of successfully attempted trials on the wheel spin task on the first day of VNS paired training was 77±4%. The same measure for the lever press task on the first day of VNS paired training was 78±4%. Microelectrode mapping techniques were used to determine the organization of the motor cortex after five days of VNS paired training on the last stage. Maps of the motor cortex were derived from 3,375 electrode penetrations (average 102 sites per animal).

Organization of the motor maps generated in the rats used in this study are consistent with the general organization of the rat motor cortex observed in previous studies (Hall and Lindholm 1974, 23-38; Neafsey et al. 1986, 77-96; Gioanni and Lamarche 1985, 49-61). In all rats tested, the anterior portion of the motor map generated movements of the rat's head, including the jaw, vibrissa, and neck (Figure 3.2a). Movements of the wrist and digits, denoted as the lower forelimb, were represented in the middle of the motor map. Movements of the hindlimb were represented posterior to the forelimb region of primary motor cortex. The organization of primary motor cortex was not significantly altered by training without VNS. The average area representing the lower forelimb, upper forelimb, head, and hindlimb were not significantly different across the naïve, wheel spin, or lever press trained rats with sham VNS cuffs, i.e. did not receive VNS (Table 1; Partially repeated measures ANOVA Main Effect of

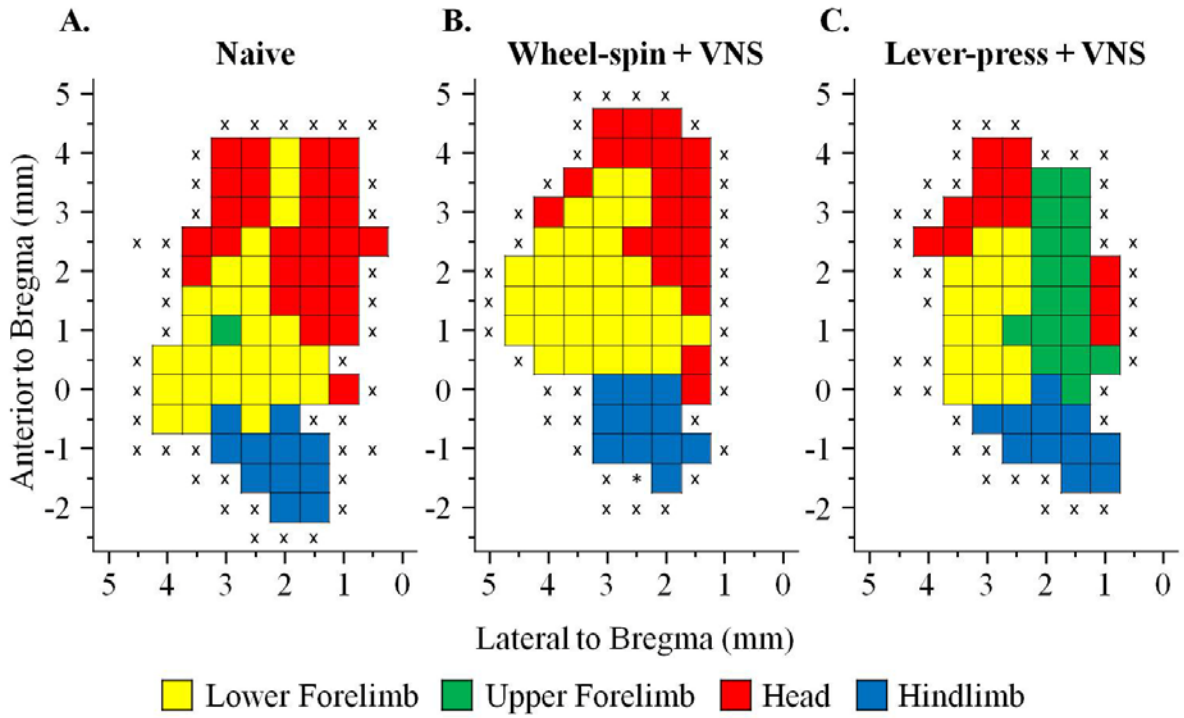

Figure 3.2
Representative motor maps of a naïve rat, a rat trained with wheel-spin paired with VNS, and a rat trained with lever-press paired with VNS. Each square represents a 0.25 mm^2 (0.5 x 0.5 mm) area. Electrode penetrations occurred in the middle of each square (±0.05 mm). Movements of the neck, face, vibrissae, and eye were classified as "head" (represented in red). Movements of the forelimb originating below the elbow were considered "lower forelimb" (represented in yellow). Movements originating between the shoulder and the elbow, including the elbow, were considered "upper forelimb" (represented in green). Any movements in the hindlimb of the rat were classified as "hindlimb" and are represented in blue. Sites eliciting no response with 200 µA of current are marked with an "x". Sites in the posterior vibrissae area are marked with an asterisk and were considered border sites to the overall somatotopic map in this study.

group, $F(2, 18)= 2.8$, $P= 0.09$; Group x area interaction, $F(6, 54)= 0.26$, $P= 0.95$). As a result, these three control groups are averaged for group analyses and referred to as the non-VNS group.

Rats that received VNS paired with the wheel spin task exhibited a significant reorganization of the motor cortex. In the non-VNS rats, the head and lower forelimb occupy approximately the same amount of cortical area (7.00 mm^2 and 6.75 mm^2 respectively in the example naïve rat shown in Figure 3.2a). Hindlimb and upper forelimb comprise a smaller region

Group	n	Lower Forelimb	Upper Forelimb	Head	Hindlimb
Naïve	8	6.3 ± 0.5	1.1 ± 0.4	5.7 ± 0.7	3.4 ± 0.3 mm^2
Wheel Spin Sham	5	5.5 ± 0.6	0.9 ± 0.4	5.6 ± 0.6	3.1 ± 0.3 mm^2
Lever Press Sham	8	5.8 ± 0.6	1.5 ± 0.3	6.5 ± 0.8	3.4 ± 0.4 mm^2
Wheel Spin + VNS	5	7.8 ± 0.2*	0.5 ± 0.3	3.7 ± 1.0*	3.4 ± 0.4 mm^2
Lever Press + VNS	7	4.5 ± 0.3*	3.1 ± 0.6*	4.2 ± 0.5*	3.0 ± 0.4 mm^2

Table 3.1
Presents the group average sizes of each area in the motor cortex followed by the standard error of the mean. Averages are reported in mm^2 followed by the standard error of the mean. Naive and sham groups were not significantly different from each other $F(2, 18)= 2.8.$, $P= 0.9$), therefore they were grouped together for further analysis. Asterisks represent a $p< 0.05$ in a t-test comparison to the naïve group.

of the motor map (2.75 mm^2 and 0.25 mm^2 respectively in the example naïve rat shown). Figure 3.2b shows a representative example of motor cortex following VNS/Wheel spin pairing. Wheel spin/VNS pairing resulted in larger lower forelimb area, smaller head area, and no upper forelimb area in this particular animal compared to the naive (7.75 mm^2 and 5.25 mm^2). These changes in cortical area for the VNS/Wheel spin paired group were significant when compared to the non-VNS group ($P< 0.05$, Figure 3.3). On average, pairing VNS with the wheel spin task caused a 32% increase in the cortical area representing the lower forelimb compared to the non-VNS group ($t(24)= 3.0$, $P= 0.003$). This increase was accompanied by a 38% smaller head area and a 63% smaller upper forelimb area ($t(24)= 2.4$, $P= 0.01$; $t(24)= 1.8$, $P= 0.04$, respectively). There was no change in the area devoted to hindlimb. These results suggest that repeatedly

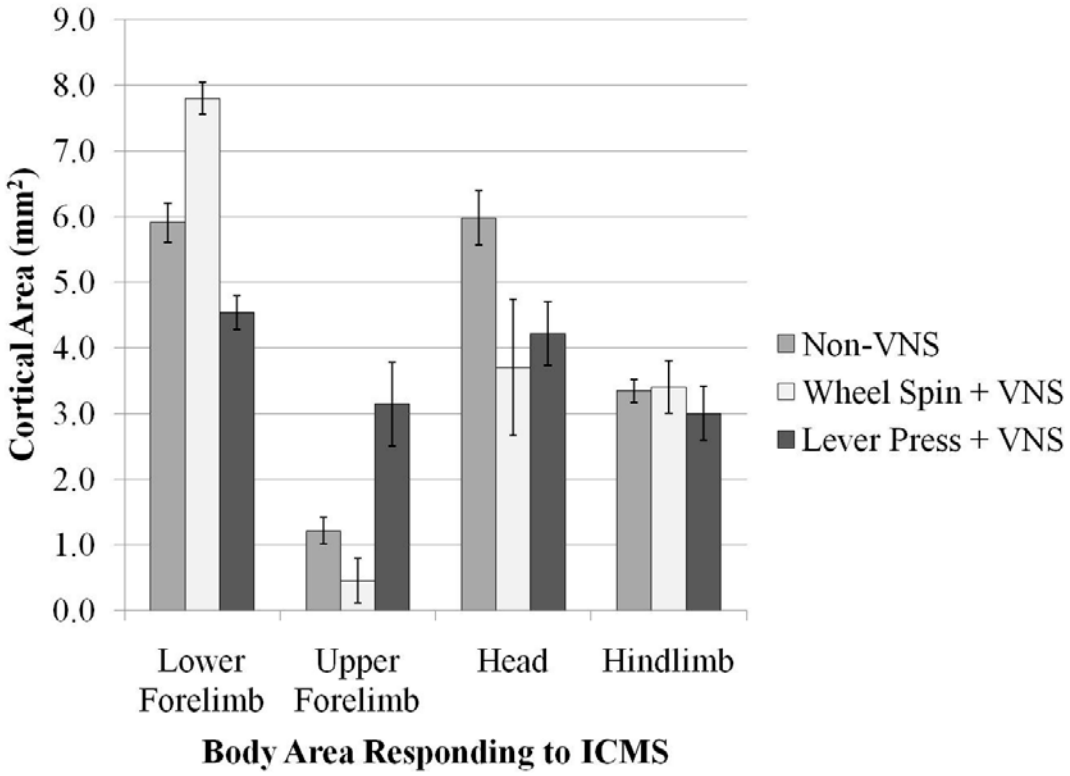

Figure 3.3
The average amount of cortical area that elicits movement of a specific body area using intracortical microstimulation for rats in the non-VNS group (n=21), rats trained on the wheel spin task paired with VNS (n=5), and the rats trained on the lever press task paired with VNS (n=7). The non-VNS group consists of the naïve rats and the rats trained on the tasks without VNS. No difference was found amongst the groups composing the non-VNS group ($F(2, 18)= 2.8.$, $P= 0.09$). Cortical area was calculated by multiplying the number of sites eliciting a response by 0.25 mm^2 (0.5 mm x 0.5 mm). Four sites are equal to 1 mm^2. Error bars represent the standard error of the mean. Brackets represent significant differences as follows between groups within a specific body area ($P< 0.05$).

pairing VNS with a particular movement can generate a specific increase in the motor cortex representation of that movement.

To confirm that the observed cortical plasticity was specific to the movement paired with VNS, we also documented the reorganization of motor cortex in rats that received VNS paired with a lever press task. Since this task primarily involves movement of the upper forelimb, we expected to see an increased upper forelimb representation after lever press/VNS pairing. A

cortical map from a representative rat shown in Figure 3.2c exhibited a dramatically larger upper forelimb area compared to the naïve rat (Figure 3.2a). The lever press/VNS rat had 4 mm^2 more area devoted to the upper forelimb area compared to the naïve rat. Pairing VNS with the lever press movement reduced the head and lower forelimb areas in this rat by 2.75 mm^2 and 4 mm^2, respectively, compared to the naïve rat. Like the wheel spin/VNS trained rat, the lever press/VNS rat had the same sized hindlimb representation as the naïve rat. These individuals suggest that the motor cortex plasticity observed following VNS-movement pairing is specific to the paired movement and not a general effect of VNS.

On average, rats that received VNS during the lever task exhibited a 159% increase in the upper forelimb area compared to the non-VNS group (Figure 3.3; $t(26)= 4.1$, $P= 0.0002$). The lever press/VNS group had a 23% smaller lower forelimb area and a 29% smaller head area than the non-VNS group ($t(26)= 2.5$, $P= 0.009$; $t(26)= 2.3$, $P= 0.01$; respectively). The most striking differences were observed between the wheel spin/VNS rats and the lever press/VNS rats. Wheel spin trained rats had a 72% larger lower forelimb area than the lever press rats and the lever press rats had a 598% larger upper forelimb area ($t(10)= 9.6$, $P= 1x10^{-6}$; $t(10)= 3.6$, $P= 0.002$; respectively). These results demonstrate that VNS-movement pairing can generate large-scale reorganization of motor cortex and confirm that the reorganization is specific to the movement repeatedly paired with VNS.

The increased representations that were observed following movement paired VNS could have potentially caused movement impairments similar to those seen in focal dystonia. We compared task performance in each group to confirm that movement paired VNS does not make the task more difficult. No behavioral differences were observed between VNS and sham groups

on the wheel spin task in the total number of successful trials (VNS=1584±73 trials, Sham= 1694±148 trials, mean ± s.e.m, $t(8)= 0.75$, $P=0.24$), the velocity at which the wheel was spun (VNS= 259±32 degrees per sec, Sham= 307±29 degrees per sec, $t(8)= 1.3$, $P=0.12$), or the percentage of successfully completed trials per session (VNS= 68±4%, Sham= 74±5%, $t(8)= 1.1$, $P= 0.14$). VNS rats showed no impairment on the lever press task and, in fact, exhibited shorter lever press intervals (VNS= 231.6±10.5 ms, Sham= 287.7±14.8 ms, $t(13)= 3.2$, $P= 0.007$) and triple pressed the lever more often than the sham rats (VNS= 107.3±25.6 trials per session, Sham= 56.3±6.2 trials per session, $t(13)= 2.2$, $P= 0.04$). Although VNS enhanced some aspects of the lever press task, the percent of successful trials and the total number of successful trials were not different between the VNS and sham rats (VNS=1023±46 trials; Sham= 926±48 trials, mean ± s.e.m; $t(13)= 1.5$, $P=0.07$; VNS= 85±3%, Sham= 81±3%, $t(13)= 1.3$, $P= 0.20$; respectively). These results indicate that VNS is unlikely to have enhanced map reorganization by making the task more difficult.

3.6 DISCUSSION

3.6.1 General

Based on a recent study in the auditory cortex (Engineer et al. 2011, 101-104), we predicted that repeatedly pairing brief stimulation of the vagus nerve with a specific movement would result in a larger representation of that movement in the motor cortex. To test this prediction, we delivered 0.5 sec of VNS each time rats used their lower forelimb to rotate a wheel. After several hundred pairings, the cortical representation of the lower forelimb was significantly larger in these rats compared to naïve rats and rats that performed the same movements without VNS. A second group of rats were trained on a motor task using a different part of their body to confirm that map reorganization was specific to the movement paired with VNS. Pairing VNS with a lever press task that required the use of the upper forelimb resulted in a significantly larger upper forelimb representation. The observations that map expansion was specific to the movement paired with VNS and that neither task generated map reorganization without VNS indicate that movement paired VNS is sufficient to direct map plasticity.

Pairing VNS with a motor event generated cortical plasticity comparable to that observed using a similar paradigm in the auditory system. Presenting a tone with a brief period of VNS causes a significant expansion of the paired tone's representation in the auditory cortex (Engineer et al. 2011, 101-104). Presenting tones or VNS alone did not alter the auditory cortex's

tonotopic organization. These two studies suggest that the plasticity enhancing mechanisms of event-paired VNS are common to the auditory and motor cortex.

3.6.2 Potential mechanisms

The exact mechanisms by which VNS directs plasticity in motor or sensory cortex are unknown. VNS causes the release of several molecules known to enhance cortical plasticity, including acetylcholine, norepinephrine, serotonin, and brain derived neurotrophic factor (Albert et al. 2009, 1042-1060; Hassert, Miyashita, and Williams 2004, 79-88; Détári, Juhász, and Kukorelli 1983, 147-154; Dorr and Debonnel 2006, 890-898; Follesa et al. 2007, 28-34). Perfusing norepinephrine into an adult cat's visual cortex produces kitten-like plasticity in a test of ocular dominance shifts following monocular deprivation (Pettigrew and Kasamatsu 1978, 761-763; Kasamatsu, Pettigrew, and Ary 1979, 163-181). Neurotoxins and reuptake inhibitors targeting serotonin block the normal ocular dominance shifts in kittens in monocular deprivation, implicating the importance of serotonin for normal plasticity (Gu and Singer 1995, 1146-1153). Blocking the release of acetylcholine prevents cortical plasticity and interferes with skill learning and recovery from brain damage (Conner et al. 2003, 819-829; Conner, Chiba, and Tuszynski 2005, 173-179; Conner, Kulczycki, and Tuszynski 2010, 2739-2748). Adding brain derived neurotrophic factors induces plastic changes in ocular dominance shifts in adult rats following monocular deprivation (Vetencourt et al. 2008, 385-388). Combining more than one of these elements can lead to greater plasticity than the influence of the elements singularly (Bear and Singer 1986, 172-176; Seol et al. 2007, 919-929). The inability of wheel-spin or lever-press training to produce cortical plasticity without VNS supports the importance of the VNS triggered release of these molecules in enhancing cortical plasticity. VNS is likely to generate cortical map

plasticity specific to the associated event through the synergistic action of multiple plasticity enhancing molecules.

3.6.3 Importance of timing

The simultaneous presentation of VNS with a specific sensory or motor event is sufficient to increase cortical representation of that movement. In this study, we used a sugar pellet to reward the animal's behavior immediately after the completion of a trial. As a result, VNS was delivered during the behavioral task as well as just a few seconds prior to the animal eating the pellets. It would not have been surprising to see an increased representation of the head and jaw in this study.

In a previous study, our lab demonstrated that changes in auditory cortex were temporally specific to tones paired with VNS (Engineer et al. 2011, 101-104). Two randomly interleaved tones were presented every 15-45 sec for several thousand trials to a rat with only one of the tones paired with VNS (Engineer et al. 2011, S2-S3). The number of sites responding to the VNS paired tone increased significantly, while the number of sites for the tone presented within tens of seconds of the VNS did not. These observations are consistent with past studies demonstrating that pairing nucleus basalis stimulations with tones only alters the tones representations when they occurred within seconds of each other (Kilgard and Merzenich 1998, 1714-1717; Weinberger 2003, 268-284).

The results from the current study found that muscles of mastication did not increase as a result of VNS just prior to chewing. This result indicates that the plasticity enhancing actions of VNS are temporally precise, lasting less than 1-2 seconds. These results demonstrate that brief pulses of VNS can be used to direct highly specific plasticity. Methods for enhancing plasticity

that rely on slow-acting mechanisms may not be as effective in generating the same accuracy of plasticity as VNS-pairing. Pharmaceuticals often elevate or diminish certain neurotransmitters for several hours. Several movements or sensory events may occur repeatedly during this time, potentially creating unwanted plasticity. The temporal precision of the VNS-pairing method for enhancing cortical plasticity should offer significant advantages in efficiency and efficacy as compared to methods with less precise actions.

3.6.4 Potential clinical uses of paired-VNS

Stroke and traumatic brain injury often damage movement-controlling areas of the motor cortex resulting in hemiparesis or hemiplegia. Following cortical injury, lost motor representations can partially regenerate in neighboring areas within motor cortex (Eysel et al. 1999, 153-164; Conner, Chiba, and Tuszynski 2005, 173-179). The size of the regenerated representations is highly correlated with the functional recovery of lost movements, but this recovered area and ability is a fraction of those seen pre-injury (Castro-Alamancos and Borrell 1995, 793-805; Ramanathan, Conner, and Tusznyski 2006, 11370-11375). Physical training in healthy animals can greatly increase cortical representation of the muscles used, but rehabilitative physical training in rats after a motor cortical injury does not generate near the same change in area (Conner, Chiba, and Tuszynski 2005, 173-179; Kleim et al. 1998, 3321; Molina-Luna et al. 2008, 1748). Movement paired VNS generates a comparable amount of cortical plasticity in approximately the same amount of time as physical training (Kleim, Barbay, and Nudo 1998, 3321-3325; Molina-Luna et al. 2008, 1748-1754; Nudo et al. 1996, 785-807). Movement paired VNS is also able to enhance plasticity where plasticity is not observed with training alone. As a result, movement paired VNS holds the possibility to enhance regenerative

plasticity for specific motor functions following cortical injury as compared to physical training alone. If cortical reorganization is representative of improved function, then pairing weakened movements with VNS in a patient with hemiparesis may increase functional recovery well beyond rehabilitative training alone.

Periodic VNS using a stimulation protocol similar to that used in treating epilepsy has improved functional recovery in rats with fluid percussion injury to the cortex (Smith et al. 2005, 1485-1502; Smith et al. 2006, 1549-1560). Periodic VNS is FDA approved as a safe and effective treatment of certain types of refractory epilepsy as well as treatment-resistant depression (Groves and Brown 2005, 493-500; Albert et al. 2009, 1042-1060; Binnie 2000, 161-169; Ben-Menachem 2001, 415-418). Protocols for treating epilepsy require 30 sec. of VNS every 5 min., using 750 times the current per stimulation compared to what was used in the current study (Groves and Brown 2005, 495-496; Albert et al. 2009, 1051). Our results demonstrate that paired VNS generates significantly more plasticity than unpaired VNS in both motor and auditory systems. It seems likely that therapies using paired VNS might be a more effective therapy. However, one needs to be very careful with this method when treating epilepsy. Pairing VNS with the detection of the onset of a seizure could strengthen those abnormal activity patterns making the patient worse.

In addition, VNS-movement pairing may possibly be used to shrink the expanded motor representations associated with spasticity (i.e. focal dystonia). The larger representations observed from the VNS paired movements were accompanied by smaller nearby cortical representations, such as movements of the head. Selectively increasing the size of surrounding muscle representations should decrease the overrepresentation of the dystonic muscles. This

selective VNS-pairing method has been used to treat tinnitus in rats (Engineer et al. 2011, 101-104). The overrepresentation of a tone was reduced by pairing VNS with tones spanning the rats hearing range except for the tone at the tinnitus frequency. This eliminated the perception of the phantom noise for at least several months past the cessation of the treatment. The strategic pairing of non-dystonic movements with VNS may relieve the symptoms of focal dystonia.

3.6.5 Conclusion

VNS paired with tone presentations previously demonstrated enhanced cortical representations for the paired tone (Engineer et al. 2011, 101-104). We have confirmed that a similar method of VNS pairing with movements causes specific plasticity for the paired movement in the motor cortex. Furthermore, both studies demonstrated the importance of the temporal precision of pairing VNS with the sensory or motor event in order to enhance cortical plasticity. These results suggest that VNS-pairing acts as a general method for causing highly specific cortical plasticity. VNS-movement pairing could provide a new approach for treating a range of movement disorders from cortical damage or disorders associated with distorted movement representations.

CHAPTER 4

CONCLUSION

The goals of this dissertation were 1) to examine the effects of lesions in an area of cortex where the resulting deficits were not well understood (Chapter 2) and 2) to explore a potential method for inducing specified cortical representation enhancements that may one day be used to treat patients (Chapter 3).

Chapter 2 documented a series of experiments aimed at determining the role of the auditory cortex in speech perception through the use of cortical lesions. Our recent paper suggested that bilateral auditory cortex lesions do not affect speech discrimination ability (Floody et al. 2010, 260-268). This conclusion seemingly conflicts with numerous other studies demonstrating that complex sounds similar to speech are impaired after lesions (Diamond, Goldberg, and Neff 1962, 223-225; Whitfield 1980, 644-647; Heffner and Heffner 1986, 683-701; Heffner and Heffner 1989, 275-285; Harrington, Heffner, and Heffner 2001, 1217-1221). We have confirmed that auditory cortex lesions do not affect speech discrimination of whole words. Rats did not demonstrate a lasting impairment for the discrimination of speech sounds even in a background noise presented 24 dB louder than the stimuli.

A previous neurophysiology study in our lab demonstrated that the behavioral discrimination of words is better correlated to differences in the neural response patterns in the first 40 ms of the response to a word than the whole response (Engineer et al. 2008, 603-608). Based on this study, rats were trained to discriminate several speech stimuli that were reduced to

the first 40 ms. The shortened stimuli reduced several potential cues that other areas in the auditory system may use to form discriminations, such as duration or amplitude differences. Rats trained on the shortened speech sounds took longer to learn the discriminations than rats trained with the full-length versions of the same sounds, but were able to discriminate the sounds equally as well as the full-length trained rats by the end of training. This result suggests that the additional acoustic cues included in the full-length stimuli facilitate the learning of the speech discrimination task, but are not required to perform the task accurately.

The rats trained on the shortened speech sounds received bilateral auditory cortex lesions and then retested on the discriminations. These rats demonstrated an immediate impairment to chance levels of performance, indicating that damaging the auditory cortex disrupted the ability of the rats to discriminate the shortened speech sounds. Lost discrimination ability of the first 40 ms of the words was not recovered, demonstrating that the auditory cortex was specialized for processing these shortened complex sounds.

Chapter 3 documented a series of experiments that paired vagus nerve stimulation (VNS) with two motor tasks. Passively listening to a tone paired with VNS resulted in increased representations of the tone in auditory cortex (Engineer et al. 2011, 101-104). Passive listening without VNS did not affect the tonotopic organization of the auditory cortex. Rats performing a wheel-spin or lever-press task without VNS also did not demonstrate significant differences in the organization of their motor cortex. VNS paired with the wheel spin task resulted in larger representations of the lower forelimb compared to rats trained without VNS and experimentally naïve rats. To confirm that the map expansion was a result of pairing VNS with the movement used to perform the task, a different group of rats performed a lever-press task paired with VNS.

These rats demonstrated significantly larger upper forelimb representations than those trained without VNS or experimentally naïve rats. This led us to the conclusion that VNS paired with a movement can selectively increase the representation of that movement.

Pairing VNS with tones surrounding a tinnitus frequency in rats caused the reversal of the overrepresented tinnitus tone and eliminated the perception of the phantom noise (Engineer et al. 2011, 101-104). We suggest that this may be a method for increasing movement representations following cortical damage to increase recovery or to selectively decrease an overrepresented movement representation, such as those seen in focal dystonia.

Work in this dissertation has demonstrated that the auditory cortex has an important role in processing the rapidly occurring acoustic transitions at the beginning of speech sounds. Additionally, we have shown that pairing VNS with a movement causes similar adaptations to the cortical representations as observed using a similar paradigm in the auditory cortex. Future experiments should focus on directing rehabilitative methods specific to the impairments observed following auditory cortex lesions. This research may have clinical implications in children with specific language impairments, whom also demonstrate difficulties in processing the rapidly occurring acoustic transitions that distinguish similar speech sounds. Movement paired VNS has potential clinical uses in stroke patients and patients with focal dystonia. Our research is event paired VNS has further demonstrated that some of the mechanisms regulating adaptations in the motor and sensory systems are the similar. Future research needs to clarify and focus on determining how similar the factors influencing cortical change are between the sensory and motor systems. Finding that these influences are more similar than different may allow

rehabilitative techniques used to treat disorders in one system to be used effectively in another system.

BIBLIOGRAPHY

Adams, F., R. Schwarting, and J. Huston. 1994. Behavioral and neurochemical asymmetries following unilateral trephination of the rat skull: Is this control operation always appropriate? *Physiology & Behavior* 55, no.55: 947-52.

Albert, G.C., C. M. Cook, F.S. Prato, and A.W. Thomas. 2009. Deep brain stimulation, vagal nerve stimulation and transcranial stimulation: An overview of stimulation parameters and neurotransmitter release. *Neuroscience and Biobehavioral Reviews* 33, no.7: 1042-60.

Bakin, J.S. and N.M. Weinberger, N. M. 1990. Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Research* 536, no.1-2: 271-86.

Baru, A.V. and G.N. Shmigidina. 1977. Role of the auditory cortex in recognition of synthesized vowels by dogs. *Neuroscience and Behavioral Physiology* 8, no.3:197-204.

Bear, M.F. and W. Singer. 1986. Modulation of visual cortical plasticity by acetylcholine and noradrenaline. *Nature* 320, no.6058: 172-6.

Ben-Menachem, E. 2001. Vagus nerve stimulation, side effects, and long-term safety. *Journal of Clinical Neurophysiology; Official Publication of the American Electroencepholographic Society* 18, no.5: 415-8.

Bertoncini, J., R. Bijeljac-Babic, S. Blumstein, and J. Mehler. 1987. Discrimination in neonates of very short CVs. *The Journal of the Acoustical Society of America* 82, no.1: 31-7.

Binnie, C.D. 2000. Vagus nerve stimulation for epilepsy: a review. *Seizure: A Journal of the British Epilepsy Association* 9, no.3: 161-9.

Blumstein, S.E. and K.N. Stevens. 1980. Perceptual invariance and onset spectra for stop consonants in different vowel environments. *The Journal of the Acoustical Society of America* 67, no.2: 648-62.

Bradlow, A.R., D.B. Pisoni, R. Akahane-Yamada, and Y. Tohkura. 1997. Training Japanese listeners to identify English /r/ and /l/: IV. Some effects of perceptual learning on speech production. *The Journal of the Acoustical Society of America* 101, no.4: 2299-310.

Butler, R.A., I.T. Diamond, and W.D. Neff. 1957. Role of auditory cortex in discrimination of changes in frequency. *Journal of Neurophysiology* 20, no.1: 108-20.

Butterworth, S., S. Francis, E. Kelly, F. McGlone, R. Bowtell, and G.V. Sawle. 2003. Abnormal cortical sensory activation in dystonia: an fMRI study. *Movement Disorders : Official Journal of the Movement Disorder Society* 18, no.6: 673-82.

Byl, N.N., M.M. Merzenich, and W.M. Jenkins. 1996. A primate genesis model of focal dystonia and repetitive strain injury: I. Learning-induced dedifferentiation of the representation of the hand in the primary somatosensory cortex in adult monkeys. *Neurology* 47, no.2: 508-20.

Castro-Alamancos, M.A. and J. Borrell. 1995. Functional recovery of forelimb response capacity after forelimb primary motor cortex damage in the rat is due to the reorganization of adjacent areas of cortex. *Neuroscience* 68m no.3: 793-805.

Clark, K.B., D.K. Naritoku, D.C. Smith, R.A. Browning, and R.A. Jensen. 1999. Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nature Neuroscience* 2, no.1: 94-8.

Clark, M.G., G.D. Rosen, P. Tallal, and R.H. Fitch. 2000. Impaired processing of complex auditory stimuli in rats with induced cerebrocortical microgyria: An animal model of develpomental language disabilities. *Journal of Cognitive Neuroscience* 12, no.5: 828-39.

Clough, R.W., S.L. Neese, L.K. Sherill, A.A. Tan, A. Duke, R.W. Roosevelt, R.A. Browning, and D.C. Smith. 2007. Cortical edema in moderate fluid percussion brain injury is attenuated by vagus nerve stimulation. *Neuroscience* 147, no.2: 286-93.

Conner, J.M., A.A. Chiba, and M.H. Tuszynski. 2005. The basal forebrain cholinergic system is essential for cortical plasticity and functional recovery following brain injury. *Neuron* 46, no.5: 173-9.

Conner, J.M., A. Culberson, C. Packowski, A.A. Chiba, and M.H. Tuszynski. 2003. Lesions of the basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning. *Neuron* 38, no.5: 819-29.

Conner, J.M., M. Kulczycki, and M.H. Tuszynski. 2010. Unique contributions of distinct cholinergic projections to motor cortical plasticity and learning. *Cerebral Cortex* 20, no.11: 2739-48.

Cooke, J.E., H. Zhang, and J.B. Kelly. 2007. Detection of sinusoidal amplitude modulated sounds: Deficits after bilateral lesions of auditory cortex in the rat. *Hearing Research* 231, no.1-2: 90-9.

Cranford, J.L., M. Igarashi, and J.H. Stramler. 1976. Effect of auditory neocortex ablation on identification of click rates in cats. *Brain Research* 116, no.1: 69-81.

Dewson, J.H. 1964. Speech sound discrimination by cats. *Science* 144, no.1: 555-6.

Dewson, J.H., K. Pribram, and J.C. Lynch. 1969. Effects of ablations of temporal cortex upon speech sound discrimination in the monkey. *Experimental Neurology* 24, no.4: 579-91.

Diamond, I.T., J.M. Goldberg, and W.D. Neff. 1962. Tonal discrimination after ablation of auditory cortex. *Journal of Neurophysiology* 25: 223-5.

Doron, N., J. LeDoux, and M. Semple. 2002. Redefining the tonotopic core of rat auditory cortex: Physiological evidence for a posterior field. *The Journal of Comparative Neurology* 453, no.4: 345-60.

Dorr, A.E. and G. Debonnel. 2006. Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *The Journal of Pharmacology and Experimental Therapeutics* 318, no.2: 890-8.

Détári, L., G. Juhász, and T. Kukorelli. 1983. Effect of stimulation of vagal and radial nerves on neuronal activity in the basal forebrain area of anaesthetized cats. *Acta Physiologica Hungarica* 61, no.3: 147-54.

Elger, G., C. Hoppe, P. Falkai, A.J. Rush, and C.E. Elger. 2000. Vagus nerve stimulation is associated with mood improvements in epilepsy patients. *Epilepsy Research* 42, no.2-3: 203-10.

Engineer, C.T., C.A. Perez, Y.H. Chen, R.S. Carraway, A.C. Reed, J.A. Shetake, V. Jakkamsetti, K.Q. Chang, and M.P. Kilgard. 2008. Cortical activity patterns predict speech discrimination ability. *Nature Neuroscience* 11, no.5: 603-8.

Engineer, N.D., J.R. Riley, J.D. Seale, W.A. Vrana, J. Shetake, S.P. Sudanagunta, M.S. Borland, and M.P. Kilgard. 2011. Reversing pathological neural activity using targeted plasticity. *Nature* 470, no. 7332: 101-4.

Evarts, E.V. 1952. Effect of auditory cortex ablation on frequency discrimination in monkey. *Journal of Neurophysiology* 15, no.6: 443-8.

Eysel, U.T., G. Schweigart, T. Mittmann, D. Eyding, Y. Qu, F. Vandesande, G. Orban, and L. Arckens. 1999. Reorganization in the visual cortex after retinal and cortical damage. *Restorative Neurology and Neuroscience* 15, no.2-3: 153-64.

Fitch, R.H., P. Tallal, C.P. Brown, C.P., A.M. Galabudra, and G.D Rosen. 1994. Induced microgyria and auditory temporal processing in rats: A model for language impairment? *Cerebral Cortex* 4, no.3: 260-70.

Floel, A. and L.G. Cohen. 2010. Recovery of function in humans: cortical stimulation and pharmacological treatments after stroke. *Neurobiology of Disease* 37, no.2: 243-51.

Floody, O.R. and M.P. Kilgard. 2007. Differential reductions in acoustic startle document the discrimination of speech sounds in rats. *The Journal of the Acoustical Society of America* 122, no.4: 1884-7.

Floody, O.R., L. Ouda, B.A. Porter, and M.P. Kilgard. 2010. Effects of damage to auditory cortex on the discrimination of speech sounds by rats. *Physiology & Behavior* 101, no.2: 260-8.

Follesa, P., F. Biggio, G. Gorini, S. Caria, G. Talani, L. Dazzi, M. Puligheddu, F. Marrosu, and G. Biggio. 2007. Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Research* 1179: 28-34.

Fregni, F., P.S. Boggio, A.C. Valle, R.R. Rocha, J. Duarte, M.J. Ferreira, T. Wagner, S. Fecteau, S.P. Rigonatti, M. Riberto, S.D. Freedman, and A. Pascual-Leone. 2006. A sham-controlled trial of a 5-day course of repetitive transcranial magnetic stimulation of the unaffected hemisphere in stroke patients. *Stroke; A Journal of Cerebral Circulation* 37, no.8: 2115-22.

Galaburda, A., J. LoTurco, F. Ramus, R. Fitch, and G. Rosen. 2006. From genes to behavior in developmental dyslexia. *Nature Neuroscience* 9, no.10: 1213-7.

Ghacibeh, G.A., J.I. Shenker, B. Shenal, B.M. Uthman, and K. Heilman. 2006. The influence of vagus nerve stimulation on memory. *Cognitive and Behavioral Neurology: Official Journal of the Society for Behavioral and Cognitive Neurology* 19, no.3: 119-22.

Gioanni, Y. and M. Lamarche. 1985. A reappraisal of rat motor cortex organization by intracortical microstimulation. *Brain Research* 344, no.1: 49-61.

Goldberg, J.M. and W.D. Neff. 1961. Frequency discrimination after bilateral ablation of cortical auditory areas. *Journal of Neurophysiology* 24: 119-28.

Goto, H. 1971. Auditory perception by normal Japanese adults of the sounds "L" and "R". *Neuropsychologia* 9, no.3: 317-23.

Groves, D.A. and V.J. Brown. 2005. Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. *Neuroscience and Biobehavioral Reviews* 29, no.3: 493-500.

Gu, Q. and W. Singer. 1995. Involvement of serotonin in developmental plasticity of kitten visual cortex. *The European Journal of Neuroscience* 7, no.6: 1146-53.

Hall, R.D. and E.P. Lindholm. 1974. Organization of motor and somatosensory neocortex in the albino rat. *Brain Research* 66, no.1: 23-38.

Harrington, I.A., R. Heffner, and H.E. Heffner. 2001. An investigation of sensory deficits underlying the aphaisa-like behavior of macaques with auditory cortex lesions. *Neuroreport* 12, no.6: 1217-21.

Hassert, D.L., T. Miyashita, and C.L. Williams. 2004. The effects of peripheral vagus nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. *Behavioral Neuroscience* 118, no.1: 79-88.

Heffner, H.E. 1978. Effect of auditory cortex ablation on localization and discrimination of brief sounds. *Journal of Neurophysiology* 41, no.4: 963-76.

Heffner, H.E. and R.S. Heffner. 1989. Cortical deafness cannot account for the inability of Japanese macaques to disciminate species-specific vocalizations. *Brain and Language* 36, no.2: 275-85.

Heffner, H.E. and R.S. Heffner. 1986. Effect of unilateral and bilateral auditory cortex lesions on the discrimination of vocalizations by Japanese macaques. *Journal of Neurophysiology* 56, no.3: 683-701.

Hinkley, L.B.N., R.L. Webster, N.N. Byl, and S.S. Nagarajan. 2009. Neuroimaging characteristics of patients with focal hand dystonia. *Journal of Hand Therapy: Official Journal of the American Society of Hand Therapists* 22, no.2: 125-34.

Hubel, D.H., T.N. Wiesel, and S. LeVay. 1977. Plasticity of ocular dominance columns in monkey striate cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 278, no.961: 377-409.

Hummel, F., P. Celnik, P. Giraux, A. Floel, W.-H. Wu, C. Gerloff, and L.G. Cohen. 2005. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain: A Journal of Neurology* 128, pt. 3: 490-9.

Kasamatsu, T., J.D. Pettigrew, and M. Ary. 1979. Restoration of visual cortical plasticity by local microperfusion of norepinephrine. *The Journal of Comparative Neurology* 185, no.1: 163-81.

Kawahara, H., I. Masuda-Katsuse, and A. de Cheveign´e. 1999. Restructuring speech representations using a pitch adaptive time-frequency smoothing and an instantaneous-

frequency-based F0 extraction: Possible role of a repetitive structure in sounds. *Speech Communication* 27, no.3: 187-207.

Kelly, J.B. and B. Masterton. 1977. Auditory sensitivity of the albino rat. *Journal of Comparative and Physiological Psychology* 91, no.4: 930-6.

Khedr, E.M., M.A. Ahmed, N. Fathy, and J.C. Rothwell. 2005. Therapeutic trial of repetitive transcranial magnetic stimulation after acute ischemic stroke. *Neurology* 65, no.3: 466-8.

Kilgard, M.P. and M.M. Merzenich. 1998. Cortical map reorganization enabled by nucleus basalis activity. *Science* 279, no.5357: 1714-7.

Kleim, J.A., S. Barbay, and R.J. Nudo. 1998. Functional reorganization of the rat motor cortex following motor skill learning. *Journal of Neurophysiology* 80, no.6: 3321-5.

Kleim, J.A., T.M. Hogg, P.M. VandenBerg, N.R. Cooper, R. Bruneau, and M. Remple. 2004. Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *The Journal of Neuroscience: The Official Journal for the Society for Neuroscience* 24, no.3: 628-33.

Kleim, J.A. and T.A. Jones. 2008. Principles of experience-dependent neural plasticity: Implications for rehabilitation after brain damage. *Journal of Speech, Language, and Hearing Research* 51, no.1: S225-39.

Kudoh, M., Y. Nakayama, R. Hishida, and K. Shibuki. 2006. Requirement of the auditory association cortex for discrimination of vowel-like sounds in rats. *Neuroreport* 17, no.17: 1761-6.

Kuhl, P.K. and J.D. Miller. 1975. Speech perception by the Chinchilla: Voiced-voiceless distinction in alveolar plosive consonants. *Science* 190, no.4209: 69-72.

Lerner, A., H. Shill, T. Hanakawa, K. Bushara, A. Goldfine, and M. Hallett. 2004.Regional cerebral blood flow correlates of the severity of writer's cramp symptoms. *NeuroImage* 21, no.3: 904-13.

Lin, P.T. and M. Hallett. 2009. The pathophysiology of focal hand dystonia. *Journal of Hand Therapy : Official Journal of the American Society of Hand Therapists* 22, no.2: 109-13.

Martin, C.O., N.L. Denburg, D. Tranel, M.A. Granner, and A. Bechara. 2004. The effects of vagus nerve stimulation on decision-making. *Cortex; A Journal Devoted to the study of the Nervous System and Behavior* 40, no.4-5: 605-12.

Meng, H., S. Smith, K. Hager, M. Held, J. Liu, R. Olson, B. Pennington, J. DeFries, J. Gelernter, T. O'Reilly-Pol, S. Somlo, P. Skudlarski, S. Shaywitz, B. Shaywitz, K. Marchione, Y.

Wang, M. Paramasivam, J. LoTurco, G. Page, and J. Gruen. 2005. DCDC2 is associated with reading disability and modulates neuronal development in the brain. *Proceedings of the National Academy of Sciences of the United States of America* 102, no.47: 17053-8.

Merzenich, M., J.H. Kaas, J. Wall, R.J. Nelson, M. Sur, and D. Felleman. 1983. Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. *Neuroscience* 8, no.1: 33-55.

Merzenich, M.M., R.J. Nelson, M.P. Stryker, M.S. Cynader, A. Schoppmann, and J.M. Zook. 1984. Somatosensory cortical map changes following digit amputation in adult monkeys. *The Journal of Comparative Neurology* 224, no.4: 591-605.

Miller, G. and P. Nicely. 1955. An analysis of perceptual confusions among some English consonants. *The Journal of the Acoustical Society of America* 27, no.2: 338-52.

Molina-Luna, K., B. Hertler, M.M. Buitrago, and A.R. Luft. 2008. Motor learning transiently changes cortical somatotopy. *NeuroImage* 40, no.4: 1748-54.

Neafsey, E.J., E.L. Bold, G. Haas, K.M. Hurley-Gius, G. Quirk, C. Sievert, and R. Terreberry. 1986. The organization of the rat motor cortex: a microstimulation mapping study. *Brain Research* 396, no.1: 77-96.

Nudo, R.J., W.M. Jenkins, and M.M. Merzenich. 1990. Repetitive microstimulation alters the cortical representation of movements in adult rats. *Somatosensory & Motor Research* 7, no.4: 463-83.

Nudo, R.J., G.W. Milliken, W.M. Jenkins, and M.M. Merzenich. 1996. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *The Journal of Neuroscience: The Official Journal for the Society for Neuroscience* 16, no.2: 785-807.

Oesterreich, R.E., N.L. Strominger, and W.D. Neff. 1971. Neural structures mediating differential sound intensity discrimination in the cat. *Brain Research* 27, no.2: 251-70.

Ohl, F.W., W. Wetzel, T. Wagner, A. Rech, A., and H. Scheich. 1999. Bilateral ablation of auditory cortex in Mongolian Gerbils affects discrimination of frequency modulated tones but not pure tones. *Learning & Memory* 6, no.4: 347-62.

Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates. 4th ed.* San Diego: Academic Press, 1998.

Pettigrew, J.D. and T. Kasamatsu. 1978. Local perfusion of noradrenaline maintains visual cortical plasticity. *Nature* 271, no.5647: 761-3.

Phatak, S., A. Lovitt, and J. Allen. 2008. Consonant confusions in white noise. *The Journal of the Acoustical Society of America* 124, no.2: 1220-33.

Plautz, E.J., G.W. Milliken, and R.J. Nudo. 2000. Effects of repetitive motor training on movement representations in adult squirrel monkeys: role of use versus learning. *Neurobiology of Learning and Memory* 74, no.1: 27-55.

Polley, D.B., H.L. Read, D.A. Storace, and M.M. Merzenich. 2007. Multiparametric auditory receptive field organization across five cortical fields in the albino rat. *Journal of Neurophysiology* 97, no.5: 3621-38.

Pons, T.P., P.E. Garraghty, A.K. Ommaya, J.H. Kaas, E. Taub, and M. Mishkin. 1991. Massive cortical reorganization after sensory deafferentation in adult macaques. *Science* 252, no.5014: 1857-60.

Quartarone, A., S. Bagnato, V. Rizzo, H.R. Siebner, V. Dattola, A. Scalfari, F. Morgante, F. Battaglia, M. Romano, and P. Girlanda. 2003. Abnormal associative plasticity of the human motor cortex in writer's cramp. *Brain: A Journal of Neurology* 126, pt.12: 2586-96.

Quartarone, A., H.R. Siebner, and J.C. Rothwell. 2006. Task-specific hand dystonia: can too much plasticity be bad for you? *Trends in Neuroscience* 29, no.4: 192-9.

Raab, D.H. and H.W. Ades. 1946. Cortical and midbrain mediation of a conditioned discrimination of acoustic intensities. *The American Journal of Psychology* 59: 59-83.

Raizada, R.D.S., F.-M.Tsao, H.-M. Liu, and P.K. Kuhl. 2010. Quantifying the adequacy of neural representations for a cross-language phonetic discrimination task: Prediction of individual differences. *Cerebral Cortex* 20, no.1: 1-12.

Ramanathan, D., J.M. Conner, and M.H. Tuszynski. 2006. A form of motor cortical plasticity that correlates with recovery of function after brain injury. *Proceedings of the National Academy of Sciences of the United States of America* 103, no.30: 11370-5.

Recanzone, G.H., M.M. Merzenich, W.M. Jenkins, K.A. Grajski, and H.R. Dinse. 1992. Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. *Journal of Neurophysiology* 67, no.5: 1031-56.

Recanzone, G.H.; C.E. Schreiner, and M.M. Merzenich. 1993. Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *The Journal of Neuroscience: The Official Journal for the Society for Neuroscience* 13, no.1: 87-103 .

Reed, P., P. Howell, S. Sackin, L. Pizzimenti, and S. Rosen. 2003. Speech perception in rats: Use of duration and rise time cues in labeling of affricate/fricative sounds. *Journal of the Experimental Analysis of Behavior* 80, no.2: 205-15.

Remple, M.S., R.M. Bruneau, P.M. VandenBerg, C. Goertzen, and J.A. Kleim. 2001. Sensitivity of cortical movement representations to motor experience: evidence that skill learning but not strength training induces cortical reorganization. *Behavioural Brain Research* 123, no.2: 133-41.

Rosenzweig, M. 1946. Discrimination of auditory intensities in the cat. *The American Journal of Psychology* 59: 127-36.

Rush, A.J., M.S. George, H.A. Sackeim, L.B. Marangell, M.M. Husain, C. Giller, Z. Nahas, S. Haines, R.K. Simpson Jr., and R. Goodman. 2000. Vagus nerve stimulation (VNS) for treatment-resistant depressions: a multicenter study. *Biological Psychiatry* 47, no.4: 276-86.

Rybalko, N., D. Suta, F. Nwabueze-Ogbo, and J. Syka. 2006. Effect of auditory cortex lesions on the discrimination of frequency-modulated tones in rats. *The European Journal of Neuroscience* 23, no.6: 1614-22.

Sanes, J.N., S. Suner, J.F. Lando, and J.P. Donoghue. 1988. Rapid reorganization of adult rat motor cortex somatic representation patterns after motor nerve injury. *Proceedings of the National Academy of Sciences of the United States of America* 85, no.6: 2003-7.

Scharlock, D.P., W.D. Neff, and N.L. Strominger. 1965. Discrimination of tone duration after bilateral ablation of cortical auditory areas. *Journal of Neurophysiology* 28: 673-81.

Seol, G.H., J. Ziburkus, S. Huang, L. Song, I.T. Kim, K. Takamiya, R.L. Huganir, H.-K. Lee, and A. Kirkwood. 2007. Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. *Neuron* 55, no.6: 919-29.

Sharma, A. and M.F. Dorman. 2000. Neurophysiologic correlates of cross-language phonetic perception. *The Journal of the Acoustical Society of America* 107, no.5: 2697-793.

Sjögren, M.J., P.T. Hellström, M.A. Jonsson, M. Runnerstam, H.C. Silander, and E. Ben-Menachem. 2002. Cognition-enhancing effect of vagus nerve stimulation in patients with Alzheimer's disease: a pilot study. *The Journal of Clinical Psychiatry* 63, no.11: 972-80.

Smith, D.C., A.A. Modglin, R.W. Roosevelt, S.L. Neese, R.A. Jensen, R.A. Browning, and R.W. Clough. 2005. Electrical stimulation of the vagus nerve enhances cognitive and motor recovery following moderate fluid percussion injury in the rat. *Journal of Neurotrauma* 22, no.12: 1485-502.

Smith, D.C., A.A. Tan, A. Duke, S.L. Neese, R.W. Clough, R.A. Browning, and R.A. Jensen. 2006. Recovery of function after vagus nerve stimulation initiated 24 hours after fluid percussion brain injury. *Journal of Neurotrauma* 23, no.10: 1549-60.

Sohn, Y.H. and M. Hallett. 2004. Disturbed surround inhibition in focal hand dystonia. *Annals of Neurology* 56, no.4: 595-9.

Threlkeld, S., M. McClure, J. Bai, Y. Wang, J. LoTurco, G. Rosen, and R. Fitch. 2007. Developmental disruptions and behavioral impairments in rats following in utero RNAi of Dyx1c1. *Brain Research Bulletin* 71, no.5: 508-14.

Vetencourt, J.F.M., A. Sale, A. Viegi, L. Baroncelli, R.D. Pasquale, O.F. O'Leary, E. Castrén, and L. Maffei. 2008. The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science* 320, no.5874: 385-8.

Wang, M. and R. Bilger. 1973. Consonant confusions in noise: A study of perceptual features. *The Journal of the Acoustical Society of America* 54, no.5: 1248-66.

Wang, X., M.M. Merzenich,K. Sameshima, and W.M. Jenkins. 1995. Remodelling of hand representation in adult cortex determined by timing of tactile stimulation. *Nature* 378, no.6552: 71-5.

Warrier, C.M., K.L. Johnson, E.A. Hayes, T. Nicol, and N. Kraus. 2004. Learning impaired children exhibit timing deficits and training-related improvements in auditory cortical responses to speech in noise. *Experimental Brain Research* 157, no.4: 431-41.

Weinberger, N.M. 2003. The nucleus basalis and memory codes: auditory cortical plasticity and the induction of specific, associative behavioral memory. *Neurobiology of Learning and Memory* 80, no.3: 268-84.

Whitfield, I.C. 1980. Auditory cortex and the pitch of complex tones. *The Journal of the Acoustical Society of America* 67, no.2: 644-7.

Winkler, I., T. Kujala, H. Tiitinen, P. Sivonen, P. Alku, A. Lehtokoski, I. Czigler, V. Csépe, R.J. Lmoniemi, and R. Näätänen. 1999. Brain responses reveal the learning of foreign language phonemes. *Psychophysiology* 36, no.5: 638-42.

Wright, B.A., L.J. Lombardino, W.M. King, C.S. Puranik, C.M. Leonard, and M.M. Merzenich. 1997. Deficits in auditory temporal and spectral resolution in language-impaired children. *Nature* 387, no.6629: 176-8.

Yu, X., S. Wang, D.-Y. Chen, S. Dodd, A. Goloshevsky, and A.P. Koretsky. 2010. 3D mapping of somatotopic reorganization with small animal functional MRI. *NeuroImage* 49, no.2: 1667-76.

VITA

Benjamin Aaron Porter was born in Leesburg, Virginia. He graduated from Plano Senior High School in Plano, TX, in 1999. He also earned the Eagle Scout award in the Boy Scouts of America in the same year. After attending Collin County Community College for a year, he transferred to Oklahoma State University in Stillwater, OK, where he earned two Bachelor of Arts in Psychology and Sociology. While at OSU, Ben earned a Lew Wentz Scholarship to support his research with Shelia Kennison, Ph. D, which was later published in the North American Journal of Psychology. He then worked as a research assistant for 2 years with Michael McClokey, Ph. D., at Johns Hopkins University in Baltimore, MD. In the summer of 2006, he married Amy Porter (Karlsson) and began work in the graduate program at the University of Texas at Dallas with Michael Kilgard, Ph. D. Ben has since earned a Master of Science in Applied Cognition and Neuroscience in 2008. In May of 2009, Ben became a father to Luke Porter.

Publications:

Porter B.A., T.R. Rosenthal, K.G. Ranasinghe, and M.P. Kilgard. 2011. Discrimination of brief speech sounds is impaired in rats with auditory cortex lesions. *Behavioural Brain Research* 219: 68-74.

Porter BA, and S.M. Kennison. In Press. Differences in men's and women's knowledge of and memory for names. *North American Journal of Psychology* 12: 433-44.

Floody O.R., L. Ouda, B.A. Porter, and M.P. Kilgard . 2010. Effects of damage to auditory cortex on the discrimination of speech sounds by rats. *Physiology and Behavior* 101: 260-8.

Bose M., P. Muñoz-Llancao, S. Roychowdhury, J.A. Nichols, V. Jakkamsetti, B. Porter, R. Byrapureddy, H. Salgado, M.P. Kilgard, F. Aboitiz, A. Dagnino-Subiabre, and M. Atzori. 2010. Effect of the environment on the dendritic morphology of the rat auditory cortex. *Synapse* 64: 97-110.

Dagnino-Subiabre A., P. Muñoz-Llancao, G. Terreros, U. Wyneken, G. Díaz-Véliz, B. Porter, M. Kilgard, M. Atzori, and F. Aboitiz. 2009. Chronic stress induces dendritic atrophy in the rat medial geniculate nucleus: effects on the auditory processing**.** *Behavioral Brain Research* 12: 88-96.