SPEECH-CODING AND TRAINING-INDUCED PLASTICITY IN

AUDITORY CORTEX OF NORMAL AND DYSLEXIA

MODEL RATS

by

Tracy Michelle Centanni

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This dissertation is dedicated to my father, who spent the last 26 years teaching me that there is always more to a question than a one sentence answer. He pushed me and helped create the inquisitive mind I have today. This dissertation would not have been possible without his love and encouragement. I love you, Dad. “Po-ta-to” doesn’t seem so stupid anymore…

SPEECH-CODING AND TRAINING-INDUCED PLASTICITY IN AUDITORY CORTEX OF NORMAL AND DYSLEXIA MODEL RATS

by

TRACY MICHELLE CENTANNI, BS, MS

DISSERTATION

Presented to the Faculty of

The University of Texas at Dallas

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY IN

COGNITION AND NEUROSCIENCE

THE UNIVERSITY OF TEXAS AT DALLAS

May 2013

ACKNOWLEDGMENTS

First, I must sincerely thank my parents for instilling in me the drive to even attempt a doctorate, and for the love and support along the way. They were both always willing to listen to me talk out a particular problem I was having, even if they didn't understand all the jargon. I am eternally grateful for that. And thank you to my sister, who helped me keep things in perspective when I was feeling frustrated. I also could not have finished this program without the love and support of my husband, Matt. He spent the entire four years living more than 1,500 miles away, visiting every 3-4 weeks and encouraging me over the phone, email, skype, and any other form of technology we could get our hands on. I cannot put into words how much that sacrifice has meant to me and I am so exceptionally lucky to have him by my side.

I am incredibly fortunate to have completed my doctoral program at UTD. My mentor, Dr. Michael Kilgard, has always encouraged me to be a better scientist. From day one, he was hounding me for project ideas, and though he time and again turned down my music-related project ideas, this process helped me become the queen of multi-tasking, running at least 4 full experiments, 4 pilot projects, and assisting in 3 other projects during my 4 years in the lab. This program has been a collaborative one between Dr. Kilgard and I, and I am a better person and a better researcher for it. Thank you for allowing me to stretch my wings and for keeping me focused when my ambitions got the better of me.

Thank you also to my committee members for their valuable advice and help along the way. Dr. Robert Rennaker has been an irreplaceable mentor from day one, helping me to develop new

behavior tasks and teaching me the art of awake recordings. Dr. Peter Assmann gave me my foundation in speech science and his advice on relating my rodent work to the human population has been enlightening. Dr. Jeff Martin was always available to give me his insights on the population of people with communication disorders, and was instrumental in always reminding me to put my work into context. The work in this dissertation is a small but important piece of a larger puzzle.

I also could not have finished this dissertation without the help of Dr. Andrew Sloan. He taught me almost everything I know about Matlab coding and never (outwardly) lost his patience with my millions of nagging questions about syntax and commands. He has also tirelessly helped me apply a new statistical method when my own knowledge fell short. I can never express how much he truly helped me and I am forever in his debt.

I have been lucky to share the lab with many wonderful colleagues. First, I must thank Dr. Crystal Engineer, who taught me the techniques of anesthetized microelectrode recording and the corresponding analysis. She and I have developed a mutual relationship of data collection and analysis and her help and advice has been crucial in this process. Secondly, thank you to Navid Khodaparast who has been a wonderful lab mate over the last 4 years. Our friendly competition pushed me, but his willingness to listen and offer advice was even more valuable. I will miss his witty comments. Also a thank you to Dr. Amanda Reed, Dr. Ben Porter, Dr. Jai Shetake, Dr. Kamalini Ranasinghe, and Dr. Navzer Engineer for being wonderful mentors as I was starting out in the lab. Thank you to Mike Borland, Will Vrana, Allison Malik, and Ryan Carraway for help with data collection throughout the years; I cannot say how much I appreciate all the late nights.

I have been fortunate to have a wonderful group of undergraduate volunteers throughout my time in the lab who were instrumental in the collection of data and the completion of this research: Nicole Lengnick, Hali Shepard, Zeenat Abdulali, Cody Mains, Nicole Moreno, Ryan Cheung, Harjoat Gill, Dez Gunter, Elizabeth Hanacik, Chris Im, Susan Mahioddin, Chanel Matney, Robert Miller, Christina May, Cal Rohloff, Christina Stelly, Lye-Yeng Wong, Akbar Afsar, Laura Baxter, Kisha Williams, Ariel Schmid, Karen Wong, Amreen Sitabkhan, Ryan Russell, Stephanie Barata, Ashley Ramdeen, Nujeen Zibari, Jacob VanNattan, and Ian Heimbuch. Finally, I would like to thank my undergraduate research advisor, Dr. Michael Wenger and my friends Dr. Jennifer Bittner and Dr. Rebecca Von Der Heide-Byrne for their continued support and advice during this process. You all helped prepare me for this crazy ride and I am eternally grateful. Go State!

March 2013

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.

SPEECH-CODING AND TRAINING-INDUCED PLASTICITY IN

AUDITORY CORTEX OF NORMAL AND DYSLEXIA

MODEL RATS

Publication No. _____

Tracy Michelle Centanni, PhD
The University of Texas at Dallas, 2013

Supervising Professor: Michael P. Kilgard, PhD

The ability to understand auditory stimuli and particularly speech sounds is a complex process involving many brain regions. Communication disorders are among the most common disabilities in the US and affect over 20% of the general population. Understanding the differences in the way the auditory cortex processes speech sounds in individuals with these disorders may elucidate the neural mechanisms behind these disorders and lead to more effective therapies. Dyslexia is the most common developmental language disorder and causes impairments in reading ability in spite of normal non-verbal IQ. Children with dyslexia have difficulty recognizing phonemes: the smallest segment of a word that, if changed, alters the meaning of the word. Dyslexic children also have altered neural responses to short auditory stimuli, such as phonemes or tones. Since humans with dyslexia have complex genetic profiles, the direct link between each of the dyslexia-associated genes and the auditory processing

impairments seen in dyslexia cannot be studied in humans and requires the precise control available in animal models. In this dissertation, I document the cortical auditory firing impairments in rats with *in utero* RNAi of *Kiaa0319*, the most well-studied candidate dyslexia gene. We document for the first time, that suppression of a candidate-dyslexia gene causes impaired phoneme processing in multiple auditory fields compared to normal controls. Many studies have shown that abnormal neural firing patterns lead to impairments in comparable behavior tasks. We report here that rats with RNAi of *Kiaa0319* also have significant behavioral impairments on phoneme discrimination tasks. Extensive behavioral training can improve speech discrimination accuracy as well as restore neural firing properties to control levels. This result provides the first evidence for a possible neural mechanism that drives improvement in dyslexic children. Finally, in an effort to develop more biologically plausible analysis tools, we also report the development and testing of a new classifier which can use auditory cortex activity to locate and identify the evoking speech stimulus in real time. The results of these studies show that the variants in the candidate dyslexia gene *KIAA0319* can cause neural and behavioral impairments in phoneme processing and provide new tools to investigate neural encoding of speech sounds in the normal and abnormal brain

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CHAPTER 1

INTRODUCTION

The ability to understand auditory stimuli and particularly speech sounds is a complex process involving many brain regions. Communication disorders are among the most common disabilities in the US and affect over 20% of the general population (asha.org). Many individuals with communication disorders experience debilitating setbacks in education which often impact the individual's quality of life (McNulty 2003; Shaywitz 1998). Understanding the differences in the way the auditory cortex processes speech sounds in individuals with these disorders compared to normal individuals may elucidate the neural mechanisms behind these disorders and lead to more effective therapies.

Dyslexia is the most common developmental language disorder and affects 5-10% of the population. This disorder causes impairments in reading ability in spite of normal non-verbal IQ. Children with dyslexia have difficulty recognizing phonemes- the smallest segment of a word that, if changed, alters the meaning of the word (Boscariol et al. 2010; Peterson and Pennington, 2012; Tallal and Piercy 1974; Vandermosten et al. 2010). Dyslexic children also have altered neural responses to short auditory stimuli, such as phonemes or tones. For example, tone-evoked EEG responses in dyslexic children were delayed and had lower amplitude compared to control children (Nagarajan et al. 1998; Tonnquist-Uhlen 1996). Individuals with dyslexia also have reduced neural responses to speech stimuli (Kraus et al. 1996; Kujala et al. 2000; Schulte-Körne et al. 2001).

Dyslexia is also highly heritable and several candidate dyslexia genes have been proposed (*KIAA0319, DCDC2, DYC1C1, ROBO1*, among others; (Bates et al. 2011; Deffenbacher et al. 2004; Francks et al. 2004; Galaburda et al. 2006; Harold et al. 2006; Luciano et al. 2007; Paracchini et al. 2006; Schumacher et al. 2006). *KIAA0319* is the most well-studied of these genes and is linked to reduced left hemisphere activation to phoneme stimuli (Pinel, 2012). We designed the studies in chapter 2 to determine the role of this specific gene in causing the neural firing deficits associated with dyslexia.

In chapter 2, we document the impaired neural firing to speech and non-speech stimuli in rats with *in utero* RNAi of *Kiaa0319.* Primary auditory cortex in these rats fired with significantly higher variability in onset latency from trial to trial of the same stimulus. An established nearest-neighbor Euclidean distance classifier can predict behavioral performance in normal rats. We used this classifier to see if the impaired firing patterns in KIA- A1 would affect the ability of neural activity to discriminate between speech sounds. Neural responses in A1 of KIA- rats were significantly impaired at discriminating between pairs of consonant or vowel speech sounds.

In chapter 2, we focused on analyzing A1 recordings in normal and KIA- rats. Other auditory fields likely contribute to speech sound processing and may provide information about specialization for certain types of auditory stimuli. In Chapter 3, we document the neural responses to speech sounds in primary (A1), anterior (AAF), ventral (VAF), and posterior (PAF) auditory fields of the normal adult rat. We observed a comparable set of speech-evoked neural response patterns in each of the four fields we examined and saw no apparent advantage for speech sound processing in other fields in the normal rat. In spite of the consistent speech-encoding across fields, we did also observe an increase in encoding diversity in the non-primary

fields. Such a result suggests differences in processing, perhaps providing the bandwidth to encode many new processing tasks.

The experiments in this chapter set the foundation for us to look in other auditory fields in our dyslexia model (as described in Chapter 2). Other auditory fields can show deficits in auditory processing even when primary auditory cortex is responding normally. For example, studies have shown that other auditory fields in autistic children respond abnormally to speech sounds even when A1 responds normally (Lai et al. 2011). Dyslexic children are treated using extensive behavioral therapy to improve impaired performance on phoneme manipulation or deletion tasks as compared to controls ('break' without the /b/ or switch the first phoneme in two words, 'dog' and 'house'; (Paulesu et al. 1996). Dyslexic individuals can also have abnormal rhyme or non-word recognition (Howland and Liederman 2012; Paulesu et al. 1996) and these deficits in phoneme processing are thought to be due to an underlying deficit in rapid auditory processing (Martino et al. 2001; Poelmans et al. 2012; Russo et al 2004; Tallal and Piercy 1974). Abnormal neural responses in dyslexic children can improve after extensive training, such as the program Fast ForWord (Scientific Learning Corporation, Oakland, CA). The neural mechanism by which it is effective, and whether other auditory fields are also benefitted by training, is unknown. We hypothesized that training may induce plasticity in primary and non-primary auditory fields of KIA- rats.

In chapter 4, we document the behavioral deficit of rats with *in utero* RNAi of *Kiaa0319*. These rats have abnormal startle response to oddball tone paradigms (Szalkowski et al., 2012) and we hypothesized that they would also have impairment on phoneme discrimination. Rats are good models of speech sound discrimination. They are able to accurately discriminate human

consonants (Engineer et al., 2008) and vowels (Perez et al., 2012) in quiet, various levels of speech-shaped and white background noise (Shetake et al., 2011) and after spectral or temporal degradation (Ranasinghe et al., 2012). We report that *in utero* RNAi of *Kiaa0319* causes significant behavioral impairments on several of these phoneme discrimination tasks. Extensive speech training can improve reliability of neural firing in KIA- A1 and improve the neural discrimination performance of this field. A similar plasticity effect was seen in KIA- PAF and in Control PAF. The results in this chapter provide the first evidence of speech training-induced plasticity in a control animal and suggest a possible mechanism by which training programs like Fast ForWord are effective.

In the previous 3 chapters, we used a nearest-neighbor two-alternative forced choice classifier to evaluate neural firing patterns in normal and dyslexia model rats. This classifier had several conditions which are biologically implausible. First, the classifier was given knowledge of the stimulus onset time, which may not be available to the animal in real world situations. Second, the classifier was forced to choose between one of two options for the evoking stimulus, which put chance level at 50%. In chapter 5, we document a new classifier which uses anesthetized or awake neural data to locate the time at which a speech stimulus was presented and identify the evoking speech sound. The benefits of this new classifier are that is it not forced to guess and is effective using a variety of previously reported stimulus sets. This classifier can also predict behavior on a relatively novel behavioral task in which speech sounds are presented in random, rapid sequences at one of 6 speeds.

In chapter 6, we discuss the implications of these experiments and how the results relate to the current literature. This dissertation consists of 6 chapters and 4 appendices that contain supplementary data and figures.

CHAPTER 2

KNOCKDOWN OF THE DYSLEXIA-ASSOCIATED GENE *KIAA0319* IMPAIRS TEMPORAL RESPONSES TO SPEECH STIMULI IN RAT PRIMARY AUDITORY CORTEX*

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* Article was published in Cerebral Cortex in February, 2013:
Knockdown of the Dyslexia-Associated Gene Kiaa0319 Impairs Temporal Responses to Speech Stimuli in Rat Primary Auditory Cortex
T. M. Centanni; A. B. Booker; A. M. Sloan; F. Chen; B. J. Maher; R. S. Carraway; N. Khodaparast; R. Rennaker; J. J. LoTurco; M. P. Kilgard
Cerebral Cortex 2013; doi: 10.1093/cercor/bht028

ABSTRACT

One in fifteen school age children have dyslexia, which is characterized by phoneme processing problems and difficulty learning to read. Dyslexia is associated with mutations in the gene *KIAA0319*. It is not known whether reduced expression of *KIAA0319* can degrade the brain's ability to process phonemes. In the current study, we used RNA interference (RNAi) to reduce expression of *Kiaa0319* (the rat homolog of the human gene *KIAA0319*) and evaluate the effect in a rat model of phoneme discrimination. Speech discrimination thresholds in normal rats are nearly identical to human thresholds. We recorded multi-unit neural responses to isolated speech sounds in primary auditory cortex (A1) of rats that received *in utero* RNAi of *Kiaa0319*. Reduced expression of *Kiaa0319* increased the trial by trial variability of speech responses and reduced the neural discrimination ability of speech sounds. Intracellular recordings from affected neurons revealed that reduced expression of *Kiaa0319* increased neural excitability and input resistance. These results provide the first evidence that decreased expression of the dyslexia associated gene *Kiaa0319* can alter cortical responses and impair phoneme processing in auditory cortex.

INTRODUCTION

Approximately 7% of children with normal intelligence have trouble learning to read (Badian 1999; Shaywitz et al. 1990; Shaywitz et al. 1992). These children typically have deficits in tasks that involve phonemic awareness (Boscariol et al. 2010; Peterson and Pennington, 2012; Tallal and Piercy 1974; Vandermosten et al. 2010). Phonemes are the smallest individual acoustic component of a word that can change that word's meaning (i.e. the "b" sound in the word /bad/). Normal individuals respond with a consistent threshold when asked to categorize sounds along a continuum. For example, in a ba-pa continuum, stimuli with a voice onset time below 25 ms are categorized as "ba," while stimuli with longer voice onset times are categorized as "pa" (Manis et al. 1997; Werker and Tees 1987). Dyslexic individuals have a less defined perceptual divide in discriminating phonemes. When asked to delete or exchange two phonemes in a spoken phrase (i.e. turn "dog house" into "hog douse"), dyslexic individuals perform significantly worse (Paulesu et al. 1996).

The phonemic deficit observed in dyslexia is theorized to be the result of temporal processing problems in the central auditory system (Martino et al. 2001; Poelmans et al. 2012; Russo et al 2004; Tallal and Piercy 1974). The impaired ability of the dyslexic brain to process phonemic stimuli likely interferes with the mapping of phonemes to the corresponding grapheme (visual letters). The observation that children with dyslexia are also impaired in rapid tone processing (Ahissar et al. 2000; Tonnquist-Uhlen 1996; Wright et al. 1997) suggests that deficits in temporal processing are not speech specific and reflect a more general dysfunction in temporal processing.

Temporal processing deficits associated with dyslexia are theorized to result from abnormal firing in the central auditory system (Ahissar et al. 2000; Boscariol et al. 2010; Tallal 1980; Temple et al. 2001, but see McLean et al., 2011 and Rosen 2003). Primary auditory cortex encodes phonemic stimuli with millisecond precision (Eimas 1985; Engineer et al. 2008). Altered cortical response properties have been found in dyslexic individuals to simple stimuli like brief tones, with longer latencies to tones and lower amplitude in dyslexics compared to controls (Nagarajan et al. 1998; Tonnquist-Uhlen 1996). Individuals with dyslexia also have reduced neural responses to speech sounds during passive exposure (Kraus et al. 1996; Kujala et al. 2000; Schulte-Körne et al. 2001) and during phoneme discrimination tasks (Flowers, Wood, Naylor 1991; Rumsey et al. 1992; Rumsey et al. 1997; Temple et al. 2001; Temple et al. 2003; Temple et al.).

Dyslexia displays both environmental and genetic risk components (Cope et al. 2005; Fisher and DeFries 2002; Nöthen et al. 1999; Pennington et al. 1991). The co-incidence rate among monozygotic twins is 50-68% (Pennington et al. 1991). Allelic variations in the gene *KIAA0319* have been consistently associated with dyslexia (Bates et al. 2011; Deffenbacher et al. 2004; Francks et al. 2004; Galaburda et al. 2006; Harold et al. 2006; Luciano et al. 2007; Paracchini et al. 2006; Schumacher et al. 2006). In addition, allelic variation in a region encompassing the *KIAA0319* gene has been associated directly with alterations in fMRI responses during reading in left superior temporal cortex in individuals with dyslexia, indicating a potentially direct role of *KIAA0319* function in cortical processing during reading (Pinel et al. 2012).

We have previously shown that neuronal responses in the primary auditory cortex of rats accurately encode human phonemes that can be difficult for dyslexic children to distinguish (Engineer et al. 2008; Perez et al. 2012; Porter et al. 2011; Ranasinghe et al. 2012a; Shetake et al. 2011). This study was designed to determine whether *in utero* RNAi of *Kiaa0319* (the rat homolog of the human gene, *KIAA0319*) can degrade the brain's ability to process phonemes.

METHODS

Animals

Subjects were Wistar rats, both male and female, that were 3-6 months old at the time of study. All rats used were subjected as embryos to *in utero* electroporation targeting lateral regions of neocortex that included the auditory cortex by methods described previously (Bai et al. 2003; Bai et al. 2008; Burbridge et al. 2008; Szalkowski et al. 2012; Threlkeld et al. 2007). The animals were transfected with either an shRNA against *Kiaa0319* which can decrease the Kiaa0319 protein expression in cell culture (Tarkar and LoTurco, unpublished observation) and to cause migration delay in neocortex in embryos that was rescued by expression of exogenous *Kiaa0319* (Parrachini et al 2006). Control transfection animals received a scrambled sequence control of *Kiaa0319* shRNA, also previously used, that contained 6 bases in the sequence scrambled to render the shRNA inactive in terms of reducing *Kiaa0319* expression (Parrachini et al 2006). *Kiaa0319* shRNA and scrambled shRNA constructs were injected at a concentration of 1.0 µg/µL. pB-GFP was co-transfected with the effective shRNA construct, and pB-mRFP was co-transfected with the scrambled *Kiaa0319* shRNA control construct to identify the experimental condition in post experimental histological analysis. Electroporation paddles were

placed in a horizontal plane and voltage pulses were discharged across the cerebrum in both polarities to achieve bilateral transfections. The experimental status of the subject remained blind to the experimenters throughout data collection. Following data collection, each subject was perfused transcardially 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. Sections were taken at 80 µm intervals and analyzed under a confocal microscope (Zeiss) to identify the experimental status of each subject (green florescent protein marked experimental subjects and red florescent protein marked control littermates). All animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee.

Anesthetized recordings

Multiunit recordings were acquired from the primary auditory cortex of 11 rats. After histological analysis, we determined that 5 were *Kiaa0319* knockdowns (KIA-, 2 females, 3 males), and 6 were littermate controls (3 females, 3 males). The recording procedure is explained in detail elsewhere (Engineer et al. 2008). In brief, animals were anesthetized with pentobarbital (50 mg kg^{-1}) and given supplemental dilute pentobarbital (8 mg ml^{-1}) as needed to maintain areflexia, along with fluids to prevent dehydration. A tracheotomy was performed to ensure ease of breathing throughout the experiment. Primary auditory cortex and several nearby auditory fields were exposed via craniotomy and durotomy. Four Parylene-coated tungsten microelectrodes (1-2 MΩ) were simultaneously lowered to layer IV of right primary auditory cortex (~600-800 µm).

Brief tones were presented at 90 randomly interleaved frequencies (1-48 kHz) at 16 intensities (1- 75 dB SPL) to determine the characteristic frequency of each site. Tones had 5 ms

cosine squared ramps and their total duration was 25 ms. Additional stimuli were randomly interleaved and presented at 20 repeats per recording site. Broad band noise was presented in trains of six 25 ms long bursts at four different presentation rates (4, 7, 10 and 12.5 Hz). Broad band stimuli contained evenly distributed frequencies between 1 and 32 kHz. We also presented 8 English consonant-vowel-consonant (CVC) speech sounds (/dad/, /sad/, /tad/, /bad/, /gad/, /dud/, /deed/, and /dood/) previously tested in our lab (Engineer et al. 2008; Floody et al. 2010; Ranasinghe et al. 2012a; Shetake et al. 2011). Sounds were shifted up 1 octave using the STRAIGHT vocoder to better match the rat hearing range (Kawahara 1997). Each sound was calibrated so that the most intense 50 ms of the stimulus length was 60 dB SPL. All sounds were presented approximately 10 cm from the left ear of the rat.

Awake recordings

Chronic awake recordings were collected from subjects implanted with 16-channel microwire electrode arrays. The implantation surgery and microwire arrays have been previously reported in detail (Rennaker et al., 2005a). Briefly, subjects were anesthetized with an intramuscular injection of a mixture of ketamine, xylazine and acepromazine (50 mg/kg, 20 mg/kg, 5 mg/kg, respectively). Atropine and dexamethazone were administered subcutaneously prior to and following surgery. A midline incision was made, exposing the top of the skull, and a section of the right temporalis muscle was removed to access primary auditory cortex. Six bone screws were fixed to the dorsal surface of the skull (two in each parietal bone and one in each frontal bone) to provide structural support for the head cap. The two middle screws had attached leads to serve as a reference wire and a grounding wire. A craniotomy and durotomy were performed to expose the cortex in the region of primary auditory cortex. The microwire array

was then inserted to a depth of 550-600 μm (layer IV/V) in primary auditory cortex using a custom built mechanical inserter (Rennaker et al, 2005b). The area was sealed with a silicone elastomer (Kwik-Cast, World Precision Instruments Inc, Sarasota, Florida) and the head cap was built with a connector secured with acrylic. Finally, the skin around the implant was sutured in the front and the back of the head cap. Subjects were given prophylactic minocycline in water ad libitum for 2 days prior and 5 days following surgery to lessen immune responses (Rennaker et al. 2007), and were also given Rimadyl tablets for 3 days after surgery to minimize discomfort. Topical antibiotic was applied to the incision to prevent infection.

Following a week of recovery, recordings were obtained from each animal in a series of daily recording sessions. During each session, the animal was unrestrained in a 30 x 30 cm cage and sounds were presented from a calibrated magnetic speaker (Tucker Davis Technologies, Alachua, FL) mounted 35 cm directly above the animal (Rennaker et al. 2005). A head-stage amplifier was directly attached to the subject's electrode connector, and neural signals were sampled at 25 kHz, amplified, and band-pass filtered from 825 to 4500 Hz using Tucker Davis Technologies System 2 hardware. Custom software was used for displaying and saving recordings and for auditory stimulus control.

Three acoustic stimulus sets were presented to awake subjects in separate recording sessions. The first stimulus set consisted of trains of broadband clicks (~1 ms duration, 3 dB points at 1.6 and 31.6 kHz) played at 13 presentation rates ranging from 1 Hz to 250 Hz. Click intensity was calibrated such that the loudest 50 ms of the fastest clicktrain had an intensity of 60 dB SPL at a distance of 5 cm from the cage floor. The second stimulus set consisted of the 5 English CVC speech sounds that were also presented to the anesthetized subjects that varied by

first consonant (/dad/, /sad/, /tad/, /bad/, and /gad/). The third stimulus set consisted of the 4 CVC speech sounds that varied by vowel (/dad/, /dud/, /deed/, and /dood/). As with the anesthetized recordings, all speech sounds were shifted up 1 octave and calibrated such that the loudest 50 ms was heard at 60 dB SPL. Since the animal was unrestrained, sound levels were measured at four locations inside the cage and then averaged to account for any change in acoustics.

Analysis of Neural Recordings

To define primary auditory cortex (A1) sites, multi-unit recording sites were manually analyzed to select the characteristic frequency of each site, as well as to obtain bandwidth, latency, peak firing and end of peak response information. From this point on, only A1 sites were analyzed.

Following selection of A1 sites, basic firing properties were calculated in response to tones. Firing latency is defined as the point in time (ms) that average firing rate (across all repeats) first exceeds 2 standard deviations above the spontaneous firing rate, threshold is defined as the lowest intensity that evoked a response from the multiunit site, and bandwidths were calculated at 10, 20, 30 and 40 dB above threshold and defined as the range of frequencies that evoked responses at the current intensity. In response to broad band click trains, normalized spike rate (number of spikes evoked by bursts 2-6, normalized by the number of spikes to the first burst) and vector strength (VS) were calculated. VS quantifies the degree of synchronization between action potentials and repeated sounds. Mean VS is calculated with the formula:

$$\mathrm{VS}=\frac{1}{n}\sqrt{x^2+y^2};\ x=\sum_{i=1}^{n}\cos\,\theta_i; y=\sum_{i=1}^{n}\sin\,\theta_i\ \theta_i=2\pi\frac{t_i}{T}$$

where n = total number of action potentials, t_i is the time of occurrence of the i'th action potential, and T is the inter-stimulus interval. Perfect synchronization would result in a value of one, whereas no synchronization would result in a value of zero. To investigate the reliability of onset latency to repetitive stimuli, we calculated the time to peak latency within the first 80 ms (the shortest inter-pulse-interval tested) of the first pulse at 4Hz and averaged across multiunit sites. The variability in this measure, as reported in variance, was compared across KIA- and controls.

Single trial response patterns to each of the isolated speech sounds were compared using a nearest neighbor classifier (Engineer et al. 2008; Foffani and Moxon 2004; Foffani and Moxon 2005; Perez et al. 2012; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Ranasinghe et al. 2012b; Shetake et al. 2011). We chose this method because our earlier studies showed that the performance of this classifier is highly correlated with rat behavioral discrimination (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011). We used Euclidean distance to compare single trial activity to the average activity (PSTH) evoked by 19 repeats each of two different stimuli. For consonants, activity was binned using 1 ms temporal precision over a 40 ms window to encompass the spike timing precision present in the initial consonant (Engineer et al. 2008; Porter et al. 2011; Ranasinghe et al. 2012a), while vowel activity was binned across a single 400 ms window so that spike count information was preserved (Perez et al. 2012; Ranasinghe et al. 2012a). The classifier then compared the response of each single trial with the average activity template (PSTH) evoked by all repeats of each of the speech stimuli presented. The current trial being considered was not included in the PSTH to avoid artifact. The classifier attempted to identify the stimulus that evoked the current

single trial activity pattern by selecting the template that was most similar to the single trial in units of Euclidean distance. ED was calculated using the formula:

$$\text{Euclidean Distance} = \sqrt{\sum_{i=1}^{\#sites} \sum_{j=1}^{\#\ bins} \left(X_{ij} - Y_{ij}\right)^2}$$

where n_{sites} is each recording site and n_{bins} is each of 40 one-millisecond bins being compared between activity evoked by speech sound X versus speech sound Y. For vowel sounds, the classifier counted the number of action potentials over a single 400 ms bin and compared the single trial response with the two PSTH templates (Ranasinghe et al. 2012a). We used t-tests for all pairwise comparisons of the accuracy of both classifiers and across experimental groups. When necessary, an α of 0.01 was used to correct for multiple comparisons.

Brain Slice recordings

Whole cell patch clamp recording were made from pyramidal neurons in acute brain slices as previously described (Maher et al. 2009). Briefly, P28-35 previously electroporated rats were deeply anesthetized with isoflurane and transcardially perfused with ice-cold oxygenated (95% O2 and 5% CO2) dissecting buffer containing (in mM): 83 NaCl, 2.5 KCl, 1 NaH2PO4, 26.2 NaHCO3, 22 glucose, 72 sucrose, 0.5 CaCl2, and 3.3 MgCl2. The rats were decapitated and the brains rapidly removed and immersed in ice-cold oxygenated dissection buffer. Coronal slices (400 μm) were cut using a vibratome (VT1200S, Leica), incubated in a dissection buffer for 30-45 min at 34°C, and then stored at room temperature. Slices were visualized using IR differential interference microscopy (DIC) (E600FN, Nikon) and a CCD camera (QICAM, QImaging). Individual layer 2/3 pyramidal cells expressing GFP or RFP were visualized with epifluourescent illumination and a 40x Nikon Fluor water immersion (0.8 numerical aperture)

objective. For all experiments, artificial cerebrospinal fluid (ACSF) was oxygenated (95% O2 and 5% CO2) and contained (in mM): 125 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 3 KCl, 25 dextrose, 1 MgCl2, and 2 CaCl2, pH 7.3. Patch pipettes were fabricated from borosilicate glass (N51A, King Precision Glass, Inc.) to a resistance of 2-5 MΩ. For current-clamp experiments pipettes were filled with (in mM): 125 potassium gluconate, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, 0.1 EGTA, 10 phosophocreatine, 0.05% biocytin, adjusted to pH 7.3 with KOH. Voltage signals were recorded and current pulses injected with a Multiclamp 700A amplifier (Molecular Devices). Data were acquired using Axograph, and data acquisition was terminated when series resistances were >15 MΩ.

RESULTS

In utero RNAi of Kiaa0319 causes degraded neural firing to phonemes

Variants in the gene *Kiaa0319* are associated with dyslexia (Bates et al. 2011; Deffenbacher et al. 2004; Galaburda et al. 2006; Harold et al. 2006; Paracchini et al. 2006; Schumacher et al. 2006). To test whether reduced expression of this gene can cause the abnormal speech-evoked potentials observed in dyslexics, we measured speech-evoked local field potentials (LFPs) derived from multi-unit recordings in awake rats that were transfected with *Kiaa0319* shRNA *in utero* (Paracchini et al. 2006). In response to the sound "dad", LFPs in transfected rats (KIA-) had a longer P1 latency than in control rats (Figure 2.1A; P1: 112.7 ± 4.3 ms vs. 75.8 ± 9.1 ms; $p < 0.01$; KIA- vs. controls respectively). Since we cannot be sure which auditory field or the exact depth our awake recordings are from, we also recorded multi-unit speech responses in anesthetized rats. Auditory responses do not differ drastically between anesthetized and awake

preparations in normal rats (Engineer et al. 2008; Shetake et al. 2011), and anesthetized recordings allow for complete control of the targeted auditory field and behavioral state. In primary auditory cortex (A1) of anesthetized rats, speech-evoked LFPs to the sound "dad" had a significantly lower N1 and P1 amplitude in KIA- sites than in controls (N1 amplitude: -44.1 ± 1.5 Hz vs. -78.2 ± 2.1 Hz; $p < 0.01$,P1 amplitude: 27.6 ± 0.9 Hz vs. 45.9 ± 1.2 Hz; $p < 0.01$; KIA- vs. controls respectively; Figure 2.1B). LFP responses to the sound "bad" (Figure 2.1 C&D) show the same pattern of response, with some slight variation. These response properties to speech sounds mimic the reduced activity seen in human dyslexic imaging studies.

We next tested whether the differences in the evoked responses were due to a reduction in the number of auditory evoked action potentials or due to differences in neural synchronization (Blau et al. 2010; Kraus et al. 1996; Lovio et al. 2010). In KIA- rats, multi-unit sites fired more spikes per stimulus than control sites. Across the length of the speech sound "da" (a 400 ms analysis window), cortical responses in KIA- rats fired 19.9 spikes as compared to 14.5 spikes from controls ($p < 0.01$). On average, KIA- sites did not fire significantly more spikes than controls in response to speech sounds (an average of 17.9 ± 4.6 spikes/vowel in KIA- sites vs. 17.1 ± 5.1 spikes/vowel in controls; $p = .10$). Since the lower amplitude in KIA- LFP recordings cannot be explained by fewer evoked spikes, we tested whether these sites fired with greater variability in onset latency across trials. In response to the sound "da," onset latency of KIA- sites was more variable trial by trial (26.8 ms^2) compared to control sites (13.2 ms^2 in controls; $p < 0.01$). This increased variability in onset latency was observed in response to all speech sounds tested (an average of 27.2 ms^2 in KIA- sites vs. 12.5 ms^2 in control sites; $p < 0.01$).

Increased variability in spike timing would be expected to decrease the amplitude of the population response to speech sounds.

In control rats, each consonant sound evoked a unique pattern of response across the tonotopic organization of A1. These different patterns can be seen by plotting the average responses to consonant sounds for each of a variety of sites and organizing those sites by characteristic frequency (low to high; Figure 2.2A). The consonants "d" and "t" evoked firing from high frequency neurons first, followed by an onset of low frequency neurons that corresponds to that consonant's voice onset time. For example, in response to the sound "d," high frequency neurons (> 6 kHz) fired first, followed by low frequency neurons approximately 20 ms later (Figure 2.2A, first panel). In response to the sound "b" (a voiced consonant), neurons fired in the opposite order; low frequency neurons fired first and high frequency neurons almost immediately after (Figure 2.2A, second panel). The observation that our control sites responded similarly to previous studies in unaffected rats (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012a; Shetake et al. 2011), suggests that the *in utero* surgery, plasmid injection and electroporation alone did not alter neural responses.

In contrast to the distinct patterns of speech responses in the controls, KIA- sites responded less precisely to speech sounds in a number of ways. As expected from the LFP data, KIA- sites responded to speech sounds more slowly, though these trends were not significant. For example, the timing of the first evoked spike to the consonant sound /d/ was slightly (but not significantly) later in KIA- sites (17.4 ± 0.2 ms) compared to control sites (15.9 ± 0.7ms; $p = 0.07$). The peak latency was significantly later for each of the speech sounds presented, firing an average of 3.9 ms later than controls (25.3 ± 0.5 vs. 21.4 ± 0.4 ms respectively; $p < 0.01$; Figure

2.3A). The variability in the onset latency across repeats of each speech sound was much higher in KIA- sites (variance of 70.1 ± 4.1 ms^2 vs. 40.6 ± 2.7 ms^2 in controls; $p< 0.01$; Figure 2.4A). In addition to the variability in latency, the number of spikes fired in the first 400 ms of each stimulus in KIA- sites was more variable across repeats (variance of 30.9 ± 0.6 $spikes^2$ vs. 24.1 ± 0.8 $spikes^2$ in controls; $p= 0.03$; Figure 2.4B). This increase in variance could have been due to an increase in mean firing rate. We measured the mean firing rate evoked by speech sounds in 40 ms and 400 ms analysis windows. On average, KIA- sites fired the same number of action potentials in response to speech sounds as control sites (Figure 2.4C). This result suggests that an increase in mean firing rate is not responsible for the increase in trial by trial variability.

The increased trial by trial variability in speech responses could interfere with the brain's ability to distinguish between similar speech sounds. We used a well validated nearest neighborhood classifier to test this hypothesis (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012a; Shetake et al. 2011). The classifier compared single trial activity patterns with millisecond precision to the average responses to two different consonant sounds. We compared single trial responses from individual recording sites to the average responses to two different stimuli. For example, the neural response (peristimulus time histogram, PSTH) of a single site in response to the sound "d" was compared to the average response of that site evoked by "d" or "b". The PSTH template that was most similar to the single trial (i.e. had the smallest Euclidean distance), was selected as the sound most likely to have elicited the single trial response. In control sites, a typical high frequency site responded very quickly after the onset of the sound "d," but with a slight delay following the onset of the sound "b" (Figure 2.5A). In a typical high frequency KIA- site, the response is less consistent from trial to trial and causes

more errors in stimulus identification (Figure 2.5B). On average, the classifier correctly identified the consonant sound 68 ± 1% of the time when using KIA- sites and 77 ± 2% of the time when using control sites ($p< 0.01$; Figure 2.5C). The degree of impairment on consonant discrimination caused by reduced KIA0319 expression is equivalent to the impairment caused by adding 60 dB SPL background noise, which resulted in a 0 dB signal to noise ratio (Shetake et al. 2011). This result indicates that *in utero* RNAi of *Kiaa0319* increases firing variability and reduces the ability of A1 neurons to discriminate different consonant speech sounds.

To test whether *in utero* RNAi of *Kiaa0319* might also impair vowel discrimination, we used a version of the neural classifier that considers only spike count (and not spike timing; Perez et al. 2012). Performance of this classifier on vowel discrimination was highly correlated with behavior observations (Perez et al. 2012). The rate based classifier used single trial responses and classified sounds based on which sound evoked the closest number of spikes on average (across 19 repeats). For example, a high frequency recording site in control rats typically fired fewer spikes in response to the vowel sound "a" (as in "dad") than in response to the vowel sound "u" (as in "dud"; Figure 2.5D). In a typical high frequency KIA- site, the variability in number of spikes fired trial-to-trial was much greater (Figure 2.5E), while the mean number of evoked spikes did not differ. Across sites, the trial by trial variability in number of evoked spikes was higher in KIA- sites vs. controls (29.9 ± 1.5 spikes^2 vs. 23.8 ± 1 spikes^2 respectively; $p<.01$). Average number of action potentials fired to each vowel was not significantly different between control and KIA- sites (to "a", 17.3 ± 1 spikes in controls vs. 17.9 ± 1 spikes in KIA- sites, $p=.49$; to "u", 19.2 ± 1 spikes in controls vs. 19.9 ± 1 spikes in KIA- sites, $p=.54$). We hypothesized that the greater trial by trial variability in spike count would lead to impaired vowel

discrimination. As expected, neural discrimination of vowel sounds using activity from KIA- rats was significantly worse than controls. Activity from sites in KIA- rats was able to correctly identify the vowel sounds 55 ± 1% of the time compared to 59 ± 1% in control sites ($p < 0.01$; Figure 2.5F). This result suggests that reduced *in utero* RNAi of *Kiaa0319* can impair both consonant and vowel discrimination.

RNAi of Kiaa0319 causes impaired neural firing to non-speech stimuli

The increased A1 response variability caused by *in utero* RNAi of *Kiaa0319* was not specific to speech sounds. In response to a noise burst, a representative control site fired consistently across twenty repeats of the stimulus. In response to the same stimulus, a representative KIA- site fired later and less consistently (Figure 2.6A). The average onset latency was significantly later in KIA- sites (16.9 ± 6.9 ms in KIA- vs. 15.3 ± 4.6 ms in controls; $p < 0.01$; Figure 2.6B). The finding that KIA- sites had longer latency to non-speech stimuli is similar to the longer latency of evoked potentials in human dyslexics (Tonnquist-Uhlen 1996). The variability in onset latency was also higher across the population of KIA- sites as compared to controls (48.7 ± 0.6 ms^2 in KIA- sites vs. 21.4 ± 1.1 ms^2 in controls; $p < 0.01$; Figure 2.6C). Similar to the reduced firing amplitude to tones seen in human EEG studies (Nagarajan et al. 1999; Tonnquist-Uhlen 1996), peak firing rate to a noise burst was significantly lower in KIA- sites as compared to controls (256.1 ± 0.4 Hz in KIA- sites compared to 383.9 ± 0.6 Hz in controls; $p < 0.01$; Figure 2.6D). The observation that the number of spikes fired to a broad band noise burst was not significantly different in KIA- sites (2.9 ± .04 spikes vs. 2.9 ± .03 spikes; $p = 0.80$), suggests that the reduced peak firing rate may be due to greater variability in onset latency. To quantify the variability in latency, we measured vector strength in response to noise

burst trains and found that KIA- sites were impaired at phase-locking compared to controls at all four presentation rates tested (Figure 2.6E; $p< 0.01$). When we compared the sites' ability to discriminate between different presentation rates (using the same classifier as used for phonemes), KIA- sites were significantly worse at identifying presentation rate (63.9 ± 1% correct vs. 80.8 ± 1% correct in control sites; $p< 0.01$). Children with dyslexia have poorer sensitivity to modulation rates compared to control children and adults (Lorenzi 2000).

To determine whether *in utero* RNAi of *Kiaa0319* impaired the sensitivity and selectivity of A1 sites, we evaluated responses at each site to a wide range of tonal stimuli (1-32 kHz, 0-75 dB SPL). The observation that average response threshold was not impaired in KIA- rats compared to controls (8.9 ± 0.6 dB SPL vs. 7.2 ± 0.6 dB SPL; $p= 0.06$) suggests that basic hearing ability was not disrupted by *Kiaa0319* RNAi. The latency of tone evoked responses was later and the response amplitude was lower in KIA- rats (Figure 2.7A), which is consistent with tone evoked responses in dyslexics (Tonnquist-Uhlen 1996). Peak latency was 27 ± 0.5 ms in KIA- rats and 22 ± 0.5 ms in control sites ($p< 0.01$). KIA- recordings had higher spontaneous firing levels than controls (16.2 ± 0.6 Hz in KIA- vs. 12.6 ± 0.6 Hz in controls; $p< 0.01$). KIA- sites also had significantly narrower bandwidths than controls. For example, BW20 (20 dB above threshold) was 1.89 ± .05 octaves in KIA- sites compared to 2.25 ± .04 octaves in control sites ($p< 0.01$). In spite of the lower peak firing rate (Figure 2.7A), KIA- sites actually fired more spikes per tone than control sites. The number of spikes evoked by tones within 0.5 octaves of the best frequency was computed for intensities from 0 to 75 dB SPL. KIA- sites fired significantly more spikes than control sites for intensities from 10 to 65 dB SPL (Figure 2.7B). For example, KIA- sites generated approximately 20% more spikes per 40 dB SPL tone than

controls (1.4 ± 0.1 vs. 1.23 ± 0.1 spikes, $p < 0.01$). The average characteristic frequency was higher in KIA- sites than in controls (13 kHz in KIA- compared to 9.7 kHz in controls; $p < 0.01$). Although *in utero* RNAi of *Kiaa0319* does not alter tone thresholds and hearing range, it does significantly alter A1 response properties which may contribute to the abnormal neural responses to speech sounds.

Firing abnormalities to non-speech stimuli contribute to poor phoneme classification

To evaluate which of the abnormal A1 response properties were most likely to contribute to degraded speech responses, we created subpopulations of sites from control rats which were selected to have the same distribution as KIA- rats for several different response properties and evaluated which subpopulations were also significantly impaired in speech discrimination (as compared to the full sample of control sites; consonant performance of 77 ± 2% and vowel performance of 59 ± 1%). Since KIA- sites fired with much higher trial by trial spike count variability (over a 40 ms window), control sites could not be found to match the distribution of KIA- sites. We analyzed the 10% of control sites with the highest variability and found that these sites' ability to discriminate consonants was significantly different than the full set of control sites sites (consonant discrimination; 68 ± 1%; $p = 0.01$) but was not different on vowel tasks; 58 ± 2%; $p=0.24$). Control sites with a spontaneous firing distribution selected to match that of KIA- rats were significantly different from the full sample of control sites at neural discrimination of consonants (68 ± 1%; $p<.01$; Figure 2.8A) and vowels (57 ± 1%; $p<.01$; Figure 2.8B). Control sites with a peak latency distribution or a bandwidth distribution selected to match that of KIA- rats were significantly different from the full set of control sites at neural discrimination of consonants (latency: 68 ± 1%; $p<.01$, bandwidth: 68 ± 1%; $p<.01$), but did not

differ at vowels (latency: 59 ± 1%; p=.67, bandwidth: 59 ± 1%; p=.46). Control sites with a CF distribution selected to match that of KIA- rats were not significantly different from the full set of control sites at consonant (73 ± 1%; p= 0.09) and vowel discrimination (59 ± 1%; p= 0.33). These results suggest that abnormal/inconsistent neural excitability and latency may contribute the impaired responses to speech sounds observed in rats transfected with *Kiaa0319* shRNA *in utero*.

Neurons with RNAi of Kiaa0319 are more excitable than control neurons

Kiaa0319 is a very large protein (1052 amino acids, 116 kDa) whose functions are poorly understood (Velayos-Baeza et al. 2010; Velayos-Baeza et al. 2008; Poon et al. 2011a). To investigate the effect of reduced expression of this gene on intracellular firing properties, we made whole-cell patch clamp voltage recordings from layer II/III pyramidal neurons expressing one of four transgenes. Cells expressing *Kiaa0319* shRNA fired many more action potentials in response to current injection compared to scramble control neurons (same control as above). For example, neurons expressing the *Kiaa0319* shRNA fired 5.5 ± 1 spikes in response to a 200pA current injection, while control (scrambled RNA) neurons responded with 0.5 ± 0.5 spikes to the same current injection ($p < 0.01$; Figure 2.9A). To confirm that the increased excitability is not due to a non-specific effect of the *Kiaa0319* shRNA, we recorded from cells that expressed both the *Kiaa0319* shRNA (which reduces *Kiaa0319* expression) and a transgene to increase *Kiaa0319* expression. The normal excitability of the rescue controls suggests that reduced *Kiaa0319* expression causes greater neural excitability. We also recorded from neurons that expressed the transgene to increase *Kiaa0319* expression (overexpression control). The normal level of excitability seen in recordings from this control confirms that the increased excitability

in the reduced *Kiaa0319* expression group (KIA-) was not due to any non-specific effect of RNAi.

One possibility for the increased excitability following *Kiaa0319* RNAi would be an increase in input resistance as cells with increased input resistance may fire more action potentials as compared to control cells. To test input resistance of individual neurons, differing amounts of current (between -200 and 500 pA in 50 pA increments) were injected into the cell and sub-threshold membrane potential of the cell was measured at each step (Figure 2.9B&C). KIA- neurons showed a significantly greater change in membrane potential for every pA of current injected as compared to scramble controls (at 100 pA of current, KIA- membrane potential changed by 18.4 ± 3.6 mV versus 8.1 ± 1.1 mV in controls; p= 0.02; Figure 2.9B), indicating a greater input resistance in KIA- neurons as compared to controls (193.7 ± 25.3 Mohm in KIA- cells vs. 103.6 ± 21.4 Mohm in scramble control; p= 0.01; Figure 2.9C&D). Neurons with reduced expression of *Kiaa0319* did not have a significant difference in gross anatomy (Galaburda et al. 2006; Peschansky et al. 2010), resting membrane potential (-71.1 ± -0.7 mV in KIA- vs. -71.5 ± -1.4 in scramble control; p= 0.70) or action potential width (0.7 ± -0.03 ms compared to 0.7± -0.02 ms in controls; p= 0.40). Our result that reduced expression of *Kiaa0319* causes increased resistance may help explain the variability in number of action potentials fired trial-to-trial in our multi-unit data.

DISCUSSION

Summary of results

This study was designed to test the hypothesis that *in utero* RNAi of *Kiaa0319* can disrupt the brain's ability to process speech sounds. Recordings in awake and anesthetized rats

demonstrate that *Kiaa0319* RNAi degrades auditory cortex responses to both speech and non-speech sounds. Increased spontaneous firing, increased latency, increased response variability and decreased frequency selectivity all contribute to the reduced ability of A1 sites to distinguish between speech sounds. Neurons with transfected with *Kiaa0319* shRNA have higher input resistance and greater excitability compared to control neurons. These results provide the first direct evidence of a neural mechanism whereby the dyslexia associated gene *Kiaa0319* could interfere with phonemic processing.

Dyslexic individuals have abnormal auditory neural responses

Dyslexics have abnormal auditory cortex responses that are similar to the abnormalities we observed in rats transfected with *Kiaa0319* shRNA *in utero* (KIA-).

Auditory-evoked potentials in dyslexic humans are later and weaker than controls in response to tones and speech sounds (Nagarajan et al. 1999; Tonnquist-Uhlen 1996). Studies using fMRI consistently show reduced cortical response to speech during passive exposure (Kraus et al. 1996; Kujala et al. 2000; Schulte-Körne et al. 2001) and during phoneme discrimination tasks (Flowers, Wood, Naylor 1991; Rumsey et al. 1992; Rumsey et al. 1997; Temple et al. 2001; Temple et al. 2003; Temple et al.). Our results suggest that this reduced response may be due to higher trial by trial variability rather than a reduced number of action potentials. Human neural responses are also less able to lock to gamma-rate modulations of white noise (Lehongre et al. 2011). The result that neural responses in rats with *in utero* RNAi of *Kiaa0319* are significantly worse at phase locking to repetitive broad band stimuli suggests that reduced expression of this gene may directly impair the ability of auditory cortex to fire consistently to speech and non-speech stimuli.

Neural responses in dyslexic humans are abnormal in several non-cortical areas. Responses in the left auditory thalamus to phoneme stimuli are weaker in dyslexics (Diaz et al. 2012). This brain region responds asymmetrically in controls but fire symmetrically in dyslexics. For example, in a phoneme task, the left auditory thalamus in controls responds more strongly than the right, and for speaker identification tasks, the right thalamus responds more strongly than left. In dyslexics, the two hemispheres show no difference relative to the task. Auditory brain stem responses (ABRs) in dyslexic humans are also weaker and fire less precisely to the timing characteristics of speech sounds (Russo et al. 2004). ABRs in dyslexics also do not adapt to repetitive stimuli as they do in controls (Chandrasekaran et al. 2009). *Kiaa0319* is expressed in many brain areas including brainstem, striatum, hippocampus and cortex (Peschansky et al. 2010; Poon et al. 2011b). It is likely that variants in the gene *Kiaa0319* disrupt neural firing properties in multiple brain regions.

Genetic basis of dyslexia

The underlying cause of dyslexia has been a matter of great debate for thirty years. Factors such as socio-economic status, birth weight, visual function, attention, and genetics have all been proposed to explain the disorder (Bates et al. 2011; Galaburda et al. 2006; Hack et al. 1991; Hari et al. 1999; Miles and Haslum 1986; Pennington et al. 1991; Ramus et al. 2003). Twin studies provided the first convincing evidence that genetics plays a major role in the development of problems with reading (Pennington et al. 1991). Genome wide association studies failed to find a single gene responsible for the majority of cases of dyslexia and instead identified a diverse set of genes (*KIAA0319*, *DCDC2*, *ROBO1*, *DYX1C1*), each one of which alone accounts for only a small percentage of the population variance (Bai et al. 2003; Bai et al.

2008; Burbridge et al. 2008; Deffenbacher et al. 2004; Fisher and DeFries. 2002; Galaburda et al. 2006; Meaburn et al. 2008; Roeske et al. 2011; Scerri et al. 2011; Threlkeld et al. 2007). All four of the dyslexia associated genes are expressed in the brain, but their contribution to reading problems remains unclear. Our study tested the earlier proposal that dyslexia is caused by poor phonemic awareness due to a degraded neural representation of speech sounds (Martino et al. 2001; Poelmans et al. 2012; Russo et al 2004; Tallal and Piercy 1974). The idea was that poor phoneme processing is usually not detected until children must explicitly relate specific speech sounds (phonemes) to specific letters (graphemes). Although it was clear that dyslexics have abnormal brain responses, it was not at all clear how dyslexia associated genes might lead to these abnormalities. Our demonstration that *in utero* RNAi of *Kiaa0319* can degrade the neural representation of speech sounds is consistent with this hypothesis.

A small sub-population of humans with dyslexia have known variants in the *KIAA0319* gene (Bates et al. 2011; Deffenbacher et al. 2004; Galaburda et al. 2006; Harold et al. 2006; Paracchini et al. 2006; Schumacher et al. 2006). Dyslexics with *KIAA0319* variants had reduced activation of the left temporal cortex in response to speech (Pinel et al. 2012). This abnormality is correlated with poor speech perception and reading ability. Dyslexics with a *KIAA0319* variant also have white matter abnormalities in left tempo-parietal cortex (Darki et al. 2012). Dyslexics with *KIAA0319* variants typically have mutations in the promoter region of the gene (Paracchini et al 2006), which causes reduced expression of *KIAA0319* (Dennis et al 2009). Our observation that *in utero* RNAi of *Kiaa0319* in rats results in degraded cortical responses to speech is consistent with observations in dyslexics with *KIAA0319* mutations.

It will be important to determine if other dyslexia genes can degrade the cortical representation of speech sounds. If reduced expression of most dyslexia genes can degrade speech sound processing, it is likely that degraded auditory processing is the primary deficit responsible for dyslexia. If reduced expression of other dyslexia genes does not degrade speech sound processing, then the auditory processing hypothesis of dyslexia would be in doubt. A recent study reported that human dyslexics with *ROBO1* mutations exhibit abnormal evoked responses in auditory cortex and the severity of this abnormality is proportional to the level of *ROBO1* gene expression (Lamminmäki et al. 2012). Additional studies in humans or animals with reduced expression of *ROBO1, DCDC2,* and *DYX1C1* are needed to determine whether auditory cortex dysfunction is a common consequence of dyslexia gene mutation.

The amount of genetic suppression present may contribute to the extent of the observed deficit. RNAi does not generate uniform suppression and does not affect every neuron. Even though this model is not a complete genetic knockout, the suppression of dyslexia associated genes can affect cells that were not transfected. Previous work has shown that RNAi of another dyslexia associated gene (*Dcdc2*) can cause non cell-autonomous effects, as demonstrated by migration abnormalities in non-transfected cells (Burbridge et al. 2008). Our results show that even though it is likely that many cells included in our multi-unit recordings were not transfected, the influence of the genetic suppression was significant enough to generate a significant impairment in cortical auditory processing. The extent of the effect on non-transfected auditory cortex neurons is unanswered and would provide insights into the multi-modal symptoms observed in dyslexics.

Future Directions

Our model of a *Kiaa0319* variant in rats is valuable for studying the direct contribution of this gene to auditory processing. The behavioral consequence of *in utero* transfection of *Kiaa0319* shRNA on speech discrimination in rats is not known. However, a recent study in these rats confirmed that they are impaired at discrimination of frequency-modulated (FM) sweeps (Szalkowski et al. 2012). Our observation that these rats exhibit impaired speech responses suggests that they may have problems discriminating between similar speech sounds. A similar degree of degradation of the neural response to speech caused by added background noise (Shetake et al. 2011) or signal degradation using a noise vocoder (Ranasinghe et al. 2012a) impaired consonant and vowel discrimination in rats. The hypothesis that *in utero* RNAi of *Kiaa0319* will impair phoneme discrimination needs to be tested.

Rats with *in utero* RNAi of *Kiaa0319* could be used to test the neural mechanisms that allow for improved phoneme processing with extensive behavioral training. Extensive therapy in dyslexics can normalize neural responses in the cortex and brainstem. For example, three months of exposure and discrimination training can improve speech evoked responses in auditory cortex and brainstem (Gaab et al. 2007; Temple et al. 2003). When interventions focus on only a small set of stimuli, improvements in cortical responses can be seen in as little as three weeks (Lovio et al. 2012; Tremblay & Kraus. 2002). Speech training can also improve timing and amplitude of speech evoked responses in the auditory brainstem (Russo et al. 2004). If neural responses in our rat model can be improved by training, recordings of action potential patterns may elucidate the mechanisms by which behavioral therapy improves speech sound processing in dyslexics.

Phoneme processing problems in dyslexia may be due to inconsistent neural firing

Many studies have documented that dyslexics have a smaller average auditory evoked response compared to control subjects. The simplest interpretation is that fewer neurons respond to sound in dyslexics. Our results suggest another explanation. It is possible that abnormal expression of dyslexia genes impairs speech processing by increasing trial by trial variability (internal noise), rather than by reducing the number of neurons that respond to sound. Several imaging studies in humans with dyslexia have suggested that poor phonological awareness is directly related to inconsistent neural responses across different stimuli (McAnally and Stein. 1996; Wible et al. 2002; Ziegler et a.. 2009). We suggest that this inconsistent firing occurs across repeats of the same stimulus as well. Rats with transfection of *Kiaa0319* shRNA *in utero* have higher trial by trial variability in the timing and the number of spikes generated by each sound. This variability appears to be responsible for the lower peak firing rate for the average population response to both speech and non speech stimuli. The classifier we used relies on single trial responses to discriminate between different sounds. Neural discrimination was impaired when *Kiaa0319* shRNA was transfected *in utero* even though the number of evoked spikes was not decreased. These results suggest the possibility that phoneme processing problems in dyslexics can be caused by increased trial by trial variability even if the average response is not reduced.

Conclusion

In utero RNAi of *Kiaa0319* increases excitability in cortical neurons and degrades the spectral and temporal fidelity of auditory cortex responses. The cortex of rats transfected with *Kiaa0319* shRNA *in utero* have delayed latency, are impaired at phase-locking to repetitive

stimuli, and show significantly poorer discrimination of both consonant and vowel stimuli. We have confirmed that the candidate dyslexia gene *Kiaa0319* is involved in phonemic processing in primary auditory cortex and our results suggest that this gene may contribute to these deficits in dyslexic humans. In addition, intracellular recordings revealed that *in utero* transfection of *Kiaa0319* shRNA increased the excitability of neocortical neurons and may account for the impaired systems level responses. Our observation that a dyslexia associated gene can degrade the neural representation of speech sounds is consistent with a prevailing theory of the biological basis for dyslexia. The rat model of speech sound processing will be useful in testing the relationship between dyslexia gene expression levels and degraded neural responses to speech. The model could also be used to elucidate the mechanism of action of current behavioral treatments for dyslexia.

Funding

This work was supported by the National Institute for Deafness and Communication Disorders at the National Institutes of Health (R01DC010433 to MPK) and the National Institute of Child Health and Human Development at the National Institutes of Health (HD055655 to JJL).

Acknowledgments

We would like to thank K. Trull and N. Wasko for help with histological analysis, M. Fink, L. Doss, R. Miller, E. Hanacik, C. Rohloff, M. Borland, N. Moreno, and C. Matney for help with *in vivo* recordings, and C. Fiondella for performing surgeries. We would also like to thank S. Hays, D.Truong, C. Engineer, K. Im, and S. del Tufo for their suggestions on earlier versions of this manuscript.

APPENDIX

CHAPTER 3 FIGURES

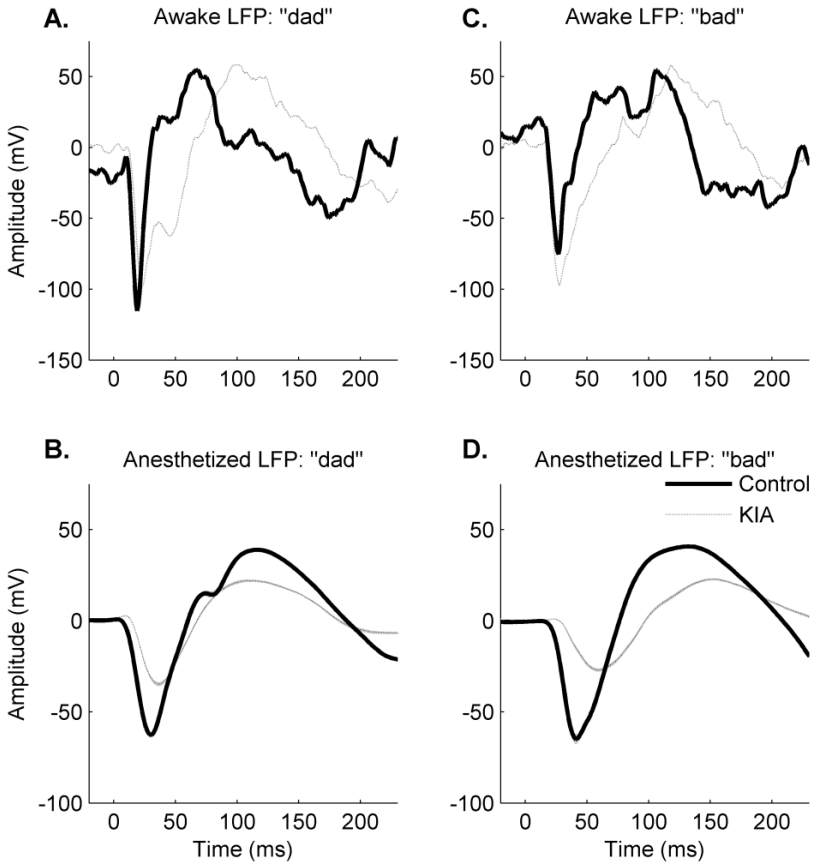

Figure 2.1. *In utero* RNAi of *Kiaa0319* (KIA-) caused delayed speech-evoked local field potentials (LFPs) in both awake and anesthetized rats. LFPs in panels **A.** and **C.** were created by averaging across 47 sites from 9 awake KIA- animals and 6 sites from 3 awake control animals. LFPs in panels **B.** and **D.** were created by averaging across Responses are averaged across all recording sites 247 sites from 5 anesthetized KIA- animals and 255 sites from 6 anesthetized control animals. Latency of the LFP was calculated by milliseconds to the peak of each component. **A.** LFPs in response to the speech sound "dad" had a lower amplitude in KIA- neurons recorded from awake animals compared to controls. **B.** These abnormal response properties were not affected by pentobarbital anesthesia. Speech evoked LFPs to the sound "dad" had longer (but not significantly longer) latency and lower amplitude in KIA- neurons recorded

Figure 2.1 continued…

from animals anesthetized with dilute pentobarbital compared to controls. **C.** LFPs in response to the speech sound "bad" had a longer P1 latency than controls. **D.** These abnormal properties were more obvious in the presence of pentobarbital anesthesia. Though the LFP response to the sound "dad" (panels A & B) were slightly different than the LFP response to the speech sound "bad" (panels C & D), the average response to these sounds was extremely similar.

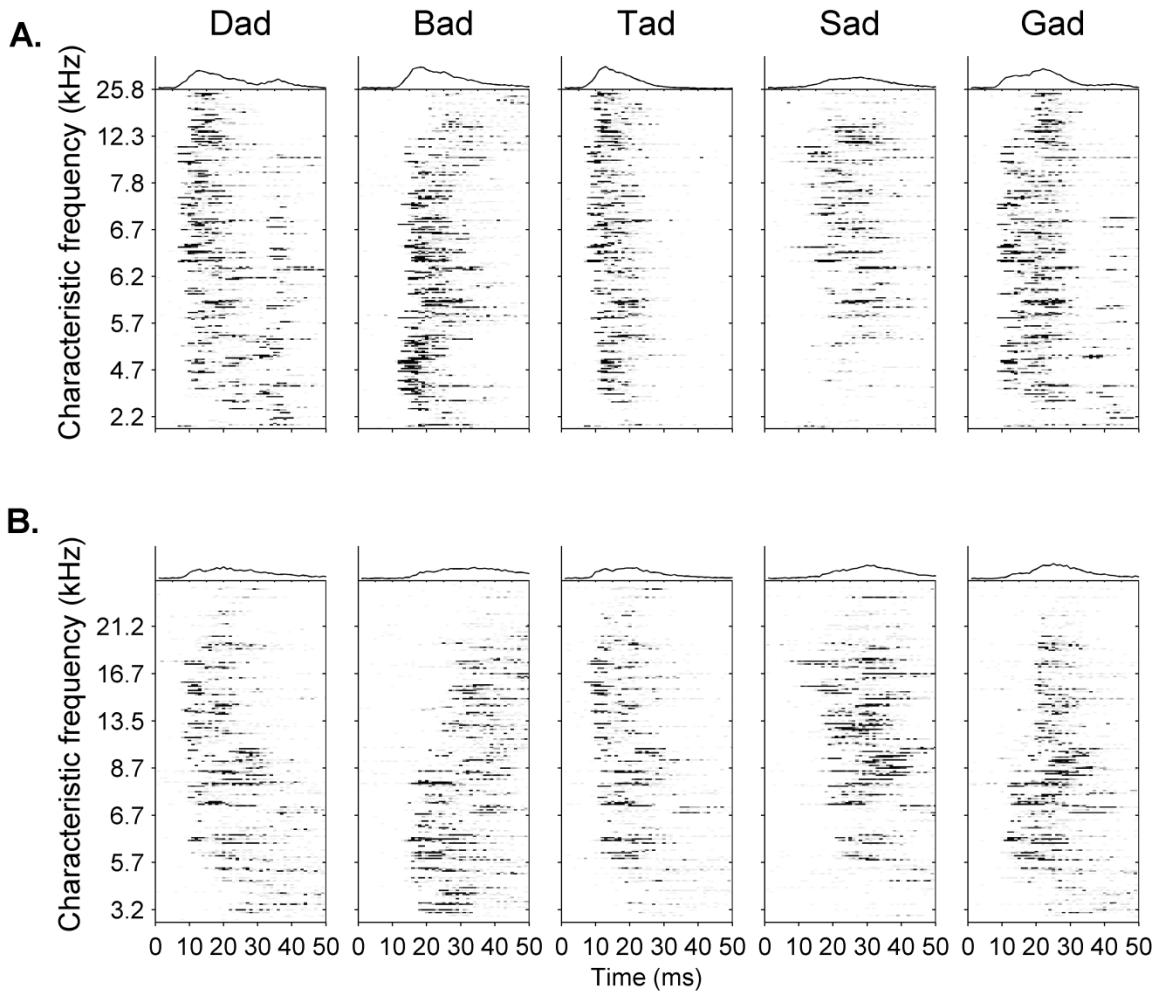

Figure 2.2. *In utero* RNAi of *Kiaa0319* caused degraded spatiotemporal response patterns to consonant speech sounds. **A.** Consonant sounds evoked unique patterns of activation across the A1 of anesthetized control rats. Each multi-unit site's average response to the speech sound over 20 repeats is organized by the characteristic frequency of the site. The average response across all sites is shown on top of each sub-panel. These patterns are similar to previous studies using unaffected rats (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012a; Shetake et al. 2011). **B.** Response patterns of multi-unit recordings taken from primary auditory cortex of anesthetized rats that had undergone *in utero* RNAi. *In utero* RNAi of *Kiaa0319* caused delayed response to speech sounds, as well as reduced precision in firing latency.

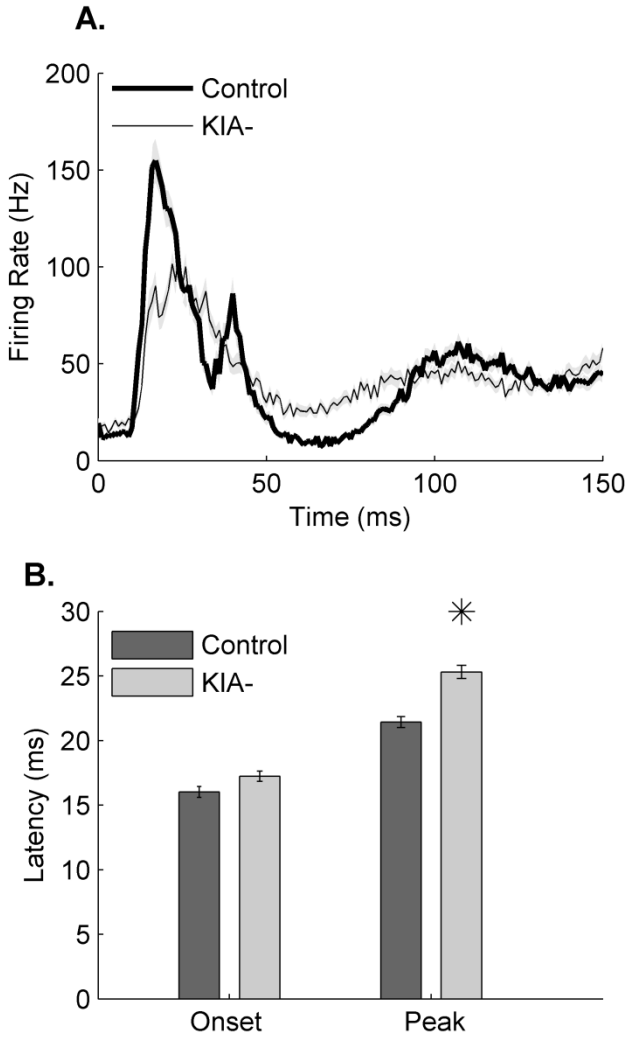

Figure 2.3. *In utero* RNAi of *Kiaa0319* caused delayed activity to speech sounds. **A.** Onset latency and peak latency in response to the consonant sound /d/ were longer in multi-unit recordings from anesthetized rats transfected with *Kiaa0319* shRNA *in utero* (* = p< 0.01). **B.** Average onset latency and peak latency to consonant stimuli were later in multi-unit recordings from anesthetized KIA- rats as compared to anesthetized control sites (* = p< 0.01).

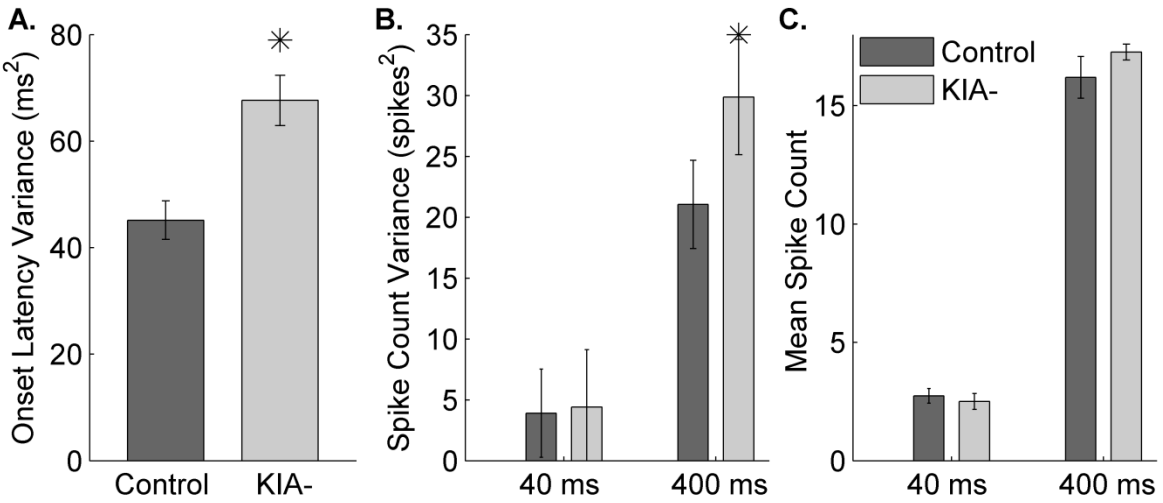

Figure 2.4. *In utero* RNAi of *Kiaa0319* caused increased variability in neural responses to speech stimuli. **A.** Onset latency to speech sounds was more variable across trials in multi-unit responses from anesthetized KIA- rats (light bar) compared to sites from anesthetized controls (dark bar; * = $p<0.01$). **B.** The number of action potentials fired during speech sounds was more variable trial-to-trial in KIA- sites (light bar) during vowels (400 ms window; * = $p< .01$). **C.** The average number of evoked action potentials to speech sounds was not significantly different between control and KIA- sites for either a 40 ms or a 400 ms analysis window ($p=.10$). This result suggests that the increased trial by trial variability is not due to an increased firing rate.

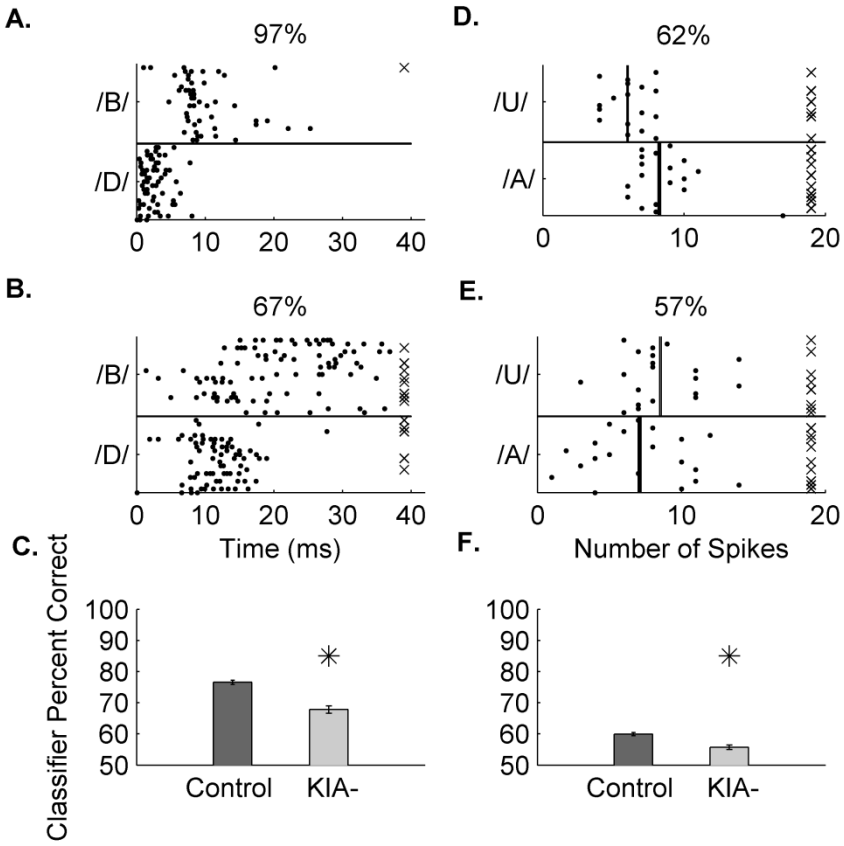

Figure 2.5. *In utero* transfection with *Kiaa0319* shRNA caused impaired neural discrimination of consonant and vowel stimuli. **A.** Single trial neural responses from a typical high frequency control site. Action potentials are plotted over the course of the consonant sound and organized by repeat (each row is a different repeat). To the sound "d", this site fired quickly after the sound onset, while this site fired with a 10 ms delay to the sound "b." Repeats during which the classifier guessed incorrectly as to the stimulus identity are marked by an 'x' on the right. Total percent correct for this pair is on top of the panel. **B.** Single trial neural responses from a typical high frequency KIA- site. To the sound "d," this site fired later than the control site, and with greater variability across repeats. To the sound "b," this site fired later than the control site, and with greater variability across repeats. This site made many more errors in identifying the source stimulus as compared to the control, due to the amount of variability across repeats. **C.** Average percent correct across ten neural consonant discrimination tasks. A two-alternative forced-choice classifier compared the Euclidean distance between a single trial response and the average response to two consonant stimuli. Using activity from a single site in 1 ms bins, the classifier guesses which sound evoked the single trial activity by picking the comparison yielding the smallest Euclidean distance. KIA- neurons were significantly impaired at discriminating between consonant sounds (* = $p < 0.01$). **D.** Single trial neural responses from a typical low frequency control site.

Figure 2.5 continued…

Average number of spikes fired per repeat is plotted by repeat. To the vowel sound “a” (as in “dad”), this site fired more spikes than in response to the vowel sound “u” (as in “dud”). Vowels were more difficult than consonants to discriminate, as the classifier compared each trial’s spike count with the average spike count for that sound (vertical lines) and this is reflected by a greater number of classifier errors, as marked by an ‘x’ on the right. **E.** Single trial neural responses from a typical low frequency KIA- site. The average number of spikes per vowel was more similar in KIA- sites (vertical lines), which makes the classifier guess incorrectly more often. **F.** Average percent correct across six neural vowel discrimination tasks. The same neural classifier used single site activity in 400 ms bins to identify which vowel sound evoked the single trial activity using Euclidean distance. KIA- neurons were significantly impaired at discriminating between vowel sounds (* = $p < 0.01$).

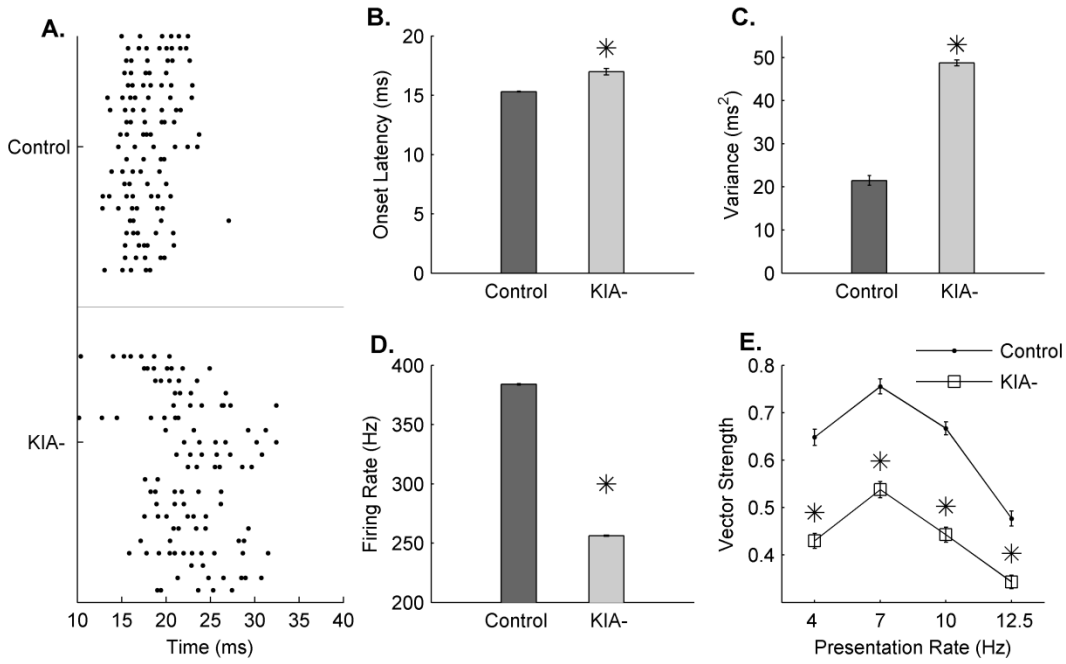

Figure 2.6. *In utero* RNAi of *Kiaa0319* impaired neural firing properties to repetitive broad band stimuli. **A.** Example of single site responses to the first broad-band noise burst. Each row represents a repeat of the stimulus, and each dot shows the location of an action potential with respect to time. The control site (top) responded consistently across repeats, while the KIA- site (bottom) responded later and with more variability across repeats. **B.** Onset latency to the first broad-band noise burst was later in KIA- neurons compared to control neurons (* = p= 0.01). **C.** Variability in peak latency across repeats was significantly longer in KIA- neurons compared to control neurons (* = p< 0.01). **D.** Firing rate to each broad-band burst was lower in KIA- neurons as compared to controls at all four presentation rates (* = p< 0.01). **E.** Vector strength was significantly lower in KIA- neurons at all four presentation rates (* = p< 0.01).

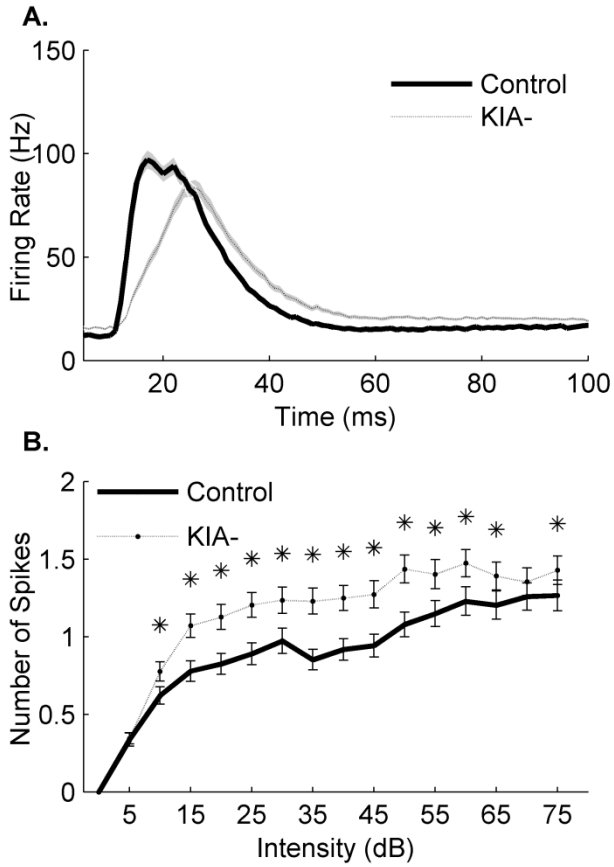

Figure 2.7. *In utero* RNAi of *Kiaa0319* caused impaired neural firing to tonal stimuli. **A.** Spontaneous levels in RNAi rats were higher than controls (* = p<0.01). Similar to speech and noise-burst stimuli, KIA- neurons had a longer peak latency to tone stimuli compared to control neurons (* = p< 0.01). We used only tones louder than 60 dB to plot the average response. This cutoff ensured that the narrower bandwidths observed in KIA- recordings did not bias the comparison. **B.** Average number of spikes to tones at each intensity tested. Number of spikes for each site was counted within the 10 sites surrounding that site's CF to account for bandwidth difference. KIA- neurons fired more spikes to tones than control neurons at most intensities tested (* = p< 0.01).

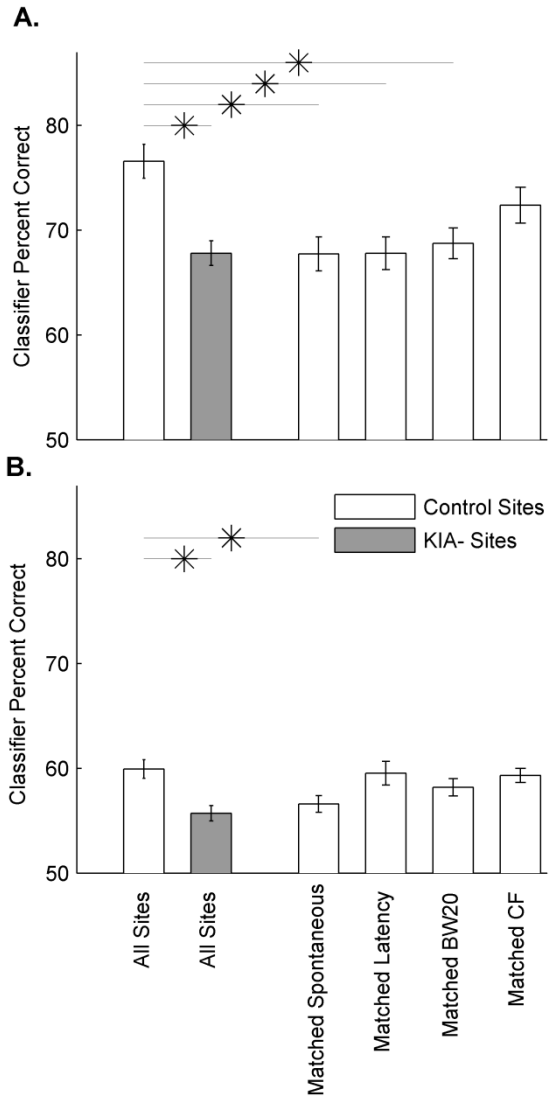

Figure 2.8. Basic neural firing impairments may contribute to poor neural classification of phonemes. We tested groups of control neurons that mimicked the distribution of KIA- neurons with respect to several neural firing properties. All * are p<.01. **A.** Several abnormal firing properties contributed to poor consonant classification. Control neurons with a distribution of spontaneous firing (third bar; 68 ± 1%; p<.01), peak latencies (fourth bar; 68 ± 1%; p<.01), and bandwidths (at 20 dB above threshold, fifth bar; 68 ± 1%; p<.01) that match the KIA- sites were significantly different than the full set of control sites on neural discrimination of consonants. **B.** Abnormal spontaneous firing levels seemed to contribute to poor vowel performance. Control neurons with a distribution of spontaneous firing rates that match the KIA- sites were significantly different from the full set of control sites on neural discrimination of vowels (57 ± 1%; p<.01).

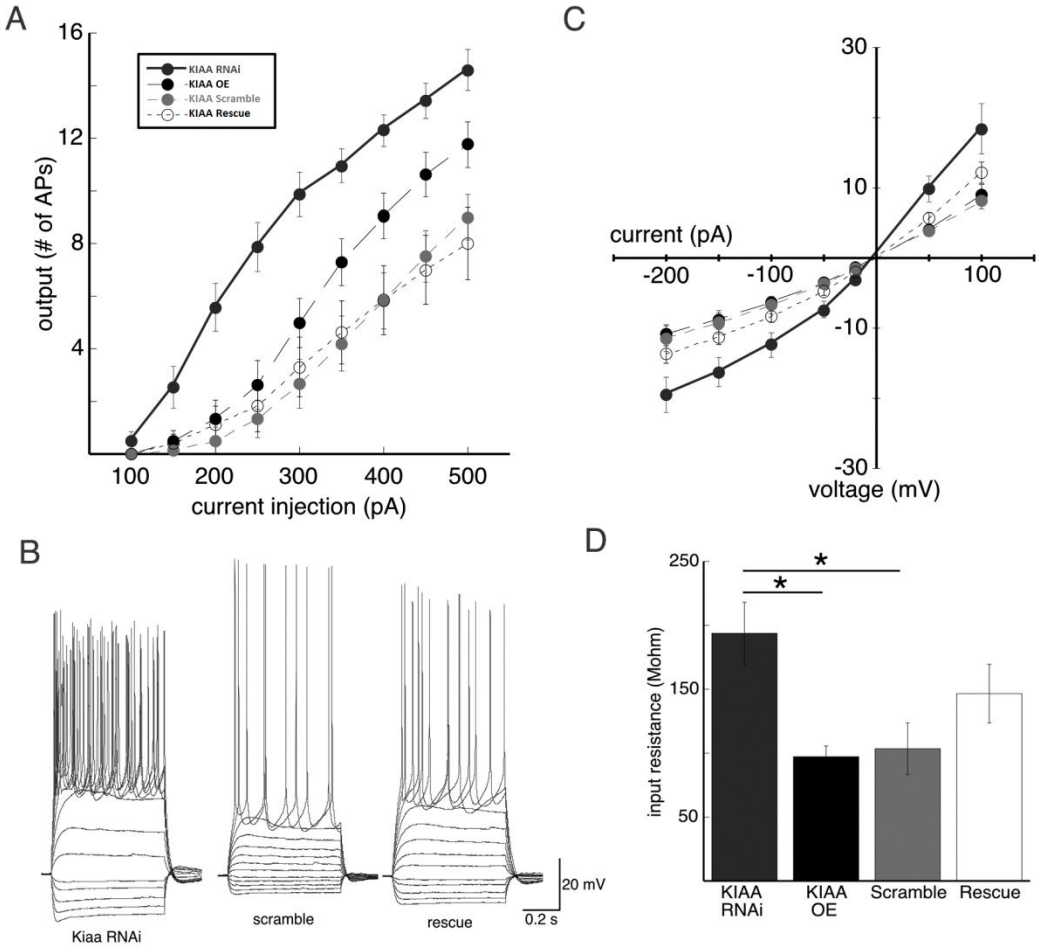

Figure 2.9. Neurons with transfection of *Kiaa0319* shRNA had higher membrane resistance than controls. Four experimental groups were used in this experiment: KIAA RNAi neurons expressed the *Kiaa0319* shRNA transgene, KIAA OE (over expression) neurons expressed a transgene which increased *Kiaa0319* expression, Scramble neurons expressed a scrambled shRNA, and Rescue neurons expressed both the *Kiaa0319* shRNA (which reduced expression) and a transgene to increase expression. **A.** KIAA RNAi neurons fired significantly more action potentials per pA of current injection than Scramble controls (at 200pA of current, KIAA RNAi neurons fire 5.5 ± 1 spike vs.0 .5 ± 0.5 spikes in controls; * = p< 0.01). **B.** Example voltage traces from one KIAA RNAi neuron and two control neurons. In response to current injection, membrane

Figure 2.9 continued…

potential of KIAA RNAi neurons changed more drastically and fired more action potentials than Scramble controls. **C.** Input resistance function for all four experimental groups tested. For each pA of current injected, the membrane potential of KIAA RNAi neurons changed significantly more than control groups (at 100 pA of current, KIAA RNAi membrane potential changed by 18.4 ± 3.6 mV versus 8.1 ± 1.1 mV in controls; * = p=0.02). **D.** KIAA RNAi neurons had a higher membrane resistance than scramble control neurons (193.7 ± 25.3 Mohm in KIA- cells vs. 103.6 ± 21.4 Mohm in scramble control; * = p= 0.01). KIAA OE and Rescue controls did not have increased membrane resistance (* = p= 0.05).

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CHAPTER 3

CORTICAL SPEECH-EVOKED RESPONSE PATTERNS IN MULTIPLE AUDITORY FIELDS ARE CORRELATED WITH BEHAVIORAL DISCRIMINATION ABILITY

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ABSTRACT

Different speech sounds evoke unique patterns of activity in primary auditory cortex (A1). Behavioral discrimination by rats is well correlated with the distinctness of the A1 patterns evoked by individual consonants, but only when precise spike timing is preserved. In this study we recorded the speech evoked responses in the primary, anterior (AAF), ventral (VAF), and posterior (PAF) auditory fields of the rat and evaluated whether activity in these fields is better correlated with speech discrimination ability when spike timing information is included or eliminated. Spike timing information improved consonant discrimination in all four of the auditory fields examined. Behavioral discrimination was significantly correlated with neural discrimination in all four auditory fields. The diversity of speech responses across recordings sites was greater in PAF and VAF compared to A1 and AAF. These results suggest that while the various auditory fields of the rat process speech sounds differently, neural activity in each field could be used to distinguish between consonant sounds with accuracy that closely parallels behavioral discrimination. Earlier observations in the visual and somatosensory systems that cortical neurons do not rely on spike timing should be reevaluated with more complex natural stimuli to determine whether spike timing contributes to sensory encoding.

INTRODUCTION

Primary auditory cortex (A1) encodes consonant sounds using precise spike timing information within 1-50 ms bins (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Schnupp et al. 2006a; Shetake et al. 2011; Wang et al. 1995). This precision generates unique patterns of activity across the tonotopic organization of this field. For example, in rat A1, the consonant sound /d/ causes an evoked response from neurons tuned to high frequencies first (higher than ~7 kHz), followed by a response from neurons tuned to lower frequencies after a short delay of ~20 ms. The consonant sound /b/ causes the opposite pattern such that low frequency neurons fire first, followed by higher frequency neurons. To account for the shifted audiogram of the rat, these stimuli are shifted up by an octave and the firing differences across frequency groups reflect the frequency information in the stimuli (Engineer et al. 2008; also see Figure 3.1). Each consonant sound evokes a unique pattern of activity and the difference in these responses can be used to identify the evoking stimulus using a pattern classifier (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012b; Shetake et al. 2011; Foffani and Moxon 2004).

The uniqueness of these patterns is correlated with behavioral ability of rats in a wide range of tasks. Rats are able to discriminate human speech sounds in quiet (Engineer et al. 2008; Perez et al. 2012), background noise (Shetake et al. 2011), and after spectral or temporal degradation (Ranasinghe et al. 2012b). The neural responses in primary auditory cortex can predict behavioral ability in all of these tasks using a Euclidean distance classifier. Sounds that create contrasting patterns of activity, such as the opposite patterns evoked by /b/ and /d/, correspond to pairs of sounds that are more easily discriminated by rats (Engineer et al. 2008;

Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011). Pairs of sounds that have similar spectrotemporal profiles, such as /r/ and /l/, cause similar neural responses and are more difficult for rats to behaviorally discriminate (Engineer et al. 2008). Degraded stimuli, like those caused by the addition of background noise or a vocoder, can cause delayed and/or weakened neural responses. The more severe the degradation to the neural response patterns, the more impaired the rats were at the corresponding behavior task (Ranasinghe et al. 2012b; Shetake et al. 2011). The preservation of the spike timing information is crucial to these correlations; if spike timing information is removed, no tasks are correlated with the differences in patterns of neural activity (Engineer et al. 2008; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011).

Other sensory systems appear to rely predominantly on spike rate, rather than spike timing for discrimination, especially in sensory fields higher up in their respective pathways. In the visual system, the primary visual cortex relies on precise spike timing to discriminate between stimuli that evoke similar number of spikes (Montemurro et al. 2008). As the information is passed to higher level regions, neurons begin to use spike rate information rather than spike timing to make behavioral decisions. For example, when monkeys were asked to identify the direction of motion of a visual stimulus after a delay, the rate of firing in the posterior parietal cortex predicted the behavioral decision of the subject (Shadlen and Newsome 2001; Shadlen and Newsome 1996). The somatosensory system functions in a similar manner. For example, when monkeys were trained to detect whether the rate of two tactile vibrations were the same, high level areas of the somatosensory system perform the task by comparing the firing rate of neurons to each stimulus (Romo and Salinas 2003). A well-received theory states

that temporal information is consistently transformed into a rate code as the information moves up the appropriate neural network (Ahissar et al. 2000). This is plausible in the auditory system as well (Buonomano and Merzenich 1995; Wang et al. 2008). The current study was designed to test the theory that neural responses to consonant speech sounds would also undergo a transformation throughout the auditory pathway. Early auditory cortex requires precise spike timing information to encode consonant sounds (Centanni et al. 2013; Engineer et al. 2008; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011), while vowel stimuli rely on spike rate, even at this early sensory level (Perez et al. 2012). We therefore used consonant stimuli in the current study to answer the following question: Do auditory fields higher in the auditory pathway than A1 use spike timing or spike rate to encode and discriminate consonant speech sounds?

METHODS

Anesthetized recordings

We acquired 1,253 multiunit recordings from the auditory cortex of 15 experimentally naïve rats. The recording procedure is explained in detail elsewhere (Engineer et al. 2008). In brief, animals were anesthetized with pentobarbital (50 mg kg^{-1}) and given supplemental dilute pentobarbital (8 mg ml^{-1}) as needed to maintain areflexia, along with fluids to prevent dehydration. A tracheotomy was performed to ensure ease of breathing throughout the experiment. Primary auditory cortex and several nearby auditory fields were exposed via craniotomy and durotomy. Four Parylene-coated tungsten microelectrodes (1-2 MΩ) were simultaneously lowered to layer IV/V of either left or right auditory cortex (~600-800 μm).

Responses were collected in four auditory fields; primary auditory cortex (A1), anterior auditory field (AAF), posterior auditory field (PAF) and ventral auditory field (VAF). These fields are widely considered core regions in the rat (Doron et al. 2002; Polley et al. 2006; Storace et al. 2010), but recent evidence suggests that these fields receive input from more than one thalamic region, which may suggest a hierarchical organization (Smith et al. 2012; Storace et al. 2012). Brief (25 ms) tones with 5 ms ramps were presented at 90 randomly interleaved frequencies (1-48 kHz) at 16 intensities (0- 75 dB SPL) to determine the characteristic frequency of each site. We also presented 7 English consonant-vowel-consonant (CVC) speech sounds (/dad/, /sad/, /tad/, /bad/, /gad/, /shad/, and /chad/) previously tested in our lab (Engineer et al. 2008; Ranasinghe et al. 2012b; Shetake et al. 2011; Floody et al. 2010). The speech stimuli were randomly interleaved and presented at 20 repeats per recording site. Sounds were shifted up 1 octave into the rat hearing range using the STRAIGHT vocoder (Kawahara 1997; Figure 3.1). Each sound was calibrated with respect to its length so that the most intense 100 ms of the stimulus length was heard at 60 dB SPL. All sounds were presented approximately 10 cm from the contralateral ear of the rat. As the effect of speaker location was beyond the scope of our study, the speaker was always located outside of the pinna and aimed directed into the ear canal. With this configuration, the sound level was always greater to the contralateral ear (which corresponded to our recording sites) versus the ipsilateral ear.

Neural data analysis

To define the borders between auditory fields, recording sites were analyzed to select the characteristic frequency of each site, as well as to obtain bandwidth, latency, peak firing and end of peak response information. Firing latency is defined as the point in time (ms) that average

firing rate (across all repeats) first crosses two standard deviations above the spontaneous firing rate, threshold is defined as the lowest intensity that evoked a response from the multiunit site, and bandwidths were calculated at 10, 20, 30 and 40 dB above threshold and defined as the range of frequencies that evoked responses at the current intensity. Each field was defined as established in the literature, using characteristic frequency gradients, gradient reversals, and tuning curve properties (Doron et al. 2002; Higgins et al. 2010; Pandya et al. 2008; Polley et al. 2006). A1 sites were defined as having sharp tuning, short onset latency (between 10-20 ms from tone onset), high firing rate (100 Hz or greater), and organized tonotopically so that characteristic frequencies (CFs) ranged from low to high in a posterior-anterior direction. AAF sites were defined using the same parameters as A1, but with a reversed tonotopy, such that CFs ranged from low to high in an anterior-posterior direction. The VAF field was located anatomically between AAF and A1. We first located the tonotopic gradient reversal at the edges of AAF and A1. Next, sites were analyzed for whether or not they exhibited non-monotonic features. Non-monotonic sites were defined as sites in which the response bandwidth at 40 dB above threshold was not wider than responses 30 dB quieter. VAF as a field also had a higher average characteristic frequency as compared to the other fields (Polley et al. 2006). PAF sites were defined as having long onset latency (greater than 30 ms) and broad tuning curves and were located immediately posterior to A1. These methods of defining site boundaries are consistent with previous work in rodent models (Doron et al. 2002; Higgins et al. 2010; Pandya et al. 2008; Polley et al. 2006). Out of the 1,253 sites we acquired, 1,116 of these were included in the subsequent analyses.

Single trial response patterns to each of the isolated speech sounds were compared using a nearest neighbor classifier (Engineer et al. 2008; Perez et al. 2012; Popescu and Polley 2010; Ranasinghe et al. 2012a; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011; Foffani and Moxon 2004; Foffani and Moxon 2005). We used Euclidean distance to compare single trial activity from 5 random sites to the average post-stimulus time histogram (PSTH) template evoked by 19 repeats each of two different stimuli. Activity was binned using 1 ms temporal precision over a 40 ms window to encompass the spike timing precision present in the initial consonant (Engineer et al. 2008; Ranasinghe et al. 2012b; Porter et al. 2011). The classifier then compared the response of each single trial with the average activity template (PSTH) evoked by all repeats of each of the speech stimuli presented. The current trial being considered was not included in the PSTH to avoid artifact. The classifier attempted to identify the stimulus that evoked the current single trial activity pattern by selecting the template that was closest to the single trial in units of Euclidean distance. ED is calculated using the formula:

$$\text{Euclidean Distance} = \sqrt{\sum_{i=1}^{\#sites} \sum_{j=1}^{\#\,bins} \left(X_{ij} - Y_{ij}\right)^2}$$

where $\#_{sites}$ is each recording site and $\#_{bins}$ is each of 40 one-millisecond bins being compared between activity evoked by speech sound X versus speech sound Y. We chose Euclidean distance as our metric for two reasons. First, it is a well established metric for this type of neural classification (Engineer et al. 2008; Perez et al. 2012; Popescu and Polley 2010; Ranasinghe et al. 2012a; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011; Foffani and Moxon 2004; Foffani and Moxon 2005). Second, this metric is inherently resistant to variations in spike rate. Since some auditory fields fire fewer spikes to auditory stimuli than others, we

wanted to ensure that this variable was accounted for in evaluating the neural encoding ability of various auditory fields. We ran the classifier so that each site was included in the classifier analysis at least once (i.e. the classifier ran once for each site recorded) and performance was calculated as the average performance across all classifier runs. For example, the classifier ran 399 times to evaluate every A1 site, 303 times to evaluate every AAF site, and so on. We used an ANOVA and t-tests to compare the accuracy of the classifier across all auditory fields. When appropriate, a Bonferroni correction was used to correct for multiple comparisons.

We then compared speech-evoked neural responses between pairs of sites in each field that had characteristic frequencies within 1/4 octave. This analysis was designed to compare the differences in neural responses between similarly tuned neural sites as a measure of firing redundancy (Chechik et al. 2006; Cohen and Kohn 2011). We counted the number of evoked spikes to each speech sound within the same 40 ms window used for the classifier. We compared the number of spikes evoked by each sound across each pair of sites and quantified the relationship using the correlation coefficient. When neural firing redundancy decreased, we refer to this as an increase in diversity.

Recordings from a subset of 4 rats were mapped with a different speaker than the remaining 11 rats (Motorola Optimus Bullet Horn Tweeter vs. TDT FF1 free field speaker). To ensure that the speaker difference did not significantly change the quality of our neural recordings, we compared the classifier's performance on four consonant tasks (/dad/ vs /bad/, /gad/, /sad/, or 'tad) using sites from three auditory fields in male rats mapped with each speaker. There were no significant differences in classifier performance across speaker (Optimus Bullet

Horn Tweeter speaker; 74.4 ± 1% correct vs. 71.2 ± 1% correct with FF1 speaker; p=0.55), so all data was combined for analysis.

Behavioral testing

We trained 10 rats to discriminate speech sounds using an operant go/no-go procedure. Each rat trained for two 1 hour sessions per day (5 days a week). Rats first underwent a shaping period in which they were taught to press a lever to hear the target sound and obtain a 45 mg sugar pellet reward. Once the rat was able to earn 100 pellets independently for two consecutive sessions, the rat was advanced to detection. During detection, the rat learned to withhold pressing the lever until the target sound was presented. Silent periods were randomly interleaved into the session to serve as catch trials. Rats were initially given 8 seconds to respond to the sound and this window was gradually decreased to 3 seconds. Once the rat achieved a d' of 1.5 or greater for 10 sessions, the rat was advanced to discrimination. During discrimination, the rat learned to press the lever for a target speech sound and not press for several distracter speech sounds. A total of 6 rats were trained to discriminate the sound /dad/ from /bad/, /gad/, /sad/, and /tad/ (data previously published in Engineer et al., 2008) and 4 rats trained on /bad/ versus /dad/, /gad/, /sad/, and /tad/, as well as /shad/ versus /sad/, /chad/, /dad/, and /gad/. Rats were only rewarded for pressing the lever to their respective target sound (presented ~44% of the time). A response to a distracter sound (presented ~44% of the time) or to a silent catch trial (presented ~11% of the time) resulted in a time out in which the cage lights were extinguished and the program paused for 6 seconds. Each discrimination task lasted for 20 training sessions over 2 weeks. Behavior percent correct was evaluated on the last day of training and is reported as the mean ± sem across rats. Training took place in soundproof, double-walled booth which was lined with

foam to reduce noise. Inside the booth, a house light, video camera and speaker were mounted outside an 8 x 8 x 8" cage which contained the lever and food dish. A pellet dispenser was mounted outside the booth to reduce noise. During the experiment, rats were food deprived to above 85% of their original body weight. Rats were housed individually and maintained on a reverse 12-hour light-dark cycle. Behavioral percent correct for correlation with classifier performance was calculated using data from the last day of training. All protocols and recording procedures were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee.

RESULTS

Topographic organization of tone frequency tuning was used to identify the four auditory fields; primary (A1), anterior (AAF), ventral (VAF), and posterior (PAF). A1 and AAF exhibited clearly ordered frequency maps (correlation between site location and characteristic frequency R= -0.77 and R= -0.69 respectively) while VAF and PAF did not (R= -0.05 and R= -0.37 respectively). Characteristic frequencies (CF) in A1 increased in a posterior to anterior direction, while CFs in AAF increased in an anterior to posterior direction (Figure 3.2). Firing latency to tones was significantly different across all four fields (one-way ANOVA, $F(3, 1112) = 181.51$, $p<0.001$; Figure 3.3). VAF had the narrowest bandwidths at 40 dB above threshold (2.4 ± 0.1 octaves), followed by A1 (2.6 ± 0.1 octaves, unpaired t-test, $p<0.01$), AAF (2.8 ± 0.1 octaves, unpaired t-tests vs. A1 and VAF, $p=0.01$), and PAF had the longest bandwidths (3.5 ± 0.1 octaves (unpaired t-tests vs. AAF, A1, and VAF, $p<0.01$; Figure 3.3C). Frequency tuning and tonotopic organization in each field was consistent with earlier reports (Polley et al. 2007;

Carrasco and Lomber 2011; Doron et al. 2002; Jakkamsetti et al. 2012; Pandya et al. 2008; Puckett et al. 2007; Takahashi et al. 2011, see Methods and Figure 3.3), so we are confident that the boundaries between fields were accurately defined. In total, we recorded from 303 multi-unit sites in AAF, 399 sites in A1, 206 sites in VAF and 208 sites in PAF.

Neural discrimination of consonants is better when spike timing information is preserved

Neural activity from each field can be used to discriminate between consonant sounds. As in previous studies, A1 responded to each speech sound differently (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011). We used this classifier to test the hypothesis that other auditory fields use spike timing information to identify speech sounds. We calculated the Euclidean distance between the single trial neural response post stimulus time histogram (PSTH) and each of two PSTH templates created from the average neural activity evoked by each of two sounds. The classifier then guessed which of the template sounds likely caused the single trial pattern of activity by choosing the template which was most similar to the single trial (i.e. had the smallest Euclidean distance; see Methods). Each run of the classifier used multi-unit activity from one to two hundred recording sites. Classifier performance most closely matched previously published behavioral performance of approximately 80% correct when the classifier was given data from five randomly selected recording sites (Engineer et al. 2008; Figure 3.4). As a result, groups of five sites were used for all classifier analyses in this paper.

We tested the ability of neural activity in each field to discriminate all possible pairs of the seven consonant sounds evaluated in this study and compared classifier performance when spike timing information was preserved or removed. We preserved spike timing information by

analyzing the data with 1 ms temporal bins (over a 40 ms analysis window) and we removed timing information by analyzing the data in a single 40 ms bin. The classifier performance was significantly different when spike timing information was preserved versus when it was not (two-way ANOVA, $F(1, 1115) = 2.07$, $p<0.001$). Across all the comparisons we used, PAF sites were significantly worse at the neural discrimination task than A1 and AAF (t-tests with Bonferroni correction, $p<0.01$; Figure 3.5). VAF performance was intermediate between AAF and PAF and was not significantly different from either field (t-tests with Bonferroni correction; $p=0.07$ and $p=0.02$ respectively; Figure 3.5). The classifier performed significantly better when spike timing information was preserved in A1 responses compared to when spike timing information was removed (1 ms bins, 94.4 ± 1.0% correct vs. a single 40 ms bin, 77.3 ± 1.5% correct; t-test, $p<0.01$; Figure 3.5), which is consistent with our earlier report (Engineer et al. 2008). The three non-primary fields also performed significantly better when spike timing information was preserved than when it was removed (AAF: 93.1 ± 1.0% v s. 72.2 ± 1.5% correct; $p<0.01$, VAF: 86.2 ± 1.3% vs. 68.2 ± 1.4% correct; $p<0.01$, PAF: 75.2 ± 1.4% vs. 66.5 ± 1.0% correct; $p<0.01$, t-tests for with and without spike timing information respectively; Figure 3.5). This result is not specific to these bin sizes. As expected, the classifier performed significantly better when spike timing information was preserved (1-10 ms bin sizes) than when spike timing information was removed (40-100 ms bin sizes). When using AAF activity, the classifier was significantly worse at consonant discrimination when 10 ms bins were used instead of 1 ms bins (89.4 ± 1.4% correct with 10 ms bins vs. 93.1 ± 1.0% correct with 1 ms bins, $p=0.02$). For all of the other fields, there was no significant difference in performance when 1 or 10 ms bins were used (A1: 92.2 ± 1.0% correct with 10 ms bins vs. 94.4 ± 1.0% correct with 1

ms bins, p=0.13; VAF: 84.3 ± 1.5% correct with 10 ms bins vs. 86.2 ± 1.3% correct with 1 ms bins, p=0.32; PAF: 75.4 ± 1.5% correct with 10 ms bins vs. 75.2 ± 1.4% correct with 1 ms bins, p=0.94). For each of the four auditory fields tested, these results suggest that spike timing can provide information about consonant identity.

Activity from non-primary fields is nearly as effective as A1 in discriminating speech sounds

As expected from earlier studies (Polley et al. 2007; Carrasco and Lomber 2011; Doron et al. 2002; Jakkamsetti et al. 2012; Pandya et al. 2008; Puckett et al. 2007; Takahashi et al. 2011), the speech evoked responses slightly differed across the four fields. Differences in the response properties to speech sounds were similar to the differences in the response properties to tones (Figure 3.3). AAF and A1 fired quickly after the onset of the speech sound, /dad/ in a single burst of activity (average latency of 14.2 ± 0.7 in AAF and 15.2 ± 0.7 ms in A1; p=0.32, Figure 3.6B&C). VAF sites responded just as quickly as AAF and A1 to the onset of each consonant sound (14.3 ± 0.8 ms; t-tests with Bonferroni correction, p=0.39, Figure 3.6D). The onset of the response in PAF sites was the latest of any of the fields (average latency of 18.1 ± 0.7 ms across all speech sounds; t-test vs. A1, $p<0.01$). The result that VAF and PAF sites responded more quickly to speech sounds than to tones may be due to the broader bandwidths in speech stimuli (Barbour and Wang 2003; Petkov et al. 2006; Rauschecker and Tian 2004; Rauschecker et al. 1995). In addition, the amplitude of response to speech sounds was highest in AAF (601.8 ± 73.2 Hz), followed by A1 (556.1 ± 70.5, t-test vs. AAF, p=0.01), VAF (414.9 ± 54.0 Hz, t-test vs. AAF and A1, $p<0.01$) and PAF (314.8 ± 38.2 Hz, t-tests vs. the other 3 fields, $p<0.01$). The representative examples shown in black in Figure 3.6 (example sites used are outlined in black in Figure 3.2) are sites tuned to ~10 kHz, but the general timing and strength of the response was

consistent across the range of characteristic frequencies in each field (average responses shown in gray; Figure 3.6).

Here, we examined responses to consonant speech sounds in A1 and three additional non-primary cortical fields. For example, the sound /d/ caused neurons tuned to high frequencies to fire first, followed by lower frequency neurons after a brief delay (Figure 3.7A). The consonant sound /g/ caused mid-frequency neurons to fire first, followed by firing from high and low neurons milliseconds later (Figure 3.7A). The spatiotemporal response patterns to speech were similar in AAF to those in A1 (Figure 3.7B). The apparent "blurring" of response in PAF may be caused by broader bandwidths in this field as sites with broad bandwidths likely responded to multiple aspects of the speech signal. In AAF and A1, some narrowly tuned sites fired to the consonant burst while other sites fired to the vowel, while PAF neurons fired to both portions of the stimulus signal. We hypothesized that these differences in response patterns may cause longer latency fields to be worse at encoding speech sounds with a short voice onset time, such as /d/ and /b/.

We calculated the similarity (using Euclidean distance) between patterns of activity evoked by all possible pairs of consonant sounds and compared these differences across auditory fields (Figure 3.8). In every field, the patterns of activity evoked by /dad/ and /bad/ were the least similar (i.e. had the largest Euclidean distance value) and /sad/ and /chad/ were the most similar (i.e. had the smallest Euclidean distance value; Figure 3.8). The difference in neural response patterns between unvoiced consonants (/t/, /s/, /sh/, and /ch/) was higher in VAF and PAF than in A1 (Figure 3.8B&C). The similarity between pairs of neural responses in VAF and PAF were lower than in A1, which suggests that these two fields may be better able to discriminate

between these sounds. In spite of this apparent advantage, classifier performance on pairs of unvoiced consonants was better in A1 than in VAF (90.1 ± 1.8% correct in A1 vs. 82.4 ± 2.6% correct in VAF, $p<0.02$) and in PAF (69.4 ± 2.2% correct, $p<0.01$). As expected from previous studies, these results did not significantly change using larger analysis windows (from stimulus onset until between 50-300 ms later), as long as spike timing information was preserved (e.g. 1-10 ms temporal bins; Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011). These results suggest that while VAF and PAF encode unvoiced consonant stimuli differently than A1, the information needed to discriminate between these patterns of activity is present in all three fields. Although the auditory fields fired to speech sounds with different latencies and bandwidths, the similarity between pairs of speech-evoked neural responses was strongly correlated across fields (Figure 3.8, not all comparisons shown).

Neural responses are correlated with behavioral discrimination of consonants

The similarity between speech-evoked patterns in A1 is strongly correlated with behavioral discrimination ability in rats (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012b; Shetake et al. 2011). We hypothesized that the similarity between patterns of speech-evoked neural responses in the three non-primary fields would also be correlated with behavior. Neural activity from all four auditory fields was correlated with behavioral discrimination ability of rats trained to discriminate several sets of consonant speech sounds. The highest performance was achieved by rats trained on tasks in which /dad/ was the target (88.3 ± 2.3% correct; see Table 3.1 for performance on each pair) and tasks in which /bad/ was the target (87.6 ± 3.7% correct; Table 3.1), followed by tasks in which /shad/ was the target (79.1 ± 9.5% correct; Table

3.1). Using the 5-site classifier described above in each field (neural data recorded in untrained rats), every field was strongly correlated with behavior (R^2=0.41, p=0.02 in AAF, R^2=0.59, p<0.01 in A1, R^2=0.39, p=0.03 in VAF and R^2=0.48, p=0.01 in PAF). Classifier performance in 3 of the 4 fields was correlated when spike timing information was preserved but not when it was removed (without spike timing in AAF, R^2=0.18, p=0.16; in A1 R^2=0.28, p=0.07; and in VAF R^2=0.10, p=0.32). Classifier performance in PAF was correlated with or without spike timing (without spike timing; R^2=0.55, p<0.01). PAF neural discrimination ability was able to correlate to rat behavioral ability without spike timing information, even though this field's classifier performance was significantly worse than the other fields.

Our earlier studies reported that the correlation between speech discrimination performance and neural responses does not depend on using a classifier to perform discrimination using neural activity (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012b; Shetake et al. 2011). The Euclidean distances between pairs of speech-evoked responses (using all neural sites from each field) was correlated to behavioral performance (R^2=0.47, p=0.01 in AAF, R^2=0.54, p=0.01 in A1, R^2=0.29, p=0.07 in VAF, and R^2=0.68; p<0.01 in PAF), as expected from our earlier studies. The patterns of evoked activity to speech in all four auditory areas were strongly correlated with each other and with behavioral discrimination. We hypothesized that the slight differences in response patterns across fields may contribute differently to the animal's performance. We used all sites from all four fields and compared the similarity between neural responses to pairs of consonants and behavioral ability. This meta-ensemble was strongly correlated to behavioral performance (R^2=0.46, p=0.02) but did not seem to perform better or worse than the individual fields. Though we cannot be sure how information

in the multiple auditory fields is used by the animal during the task, these results suggest that each auditory field contains comparable information about consonant stimuli.

Response diversity to speech stimuli is higher in non-primary fields

Diversity was quantified as the strength of the correlation between the responses evoked in similarly tuned neurons to a variety of speech sounds. Since VAF and PAF are not as clearly organized by characteristic frequency as AAF and A1, we could not simply analyze pairs of sites that were anatomically close to each other. For this study, we restricted the analysis to pairs of sites that were within ¼ octaves of each other. Similarly tuned sites in A1 and AAF encode speech stimuli more similarly than similarly tuned sites in VAF or PAF. For example, in a pair of similarly-tuned AAF sites, the speech sound /dad/ evoked more spikes in site #2 than in site #1, but the relationship was still significantly correlated (R^2=0.77, p=0.01; Figure 3.9A). In A1, two sites with a CF of 5 kHz fired the most spikes to the sounds /bad/ and /dad/ and fewest to the sound /sad/ and the relationship between the firing strength of these two sites to all speech sounds was significantly correlated (R^2=0.86, p<0.01; Figure 3.9B). These results suggest that similarly tuned sites in both A1 and AAF encode speech stimuli with a significant level of redundancy. In PAF and VAF, spike rate among pairs of sites is also significantly correlated (R^2=0.61, p=0.04 in VAF and R^2=0.63, p=0.04 in PAF; Figure 3.9C&D), but several consonant sounds evoke different responses within each pair. For example, in PAF, site #1 fired almost no spikes to the sounds /sad/, /gad/ and /bad/, while site #2 fired strongly to each of these sounds (Figure 3.9D). The trends in these single pair examples are representative of the population of pairs in each of the four auditory fields we tested.

The highest average correlation across pairs of sites with the same CF (±¼ octave) was observed in A1, followed by AAF (R= 0.36 and R= 0.33 respectively; $p<0.01$). In these two fields, 10% of site pairs were in the 75th percentile of their respective distributions (R value above 0.6; Figure 3.10A&B). VAF and PAF were less correlated than AAF or A1 (t-tests with Bonferroni correction, $p<0.01$), but were not significantly different from each other (in VAF; R= 0.17 and in PAF; R= 0.18, $p=0.04$). In VAF and PAF, the distribution shifts so that only 8% of pairs were in the 75th percentile (R value above than 0.5; Figure 3.10C&D, respectively). These results suggest that pairs of sites in VAF and PAF are encoding the same speech sound with less redundancy than in AAF or A1. The encoding redundancy in A1 may increase the efficacy of this field in driving downstream neurons (Eggermont 2007).

These results suggest that similarly tuned neurons encode speech stimuli with various levels of redundancy across the auditory fields we recorded from. This difference in redundant firing across similarly tuned neurons supports the hypothesis that information is transformed across the synapses of the auditory pathway. In spite of this increased diversity, no auditory field was better correlated to behavioral performance than any other. It is unlikely that neural circuits use this method of calculating similarity between individual neural responses. We report that neural firing patterns in each of four auditory fields can be used to achieve comparable levels of performance on a consonant discrimination task using Euclidean distance as a metric. This result suggests that the information needed to accomplish such a task is encoded in each of the fields we investigated.

Neural activity from multiple fields can be used to identify consonant sounds.

Identifying sounds from a list of many possibilities is different than simply categorizing sounds into two categories ("go" and "no go"). To determine whether neural responses in multiple fields would able to identify consonant speech sounds, we tested the classifier on a seven-alternative forced-choice task with and without spike timing information. The classifier compared single trial activity to the average template of each of seven sounds instead of two sounds used above and in previous studies (Engineer et al. 2008; Centanni et al. 2013; Perez et al. 2012; Ranasinghe et al. 2012a; Ranasinghe et al. 2012b; Shetake et al. 2011). The classifier was able to identify seven different sounds at well above chance level using activity from any of the auditory fields tested (chance is 14%; t-tests for all fields' performance vs. chance, $p<0.001$; Figure 3.11). Spike timing information improved classifier performance for each field ($p<0.001$). The classifier performed best when using A1 activity, followed by AAF (t-test vs. A1, $p<0.01$), VAF (t-tests vs. AAF and A1, $p<0.01$), and PAF (t-tests vs. all other fields, $p<0.01$). Spike timing improved classifier performance more in some fields than others. The classifier benefited most from having spike timing when AAF activity was used (increase of $40.6 \pm 0.7\%$), followed by A1 ($36.6 \pm 0.7\%$), VAF ($30.8 \pm 1.2\%$), and PAF ($13.6 \pm 1.2\%$). These results suggest that neural activity patterns in all four fields can be used to accurately identify different consonant sounds.

DISCUSSION

Summary of results

This study was designed to test whether spike timing information contributes to speech processing in non-primary auditory fields. We used an established nearest neighbor classifier to demonstrate that spike timing information improves accuracy on a neural discrimination task in all auditory fields. The classifier performance in each field was correlated with behavioral ability of rats trained to discriminate the same sounds in an operant go/no-go paradigm. The response to speech sounds between recording sites with the same characteristic frequency is less redundant in long latency fields of the auditory pathway. Our results suggest that while the various auditory fields process speech sounds differently, each fields' neural discrimination ability is strongly and independently correlated with the behavioral performance of rats.

Anesthesia may affect neural responses

Our recordings were obtained from the auditory cortex of anesthetized adult rats. Neural recordings in awake animals differ from recordings in anesthetized animals, especially to repetitive stimuli, and may have affected our recordings using speech stimuli. Auditory cortex neurons in awake animals respond strongly to repetitive noise burst stimuli and encode more information about these sounds than the anesthetized cortex (Dong et al. 2011; Anderson et al. 2006). Basic tuning properties in the rat auditory cortex change under anesthesia, including a reduction in the number of active neurons and sharper tuning curves in those active neurons (Gaese and Ostwald 2001). Although response properties of neurons can differ when animals are awake compared to anesthetized, discrimination between similar sounds using cortical activity from awake and anesthetized animals is comparable (Engineer et al. 2008; Hromádka et al. 2008;

Huetz et al. 2009; Huetz et al. 2009). In awake rats and monkeys, response patterns evoked by speech sounds are just as accurate at encoding the stimulus as in anesthetized cortex (Engineer et al. 2008; Centanni et al. 2013; Steinschneider et al. 1994). In spite of the firing differences to tones and repetitive stimuli caused by anesthesia, speech sound responses in A1 are not qualitatively different in the awake versus the anesthetized rat. The ability to record speech sound responses in the anesthetized animal ensures a low spontaneous firing rate (Anderson et al. 2006; Rennaker et al. 2007). The reduction of spontaneous firing makes the evoked responses easily visible and reduces the variability in identifying driven recordings. Responses in non-primary visual cortex to complex stimuli are similar in anesthetized and awake monkeys (Jazayeri et al. 2012; Stoner and Albright 1992; Schmolesky et al. 1998), which suggests that responses in non-primary auditory cortex of awake and anesthetized subjects may also be similar. In human subjects with intra-cranial electrodes, anesthesia diminishes activity in non-primary auditory fields, but the general pattern of activity is comparable (Howard et al. 2000). Additional studies are needed to determine how anesthesia affects neural encoding of speech sounds in non-primary auditory fields during behavioral tasks and in passive listening conditions.

The effect of training on neural responses to speech sounds

The speech-evoked responses in untrained rats is correlated with behavioral ability in several different tasks, including consonant and vowel discrimination in quiet (Engineer et al. 2008; Perez et al. 2012), in various levels of background noise (Shetake et al. 2011) and after spectral or temporal degradation (Ranasinghe et al. 2012b). Extensive behavioral training does change neural firing patterns in auditory cortex (Takahashi et al. 2011; Reed et al. 2011) and may therefore affect the ability of the classifier to predict stimulus identity. Training in ferrets

increased the amount of information encoded (as measured by bits), but did not seem to affect the fundamental nature of the spatiotemporal response patterns (Schnupp et al. 2006b). Additional studies are needed to determine whether speech sound training alters speech responses in primary and non-primary cortex of non-human animals. Human imaging studies suggest that speech training will enhance neural responses (Kraus et al. 1995; Tremblay et al. 2001).

Diversity increases throughout the auditory pathway

Diversity in the speech-evoked responses in cortical auditory fields may contribute to the ability of an animal to generalize to stimuli or accurately perceive stimuli in adverse listening conditions (Kilgard 2012). The redundancy of encoded information decreases significantly as information is passed from the cochlea to the non-primary cortical fields. In the inferior colliculus (IC), neural responses in neurons tuned to the same frequency are highly correlated with each other (Chechik et al. 2006). In the thalamus and the primary auditory cortex, the response patterns in similarly tuned neurons are already substantially different, likely due to the transformation of information to reflect different stimulus characteristics (Chechik et al. 2006; Sen et al. 2001; Spitzer and Semple 1998; Winer et al. 2005). Earlier studies have indicated that greater diversity results in a more robust representation of sensory information (Morisset and Ghallab 2008; Lyons-Warren et al. 2012; Schnupp 2006a; Sharpee et al. 2011; Shimizu et al. 2000). Novel natural stimuli, such as songbird vocalizations, evoke highly redundant patterns of activity in the cat inferior colliculus (IC) as compared to A1 (Chechik et al. 2006; Tishby et al. 2002). Both the spike rate and the spike timing information of a stimulus encoded by IC neurons are strongly correlated across neurons with the same characteristic frequency, while similarly

tuned A1 neurons encode less redundant information about the same stimulus. This result is likely due to the longer integration time in A1 as compared to IC (Chen et al. 2012). We show that the non-primary field, PAF, has significantly longer integration times than A1, and may represent a continuation of this hierarchical organization. Classifier performance using PAF activity was correlated with behavior both with and without spike timing information. With larger numbers of sites, PAF is able to perform the neural discrimination task with higher accuracy (Figure 3). Since the brain has access to the entire field of neurons, the performance difference we show here may not accurately reflect functional differences across fields. Activity from more PAF neurons (compared to the other fields) may be needed to complete the same tasks. Our results support these earlier results as we have shown an increase in firing diversity throughout the cortical auditory fields we tested.

The ability of multiple fields to identify complex auditory stimuli may be beneficial in ensuring the processing of important auditory cues. The presence of background noise dramatically alters the neural responses in the primary auditory cortex in rats. When 60 dB of speech shaped background noise is added to a speech sound stimulus, A1 sites fire with a delayed latency and lower amplitude in comparison to speech sounds presented in quiet (Shetake et al. 2011). In spite of the severe degradation to the neural response, rats are still able to behaviorally discriminate speech sounds with this level of background noise significantly above chance levels. If non-primary auditory fields are encoding different aspects of the speech stimulus, as has been previously suggested (Rauschecker et al. 2009), this may explain the robust speech discrimination ability of rats at this signal to noise ratio. Similarly, spectral or temporal degradation with a vocoder causes degraded neural firing patterns in rat A1, while the behavioral

performance remains significantly above chance (Ranasinghe et al. 2012b). Other auditory fields may encode the speech stimuli in a way that is more robust to such interference, allowing the rat to accomplish the behavioral task in adverse listening environments. Additional cortical deactivation experiments are needed to evaluate whether each auditory field is capable of compensating for the loss of other auditory field activity.

Evidence for an integrated and parallel hierarchical organization

There are two opposing theories for sensory organization in the brain that are currently being debated. The first suggests that the auditory system is organized into separate streams of information processing. Similar to the visual system, a "what" and a "where" pathway may also exist in the auditory system (Lomber and Malhotra 2008; Recanzone 2000; Rauschecker and Scott 2009). Deactivation of AAF in cats causes selective impairment on pattern discrimination tasks while deactivation of PAF causes impairment on spatial awareness tasks (Lomber and Malhotra 2008). The second theory suggests that the auditory system functions as an arrangement of integrated but parallel groups of neurons (Sharpee et al. 2011; Recanzone 2000). Our results, as well as previous work in rats, show that as information moves farther up the auditory pathway, onset latency significantly increases and suggests an order of processing (Jakkamsetti et al. 2012; Pandya et al. 2008; Polley et al. 2007; Puckett et al. 2007; Storace et al. 2012). The encoding of different stimulus features across different levels of the auditory system may help the brain to better encode spatial location (Recanzone 2000; Walker et al. 2011) or help the brain process information in adverse listening environments (Ranasinghe et al. 2012b; Shetake et al. 2011). Our data suggest that while multiple auditory fields encode speech sounds in a similar but not identical manner, each field is highly correlated with behavioral

discrimination ability. The similarity in correlative ability across all fields supports the view that processing of novel sounds involves neural activity that is distributed across multiple auditory cortex fields.

Acknowledgments

The authors would like to thank K. Im and N. Moreno for help with acquiring microelectrode recordings. We would also like to thank D. Polley, N. Khodaparast, and A. Sloan for their comments on earlier versions of this manuscript. This work was supported by the National Institute for Deafness and Communication Disorders at the National Institutes of Health (R01DC010433).

APPENDIX

CHAPTER 3 FIGURES AND TABLES

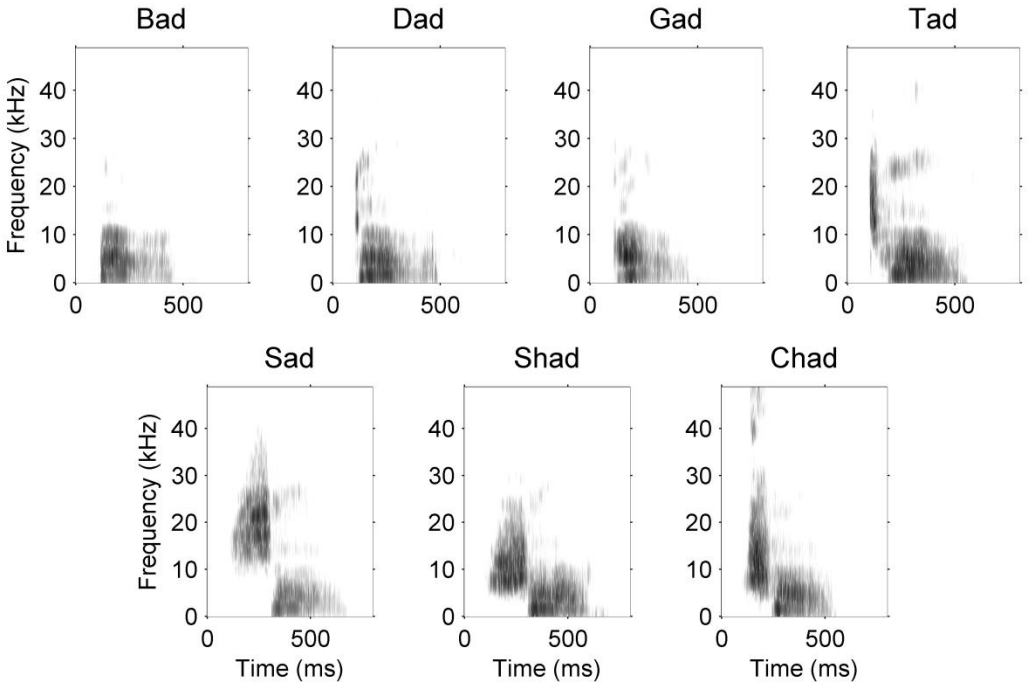

Figure 3.1.Speech sound stimuli were shifted up by an octave. Spectrograms of the seven consonant speech sounds that we used in the present study. Since the rat audiogram is considerably higher than the human audiogram, we shifted speech sounds up by an octave, preserving all other spectral and temporal information using the STRAIGHT vocoder (Kawahara 1997; see Methods).

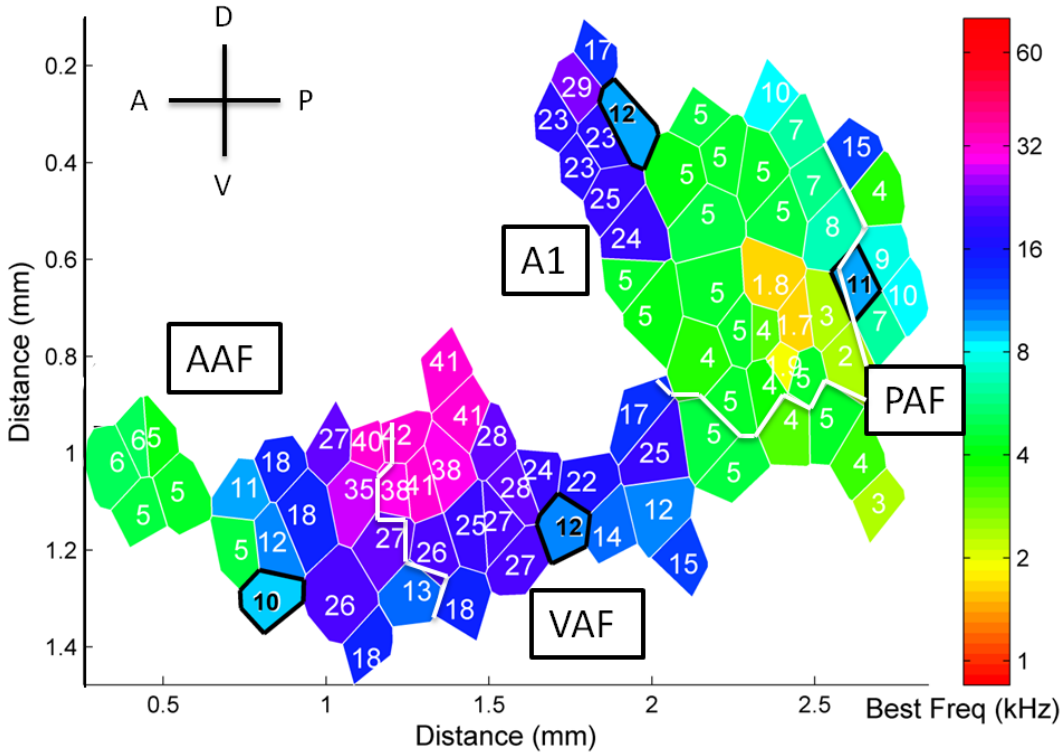

Figure 3.2. Example of an auditory cortex map from one anesthetized, adult rat. Microelectrode recordings were acquired from layer IV/V of 15 experimentally naïve rats. We recorded responses from each of four fields; anterior, primary, ventral and posterior auditory fields. Tonotopic organization and latency were used to identify boundaries between fields. Anterior auditory field was organized from low frequency sites to high frequency in an anterior-to-posterior direction, while primary auditory field was organized from low to high in a posterior-to-anterior direction. Ventral auditory field was located anatomically between the two fields but had no tonotopic gradient. Posterior auditory field was located posterior to the primary auditory field and also had no tonotopic gradient. Sites outlined in black and with black text represent the individual examples shown in Figure 3.6.

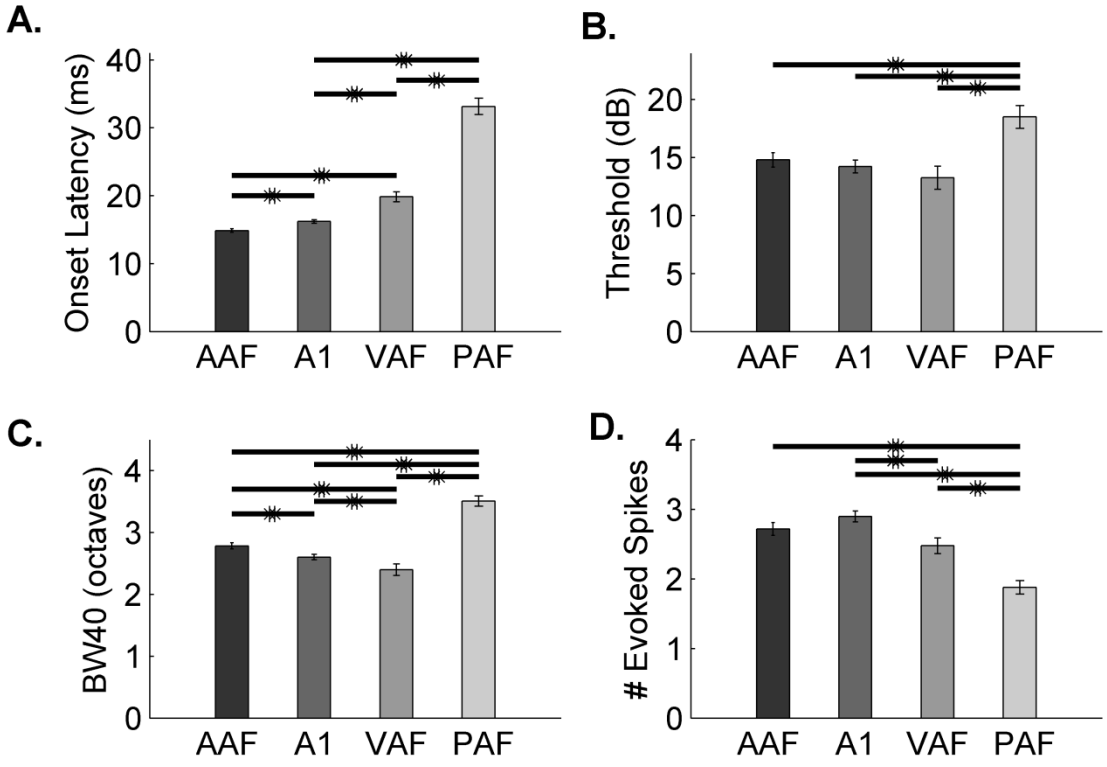

Figure 3.3. Tone response properties in anterior, primary, ventral, and posterior auditory fields mimic previous studies. **A.** AAF and A1 responded to tones with the shortest onset latency (14.8 ± 0.6 ms and 16.2 ± 0.2 ms; $p<0.01$), followed by VAF (19.8 ± 0.8 ms; t-test vs. A1, $p<0.01$). PAF fired with the longest onset latency of any field and was significantly different from every other field (33.1.2 ± 1.2 ms; t-test vs. VAF, $p<0.01$). **B.** AAF, A1 and VAF responded to tones with the same threshold (14.2 ± 0.5 dB, 14.8 ± 0.6 dB, and 3.2 ± 1 dB respectively), while PAF sites had a significantly higher threshold than the other three fields (18.1 ± 1.0 dB; t-tests with Bonferroni correction, $p <0.01$). **C.** VAF had the narrowest bandwidths at 40 dB above threshold (2.4 ± 0.1 octaves), followed by A1 (2.6 ± 0.1 octaves, t-test vs. VAF, $p<0.01$). AAF had broader bandwidths than VAF and A1 (2.8 ± 0.1 octaves (unpaired t-tests with Bonferroni correction, $p=0.01$) and PAF had the broadest bandwidths at this intensity level (3.5 ± 0.1 octaves (unpaired t-tests with Bonferroni correction, ($p<0.01$). **D.** AAF and A1 fired the most driven spikes to tones (2.8 ± 0.1 spikes and 2.7 ± 0.1 spikes respectively); t-test, $p=0.14$). VAF fired significantly fewer spikes than AAF (2.4 ± 0.1 spikes, $p<0.01$), and PAF fired the least amount of driven spikes of any field (1.9 ± 0.1 spikes, respectively; t-tests with Bonferroni correction, $p<0.01$).

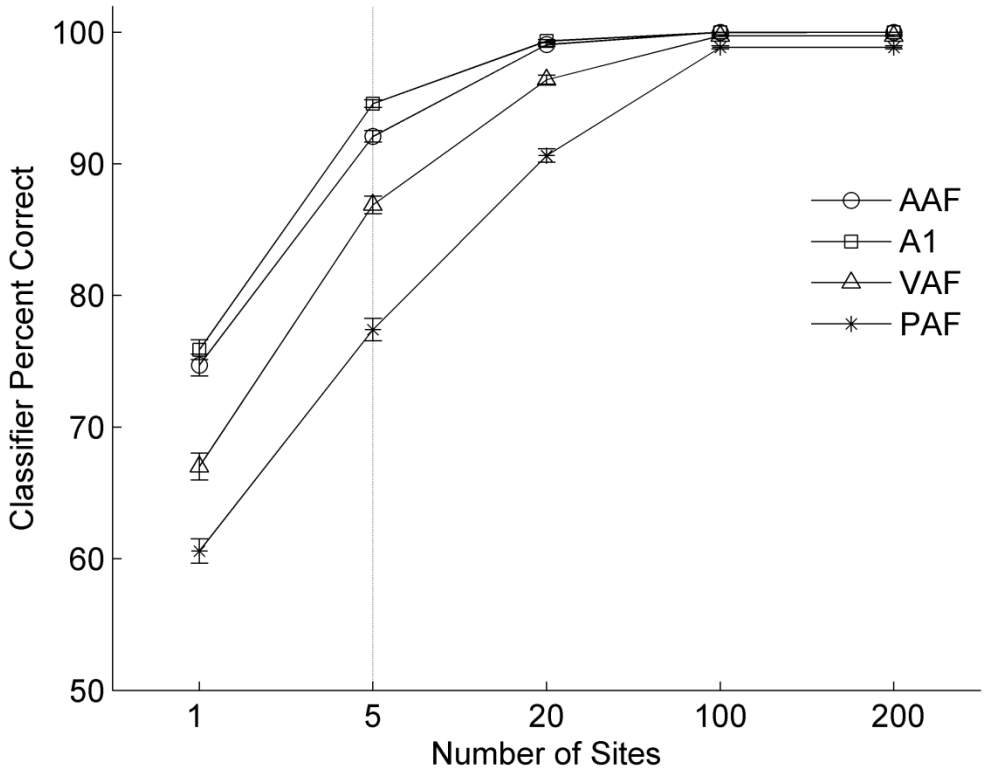

Figure 3.4. Classifier performance by auditory field as a function of number of sites. The two-alternative forced choice classifier reaches ceiling performance in all fields when greater than 20 sites are used, while performance is close to floor when single sites are used. For the analyses in this report, we used 5 sites per classifier run (marked by the vertical line) to achieve performance well above chance level while avoiding ceiling performance. Classifier was run in all instances using spike timing information: 1 ms temporal bins across a 40 ms analysis window.

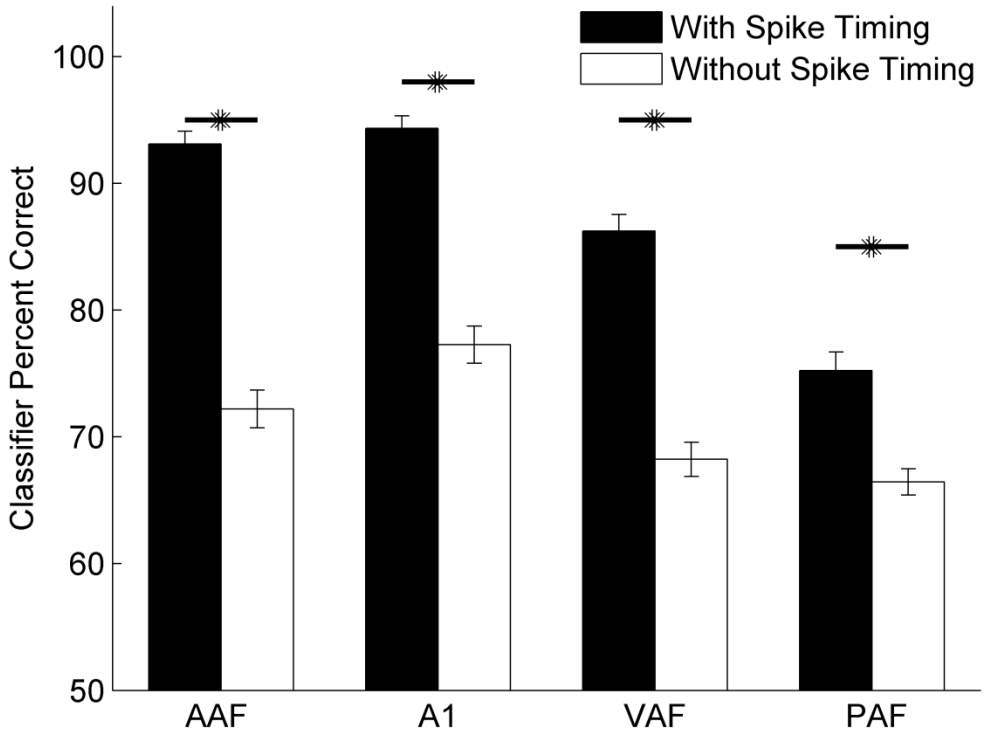

Figure 3.5. Neural classifier performance in each auditory field with and without spike timing information. Neural activity in anterior, primary, ventral, and posterior auditory fields were all better able to discriminate pairs of consonant speech sounds when spike timing information was preserved than when spike timing information was removed. Classifier performance plotted is the average of many groups of 5 random sites performing neural discrimination of 21 different consonant pairs (see Methods). In AAF, the classifier achieved 91.3 ± 2.3% correct when spike timing information was preserved versus 67.7 ± 2.2% correct when spike timing information was removed ($p<0.01$). In A1, the classifier achieved 92.6 ± 2.1% correct vs. 72.9 ± 2.2% ($p<0.01$). In VAF, the classifier achieved 84.6 ± 2.9% correct vs. 65.0 ± 2.2%, $p<0.01$. In PAF, the classifier achieved 75.0 ± 2.9% correct vs. 62.8 ± 1.6%, $p<0.01$. All t-tests reported tested classifier performance with and without spike timing respectively. Error bars represent standard error of the mean across groups of 5 recording sites.

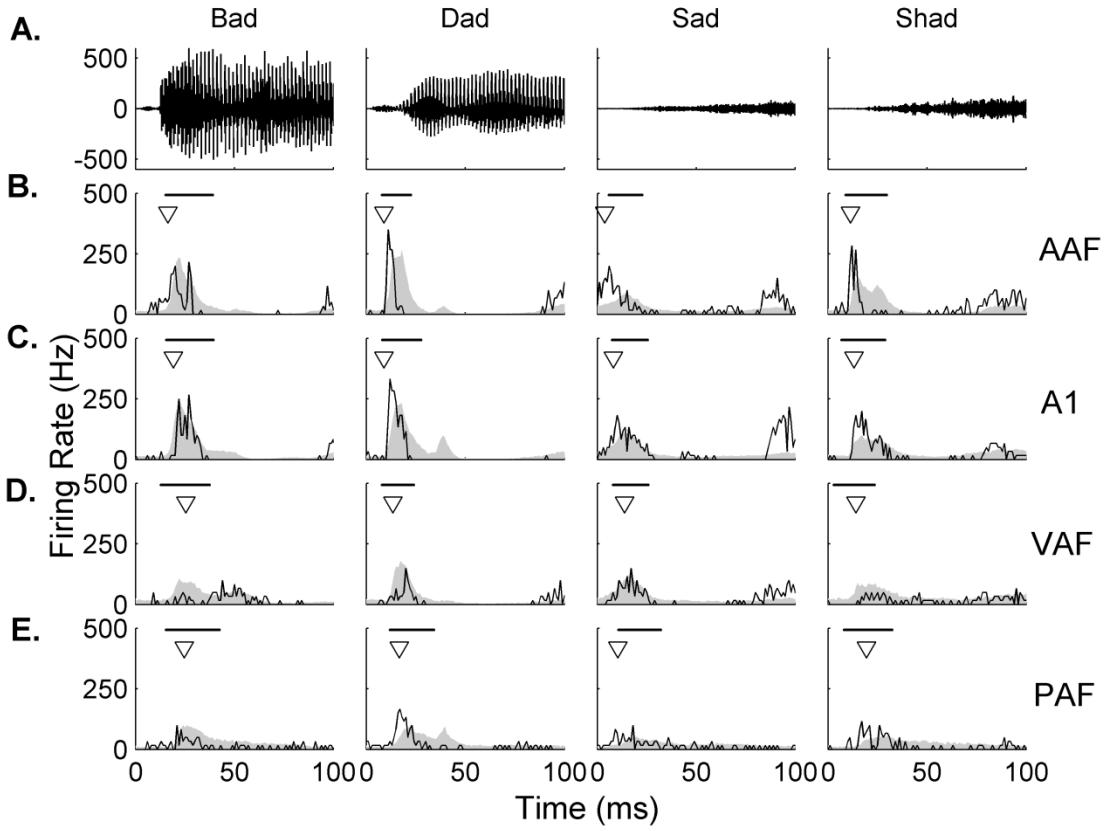

Figure 3.6. Single site examples of the evoked-response to consonant speech sounds in each auditory field. Average response (from a ~10 kHz site in each field) to 20 repeats of each of four consonant speech sounds as compared to the average post-stimulus-response-histograms (PSTH) response in each field. The individual site examples are plotted in black and the population PSTH for the entire field is plotted in gray for comparison. Onset latency for the individual site is marked by a triangle and the mean ± std of the latencies for each site in the population is marked by the black bar. **A.** Waveforms of four example consonant speech sounds; two voiced consonants; /b/ and /d/, and two unvoiced consonants; /s/ and /sh/. **B.** Single site PSTH of a representative AAF site. AAF sites responded quickly to the onset of a speech stimulus in a well defined peak of activity (average onset latency of 14.2 ± 0.7ms; mean ± sem). **C.** PSTH responses from a representative A1 site. Like AAF sites, A1 sites responded quickly to the onset of a stimulus and had a short peak response (15.2 ± 0.7 ms in A1; t-test vs. AAF, p=0.32). **D.** PSTH responses from a representative VAF site. This result was similar to the longer latency seen in response to tones. **E.** PSTH responses from a representative PAF site. Just as PAF sites responded last to tones (compared to the other three fields), this field also responded last to speech sounds (18.1 ± 0.7 across all speech sounds; t-test vs. A1, $p<0.01$).

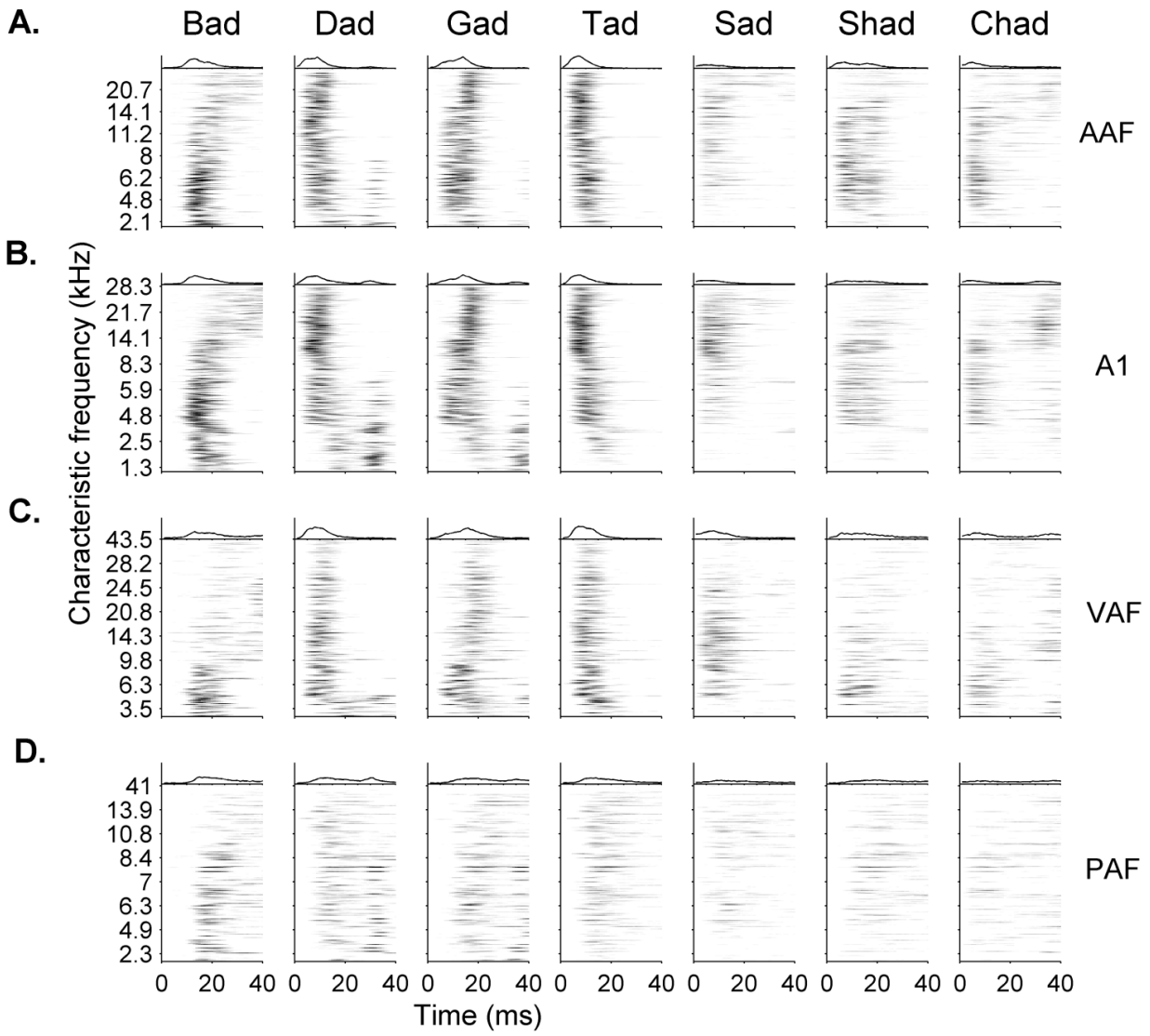

Figure 3.7. Spatiotemporal response patterns to all consonant speech sounds tested in anterior, primary, ventral, and posterior auditory fields. Average response to speech sounds from each site in each field, organized by characteristic frequency. The average response across all sites is shown on top of each sub-panel. The average response plotted is the same as is shown in gray in Figure 3.6. **A.** AAF sites responded strongly to all speech sounds, but responded less strong for non-stop consonants (/s/, /sh/, and /ch/). Each speech sound evoked a unique pattern of response. For example, the sound /b/ caused low frequency sites to fire first, followed by high. The consonant /d/ caused the opposite firing pattern. **B.** A1 responses to speech sounds were similar to AAF and mimic previous recordings in A1. Like AAF, A1 sites responded more strongly to stop consonants. **C.** VAF did not have as many low frequency sites as AAF or A1, which caused the response patterns to appear more similar. In spite of the bias in characteristic frequency, VAF

Figure 3.7 continued…

sites tuned below 6 kHz did respond to the vowel portion of the speech sounds in a manner that mimicked AAF and A1 responses. **D.** PAF sites were more broadly tuned than the other three fields. As a result, each site responded to both the consonant onset as well as the vowel onset.

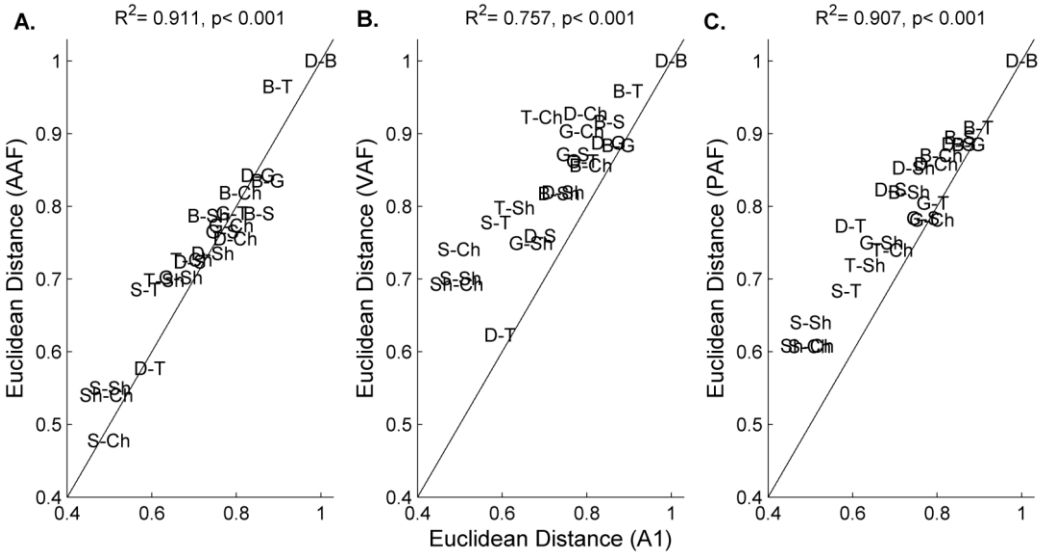

Figure 3.8. Similarity between neural responses to speech sounds is highly correlated across fields. Euclidean distance was calculated between the neural responses in each field to every pair of consonant sounds. The similarity between neural responses to speech sounds was then compared between every combination of fields. In general, /d/ and /b/ were the most distinct and /s/ and /ch/ were the least distinct. Despite some variation, the correlation between fields was high and significant. Not all comparisons shown. **A.** The similarity between the neural response to pairs of speech-evoked responses is highly correlated between A1 and AAF (R^2=0.91, p<0.01). These two fields perform the neural discrimination task with comparable accuracy. **B.** The similarity between pairs of speech-evoked neural responses between A1 and VAF is highly correlated (R^2=0.76, p<0.01), but the correlation contains more outliers than the AAF/A1 comparison. VAF is better able to discriminate between unvoiced consonants (for example, S/T and S/Ch) than A1. This difference in the similarity between response patterns does not give VAF an advantage for these tasks in the neural discrimination task. **C.** The similarity between pairs of speech-evoked neural responses between A1 and PAF is as strongly correlated as A1 and AAF (R^2=0.91, p<0.01). The neural responses in PAF are more distinct than in A1. This difference does not give VAF and advantage for these tasks in the neural discrimination classifier.

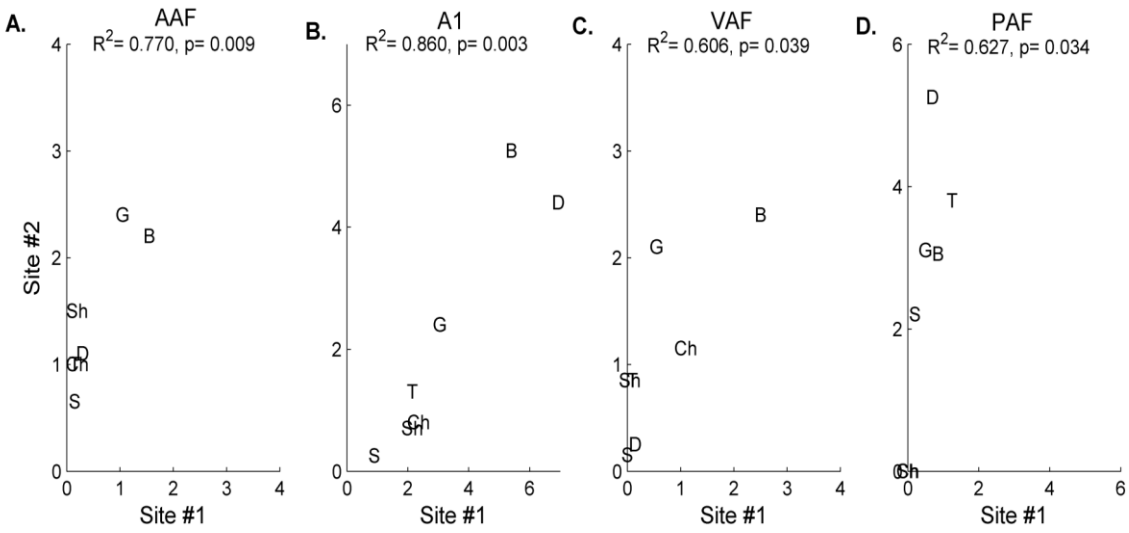

Figure 3.9. Example correlations from one pair of sites in each field. We counted the number of evoked spikes to each speech sound fired from each of two sites that were tuned within ¼ octave of each other and quantified these pairs using the correlation coefficient. These examples represent pairs in the 75th percentile in each field. **A.** AAF sites fire with a similar number of spikes per sound. For example, both of these sites fired the most spikes to /b/ and /g/, and the least number of spikes to /s/. This example had an R^2 of 0.77 (p=0.01). **B.** A1 pairs had the highest correlation, suggesting the largest amount of redundant information. This pair had an R^2 of 0.86 (p<0.01). **C.** VAF (R^2 of 0.61, p=0.04) and **D.** PAF (R^2 of 0.63, p=0.03) pairs had weak correlations, suggesting that these fields had less redundancy in information encoding. For example, in both **C.** and **D.**, one site in the pair fired more spikes to /g/ than the other site in the pair.

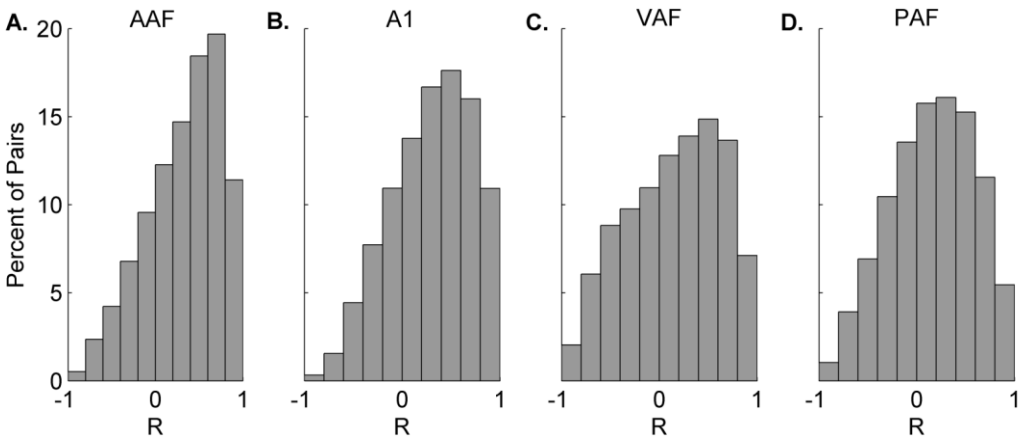

Figure 3.10. Distribution of correlation coefficients between speech evoked responses in pairs of recording sites. In each field, we found pairs of sites tuned within ¼ octave of each other and compared the number of evoked spikes to each of the seven consonant sounds presented. We quantified these comparisons using the correlation coefficient. **A.** Pairs of sites in AAF were strongly correlated with each other when comparing the number of evoked spikes to speech sounds. AAF site pairs had an average R value of 0.33. **B.** A1 pairs were the most correlated with each other (an average R value of 0.36, t-test vs. AAF, $p<0.01$). **C.** VAF and **D.** PAF pairs were the least correlated, with an average R value of 0.17 in VAF and 0.18 in PAF. Both of these fields were less correlated than AAF or A1 (t-tests with Bonferroni correction, $p<0.01$), but were not different from each other ($p=0.04$).

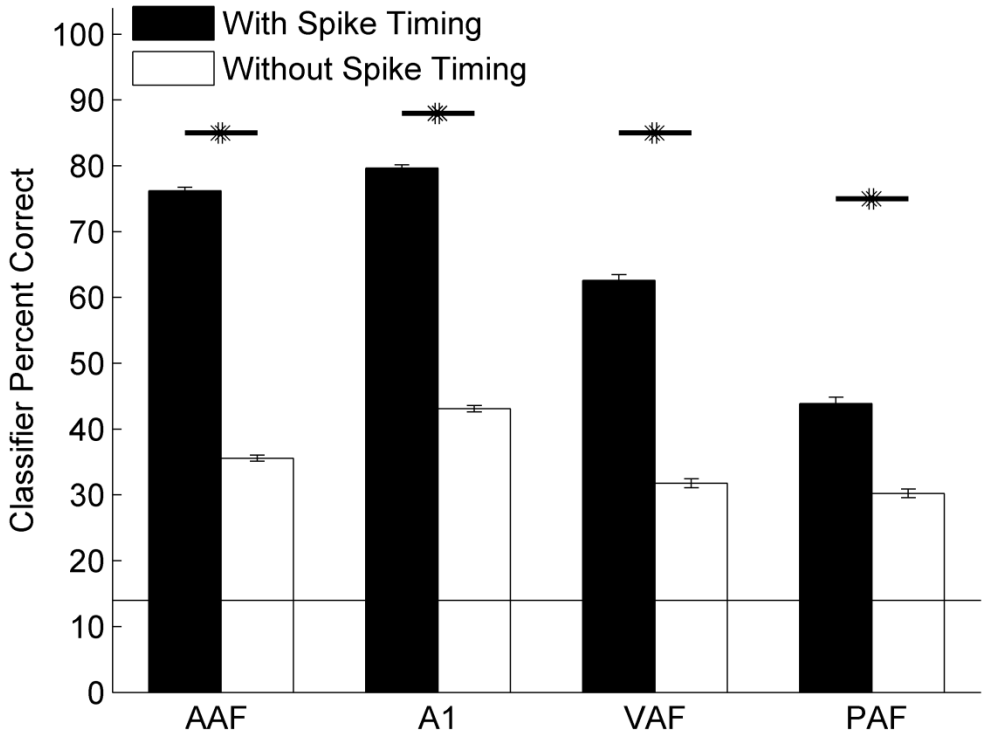

Figure 3.11. Neural activity from multiple fields can identify consonant sounds better when spike timing information is preserved. We ran the classifier using templates for all 7 sounds simultaneously to test the ability of neural activity to identify the 7 sounds with and without spike timing information. All four fields were significantly better at the identification task when spike timing information was preserved (black bars) than when spike timing information was removed (white bars; * = $p<0.001$). AAF was most affected by the removal of spike timing (i.e. had the greatest difference in performance across the two conditions), followed by A1, VAF, and PAF ($p<0.001$).

Table 3.1. Behavioral speech sound discrimination performance of rats. Rats were trained to discriminate speech sounds in one of three tasks; /dad' versus /bad/, /gad/, /sad/, /tad/ (n=10 rats, data previously published in Engineer et al. 2008), /bad/ versus /dad/, /gad/, /sad/, /tad/ (n=4 rats), or /shad/, versus /dad/, /sad/, /chad/, /gad/ (the same 4 rats trained on the /bad/ task). Mean performance across rats on the last day of training is reported ± sem.

Task:	**/d/ vs. /b/**	**/d/ vs. /g/**	**/d/ vs. /s/**	**/d/ vs. /t/**	**Total % correct**
Percent correct:	90.7 ± 2.0%	86.9 ± 2.9%	92.5 ± 0.8%	83.1 ± 3.3 %	88.3 ± 2.3%
Task:	**/b/ vs. /d/**	**/b/ vs. /g/**	**/b/ vs. /s/**	**/b/ vs. /t/**	**Total % correct**
Percent correct:	89.4 ± 3.4 %	80.6 ± 6.1%	85.5 ± 4.8%	91.1 ± 5.9%	86 .6 ± 2.7%
Task:	**/sh/ vs. /d/**	**/sh/ vs. /s/**	**/sh/ vs. /ch/**	**/sh/ vs. /g/**	**Total % correct**
Percent correct:	90.7 ± 2.7%	85.3 ± 4.5%	57.6 ± .1%	82.9 ± 5.4%	79.1 ± 8.5%

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CHAPTER 4

SPEECH SOUND PROCESSING DEFICITS AND TRAINING-INDUCED NEURAL PLASTICITY IN RATS WITH DYSLEXIA GENE KNOCKDOWN

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ABSTRACT

Reduced expression of the dyslexia associated gene *Kiaa0319* in rats (KIA-) causes degraded responses to phoneme stimuli as well as increased trial-by-trial variability in onset latency. Here we report that *in utero* RNAi of this gene causes significant behavioral speech sound processing impairments in rats. KIA- rats needed twice as much practice on a speech discrimination task to perform at control levels. The percentage of neurons affected by RNAi is strongly correlated with speech discrimination ability. Extensive behavioral training (>10 weeks) was able to restore trial by trial neural firing variability. This amount of training also restored the performance of neural activity patterns to predict stimulus identity. KIA- rats are able to learn difficult speech discrimination tasks, but require long training periods. These results provide the first direct evidence that *in utero* suppression of the dyslexia associated gene *KIAA0319* can cause behavioral phoneme processing impairments.

INTRODUCTION

Dyslexia is the most common developmental language disorder and affects approximately 7% of the population (Shaywitz et al. 1992; Shaywitz et al. 1998). Individuals with this disorder have normal nonverbal intelligence, but score at least 1 standard deviation below their peers on reading tests (Shaywitz et al. 1992; Tallal 1980; Ziegler and Goswami 2005). Children with dyslexia typically have deficits in phoneme perception and manipulation (Boscariol et al. 2010; Peterson and Pennington 2012; Tallal and Piercy 1974; Vandermosten et al. 2010). For example, dyslexic children are less accurate at identifying consonants along a continuum (Manis et al. 1997; Vandermosten et al. 2010; Werker and Tees 1987), at exchanging or manipulating phonemes (i.e. say "break" without the /b/ sound; (Elbro, Nielsen, Petersen 1994; Paulesu et al. 1996), or at identifying pairs of rhyming words (Howland and Liederman 2012; Kovelman et al. 2012). Dyslexia is highly heritable (Cope et al. 2005; Fisher and DeFries 2002; Nöthen et al. 1999; Pennington et al. 1991) and at least four candidate dyslexia genes have been identified (*KIAA0319, DYX1C1, DCDC2 and ROBO1;* (Bates et al. 2011; Burbridge et al. 2008; Galaburda et al. 1985; Galaburda et al. 2006; Harold et al. 2006; Scerri et al. 2011; Threlkeld et al. 2007). *KIAA0319* variants are consistently associated with dyslexia and are directly related to poor speech perception and reading ability (Pinel et al. 2012). The auditory temporal processing theory hypothesizes that the behavioral deficits seen in dyslexics are caused by abnormal neural firing to rapid auditory stimuli (Ahissar et al. 2000; Boscariol et al. 2010; Tallal 1980; Temple et al. 2000) but see McLean 2011 and Rosen 2003). Variants in *KIAA0319* are linked with reduced left temporal lobe activation to speech sound stimuli (Pinel et al. 2012) and may contribute to abnormal auditory processing.

Rats with *in utero* RNA interference (RNAi) of the rat homolog of this gene (*Kiaa0319*) have significantly higher trial-by-trial variability in the timing of neural responses to tones and speech stimuli (Centanni et al. 2013). This result suggests that this specific candidate dyslexia gene causes unreliable neural firing in auditory cortex and may also contribute to behavioral auditory processing impairments. Neural activity patterns are correlated with behavioral ability of rats on discrimination tasks using human consonants and vowels in quiet (Engineer et al. 2008b; Perez et al. 2012), in various levels of background noise (Shetake et al. 2011), and with temporal or spectral degradation (Ranasinghe et al. 2012a). Rats with *in utero* RNAi of *Kiaa0319* have difficulty discriminating short non-speech stimuli, such as frequency-modulated sweeps (Szalkowski et al. 2012) and we hypothesized that these rats would also have impaired speech sound discrimination.

Extensive and targeted behavioral therapy is currently the most effective intervention for children with dyslexia. Many programs focus on using synthetically stretched or otherwise modified speech stimuli to improve phoneme awareness (Lovio et al. 2012; Penolazzi et al. 2010; Russo et al. 2005; Temple et al. 2003). Such training can cause changes in neural responses at multiple stages of the auditory pathway (Penolazzi et al. 2010; Tremblay and Kraus 2002). For example, an 8-week training program for phonemic awareness (*Earobics*, Evanston, IL) improved speech in noise responses in the auditory brainstem (Russo et al. 2005). A similar remediation program (Fast ForWord (Scientific Learning Corporation, Oakland, CA) increased fMRI activation in several cortical brain regions, including the left temporo-parietal cortex and inferior frontal gyrus (Temple et al. 2003). These studies support the hypothesis that behavioral training can induce neural plasticity in a genetic disorder. However, some studies suggest that

behavioral training is not completely effective (Eden and Moats 2002; Lovett et al. 1989; Schulte-Körne 2010) and the cause of this discord is unknown. The specific genetic variants of the participants in those studies are unknown and it is possible that such behavioral training may not induce changes for people with specific genetic variants.

In the current study, we trained rats with *in utero* RNAi of *Kiaa0319* on a variety of speech sound discrimination tasks designed to mimic known deficits in human dyslexics including speech in noise, rapid speech sounds, and phonemes. We also recorded multiunit neural activity in primary (A1) and posterior auditory fields (PAF) to evaluate whether extensive behavioral training improved neural responses to speech and non-speech stimuli.

METHODS

Animals

Subjects were Wistar rats, both male and female, that were 4-8 months old at the time of study. All rats used were subjected as embryos to *in utero* electroporation targeting lateral regions of neocortex that included the auditory cortex by methods described previously (Bai et al. 2003; Bai et al. 2008; Burbridge et al. 2008; Centanni et al. 2013; Szalkowski et al. 2012; Threlkeld et al. 2007)(Bai et al. 2003; Bai et al. 2008; Burbridge et al. 2008; Szalkowski et al. 2012; Threlkeld et al. 2007). The animals were transfected with either an shRNA against *Kiaa0319* which can decrease the Kiaa0319 protein expression in cell culture (Tarkar and LoTurco, unpublished observation) and to cause migration delay in neocortex in embryos that was rescued by expression of exogenous *Kiaa0319* (Paracchini et al. 2006). Control transfection animals received a scrambled sequence control of *Kiaa0319* shRNA, also previously used, that

contained 6 bases in the sequence scrambled to render the shRNA inactive in terms of reducing *Kiaa0319* expression (Paracchini et al. 2006). *Kiaa0319* shRNA and scrambled shRNA constructs were injected at a concentration of 1.0 µg/µL. pB-GFP was co-transfected with the effective shRNA construct, and pB-mRFP was co-transfected with the scrambled *Kiaa0319* shRNA control construct to identify the experimental condition in post experimental histological analysis. Electroporation paddles were placed in a horizontal plane and voltage pulses were discharged across the cerebrum in both polarities to achieve bilateral transfections. The experimental status of the subject remained blind to the experimenters throughout data collection. Following data collection, each subject was perfused transcardially 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. Sections were taken at 80 µm intervals and analyzed under a confocal microscope (Zeiss) to identify the experimental status of each subject (green florescent protein marked experimental subjects and red florescent protein marked control littermates). All animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee.

Behavioral Training

We trained 26 rats to discriminate a target speech sound (/dad/) in four different contexts. Of these rats, 16 received RNAi of *Kiaa0319* (KIA-) and 10 received scrambled RNAi and served as controls. The behavior tasks we tested are described in detail elsewhere (Engineer et al. 2008b; Perez et al. 2012; Porter et al. 2011; Sloan, Dodd, Rennaker II 2009). Briefly, rats were trained to respond to a target sound /dad/ using either a lever press or withdrawal from an infra-red nose poke. Once rats understood the mechanism of response was either a lever press or a withdrawal from the nose poke, rats were trained to wait for the presentation of a target sound

prior to making a response. Once rats reached a d' of >= 1.5 for 10 sessions, they were moved on to 20 sessions of each of four discrimination tasks.

The isolated speech task consisted of a go-no go paradigm in which rats were trained to press a lever in response to the target sound and to reject 7 distracters: /dad/ versus /bad/, /gad/, /sad/, /tad/, /dud/, /deed/, /dood/ (Engineer et al. 2008b). The speech in noise task involved a similar lever press discrimination task where the same set of speech sounds described above were presented in four levels of background speech shaped noise: 0, 48, 60, 72 dB speech shaped background noise (Shetake et al. 2011). The phoneme task presented only the first 40 ms of the consonant information and rats were required to press the lever in response to 'da' and ignore the distracters (Porter et al. 2011). The rapid speech task presented a random sequence of distracter sounds (/bad/, /gad/, /sad/, /tad/), with the target sound (/dad/) inserted randomly between 2-7 seconds from the start of the trial. Responses were registered in this task by removal from an infra-red nose poke (Sloan, Dodd, Rennaker II 2009).

We trained half of the rats using one task order (isolated speech, speech in noise, rapid speech, phonemes) and the other half using a second task order (phonemes, isolated speech, speech in noise, rapid speech). This change in order was to determine if KIA- rats were able to learn the difficult short speech task if given extensive training. Since there were only minor differences across groups in the neural responses following training, neural activity from both groups was combined for analysis. Percent correct is average hits-false alarms across all tasks.

Acute neural recordings

Following the approximately 4 months of training needed to complete all four tasks; rats were anesthetized with pento barbital and mapped. The techniques used for acute recordings are

described in detail elsewhere (Centanni et al. 2013; Engineer et al. 2008a; Ranasinghe et al. 2012a; Shetake et al. 2011). In brief, animals were anesthetized with pentobarbital (50 mg kg^{-1}) and were given supplemental dilute pentobarbital (8 mg ml^{-1}) as needed to maintain areflexia, along with a 1:1 mixture of dextrose (5%) and standard Ringer's lactate to prevent dehydration. A tracheotomy was performed to ensure ease of breathing throughout the experiment and filtered air was provided through an air tube fixed at the open end of the tracheotomy. A craniotomy and durotomy was performed, exposing right primary auditory cortex. Four Parylene-coated tungsten microelectrodes (1-2 MΩ) were simultaneously lowered to layer (4/5) of right primary auditory cortex (~600-800 μm). Electrode penetrations were marked using blood vessels as landmarks. In addition, we recorded multiunit responses from 11 experimentally naïve rats to evaluate the effect of training on neural responses (5 KIA-, 6 controls, previously published in Centanni et al. 2013).

All animals were exposed to the same set of auditory stimuli. Brief (25 ms) tones were presented at 90 randomly interleaved frequencies (1-47 kHz) at 16 intensities (1- 75 dB SPL) to determine the characteristic frequency of each site. Trains of six 25 ms tones were presented, with a frequency change between the 5^{th} and 6^{th} tones. This set of stimuli was used to evaluate the presence of mismatched negativity response between a repeated standard and a deviant frequency. Next, broad band noise burst trains were presented at four different speeds (4, 7, 10 and 12.5 Hz) to evaluate following ability of A1 neurons. Each train consisted of 6 bursts with duration of 25 ms for each burst. We also presented a series of speech stimuli identical to those used in our lab previously (Engineer et al. 2008b; Porter et al. 2011; Shetake et al. 2011). The sounds were recorded in a double-walled, soundproof booth and were spoken by a female native-

English speaker. The spectral envelope was shifted up in frequency by a factor of two using the STRAIGHT vocoder (Kawahara 1997) to better accommodate the rat hearing range. Eight individual speech sounds that were the same as those discriminated during the isolated speech task (see Behavior Training section). Finally, we presented a sequence of speech sounds to mimic the stream task. /Bad-bad-gad-sad-tad-dad/ was played at six speeds, measured in syllables per second (2, 4, 5, 6.667, 10 and 20 sps). Each set of stimuli was randomly interleaved, and presented at 20 repeats per recording site. All sounds were presented approximately 10 cm from the left ear of the rat. Stimulus generation, data acquisition and spike sorting were performed with Tucker-Davis hardware (RP2.1 and RX5) and software (Brainware). All animal protocols were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee.

Analysis of neural recordings

Though behavior did not differ between groups, we analyzed tuning curves for each group to see if the training order caused differences in the neural responses. No major differences were seen, so all neural data was also pooled for analysis. To define primary (A1) and posterior (PAF) auditory cortex sites, multi-unit recording sites were manually analyzed to select the characteristic frequency of each site, as well as to obtain bandwidth, latency, peak firing and end of peak response information. From this point on, only A1 and PAF sites were analyzed.

Following selection of A1 and PAF sites, basic firing properties were calculated in response to tones. Firing latency is defined as the point in time (ms) that average firing rate (across all repeats) first exceeds 2 standard deviations above the spontaneous firing rate, threshold is defined as the lowest intensity that evoked a response from the multiunit site, and bandwidths

were calculated at 10, 20, 30 and 40 dB above threshold and defined as the range of frequencies that evoked responses at the current intensity. In response to broad band click trains, normalized spike rate (number of spikes evoked by bursts 2-6, normalized by the number of spikes to the first burst) and vector strength (VS) were calculated. VS quantifies the degree of synchronization between action potentials and repeated sounds. Mean VS is calculated with the formula:

$$\mathrm{VS}=\frac{1}{n}\sqrt{x^2+y^2};\ x=\sum_{i=1}^{n}\cos\,\theta_i; y=\sum_{i=1}^{n}\sin\,\theta_i\ \theta_i=2\pi\frac{t_i}{T}$$

where n = total number of action potentials, t_i is the time of occurrence of the i'th action potential, and T is the inter-stimulus interval. Perfect synchronization would result in a value of one, whereas no synchronization would result in a value of zero. To investigate the reliability of onset latency to repetitive stimuli, we calculated the time to peak latency within the first 80 ms (the shortest inter-pulse-interval tested) of the first pulse at 4Hz and averaged across multiunit sites. The variability in this measure, as reported in variance, was compared across KIA- and controls.

Single trial response patterns to each of the isolated speech sounds were compared using a nearest neighbor classifier (Centanni et al. 2013; Engineer et al. 2008b; Foffani and Moxon 2004; Foffani and Moxon 2005; Perez et al. 2012; Ranasinghe et al. 2012b; Shetake et al. 2011). We chose this method because our earlier studies showed that the performance of this classifier is highly correlated with rat behavioral discrimination. We used Euclidean distance to compare single trial activity to the average activity (PSTH) evoked by 19 repeats each of two different stimuli (Centanni et al. 2013; Engineer et al. 2008b; Foffani and Moxon 2004; Foffani and Moxon 2005; Perez et al. 2012; Ranasinghe et al. 2012b; Shetake et al. 2011). For consonants,

activity was binned using 1 ms temporal precision over a 40 ms window to encompass the spike timing precision present in the initial consonant (Engineer et al. 2008b; Porter et al. 2011; Ranasinghe et al. 2012a), while vowel activity was binned across a single 400 ms window so that spike count information was preserved (Perez et al. 2012; Ranasinghe et al. 2012a). The classifier then compared the response of each single trial with the average activity template (PSTH) evoked by all repeats of each of the speech stimuli presented. The current trial being considered was not included in the PSTH to avoid artifact. The classifier attempted to identify the stimulus that evoked the current single trial activity pattern by selecting the template that was most similar to the single trial in units of Euclidean distance. ED was calculated using the formula:

$$\text{Euclidean Distance} = \sqrt{\sum_{i=1}^{\#sites} \sum_{j=1}^{\#\ bins} \left(X_{ij} - Y_{ij}\right)^2}$$

where n_{sites} is each recording site and n_{bins} is each of 40 one-millisecond bins being compared between activity evoked by speech sound X versus speech sound Y. For vowel sounds, the classifier counted the number of action potentials over a single 400 ms bin and compared the single trial response with the two PSTH templates (Perez et al. 2012)(Ranasinghe et al. 2012a). We used t-tests for all pairwise comparisons of the accuracy of both classifiers and across experimental groups. 1-way ANOVA was used to compare vector strength across groups. When necessary, Bonferroni correction was used to correct for multiple comparisons.

RESULTS

In utero RNAi of Kiaa0319 causes speech sound discrimination impairment

Rats with *in utero* RNA interference (RNAi) of the dyslexia-associated gene *Kiaa0319* (KIA-) have significantly higher trial-by-trial variability in neural firing to speech and non-speech stimuli (Centanni et al. 2013) and we hypothesized that they would also have deficits on behavioral speech sound discrimination tasks. We trained KIA- rats to discriminate a target speech sound from a variety of speech sound distracters. Rats with *in utero* RNAi of *Kiaa0319* were impaired at this task on the first couple days, but were not impaired in their ability to reach the performance criterion compared to controls (10 sessions with a d'≥1.5; Figure B4.1). Both groups reached this criterion in approximately 5 days (Controls: 4.4 ± 0.8 days vs. KIA-: 5.4 ± 5.4 days; unpaired t-test, p=0.35; Figure B4.1). We tested 8 KIA- rats and 5 control rats on a full speech task in which they were required to press the lever to the target sound /dad/ and withhold pressing to the distracter sounds (/bad/, /gad/, /sad/, /tad/, /dud/, /deed/, /dood/). On the first day, both controls and KIA- rats performed at chance (51.9 ± 3.1% correct by controls vs. 44.8 ± 2.4% correct by KIA- compared to 50% chance; unpaired t-tests, p=0.84 and p=0.59 respectively; Figure A4.1A). On the following 4 days of the testing period, KIA- rats were significantly worse than controls at performing this task (unpaired one-tailed t-tests, p<0.01; Figure A4.1A), while control rats significantly improved during this time period (last day performance was 76.5 ± 3.0% correct, paired t-test vs. first day, p=0.01; Figure A4.1A). On the last day of testing, KIA- rats were better at vowel tasks than consonant tasks (58.9 ± 7.8% on vowel tasks vs. 46.7 ± 5.0% correct on consonant tasks, paired t-test, p=0.01) and KIA- rats false alarmed to distracter sounds more than control rats (61.3 ± 9.3% false alarms by KIA- rats vs.

32.7 ± 8.2% false alarms by control rats; unpaired t-test, p=0.04, Figure A4.1B&C). The result that KIA- rats were impaired at speech sound discrimination tasks suggests that a variant in the candidate dyslexic gene *KIAA0319* can cause behavioral phoneme awareness deficits. The observation that consonant sounds were more difficult than vowels for KIA- rats is consistent with the phoneme impairments seen in humans with dyslexia (Tallal and Piercy 1974).

Rats with in utero RNAi of Kiaa0319 can improve on certain tasks with extensive speech training

The most effective therapy for dyslexic children is extensive behavioral training using modified speech sounds (Lovio et al. 2012; Penolazzi et al. 2010; Russo et al. 2005; Temple et al. 2003). Training programs often focus on intensive practice on phoneme awareness and can lead to improved speech perception (Lovio et al. 2012; Penolazzi et al. 2010; Russo et al. 2005; Temple et al. 2003). We hypothesized that with additional training, KIA- rats would also improve. KIA- rats did reach 80% correct on the full speech task (81.4 ± 2.3% correct on the last day of training), but it took significantly longer to reach this point compared to controls (9.6 ± 0.6 days of training vs. 6.2 ± 0.6 days for control rats, unpaired t-test, $p<0.01$; Figure A4.2A).

We hypothesized that if the RNAi of *Kiaa0319* was responsible for the impaired performance in KIA- rats, that rats with a greater number of neurons affected by the RNAi transfection would perform worse at the task than rats with fewer transfected neurons. We counted the percentage of pyramidal neurons which were co-transfected with fluorescent protein (see Methods) in layer 2/3 of primary auditory cortex bilaterally and compared the extent of the transfection with the animals' last day performance on the full speech discrimination test. In rats with *in utero* RNAi of *Kiaa0319*, the percent of affected neurons was strongly correlated with

last day speech discrimination performance. Rats with a greater percentage of affected neurons were more impaired at the task than rats with fewer affected neurons (R^2= 0.77, p<0.01; Figure A4.3A). The percentage of transfected neurons in control rats (injected with a scramble mRNA and a different color protein) did not correlate with behavioral performance (R^2= 0.61, p=0.12; Figure A4.3B). The lack of correlation in our control rats suggests that the surgery itself did not cause the behavioral impairment, but that the deficit seen in KIA- rats was due to RNAi of the candidate dyslexia gene *Kiaa0319.* Our results suggest that the degree of *in utero* suppression of this gene is related to each rats' aptitude for learning the full speech sound task.

Following this additional week of training on full speech, we trained all rats for 2 weeks on a speech in noise task. The same target and distracters from the full length task were used and were presented with speech-shaped background noise at one of 4 intensities (0, 48, 60, or 72 dB SPL; Shetake et al. 2012). We hypothesized that KIA- rats would be less able to learn a more difficult speech discrimination task, such as speech in background noise. Children with dyslexia can often perform speech discrimination in quiet at control levels, but have significant impairment on the same task in background noise (Chandrasekaran et al. 2009; Nagarajan et al. 1999; Ziegler and Goswami 2005; Ziegler et al. 2009). On the first day of speech in noise training, KIA- rats and control rats both experienced a drop in overall performance levels compared to their last day of full speech training (first day of speech in noise by KIA- rats was 64.3 ± 6.3% correct vs. 81.1 ± 3.1% correct on the last day of full speech; p<0.01, and first day of speech in noise by controls was 67.2 ± 8.0% correct vs. 84.2 ± 2.1% correct on the last day of full speech; p<0.01; Figure A4.2A). The drop in performance seen from the last day of full speech to the first day of speech in noise is likely due to the stress of switching from a relatively

easy task (speech in silence) to a more difficult task (speech sounds in a variety of background noise). Control rats were able to improve over the course of 10 training days at 3 of the 4 noise levels (last day performance and paired t-test of last day performance vs. first day; 0 dB 91.2 ± 1.5% $p<0.01$; 48 dB 86.9 ± 2.3% $p<0.01$; 60 dB 72.2 ± 2.3% $p<0.01$; 72 dB 51.9 ± 1.5% $p=0.38$; Figure A4.2B). The inability to improve on the loudest intensity noise mimics previous chance level performance at this noise level using control rats (Shetake et al. 2011). KIA- rats were also able to improve over the course of training on two of the four noise levels (last day performance and paired t-test of last day performance vs. first day; 0 dB 83.5 ± 3.0% $p<0.01$; 48 dB 78.9 ± 2.8% $p<0.01$; 60 dB 62.0 ± 2.1% $p=0.11$; 72 dB 51.2 ± 1.2% $p=0.96$; Figure A4.2B), but remained significantly worse than control rats at three of the four noise levels (unpaired t-tests, 0 dB $p=0.02$, 48 dB $p=0.03$, 60 dB, $p<0.01$, 72 dB $p=0.72$). The improved performance caused by full speech training did not seem to generalize to other conditions. This result suggests that training with clear speech in quiet conditions may be the key to improving performance.

To test whether KIA- rats could learn an equally difficult task using clear speech in quiet, we next trained rats for 40 days on a speech sequence task in which a target speech sound (/dad/) was inserted into a random string of distracter sounds. This task was designed to be a difficult task for rats to learn and to evaluate whether KIA- rats are simply unable to learn a challenging speech task. Rats responded to the target sound by removing their noses from an infra-red nosepoke (Sloan, Dodd, Rennaker II 2009). Since this task required a different response mechanism than the previous tasks (infra-red nosepoke vs. lever press), several days of training were required to teach rats the new task. There was no difference in the amount of time needed to learn the task between control and KIA- rats. Performance between these two groups did not

differ on any day of the 40 day training period (Figure A4.2A). During the last 10 days of training, rats were trained on all six presentation rates within a single session (in random blocks of 20 trials per presentation rate; see Methods). Control and KIA- rats performed at the same level on all six presentation rates (control and KIA- rats last day performance respectively; at 2 sps, 64.6 ± 13.9% correct vs. 74.6 ± 6.9%, unpaired t-test, p=0.45; 4 sps, 74.4 ± 10.7% vs. 68.8 ± 7.4%, p=0.68; 5 sps, 79.4 vs. 4.7% vs. 71.9 ± 3.6%, p=0.27; 6.67 sps, 60.9 ±11.5% vs. 67.2 ± 7.9%, p=0.65; 10 sps, 47.6 ±14.2% vs. 47.8 ±8.7%, p=0.99; 20 sps, 21.4 vs. 9.6% vs. 17.9 ±4.9%, p=0.74). These results suggest that KIA- rats are not impaired at their ability to learn complex speech discrimination tasks, as long as the task occurs in a quiet setting.

KIA- rats are impaired at short speech discrimination

The ability to discriminate phoneme sounds without additional cues such as pitch or duration is extremely difficult for normal rats. We hypothesized that the months of prior training would help KIA- rats learn this difficult task. We truncated the speech sounds so that only the consonant and the beginning of the transition to the vowel remained (each stimulus was exactly 40 ms; Porter et al. 2011) and tested KIA- and control rats on this task for 2 weeks. On the first day of testing, control rats were significantly better than KIA- rats (89.7 ± 0.6% correct by controls vs. 82.4 ± 0.6% correct by KIA- rats; unpaired t-test, $p<0.01$; Figure A4.2A) and KIA- rats remained significantly impaired until the final day of training (unpaired t-tests, day 1-9, $p<0.01$; day 10, p=0.08; Figure A4.2A). This result suggests that any behavioral benefit caused by speech training with full speech tasks in quiet does not generalize to more difficult listening environments. We hypothesized that more focused training using the short speech sounds in quiet, would better generalize to the other behavior tasks.

KIA- rats can learn phoneme discrimination with extensive training

We trained a second group of 8 KIA- and 5 control rats on the short speech task for 28 days (the length of time needed for control rats' performance to reach ceiling; Porter et al. 2010). All rats were trained on shaping and detection as described above. Following detection, group 2 rats were trained on discrimination using the short speech stimuli. As seen previously, this task was extremely difficult to learn; both control and KIA- rats needed 7 days of training to perform above chance levels. Controls were able to perform the task at 80% correct after 12 ± 1.2 days of training. KIA- rats were only slightly impaired at reaching this level of performance and needed 16.3 ± 2.1 days (unpaired 1-tailed t-test, p=0.07; Figure A4.4A). KIA- rats were not significantly different from controls by the end of the 28 day training period (94.5 ± 1.6% correct on day 28 by controls vs. 90.8 ± 1.9% correct on day 28 by KIA- rats; unpaired 1-tailed t-test, p=0.11; Figure A4.4A). These results suggest that with focused training, KIA- rats are able to learn the short speech task and perform at control levels. We then tested these rats on the full speech task to determine whether or not the focused short speech training would generalize to performance on the other tasks using full speech sounds. KIA- rats were able to perform the full speech task as well as control rats throughout the 10 day testing period (average percent correct in KIA- rats was 87.7 ± 3.1% vs. 93.9 ± 2.2% in controls, unpaired t-test, p=0.37; Figure A4.4A). KIA- rats were significantly below controls on only one of the four noise levels (average performance at 0 dB noise in KIA- rats was 89.5 ± 0.6% vs. 92.9 ± 0.9% correct in controls, $p<0.01$, at 48 dB, KIA- rats achieved 90.1 ± 1.2% correct vs. 89.9 ± 2.3% by controls, p=0.97, at 60 dB, KIA- rats achieved 76.6 ± 1.8% correct vs. 78.2 ± 3.6% correct by controls, p=0.71, at 72 dB, KIA- rats achieved 49.6 ± 0.9% correct vs. 49.8 ± 1.2% correct by controls, p=0.53; Figure A4.4B) or

during the speech sequence task (average performance at 2 sps in KIA- rats was 76.0 ± 7.7% correct vs. 71.1 ± 9.0% correct by controls; p=0.69, at 4 sps, KIA- rats achieved 69.8 ± 10.1% correct vs. 63.1 ± 12.6% correct by controls; p=0.69, at 5 sps, KIA- rats achieved 78.1 ± 6.9% correct vs. 74.7 ± 7.5% correct by controls; p=0.75, at 6.67 sps, KIA- rats achieved 73.6 ± 5.6% correct vs. 69.4 ± 7.0% correct by controls; p=0.66, at 10 sps, KIA- rats achieved 41.7 ± 6.2% correct vs. 44.4 ± 5.3% correct by controls; p=0.75, at 20 sps, KIA- rats achieved 15.6 ± 2.8% correct vs. 21.0 ± 7.7% correct by controls; p=0.53; Figure A4.4C). Extensive behavioral training in normal rats and in human dyslexics can improve neural responses to speech and non-speech stimuli (Engineer et al. 2012; Habib et al. 2002; Jakkamsetti, Chang, Kilgard 2012; Penolazzi et al. 2010; Takahashi et al. 2011). We hypothesized that the extensive training programs our rats completed would also improve neural responses.

Extensive behavioral training restores neural firing patterns in KIA- auditory cortex

After 4 months of training and testing on the speech sound discrimination tasks described above, all rats were anesthetized and neural recordings were acquired from primary (A1) and posterior (PAF) auditory fields. Neural responses in untrained KIA- rats were significantly later than control rats (Centanni et al. 2013). In response to tones and speech sounds, control A1 and PAF sites fired faster after training. For example, untrained KIA- A1 sites fired 25.8 ± 0.6 ms after tone onset versus 17.7 ± 0.6 ms after training (unpaired t-test, $p<0.01$) and PAF sites fired 45.6 ± 7.1 ms after tone onset vs. 29.5 ± 2.2 ms after training (unpaired t-test, $p<0.01$; Figure B4.4A). Training also shortened control responses to tones in A1 (22.3 ± 0.7 ms in untrained A1 vs. 17.2 ± 0.6 ms in trained A1; unpaired t-test, $p<0.01$) but was less effective at shortening latency in PAF (42.5 ± 7.2 ms in untrained PAF vs. 31.8 ± 2.7 ms in trained PAF; unpaired t-test,

p=0.11; Figure B4.4A). In addition to the significantly shorter onset latency, the trial by trial variability in onset latency was significantly decreased in KIA- A1 (70.2 ± 4.1 ms^2 in untrained vs. 27.9 ± 4.4 ms^2 after training; unpaired t-test, p<0.01; Figure A4.5A and Figure A4.6F) and PAF (103.2 ± 3.9 ms^2 in untrained vs. 44.5 ± 3.2 ms^2 after training; unpaired t-test, p<0.01; Figure A4.5A and Figure A4.6H). Training also decreased trial-by-trial variability in control A1 (40.6 ± 2.7 ms^2 in untrained vs. 31.8 ± 3.3 ms^2 after training; unpaired t-test, p=0.04; Figure A4.5A and Figure A4.6E) and control PAF (103.2 ± 3.9 ms^2 in untrained vs. 55.3 ± 3.5 ms^2 after training; unpaired t-test, p<0.01; Figure A4.5A and Figure A4.6G). The additional 4 weeks of training by group 2 rats caused control A1 and PAF variability to significantly decrease compared to the group 1 control rats (A1: 65.3 ± 2.1% correct by group 1 vs. 77.2 ± 5.5% correct by group 2; unpaired t-test, p<0.01, PAF: 59.4 ± 4.3 ms^2 in group 1 vs. 29.5 ± 2.6 ms^2 in group 2; unpaired t-test, p<0.01; Figure B4.5A), but had no effect on KIA- neural variability (A1: 27.3 ± 4.6 ms^2 in group 1 vs. 29.4 ± 4.5 ms^2 in group 2; p=0.72, PAF: 44.5 ± 3.6 ms^2 in group 1 vs. 44.5 ± 3.9 ms^2 in group 2, p=0.99; Figure B4.5A). The improved reliability in trial-by-trial neural firing to auditory stimuli may generalize to improved neural discrimination ability.

We hypothesized that the training-induced neural plasticity would also extend to the ability of KIA- neural recordings to identify speech stimuli. Using the nearest-neighbor classifier described above, we compared neural classifier performance in trained versus untrained neural recordings. Control A1 sites did not improve on the neural discrimination task as a result of speech training (consonant tasks: 76.6 ± 0.9 correct vs. 76.4 ± 0.9% correct, using untrained or trained A1 sites respectively; unpaired t-test, p=0.94; vowel tasks: 59.9 ± 0.9% vs. 58.9 ± 0.8% correct using trained or untrained PAF sites, respectively; unpaired t-test, p=0.33; Figure

A4.5B&C and Figure B4.3E&G). The lack of training-induced plasticity in control rats supports earlier studies showing an absence of plasticity after tone (Reed et al. 2011) in the A1 of normal animals. PAF sites in control rats did significantly improve after training on neural discrimination of consonant sounds (59.7 ± 3.2% correct in untrained sites vs. 66.9 ± 1.9% correct after training; unpaired t-test, $p<0.01$; Figure A4.5B and Figure A4.6G). Training did not improve control PAF sites' ability to identify vowel stimuli (56.2 ± 3.4% correct in untrained sites vs. 56.2 ± 1.6% correct after training; unpaired t-test, $p=0.97$; Figure A4.5C and Figure B4.3G). A1 sites in KIA- rats were significantly better at the neural discrimination task following behavioral training. KIA- A1 sites were significantly better after training at consonant discrimination (67.8 ± 0.9% correct in untrained vs. 74.6 ± 0.8% correct after training; unpaired t-test, $p<0.01$; Figure A4.5B and Figure A4.6F). Training was less effective at improving KIA- A1 sites on neural discrimination of vowels (55.7 ± 1.1% correct in untrained vs. 57.4 ± 0.7% correct after training; unpaired t-test, $p=0.09$; Figure A4.5C and Figure B4.3F). Trained KIA- A1 sites were not significantly different from trained control A1 sites (consonants $p=0.46$; vowels $p=0.13$). Training improved KIA- PAF sites' performance on neural consonant discrimination (58.8 ± 2.0% correct in untrained sites vs. 67.7 ± 2.0% correct after training, $p<0.01$; Figure A4.5B and Figure A4.6H) but not neural discrimination of vowels (56.4 ± 1.9% correct in untrained sites vs. 55.4 ± 1.9% correct after training; unpaired t-test, $p=0.52$; Figure A4.5C and Figure B4.3H). As with neural variability, we saw an increase in consonant classifier performance in group 2 control PAF as compared to group 1 controls (65.3 ± 2.1% correct by group 1 vs. 77.2 ± 5.5% correct by group 2; unpaired t-test, $p<0.01$; Figure B4.5). This result

may suggest that there is a ceiling to the amount of training-induced neural plasticity in a brain with *in utero* knockdown of *Kiaa0319* as compared to control rats.

We expected that extensive training would improve the ability of KIA- neurons to fire to repetitive stimuli as well as to brief tones. Auditory cortex in untrained KIA- rats had significantly lower vector strength than control rats (Centanni et al. 2013, Figure A4.7A and Figure B4.4). After 4 months of speech discrimination training, vector strength in KIA- A1 was no longer significantly different from control rats at any speed we tested (an average of 0.65 ± 0.1 in controls vs. 0.62 ± 0.1 in KIA- rats; 1-way ANOVA, $F(1,6)=0.18$, $p=0.68$; Figure A4.7A&C). There were no significant differences in vector strength in PAF across control and KIA- groups (unpaired t-tests; 4 Hz $p=0.67$, 7 Hz $p=0.24$, 10 Hz $p=0.06$, 12.5 Hz $p=0.39$). Training did significantly improve vector strength in KIA- PAF (0.32 ± 0.1 in untrained KIA- PAF vs. 0.51 ± 0.1 in trained KIA- PAF; 1-way ANOVA, $F(1,6)=16.1$, $p<0.01$; Figure A4.7C&D), but did not affect vector strength in control PAF (0.45 ± 0.1 in untrained control PAF vs. 0.55 ± 0.1 in trained PAF; 1-way ANOVA, $F(1,6)=4.52$, $p=0.08$; Figure A4.7C&D). Untrained KIA- A1 sites fired more action potentials both spontaneously as well as to auditory stimuli, such as tones, broad band bursts, and speech sounds (Centanni et al. 2013; Figure B4.3). Training also changed these basic firing properties in both KIA- A1 and PAF (Figure B4.2). These results suggest that extensive speech training also improves neural firing to non-speech stimuli.

EEG and fMRI recordings from dyslexic children before and after training demonstrate an improvement in the amplitude and latency of auditory-evoked responses (Hornickel et al. 2012; Penolazzi et al. 2010; Russo et al. 2005; Temple et al. 2003; Tremblay and Kraus 2002). We

hypothesized that the average improvement in our multi-unit recordings (as local field potentials, or LFPs) would also show improvements in amplitude and onset latency. After speech discrimination training, local field potentials (LFPs) in control A1 responded faster to the onset of the speech sound /dad/ (40.9 ± 0.4 vs. 35.9 ± 0.6 ms, untrained vs. trained, respectively; unpaired t-test, $p<0.01$; Figure A4.8A). Trained KIA- A1 LFPs also responded faster compared to untrained recordings (43.7 ± 0.8 vs. 32.9 ± 0.7 ms; unpaired t-test, $p<0.01$; Figure A4.8B). N1 amplitude was significantly increased as a result of training in control A1 (-72.4 ± 2.0 mV in untrained control A1 vs. -151.3 ± 4.2 mV after training; unpaired t-test, $p<0.01$; Figure A4.8A) and in KIA- A1 (-41.3 ± 1.5 mV in untrained KIA- A1 vs. -100.6 ± 2.9 mV after training; unpaired t-test, $p<0.01$; Figure A4.8B). Latency of the N1 in control PAF was not significantly affected by training (46.1 ms ± 1.3 ms in untrained control PAF vs. 42.4 ± 1.1 ms after training; unpaired t-test, $p=0.07$; Figure A4.8C). KIA- PAF LFP had a longer latency following training (20.8 ± 14.5 ms in untrained KIA- PAF vs. 42.0 ± 0.9 ms after training; unpaired t-test, $p<0.01$; Figure A4.8D). The longer latency may be due in part to the significant increase in N1 amplitude in KIA- PAF (-18.9 ± 3.5 mV in untrained KIA- PAF vs. -73.8 ± 5.1 mV after training; unpaired t-test, $p<0.01$; Figure A4.8D), which sharpened the peak response. N1 amplitude in control PAF was also significantly increased by training (-42.1 ± 5.2 mV in untrained control PAF vs. -94.6 ± 10.4 mV after training; unpaired t-test, $p<0.01$; Figure A4.8C). Our observation that training induced plasticity improved neural discrimination performance of KIA- A1 and PAF sites suggests a possible neural basis for the success of current therapeutic options for humans with dyslexia. The result that control PAF improved after training supports the idea that auditory

fields higher than A1 may support auditory learning tasks in a unique and meaningful way (Lai et al. 2011; Takahashi et al. 2011).

DISCUSSION

Summary of results

This study was designed to test the hypothesis that *in utero* RNAi of the candidate dyslexia gene *Kiaa0319* would cause impaired speech sound discrimination in adult rats. KIA- rats were significantly impaired at discriminating a target speech sound from distracter speech sounds in a variety of contexts and required twice as much training to perform at control levels. KIA- rats were able to learn more difficult speech tasks, such as sequences and short speech. Extensive training significantly reduced the variability in KIA- auditory cortex responses and improved the neural encoding of speech sounds. Our results provide the first direct evidence that the candidate-dyslexia gene *KIAA0319* is directly related to phoneme perception. We provide evidence that the impairments caused by specific developmental genetic variants may be mediated by training.

Effect of anesthesia on neural responses

The majority of our neural recordings were made in the auditory cortex of anesthetized rats. There is evidence that anesthesia affects basic firing properties of auditory cortex to tonal stimuli. For example, neural responses in anesthetized animals can have sharper tuning curves (Gaese and Ostwald 2001) and fire less spontaneous action potentials than when the animal is awake (Capsius and Leppelsack 1996). In spite of these basic firing differences, neural encoding of natural sounds, specifically human speech sounds, are not significantly different as a result of

anesthesia (Engineer et al. 2008b; Hromádka, DeWeese, Zador 2008; Huetz, Philibert, Edeline 2009; Steinschneider et al. 1994). In the current study, we recorded speech sounds from anesthetized animals, but did not record from trained, awake rats. In our previous study, we reported that anesthesia did not significantly affect neural responses to speech sounds in experimentally-naïve rats (Centanni et al. 2013). Training on auditory tasks can affect neural responses. For example, when rats are trained to identify a low frequency tone, there is a corresponding expansion in the amount of auditory cortex neurons that preferentially respond to the target frequency (Takahashi et al. 2011). It is possible that extensive training on speech sound stimuli may also cause changes in the organization or firing patterns of awake rats. Additional studies are needed to evaluate whether the auditory cortex of trained KIA- rats fires differently following training and how those responses compare to the anesthetized responses reported here.

Training effects may be larger in non-primary auditory fields

Extensive training can change firing patterns in primary auditory cortex. For example, after rats are trained to discriminate sequences of auditory sounds, the bandwidth of primary auditory cortex responses was significantly narrowed and response latencies were significantly shorter (Engineer et al. 2012). Responses in other auditory fields can also be affected by extensive training. After more than 20 days of behavioral training, the percentage of neurons firing to the target frequency was significantly increased compared to naïve controls, and this effect is just as big or bigger in other auditory fields, such as the ventral auditory field (Takahashi et al. 2011).

Our results show that the trial-by-trial variability was reduced more after training in control and KIA- PAF than in A1. There are several explanations for this observation. It is possible that non-primary auditory fields are more sensitive to insult and are therefore more affected in communication disorders than the primary field. When autistic children listen to speech sounds, there is less evoked activity in the superior temporal gyrus compared to controls, while primary auditory cortex responses do not differ (Lai et al. 2011). Another explanation could be that this effect is merely an artifact of the degree of firing differences across fields. In experimentally naïve posterior auditory field, tone-evoked responses are significantly later than A1 and PAF has a significantly higher threshold, broader bandwidths and fewer evoked spikes than A1, anterior, or ventral auditory fields (Polley et al. 2007). The observation that PAF seems to benefit more from training than A1 does may be due to the smaller beginning impairment in A1 causing a floor effect after training. Additional studies should be conducted in other auditory fields to see if the other non-primary fields experience greater training-induced plasticity than A1.

Effect of training time on neural improvement

Neural plasticity is likely a transient phenomenon, causing observable changes in the cortical organization of auditory cortex during the learning phase of behavioral training, but disappearing once the task is mastered (Reed et al. 2011; Takahashi et al. 2011). Such plasticity is necessary for learning, but does not seem to be needed for continued accuracy at the task (Reed et al. 2011). The degree of neural plasticity is related to task difficulty. Tasks that are easy for an animal to acquire do not cause an obvious expansion in neurons dedicated to the task (Engineer et al. 2012). Our observation that KIA- animals with a greater percentage of

transfected neurons were worse at the speech discrimination task suggests that animals with more transfection may need more training to achieve high levels of accuracy than animals with less transfection. The degree of impairment is likely related to the amount of training needed to cause the improvement in neural response we report here. Future experiments should investigate a link between the amount of transfection and the amount of training needed to normalize both behavioral and neural responses to speech sounds.

Are these training effects specific to this candidate dyslexia gene?

We report here that *in utero* RNAi of the candidate dyslexia gene *Kiaa0319* in rats causes impaired speech discrimination performance. Humans with variants in this gene also have impairments in phoneme awareness which correlates to reduced activation in left auditory cortex (Pinel et al. 2012). *KIAA0319* is not the only candidate dyslexia gene and accounts for a small percentage of all dyslexia cases. The candidate dyslexia gene *DCDC2* has been linked to visual and spatial impairments in both rat models and in human dyslexics (Lind et al. 2010; Scerri et al. 2011), and knockout mice show some signs of rapid auditory processing impairment (Truong 2009). The dyslexia-associated gene *ROBO1* has been linked to phonological impairments, but only in one subpopulation of humans with the disorder (Bates et al. 2011). In addition, rats with *in utero* RNAi of the gene *DYX1C1* have small deficits in the ability to process rapid tonal stimuli (Threlkeld et al. 2007). No studies to date have investigated the effect of *in utero* RNAi of any of the other three dyslexia-associated genes on neural or behavioral speech processing. Future studies are needed to evaluate these genes on tasks using speech sounds as stimuli. This work will be critical to understanding the role variants in these other genes play on creating the variety of behavioral symptoms seen in dyslexic individuals.

Improved variability may be the mechanism for therapy in human dyslexics

We report that extensive training of rats with *in utero* knockdown of *Kiaa0319* can improve both behavioral and neural processing of speech sound stimuli. Behavioral training programs can improve speech processing in humans with dyslexia (Lovio et al. 2012; Penolazzi et al. 2010; Russo et al. 2005; Temple et al. 2003), but the mechanism by which this training is effective is still unknown. Our results show that extensive training was able to significantly reduce trial-by-trial variability in onset latency to both speech and non-speech stimuli. Recent studies suggest that this variability may be an underlying cause of the auditory phoneme awareness deficits in dyslexic people. Auditory brain stem responses in children with dyslexia are significantly more variable in response to speech sounds than healthy control children (Hornickel and Kraus 2013). Neural responses in people with other disorders, such as autism, can also be unreliable to a variety of sensory stimuli. Variability in the neural encoding of visual, auditory, and somatosensory stimuli is significantly higher in autistic individuals compared to controls (Dinstein et al. 2012). Interventions for dyslexic children can improve the latency and firing amplitude of speech-evoked neural responses (Hornickel et al. 2012; Lovio et al. 2012; Penolazzi et al. 2010; Russo et al. 2005; Temple et al. 2003), but it is not yet known what effect this training has on the trial-by-trial variability in onset response. As individuals age, the neural onset to auditory stimuli becomes less precise and reliable. With auditory training, the onset latency to speech sounds is significantly reduced and corresponds to an increase in behavioral ability (Anderson et al. 2013) . We suggest that trial-by-trial variability may be a mechanism for the auditory processing impairments seen in dyslexia and the significant reduction in variability caused by training may be a possible mechanism by which such training is effective.

Additional studies are needed in both human dyslexics as well as in animal models to further evaluate this claim.

Conclusion

In utero RNAi of the candidate dyslexia gene *Kiaa0319* causes behavioral impairments on speech sound discrimination tasks by rats. These rats are able to learn complex tasks, such as identifying a target sound in a rapid sequence, but still have difficulty identifying phonemes without additional cues like duration or pitch. This result provides the first evidence that the suppression of the gene *KIAA0319* directly causes speech sound discrimination deficits. The extent of the deficit is strongly correlated to the percentage of affected neurons and suggests a possible mechanism for the large degree of variance seen in the symptoms of human dyslexics. Extensive training reduces trial-by-trial onset variability and improves the accuracy of neurons to encode speech sounds. This improvement in neural firing suggests that impairments caused by developmental genetic variants may not be permanent.

Acknowledgments

The authors would like to thank K. Im, N Moreno, K Rahebi, E Hancik, M Borland, E Buell, M Fink, and R Carraway for help with microelectrode recordings, as well as N Khodaparast, L Baxter, K Trull, and N Wasko for help with histology. This work was supported by the National Institute for Deafness and Communication Disorders at the National Institutes of Health (R01DC010433 to MPK) and the National Institute of Child Health and Human Development at the National Institutes of Health (HD055655 to JJL).

APPENDIX A

CHAPTER 4 FIGURES

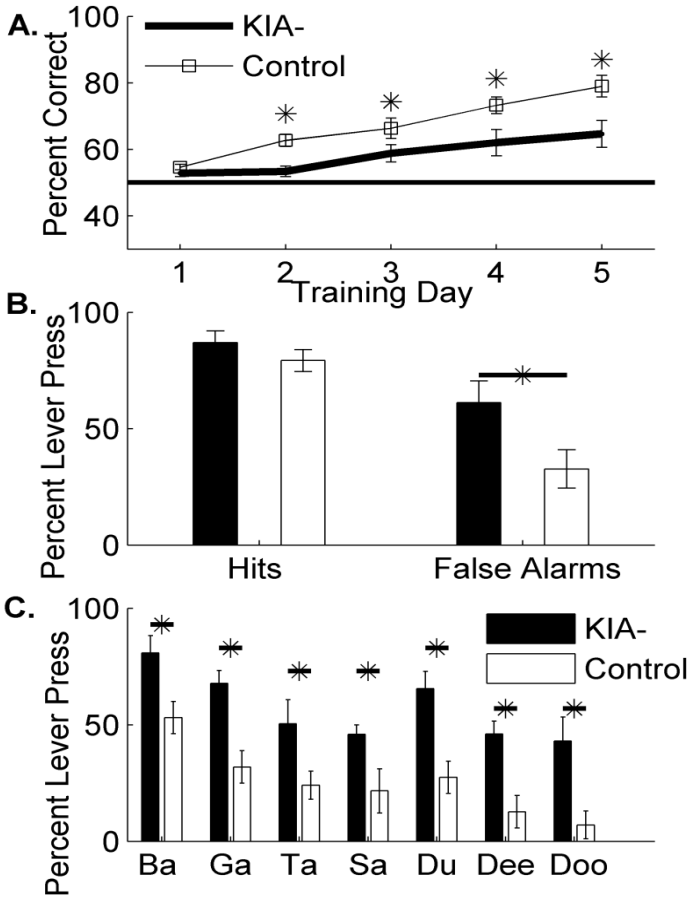

Figure A4.1. Rats with in utero RNAi of Kiaa0319 are impaired at speech discrimination tasks. **A.** On the first day of testing, KIA- and control rats both performed at chance level (unpaired t-tests, p=0.84 and p=0.59 respectively). KIA- rats were significantly worse than control rats on the full speech discrimination task for the remainder of testing (unpaired, one-tailed t-tests, *=p<0.01). **B.** KIA- rats hit to the target sound dad at the same rate as control rats (unpaired t-test; p=0.33), but false alarmed to the distracter sounds significantly more than control rats

Figure A4.1 continued…

(*=p=0.04). **C.** Break down of lever press rates to each of the distracter sounds. KIA- rats responded to every sound significantly more than control rats (unpaired t-tests, *=p<0.01).

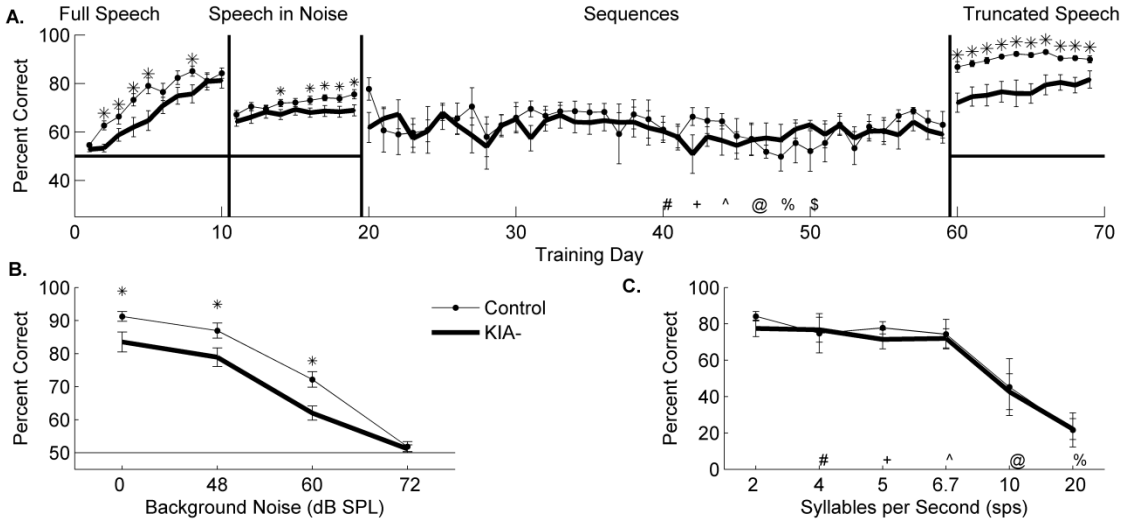

Figure A4.2. Extensive speech discrimination training can improve on clear speech tasks. **A.** After an additional week of training, 8 KIA- rats were able to perform the full speech task at the same level as 5 control rats (unpaired t-test, p=0.24). Rats were then trained for 2 weeks on a speech in noise task. On the first day, control and KIA- rats experienced a drop in performance compared to full speech (paired t-tests; p<0.01). By the last day of training, control rats had significantly improved on 3 of the 4 noise levels (last day performance and paired t-test of last day performance vs. first day; 0 dB p<0.01; 48 dB p<0.01; 60 dB p<0.01; 72 dB p=0.38). KIA- rats remained significantly below control levels at the end of training (unpaired t-tests, 0 dB p=0.02, 48 dB p=0.03, 60 dB, p<0.01, 72 dB p=0.72). Rats were next trained on a sequence task in which a target sound was inserted into a sequence of random distracter speech sounds. There were no significant differences between control and KIA- rats during this 40 day training period (at 2 sps; p=0.41, at 4 sps; p=0.90, at 5 sps; p=0.50, at 6.67 sps; p=0.85, at 10 sps; p=0.91, at 20 sps; p=0.97). For this task, chance performance was 0% rather than 50% because in this task, distracters were presented significantly more often than targets. Finally, rats were trained for 2 weeks on a short speech task in which only the first 40 ms of the speech stimulus was presented. KIA- rats were significantly impaired at this task compared to controls until the final day of training (unpaired t-tests, day 1-9, p<0.01; day 10, p=0.08). **B.** Breakdown of the last day performance of rats on the speech in noise task. Both control and KIA- rats performed at chance level at the highest noise intensity (72 dB; unpaired t-test, p=0.72). KIA- rats were significantly impaired at the remaining 3 noise levels compared to controls (0 dB; p=0.02, 48 dB; p=0.03, 60 dB; p<0.01). **C.** Breakdown of last day performance of rats on the sequence task. There were no significant differences between control and KIA- rats at any presentation rate tested (2 sps, p=0.45; 4 sps, p=0.68; 5 sps, p=0.27; 6.67 sps, p=0.65; 10 sps, p=0.99; 20 sps, p=0.74).

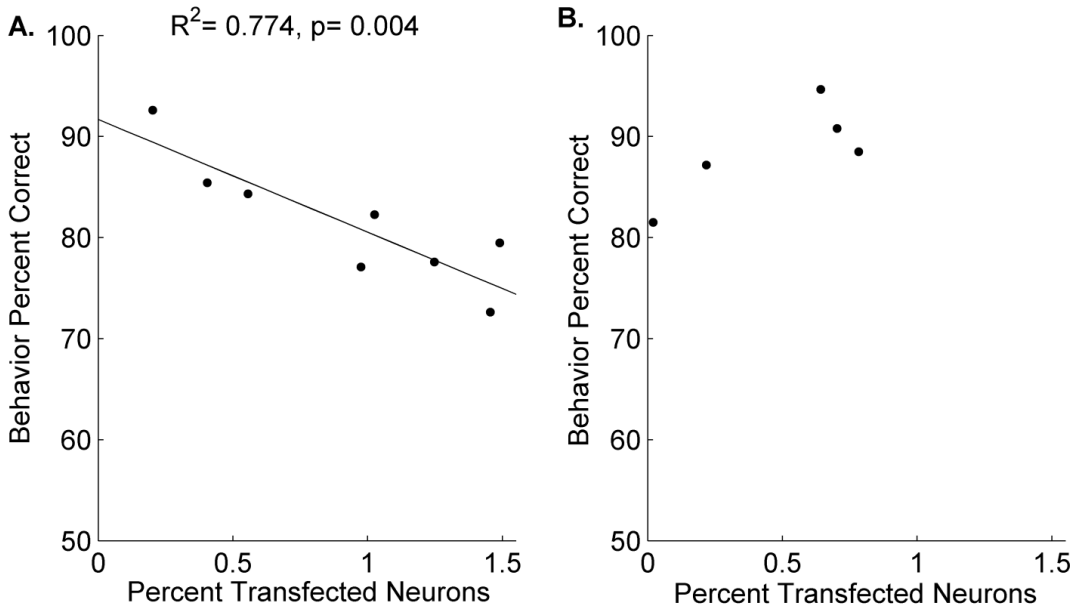

Figure A4.3. Percentage of transfected neurons predicts behavioral aptitude in KIA- rats. **A.** The percentage of layer 2/3 pyramidal neurons affected by the transfection was calculated in A1 bilaterally. Affected neurons were co-transfected with GFP (green florescent protein) to make the cells easily visible and the number of labeled neurons was counted. In KIA- rats, a higher percentage of transfected neurons was strongly correlated with impaired behavioral performance on the last day of full speech training (R^2=0.77, p<0.01). **B.** The percentage of transfected neurons in control animals was not correlated with performance (R^2= 0.61, p=0.12), which suggests that the surgery itself did not affect performance.

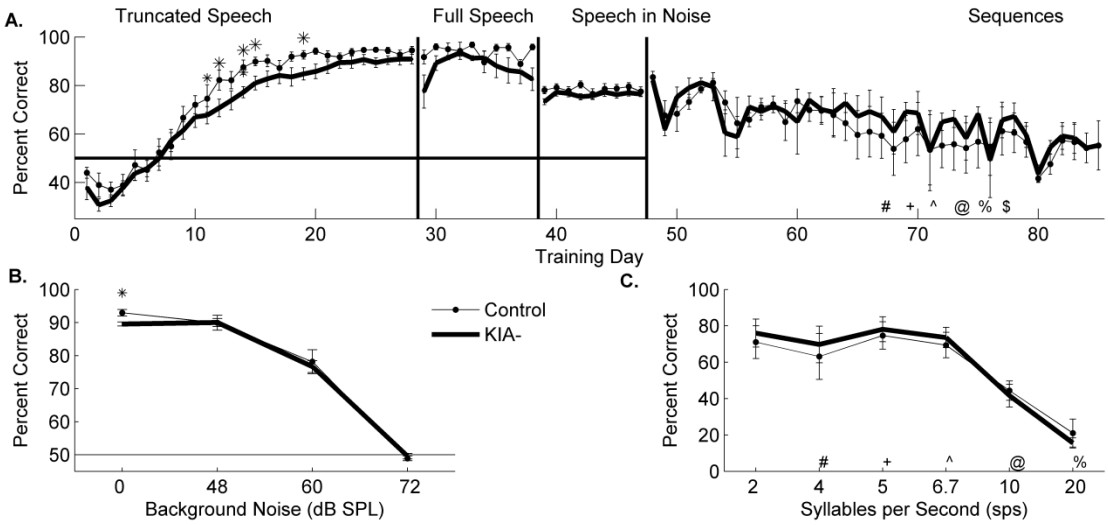

Figure A4.4. Extensive short speech training improves full speech and speech in noise performance in KIA- rats. **A.** 8 KIA- rats and 5 control rats were trained for 28 days on the short speech task. KIA- rats needed slightly longer to reach 80% correct compared to controls (unpaired 1-tailed t-test, p=0.07). At the end of training, there was no significant difference in performance across groups (unpaired 1-tailed t-test, p=0.11). Rats were then trained on the full speech task for 2 weeks, and we saw no difference in performance across the 10 days of testing (unpaired t-test, p=0.37). We also saw no difference in performance during the 10 days of speech in noise training that followed (0 dB p=0.06, at 48 dB, p=0.51, at 60 dB, p=0.39, at 72 dB, p=0.52). KIA- rats and control rats performed equally well on all 40 days of the sequence task, which came next (unpaired t-test, p=0.18). For this task, chance performance was 0% rather than 50% because in this task, distracters were presented significantly more often than targets. **B.** Breakdown of the last day of speech in noise performance by control and KIA- rats. We saw no significant difference in performance at 3 of the 4 noise intensities (at 0 dB; $p<0.01$, at 48 dB; p=0.97, at 60 dB; p=0.71, at 72 dB; p=0.53). **C.** Breakdown of the last day of speech in noise performance. We saw no significant difference in performance at any presentation rate we tested (at 2 sps: p=0.69, at 4 sps; p=0.69, at 5 sps; 0.75, at 6.67 sps; p=0.66, at 10 sps; p=0.75, at 20 sps; p=0.53).

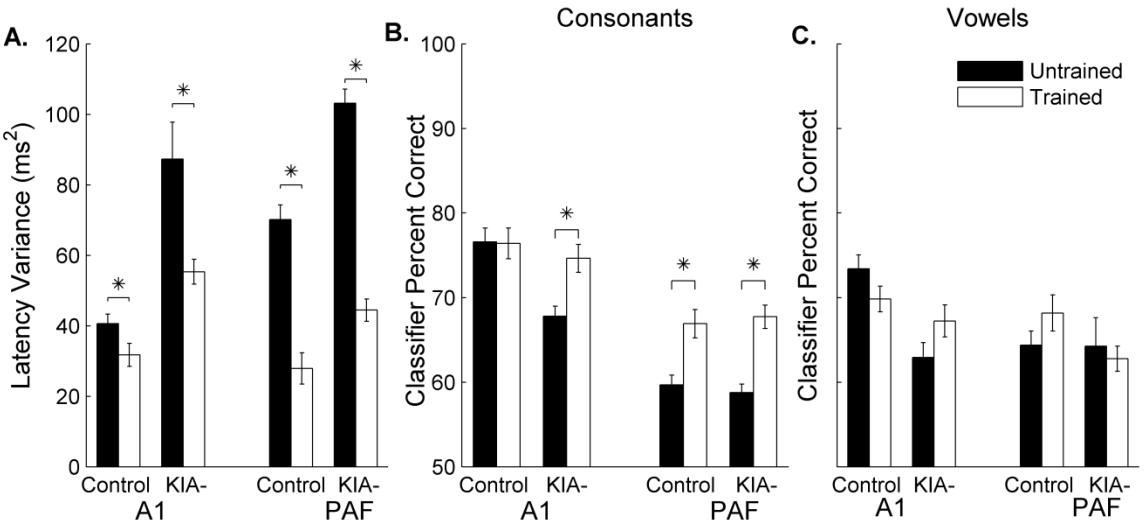

Figure A4.5. Extensive behavioral training improves reliability of neural firing and neural discrimination performance. **A.** Trial-by-trial variability was compared between trained and untrained neural recordings in control A1, KIA- A1, control PAF, and KIA- PAF. Training significantly decreased the variability in onset latency in KIA- A1 (unpaired t-test,*= $p<0.01$) and KIA- PAF (unpaired t-test, *=$p<0.01$). Training also decreased variability in control A1 (unpaired t-test, *=$p=0.04$) and control PAF (unpaired t-test, *=$p<0.01$). **B.** Extensive behavioral training improved the ability of neural responses to discriminate consonant speech sounds in every field except control A1. Control PAF sites significantly improved after training (unpaired t-test, *=$p<0.01$), as did KIA- A1 (unpaired t-test,*= $p<0.01$) and KIA- PAF (unpaired t-test, *=$p<0.01$). **C.** Training did not improve neural discrimination performance on vowel tasks in any field: in control A1 (unpaired t-test, $p=0.33$), control PAF (unpaired t-test, $p=0.97$), KIA- A1 (unpaired t-test, $p=0.09$), or KIA- PAF (unpaired t-test, $p=0.52$).

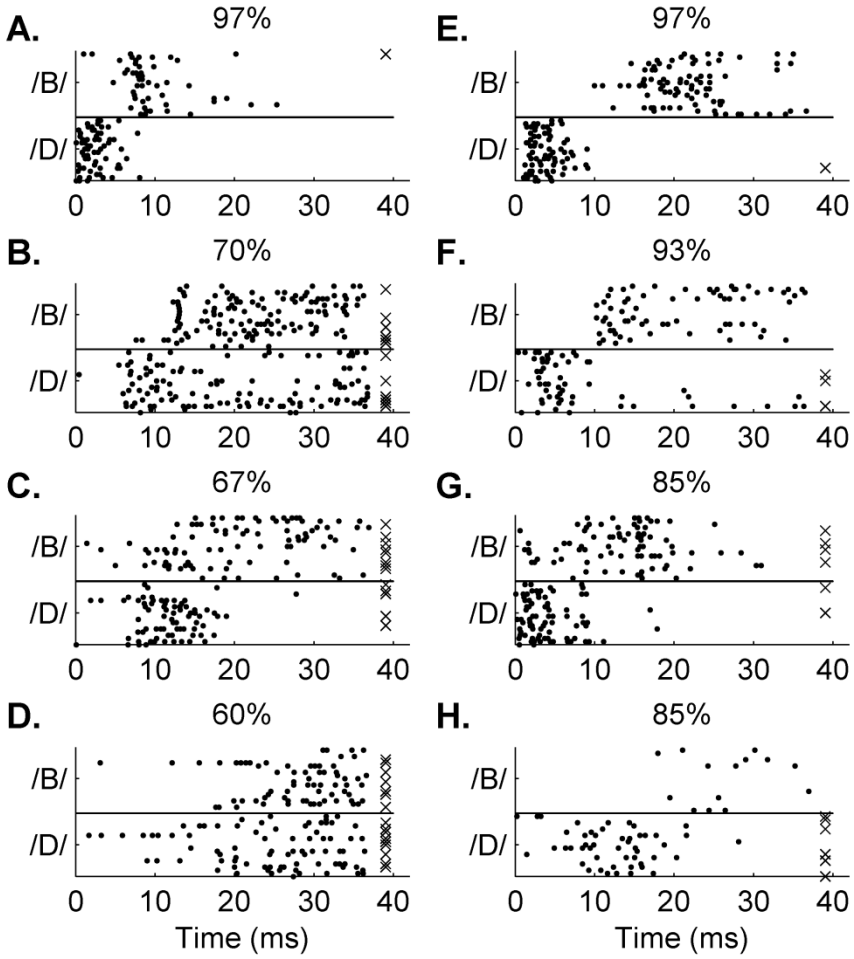

Figure A4.6. Training improves firing reliability in response to consonant speech sounds. Single site examples of neural responses to the consonant sounds /d/ and /b/ in every field before and after training. Classifier performance for each site is plotted on top of each panel, and trials which the classifier guessed incorrectly are marked by an 'x'. **A.** A representative single site in untrained control A1. This data was originally collected for and reported in Centanni et al. 2013. **B.** A representative single site in untrained KIA- A1. This data was originally collected for and reported in Centanni et al. 2013. **C.** A representative single site example in untrained control PAF. Control PAF sites had longer onset latency to tones than A1 sites and had a higher variability in onset latency (unpaired t-tests, $p<0.01$ and $p<0.01$ respectively). **D.** A representative single site example in untrained KIA- PAF. KIA- PAF sites had longer onset latency to tones than A1 sites and had a higher variability in onset latency (unpaired t-tests, $p<0.01$ and $p<0.01$ respectively). **E.** A representative single site example in trained control A1. Training did not significantly affect the ability of control A1 sites to encode consonant stimuli. **F.** A representative single site example in trained KIA- A1. Training significantly improved the

Figure A4.6 continued…

trial by trial variability in KIA- A1 sites (unpaired t-test, $p<0.01$) as well as reduced the number of action potentials fired (unpaired t-test, $p<0.01$; Figure B4.2). These aspects of neural plasticity likely contributed to the increased classifier performance. **G.** A representative single site example in trained control PAF. Training significantly improved trial by trial variability in this field (unpaired t-test, $p<0.01$) and likely contributed to the improved classifier performance. **H.** A representative single site example in trained KIA- PAF. Training shortened onset latencies (unpaired t-test, $p<0.01$) and reduced trial-by-trial variability in this field (unpaired t-test, $p<0.01$). These properties likely contributed to the improved classifier performance.

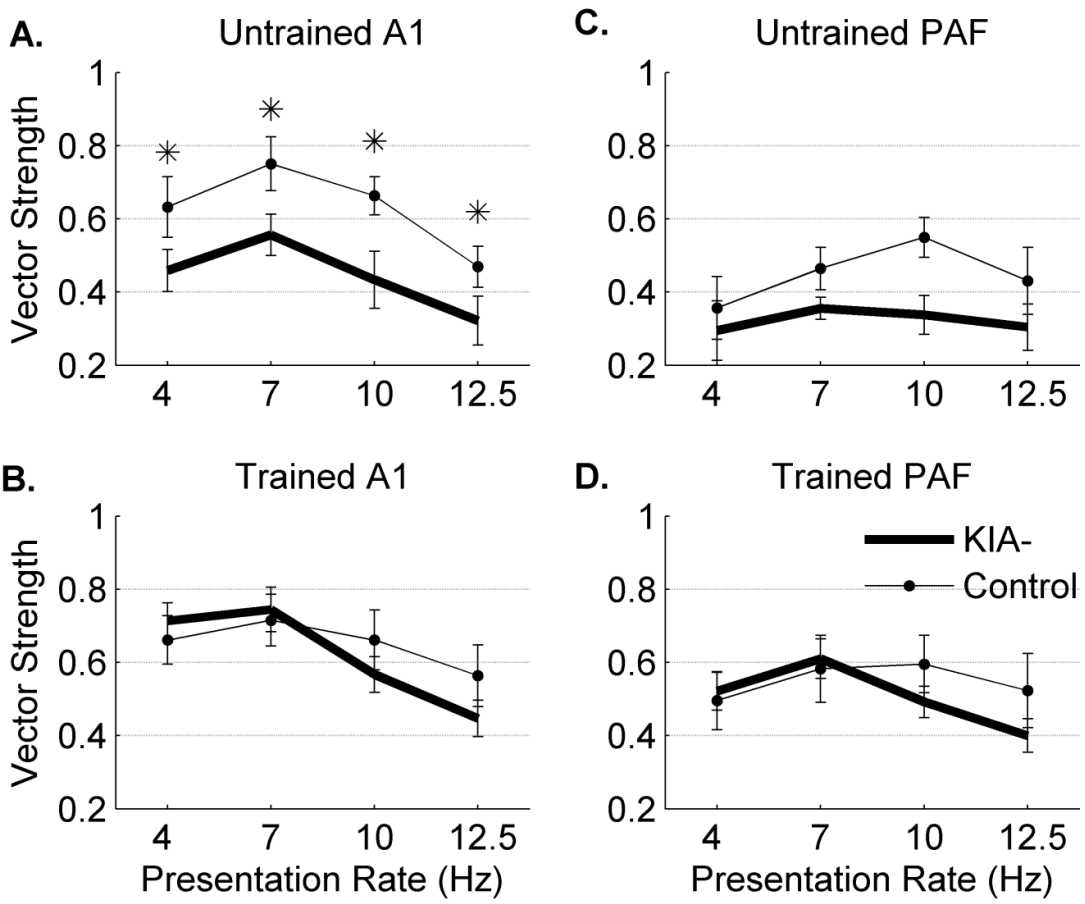

Figure A4.7. Training improves the ability of KIA- A1 and PAF sites to fire reliably to repetitive stimuli. **A.** Untrained KIA- A1 sites are significantly worse at following repetitive stimuli as measured by vector strength (*=$p<0.01$). Data was originally collected for and reported in Centanni et al. 2013. **B.** There was no significant difference in A1 vector strength between control and KIA- rats after extensive behavioral training (one-way ANOVA $F(1,6)=0.18$, $p=0.68$). **C.** Prior to training, there were no significant differences in vector strength between control and KIA- PAF sites (unpaired t-tests; 4 Hz $p=0.67$, 7 Hz $p=0.24$, 10 Hz $p=0.06$, 12.5 Hz $p=0.39$). **D.** Extensive training significantly improved KIA- PAF vector strength (one-way ANOVA $F(1,6)=16.1$, $p<0.01$) but did not affect control PAF vector strength ($F(1,6)=4.52$, $p=0.08$).

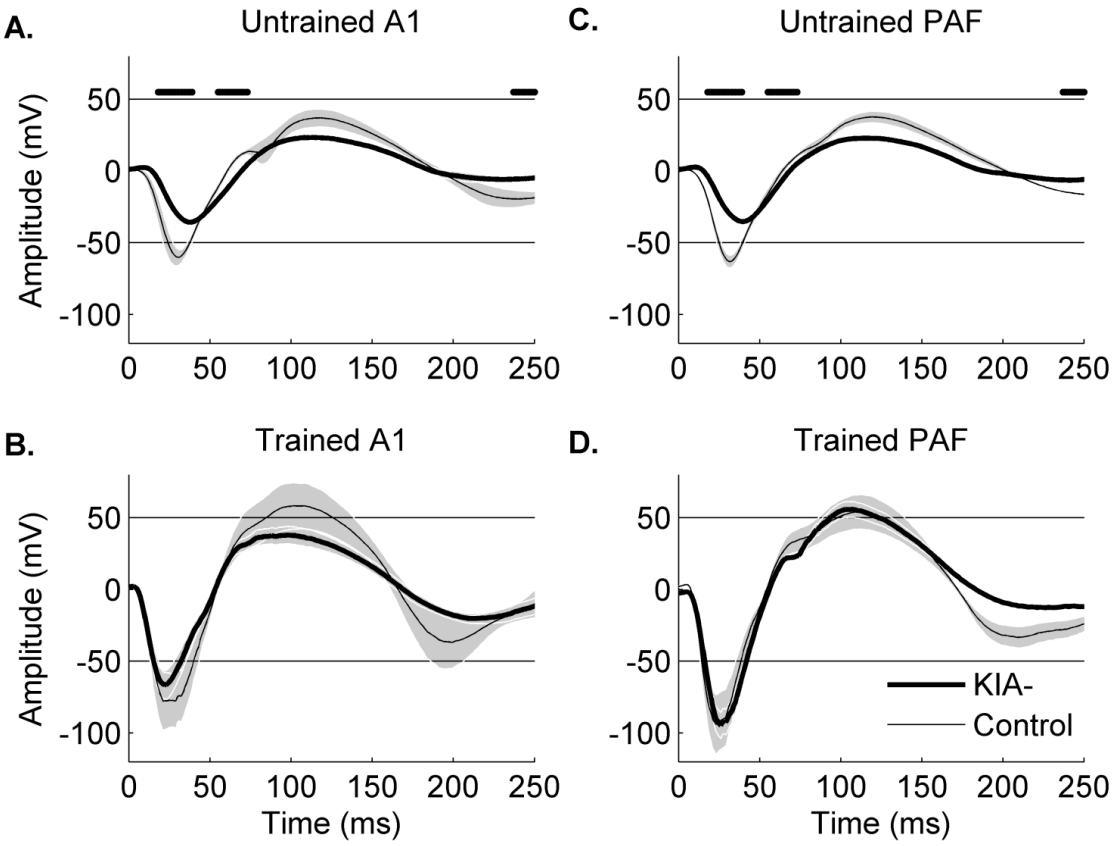

Figure A4.8. Extensive behavioral training shortens latency and amplitude of N1 component of LFP response to the speech sound /dad/. Responses are plotted with gray markers at -50 and 50 mV to help visualize differences across plots. Significant differences are marked by a black line. **A.** LFP response to the sound /dad/ in untrained control and KIA- A1. This data was originally collected for and reported in Centanni et al. 2013. **B.** Extensive training improves onset latency and amplitude of the LFP response in KIA- A1. After this training, there were no differences in LFP response between control and KIA- A1. **C.** LFP response to the sound /dad/ in untrained control and KIA- PAF sites. As was seen in A1 recordings, latency and amplitude of KIA- PAF responses were significantly different from control recordings. **D.** Following training, there were not significant differences in the LFP response to the sound /dad/ between control PAF and KIA- PAF.

APPENDIX B

CHAPTER 4 SUPPLEMENTARY FIGURES

A. Figure B4.1, related to Figure A4.1.

Rats were first trained to press a lever, which triggered the presentation of the target sound (/dad/) and a sugar pellet reward. KIA- rats learned this task in the same amount of time as control rats (to criterion of 2 sessions of 100 self presses; 113.4 ± 14.2 minutes for KIA- rats vs. 141.5 ± 27.2 minutes for controls; unpaired t-test, p=0.30). After learning to press the lever, rats were transitioned to detection in which they were required to press the lever only when the target sound /dad/ was presented. Rats with *in utero* RNAi of *Kiaa0319* were not impaired in their ability to switch from free pressing to waiting for the target sound. Both groups were able to reach the performance criterion (10 sessions with a d'≥1.5) in approximately 5 days (Controls: 4.4 ± 0.8 days vs. KIA-: 5.4 ± 5.4 days; unpaired t-test, p=0.35; Figure B4.1A). To determine whether KIA- rats also had attention impairments, we analyzed reaction times as well as hit and false alarm rates. KIA- rats did not take longer to respond to speech sounds as compared to controls. On the first day of training, KIA- rats actually responded faster than controls (1.7 ± 0.1 s vs. 2.2 ± 0.1 s, KIA- and controls respectively, one tailed t-test, p<0.01; Figure B4.1B). KIA- rats did not false alarm to silent catch trials more than control rats at any point during detection training (one tailed t-tests, p=0.51; Figure B4.1C). KIA- rats did miss more target sounds than controls did early in training, but were not significantly different from controls throughout the remainder of detection training (days 2 and 3 of detection training, one tailed t-test, p<0.01; Figure B4.1C). *In utero* RNAi of *Kiaa0319* did not impair rats' ability to learn to press a lever in response to a target sound, and were then able to transition to a more difficult discrimination task (Figure

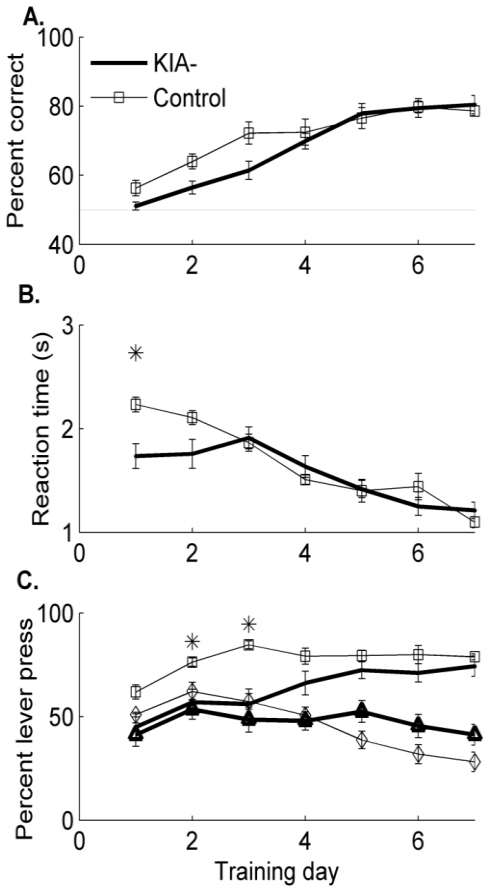

Figure B4.1 continued…

A4.1, Main text). This observation supports earlier reports that these animals can hear speech sounds and can learn behavioral tasks (Centanni et al. 2013; Szalkowski et al. 2012).

Figure B4.1. Rats with in utero RNAi of Kiaa0319 are able to learn a simple lever pressing task. **A.** KIA- rats were not impaired at their ability to reach a criterion of 10 sessions with a d'≥1.5 as compared to control rats. Both groups were able to learn this task within 7 days of training (14 sessions). **B.** KIA- rats responded to the target sound later than control rats on the first day of training ($p<0.01$). By the following day, and for the rest of the training period, there was no difference in response times across groups. **C.** KIA- rats respond to the target sound less than control rats do during the first 3 days of training (*=$p<0.01$). Performance was not significantly different overall due to the "normal" false alarm rate to silent catch trials on these days. By the middle of training, KIA- rats were hitting to the target and withholding response to the silent catch trials at the same rate as control rats.

B. Figure B4.2, related to Figures A4.3 & A4.4.

We trained rats for 4 months on a variety of speech discrimination tasks (Figures A4.3& A4.4, Main Text) and evaluated the effect of such training on neural firing properties. Training reduced onset latency in both KIA- A1 (25.8 ± 0.6 ms in untrained vs. 17.7 ± 0.7 ms after training; $p<0.01$) and PAF (45.6 ±7.1 ms in untrained vs. 29.5 ± 2.2 ms after training, $p=0.01$; Figure B4.2A), and KIA- A1 also fired fewer evoked spikes after training (2.9 ± 0.1 spikes in untrained vs. 2.4 ± 0.1 spikes after training, $p<0.01$; Figure B4.2D). This reduction in action potentials may be related to the decrease in neural variability we observed (Figure A4.5, Main Text). Control A1 sites had shorter latencies (22.3 ± 0.7 ms in untrained vs. 17.2 ± 0.6 spikes after training, $p<0.01$; Figure B4.2A), narrower bandwidths (2.3 ±0.1 octaves in untrained vs. 1.9 ± 0.1 octaves after training, $p<0.01$; Figure B4.2B), and had a greater number of driven action potentials to tones (2.8 ± 0.1 spikes in untrained vs. 3.2 ± 0.1 spikes after training, $p<0.01$; Figure B4.2D). Thresholds were not affected by training in any group (Figure B4.2C).

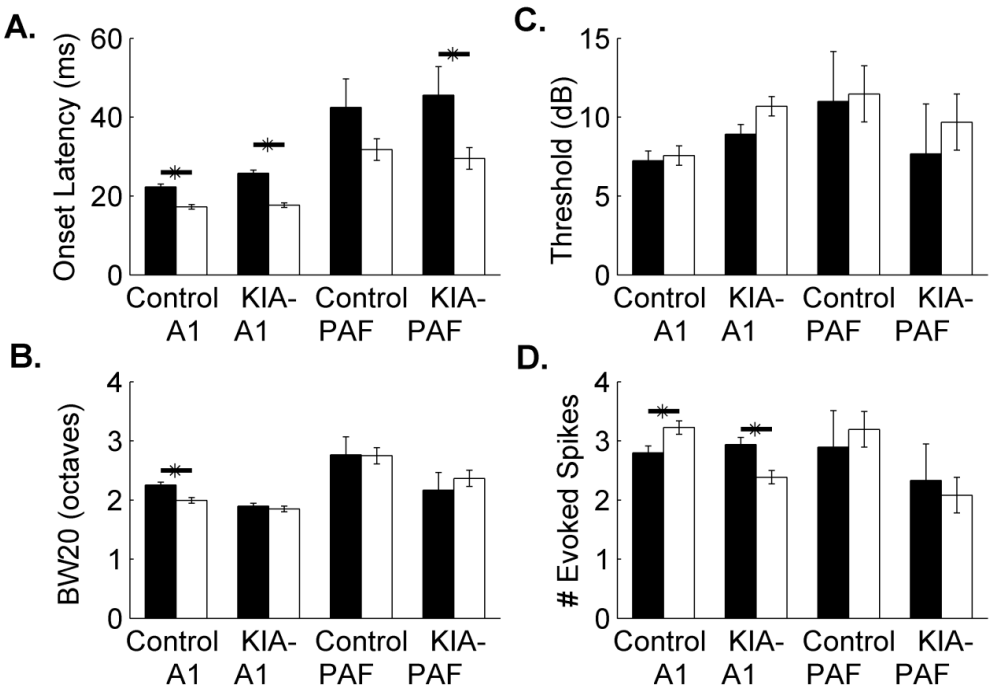

Figure B4.2. Training affects basic neural firing properties to tonal stimuli in KIA- and Control rats. **A.** Training significantly shortened the onset latency in Control A1 (22.3 ± 0.7 ms in untrained vs. 17.2 ± 0.6 spikes after training, $p<0.01$), KIA- A1 (25.8 ± 0.6 ms in untrained vs. 17.7 ± 0.7 ms after training; $p<0.01$), and KIA- PAF (45.6 ±7.1 ms in untrained vs. 29.5 ± 2.2 ms after training, $p=0.01$). No significant differences were seen in control PAF sites. **B.** Extensive behavioral training shortened bandwidths in Control A1 (2.3 ±0.1 octaves in untrained vs. 1.9 ± 0.1 octaves after training, $p<0.01$), but had no effect on bandwidths in the other fields. **C.**

Figure B4.2 continued…

Extensive behavioral training had no effect on auditory thresholds in any group or field (control A1; $p=0.72$, KIA- A1; $p=0.06$, control PAF; $p=0.90$, KIA- PAF; $p=0.53$). **D.** Extensive behavioral training increased the number of tone-evoked action potentials fired in control A1 (2.8 ± 0.1 spikes in untrained vs. 3.2 ± 0.1 spikes after training, $p<0.01$), but reduced the number of tone-evoked spikes fired in KIA- A1 (2.9 ± 0.1 spikes in untrained vs. 2.4 ± 0.1 spikes after training, $p<0.01$). The reduction in spikes in KIA- A1 may contribute to the improved neural encoding of consonants, since the spontaneous rate of firing and the number of evoked spikes in untrained KIA- was significantly higher than controls (Centanni et al., 2013).

C. Figure B4.3, related to Figure A4.6.

After training, trial-by-trial variability in onset latency across sites in KIA- A1 and PAF as well as control PAF were significantly reduced (Figure A4.5, Main Text). Responses to consonant speech sounds were significantly more precise following training and were better able to encode the differences between consonant sounds (Figure A4.6, Main Text). We saw a similar effect in the encoding of vowel sounds following training. Vowel sounds are encoded using spike count over a single 400 ms analysis window (Perez et al. 2012). As reported previously, untrained control and KIA- A1 responded to vowel sounds with a high degree of variability, and these two sites performed worse at the vowel task than at the consonant task (Figure B4.3 A&B; data originally collected for and reported in Centanni et al. 2013). Untrained PAF in control animals was slightly worse at the vowel task than A1 in each group. Average performance by untrained control PAF sites was 64.4 ± 2.4% correct vs. 73.4 ± 0.6% correct in untrained control A1 ($p<0.01$; Figure B4.3C). Performance in untrained KIA- PAF sites was not significantly worse than untrained KIA- A1 sites (64.3 ± 1.4% correct in PAF vs. 62.9 ± 0.1% correct in A1; $p=0.71$; Figure B4.3D). Following training, we noticed a slight (but not significant) improvement in the neural encoding of vowels. Trial-by-trial variability was reduced in every field (Figure B4.3 E-H and Figure A4.5, Main Text), which slightly improved the ability of each site to encode differences in vowel sounds. This result suggests that the specific training tasks we used benefitted consonant processing more effectively than vowel processing.

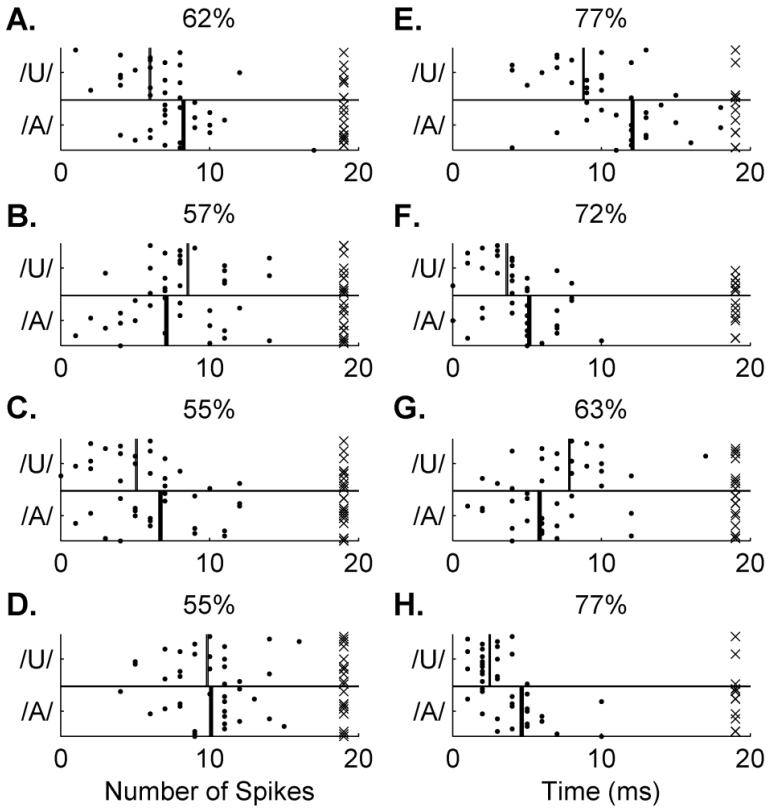

Figure B4.3. Extensive behavioral training improves neural encoding of vowel sounds in control and KIA- auditory cortex. **A.** A representative site from untrained control A1. The number of

Figure B4.3 continued…

spikes encoded in response to each vowel sound was used to predict which sound evoked each single trial response. Data originally collected for and reported in Centanni et al. 2013. **B.** A representative site from untrained KIA- A1. The variability in neural firing was significantly higher in KIA- sites, which significantly impaired the ability of these sites to perform the vowel discrimination task. Data originally collected for and reported in Centanni et al. 2013. **C.** A representative site from untrained control PAF. **D.** A representative site from untrained KIA- PAF. **E.** A representative site from trained control A1. Though training did not have a significant impact on the classifier performance, the reduced variability in this field following training did provide some improvement on neural processing of vowels in this field. **F.** A representative site from trained KIA- A1. The improved variability in KIA- neurons after training did improve classifier performance on the vowel tasks, though this improvement was not significant. **G.** A representative site from trained control PAF. There was significant reduction in trial-by-trial variability in this field after training, and there was slight (but not significant) improvement in this fields' vowel classifier performance. **H.** A representative site from trained KIA- PAF. There was significant reduction in trial-by-trial variability in this field after training, and there was slight (but not significant) improvement in this fields' vowel classifier performance.

D. Figure B4.4, related to Figure A4.7.

Rats were implanted with a chronic array of 16 microelectrodes into right primary auditory cortex. Neural recordings were acquired in a single session while the animals were awake, unrestrained, and passively listening to a variety of stimuli. This data was collected for and partially reported in Centanni et al. 2013. In response to click trains at a variety of speeds, 55 sites in 9 KIA- rats were less able then controls (16 sites in 3 animals) to phase-lock to these stimuli as measured by vector strength (Figure B4.4). The difference between vector strength in KIA- rats versus control rats is not significant at speeds less than 76 Hz in the awake animals (Figure B4.4), while it was significant at 4, 7, 10, and 12.5 Hz in the anesthetized condition (Figure A4.7, Main Text). Vector strength is a metric that relies on spike count for the calculation. Since recordings from awake animals have higher spontaneous firing than anesthetized recordings, this discrepancy in spike count may be driving the lack of a significant effect at the slower speeds in the awake recordings.

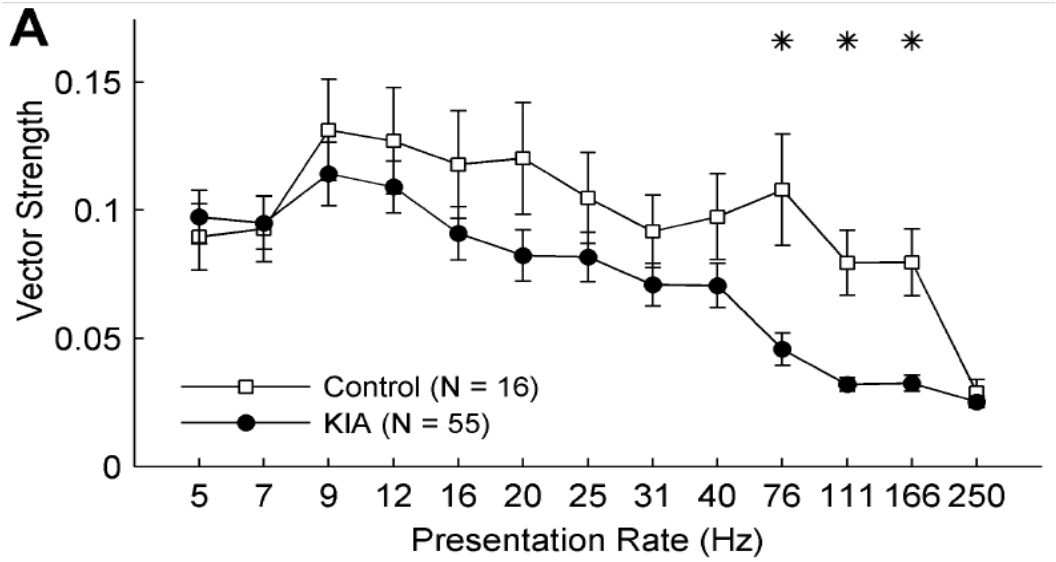

Figure B4.4. Untrained, awake KIA- neurons are impaired at following repetitive stimuli. Primary auditory cortex responses to repetitive click trains from 9 KIA- rats and 3 control rats chronically implanted with a 16 microelectrode array. Vector strength in KIA- rats was significantly worse than control rats at speeds between 76 and 166 Hz (*=$p<0.01$). At speeds of 9 through 40 Hz, KIA- rats have slightly (but not significantly) lower vector strength. The higher spontaneous firing rate in awake rats is likely the factor keeping this trend from reaching significance.

E. Figure B4.5, related to Figure A4.5.

The 4 weeks of additional training was also able to further reduce the trial-by-trial onset latency variability in control rats, but not KIA- rats as compared to group 1. In control A1, neural recordings from group 2 rats had lower trial-by-trial variability compared to group 1 (34.6 ± 3.3 ms^2 in group 1 vs. 20.3 ± 3.2 ms^2 in group 2; unpaired t-test, p=0.01; Figure B4.5A and Figure A4.6, Main Text). Control PAF in group 2 was also less variable trial-by-trial as a result of the additional training (59.4 ± 4.3 ms^2 in group 1 vs. 29.5 ± 2.6 ms^2 in group 2; unpaired t-test, p<0.01; Figure B4.5A). Trial-by-trial variability in KIA- rats did not decrease with additional training (A1: 27.3 ± 4.6 ms2 in group 1 vs. 29.4 ± 4.5 ms2 in group 2; p=0.72, PAF: 44.5 ± 3.6 ms2 in group 1 vs. 44.5 ± 3.9 ms2 in group 2, p=0.99; Figure B4.5A). We observed an increase in neural discrimination (as measured by the nearest-neighbor classifier) ability selectively in control PAF. Neural activity from group 2 control PAF sites were better able to discriminate between pairs of consonants than group 1 control PAF (65.3 ± 2.1% correct by group 1 vs. 77.2 ± 5.5% correct by group 2; unpaired t-test, p<0.01; Figure B4.5B). Control and KIA- A1 and KIA- PAF sites did not improve on the neural consonant discrimination task as a result of additional training (unpaired t-tests; p=0.29, p=0.16, and p=0.88, respectively; Figure B4.5B). Similarly, no group experienced an increase in neural vowel discrimination performance as a benefit of additional training (Control A1, p=0.05; Control PAF, p=0.36; KIA- A1, p=0.42; KIA- PAF, p=0.70; Figure B4.5C). The result that additional training did not provide additional neural plasticity in KIA- rats suggests that there may be a limit in how beneficial behavioral therapy can be in mediating the impairment caused by variants in *Kiaa0319.*

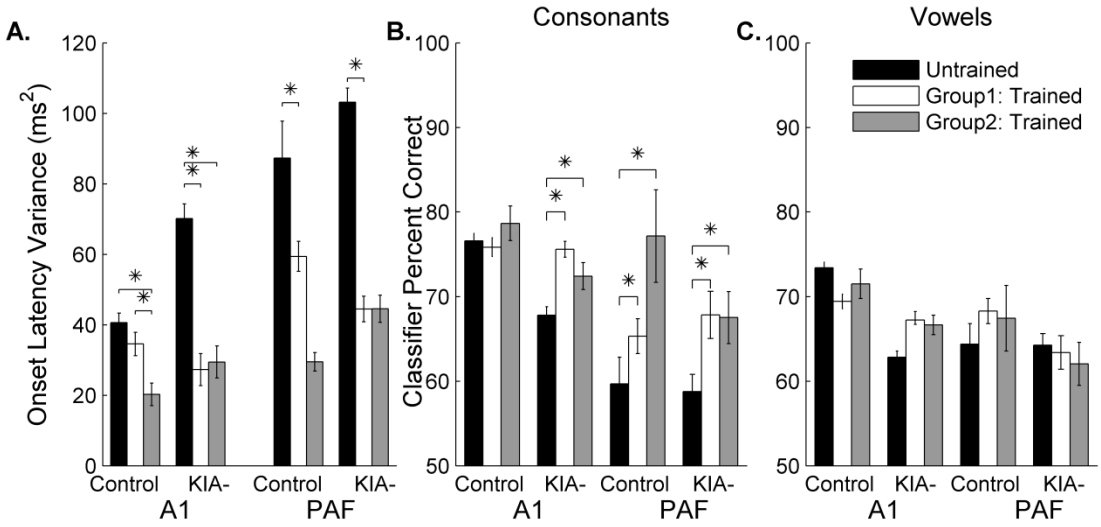

Figure B4.5. An additional 4 weeks of behavior training causes additional plasticity in control rats. **A.** The additional training received by group 2 caused a significant reduction in trial-by-trial variability in control A1 (p=0.01) and control PAF (p<0.01). No significant changes were seen in

Figure B4.5 continued…

either field in KIA- rats (p=0.72 and p=0.99 in A1 and PAF respectively). **B.** Additional training improved the ability of control PAF sites to perform the consonant neural discrimination task ($p<0.01$), but this training did not improve classifier performance in control A1 (p=0.29), KIA- A1 (p=0.16), or KIA- PAF (p=0.88). **C.** Additional training did not improve the ability of neural activity in any group or field to perform the neural discrimination task using vowel stimuli. Control A1; p=0.05, KIA- A1; p=0.42, control PAF; p=0.36, KIA- PAF, p=0.70.

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CHAPTER 5

REAL TIME IDENTIFICATION OF SPEECH SOUNDS USING CORTICAL ACTIVITY PATTERNS

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ABSTRACT

We developed a classifier that can accurately identify nine English consonants from neural activity in real time. The classifier searches primary auditory cortex activity for action potential patterns that indicate that a speech sound has occurred and identifies the speech sound within 40 milliseconds of stimulus onset. The classifier can accurately identify the occurrence of nine consonants without prior knowledge of the stimulus onset times. The classifier performs as well as rats trained on several previously reported consonant discrimination tasks. To test the temporal limits of the classifier, we developed a novel task that requires rats to identify individual consonants from a stream of distracter consonants. The classifier successfully predicted the ability of rats to accurately identify speech sounds when the syllable presentation rate was at or below 10 syllables per second. These results suggest a novel method to read out detailed information from cortical networks in real time.

INTRODUCTION

Speech sounds evoke unique spatiotemporal patterns in the auditory cortex of many species (Eggermont 1995; Engineer et al. 2008; Kuhl and Miller 1975). Consonant sounds evoke transient bursts of neural activity in primary auditory cortex (A1). A1 neurons fire to all consonants, but fire at different times for different sounds. For example, the consonant /d/ evokes activity first in neurons tuned to high frequencies, followed by neurons tuned to lower frequencies. The sound /b/ causes the opposite pattern such that low frequency neurons fire first, followed by high (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012; Shetake et al. 2011). This pattern of activity can be used to identify auditory stimuli. EEG recordings in patients listening to isolated words (Chang et al. 2010; Pasley et al. 2012) can be used to identify which word was presented, provided that the recordings encompass a large frequency range. Since consonant sounds are transient and occur on a millisecond time scale, microelectrode recordings in rats provide the high level of spectral and temporal precision needed to visualize the neural patterns evoked by these complex stimuli.

These unique patterns of activity predict behavioral discrimination accuracy in rats. Rats are a good model of human speech sound discrimination as these rodents have neural and behavioral discrimination thresholds that are similar to humans. Rats can discriminate isolated human speech sounds with high levels of accuracy (Engineer et al. 2008; Perez et al. 2012). Rats and humans can discriminate speech sounds with as few as 4 bands of spectral information (Ranasinghe et al. 2012). Rats and humans are also able to discriminate speech sounds when presented at 0 dB signal to noise ratio (Shetake et al. 2011). Sounds that evoke different patterns of neural activity are more easily discriminated than two sounds that evoke similar patterns of

activity (Engineer et al. 2008; Ranasinghe et al. 2012; Shetake et al. 2011). Speech sounds presented in background noise evoke neural response patterns with longer latency and lower firing rate than speech presented in quiet and the extent of these differences is correlated to behavioral ability (Martin and Stapells 2005; Shetake et al. 2011). Neural activity patterns in anesthetized rats also predict behavioral discrimination ability of spectrally and/or temporally degraded speech stimuli (Ranasinghe et al. 2012).

The classifier used in the aforementioned studies was provided the stimulus onset time, which may not be readily available to the brain during discrimination tasks. Previous classifiers in our lab performed as a two-alternative forced choice classifier in which the correct answer was always one of the two choices. In the current study, we designed a classifier that could identify any of nine consonant sounds, but was not forced to guess if a decision criterion was not met. We hypothesized that neural activity patterns should be sufficient to identify the evoking stimulus in real-time and report a novel behavioral paradigm to test this hypothesis.

METHODS

Speech Stimuli

For this study, we used the same stimuli as several previous studies in our lab (Engineer et al. 2008; Floody et al. 2010; Porter et al. 2011; Ranasinghe et al. 2012; Shetake et al. 2011). We used nine English consonant-vowel-consonant (CVC) speech sounds differing only by the initial consonant: (/bad/, /dad/, /gad/, /kad/, /pad/, /sad/, /tad/, /wad/, and /zad/), which were recorded in a double-walled, soundproof booth by a female native- English speaker. The spectral envelope was shifted up in frequency by a factor of two while preserving all spectral information using the

STRAIGHT vocoder (Kawahara 1997) to better accommodate the rat hearing range. The intensity of each sound was calibrated with respect to its length, such that the loudest 100 ms was presented at 60 dB SPL and 5 ms on and off ramps were added to prevent any artifacts.

Surgical procedure- anesthetized recordings

Multiunit recordings were acquired from the primary auditory cortex of anesthetized, experimentally-naïve Sprague-Dawley rats. Recording procedures are described in detail elsewhere (Engineer et al. 2008; Ranasinghe et al. 2012; Shetake et al. 2011). In brief, animals were anesthetized with pentobarbital (50 mg kg^{-1}) and were given supplemental dilute pentobarbital (8 mg ml^{-1}) as needed to maintain areflexia, along with a 1:1 mixture of dextrose (5%) and standard Ringer's lactate to prevent dehydration. A tracheotomy was performed to ensure ease of breathing throughout the experiment and filtered air was provided through an air tube fixed at the open end of the tracheotomy. A craniotomy and durotomy was performed, exposing right primary auditory cortex. Four Parylene-coated tungsten microelectrodes (1-2 MΩ) were simultaneously lowered to layer (4/5) of right primary auditory cortex (~600 μm). Electrode penetrations were marked using blood vessels as landmarks.

Brief (25 ms) tones were presented at 90 randomly interleaved frequencies (1-47 kHz) at 16 intensities (1- 75 dB SPL) to determine the characteristic frequency of each site. A set of four stimuli were created using Adobe Audition for comparison to our behavioral task (described below). Each stimulus consisted of a train of six individual speech sounds such that across all four sequences, 24 possible sound pairs were presented once (/bad bad gad sad tad dad/, /tad tad sad gad bad dad/, /gad gad tad bad sad dad/, /sad sad bad tad gad dad/). The temporal envelope of the stimuli was compressed so that when presented with a 0 second inter-stimulus interval,

sounds were presented at 2, 4, 5, 6.667, 10 and 20 syllables per second (sps). All speech stimuli were randomly interleaved, and presented at 20 repeats per recording site. All sounds were presented approximately 10 cm from the left ear of the rat. Stimulus generation, data acquisition and spike sorting were performed with Tucker-Davis hardware (RP2.1 and RX5) and software (Brainware).

Surgical procedure- awake recordings

Rats were anesthetized and implanted with a chronic array of 16 polyimide-insulated 50 μm diameter tungsten micro wires. The implantation surgery and microwire arrays have been previously reported in detail (Rennaker et al. 2005). Briefly, subjects were anesthetized with an intramuscular injection of a mixture of ketamine, xylazine and acepromazine (50 mg/kg, 20 mg/kg, 5 mg/kg, respectively). Atropine and dexamethazone were administered subcutaneously prior to and following surgery. A midline incision was made, exposing the top of the skull, and a section of the right temporalis muscle was removed to access primary auditory cortex. Six bone screws were fixed to the dorsal surface of the skull (two in each parietal bone and one in each frontal bone) to provide structural support for the head cap. The two middle screws had attached leads to serve as a reference wire and a grounding wire. A craniotomy and durotomy were performed to expose the cortex in the region of primary auditory cortex. The microwire array was then inserted to a depth of 550-600 μm (layer IV/V) in primary auditory cortex using a custom built mechanical inserter (Rennaker et al. 2005). The area was sealed with a silicone elastomer (Kwik-Cast, World Precision Instruments Inc, Sarasota, Florida) and the head cap was built with a connector secured with acrylic. Finally, the skin around the implant was sutured in the front and the back of the head cap. Subjects were given prophylactic minocycline in water ad

libitum for 2 days prior and 5 days following surgery to lessen immune responses (Rennaker et al. 2005), and were also given Rimadyl tablets for 3 days after surgery to minimize discomfort. Topical antibiotic was applied to the incision to prevent infection. After a minimum of 5 days of recovery, neural activity was collected in a single 2.5 hour session and saved using custom MATLAB programming. The session included an abridged tuning curve (to assess each site's best frequency) and the same set of speech sequence stimuli presented to the anesthetized animals. All passive sound sets were created and run through custom MATLAB programming.

Neural analysis and classifier

To identify stimulus identity in real-time using neural activity, we modified a well established classifier (Engineer et al. 2008; Foffani and Moxon 2004; Perez et al. 2012; Ranasinghe et al. 2012; Schnupp et al. 2006; Shetake et al. 2011). The classifier searched neural activity (from a random selection of a subgroup of sites) for the onset of a sound by locating a pattern of activity. We trained the classifier to recognize nine patterns of activity, corresponding to each consonant sound in the English language, by providing the mean activity across 19 repeats of each stimulus. 150 random sites was enough to capture the range of characteristic frequencies sampled and show the unique pattern of activity evoked by each consonant. The classifier then analyzed a single repeat of neural activity and identified if a pattern was located, when it occurred, and which sound caused the pattern of activity. We calculated decision thresholds for each possible consonant sound which allowed the classifier to determine which consonant sound most likely caused the activity, and when that sound was likely presented. To calculate the thresholds, we compared the similarity between each average pattern of activity, or template, to the response of each single repeat (20 repeats x 20 speech sounds). To reduce the

influence of uncorrelated spontaneous activity, we smoothed the data using a Gaussian filter with a half width of approximately 15% of the total number of sites to smooth sites across bins with similar characteristic frequency. Euclidean distance was used to measure the similarity between the single trial and each template and is calculated by taking the square root of the sum of the squared differences between two patterns of neural activity. The threshold was set to maximize the sensitivity index for each sound to ensure the maximum number of correct responses while minimizing the number of false alarms (Figure 5.1).

Once all thresholds were calculated, we normalized the single trials and templates so that the comparison between spontaneous activity and any template equaled 0. A normalized metric of Euclidean distance values for each single trial were calculated so that the values centered on 0 and templates similar to the single trial returned positive values while templates less similar to the single trial returned negative values. This was done using the equation:

$$NM_c = -\left(\frac{ED_c - ED_{sp}}{(t_c/mt)} \right)$$

where c is the window currently being analyzed, ED_c is the raw Euclidean distance at that point, ED_{sp} is the Euclidean distance between this template and spontaneous activity, t_c is the threshold for this template and mt is the minimum threshold across all templates. Once this normalized metric (NM) was calculated for the entire single trial, the classifier then searched each single trial recording sweep and identified when a pattern of activity occurred (when a threshold was crossed) and which stimulus caused that pattern of activity. If more than one template crossed the threshold, the classifier guessed the template with the highest NM value, or the template that was

closest to the single trial being analyzed. Since the classifier runs in real time, the first crossing is counted as the guess, even if the threshold would have been met again later in the sequence. The classifier was run thirty times, with a different randomly sampled neural population for each run. The average percent correct for each compression level was calculated and plotted with behavior. The strength of the correlation was measured using the correlation coefficient.

Behavioral Paradigm

Sprague-Dawley albino rats were trained using either an established lever press paradigm for the isolated speech task (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012; Shetake et al. 2011) or an operant paradigm with an infrared nose poke (for the speech sequence task), developed by Dr Robert Rennaker (Sloan, Dodd, Rennaker II 2009). Each rat trained for two 1-hour sessions per day, 5 days per week. For the isolated speech task, the behavioral training procedures and data reported here were the same as was reported in Engineer et al. (2008). In brief, 6 rats were trained to press a lever when a target speech sound was presented and to withhold pressing when a distracter sound was presented (/d/ vs. /s/, /d/ vs. /t/, and /d/ vs. /b/ and /g/). Rats were trained for 2 weeks on the tasks in the order given and performance was assessed after training on each task to obtain the values reported in Engineer et al. (2008) and the current study.

For the speech sequence task, all animals were first trained to associate the infrared (IR) nose poke with the sugar pellet reward. Each time the rat broke the IR beam, the target speech sound (/dad/) was played and a 45 mg sugar pellet was dispensed. After each animal earned over 100 pellets, each rat was then trained to wait patiently in the nose poke and withdraw their nose from the nose poke after hearing the target. During training stages, d’ was used as a criterion for

advancing to the next stage (Green and Swets 1966). This stage lasted until the animal performed with a d' greater than 1.5 for 10 sessions**.** For these first two stages, the animal had a response window of 800 ms to withdraw their nose in response to the target.

Rats were then introduced to the four possible distracters by presenting a string of repeats of a single distracter prior to the presentation of the target. The inter-stimulus-interval (ISI) was 1 second and the response window was also reduced to 650 ms during these stages. Since the task involved random patterns of distracters, we trained the animals on a fixed pattern of distracters to introduce the concept of multiple distracters per trial. For each trial in this stage, two or three of the four CS- were randomly selected and alternated. In the final two training stages, a sequence for each trial was randomly generated using all four possible distracters and presented to ensure that the rat could not memorize the pattern or time their responses. In addition, the ISI was reduced to 0 seconds and the response window was reduced to 500 ms. Once rats performed with a d' > 1.5 for at least two sessions, they were introduced to each compression level. During this period of training, rats were presented with blocks of 20 trials each. Each trial contained a random hold time (the time before the onset of the target sound) between 2 and 7 seconds, with the sounds prior to the target consisting of randomly selected distracters (Figure 5.5). The presentation rate of each block was either 2 sps or one of the additional presentation rates. These blocks were presented in random order. 20% of trials were catch trials in which no target was presented to ensure the rats were listening for the target and not attempting to time the target location (Figure 5.5).

Animals were tested for a minimum of 10 sessions during which all six presentation rates were randomly interleaved. The animals were individually housed with free access to water and

were food deprived to no less than 85% body weight while experimenting. When not experimenting, they were given free access to water and food and housed on a reverse 12:12 light/dark schedule. The behavioral paradigm was written and executed via a custom-designed MATLAB (The Mathworks Inc, Natick, Massachusetts) program and run through a PC computer with an external real-time digital-to-analog processor (RP2.1; Tucker-Davis Technologies), which monitored the infrared nose poke and controlled the stimuli presentation and lights. Each of the 5 sounds was analyzed for response rate (number of responses/number of presentations *100). Target responses are referred to as hits and the summed response to all four distracters is referred to as false alarm rate. Overall performance is reported in terms of hits-false alarms per presentation rate. All protocols and recording procedures were approved by the University of Texas Institutional Animal Care and Use Committee.

RESULTS

Neural activity patterns predict stimulus identity in real time

Speech sounds evoke unique spatiotemporal response patterns in primary auditory cortex (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012; Shetake et al. 2011). Previous classifiers have been able to use these patterns to identify the evoking stimulus when the stimulus onset time was provided. We have developed a neural classifier that can identify a wide range of speech sounds from continuous activity recorded in the primary auditory cortex (A1) of anesthetized rats, without prior knowledge of the stimulus onset time. Previous classifiers were forced to guess (in a two-alternative paradigm), while our classifier did not need to guess if a decision criterion was not met. The classifier was trained to recognize nine different

English consonants. Decision thresholds were determined by calculating the Euclidean distance between the average response to each consonant and the single trial responses evoked by each sound (all using 2 ms temporal bins over an 80 ms analysis window). We then compared the Euclidean distances that occurred when the single trial matched the template (i.e. when the single trial and the template were both evoked by the same sound) to the Euclidean distance that occurred when the single trial and the template were evoked by different sounds. The threshold was set at the Euclidean distance value that optimized the sensitivity index for each sound. This value ensured the maximum number of correct responses while minimizing the number of false alarms (Figure 5.1). The classifier then searched single trials of neural activity for a location in which one of the decision thresholds was crossed. A normalized metric of Euclidean distance values for each single trial were calculated so that the values centered on 0 and templates similar to the single trial returned positive values while templates less similar to the single trial returned negative values. We then tested both the detection and discrimination abilities of this new classifier.

Evoked patterns of neural activity are identifiable when neurons with a variety of characteristic frequencies (CFs) are recorded. For example, if the stimulus onset time was unknown and only one recording site was available for analysis, it would be impossible to identify when a speech sound occurred compared to spontaneous activity. Small numbers of sites could identify if a sound occurred, but performed at chance when asked to identify the sound (60 site detection at 53.4 ±22.8% correct, discrimination performance at 31.8 ± 13.1% correct; Figure 5.2A). This inability to identify stimuli using 60 sites corresponds with the amount of bits encoded with this number of sites (0.8 bits of information) compared to almost 3 bits of

information in a group of 400 sites (Figure 5.2B). This increase in bits could be due to a larger frequency range included in larger site groups. If a large number of sites are used, synchronized activity across those sites could be used to identify when a sound occurred. For example, in response to the sound /dad/, sites with a characteristic frequency above ~7 kHz responded to the consonant /d/ first, while lower frequency sites fired only to the onset of the vowel (Figure 5.3A). To the sound /bad/, the opposite pattern occurred; low frequency sites fired first, followed by high frequency sites (Figure 5.3A, third panel). Some sounds evoked neurons in the same order, such as /d/ and /t/, which both evoked responses from high frequency neurons first, followed by low frequencies. We used these distinct patterns of activity to train our classifier to recognize the evoked response to each sound. Using large numbers of sites, this entire frequency range was represented and the classifier was able to perform the task well above chance level.

Our recordings were not acquired simultaneously (4 electrodes were inserted at a time) and the uncorrelated spontaneous activity seemed to significantly impair the classifier's performance. The synapses in the sensory cortex likely have ways of strengthening the weight of evoked responses and dampening the weight of spontaneous neural firing. To diminish the influence of uncorrelated spontaneous firing, we ran the data through a Gaussian filter to strengthen the influence of the evoked signal (Giraud et al. 2000; Langers, Backes, Dijk 2003; Langers, Backes, Van Dijk 2007). Since our classifier counts every spike equally, this technique of spectral smoothing allowed us to strengthen the evoked activity in a biologically plausible manner (Figure 5.3B; Bear, Cooper, Ebner 1987; Hao et al. 2009; Poirazi, Brannon, Mel 2003a; Poirazi, Brannon, Mel 2003b; Sengpiel and Kind 2002). While various amounts of spectral smoothing were adequate to dampen the spontaneous activity (10-20% of the total number of

sites), peak performance was obtained using data from 400 sites that had been smoothed with a Gaussian filter with a half width of approximately 15% of the total number of sites (data collected for Engineer et al. 2008). Even with a smaller group of 150 sites, this classifier was able to identify the onset of a speech sound as well as identify the stimulus with high levels of accuracy (64.7 ± 6.0%; Figure 5.2A). These results validate that our new classifier is able perform the task using neural activity without specific knowledge of the stimulus onset time. In addition, our results show that a Euclidean distance classifier can perform with high levels of accuracy without being forced to guess.

To test the temporal limits of this new classifier, we created four sequences of speech sounds (/bad bad gad sad tad dad/, /tad tad sad gad bad dad/, /gad gad tad bad sad dad/, /sad sad bad tad gad dad/; neural responses acquired from a different set of four rats). We applied the same spectral smoothing as was used for the classification of isolated speech sounds. Following smoothing, the classifier was able to guess the location of the target sound with an accuracy of 70.0 ± 0.5% using random groups of 150 sites. Figure 5.4 shows a single trial example of the classifier's performance at the sequence /bad-tad-gad-dad/. As described earlier, the Euclidean distance values are normalized to a scale from 0-1 where values close to 0 indicate that the template does not match the single trial and values equal to 1 indicate that the template matches the single trial activity. Note in this example that the values for more than one template are close to 1 (Figure 5.4). If multiple templates reach a value of 1, the classifier uses the template with the highest value as a tie-breaker. This method takes into account that some sounds are similar and may therefore account for some common errors made by humans in difficult listening environments.

It was possible that our classifier would perform differently using awake recordings, due to differences in spontaneous activity or attention effects (Steinmetz et al. 2000; Treue 2001). A different group of rats were implanted with a chronic array of 16 micro-electrodes. After recovery, we presented four speech sound sequences during a single passive recording session. Awake recordings had a higher spontaneous firing rate than anesthetized recordings (64.2 ±1.8 Hz compared to 23.4 ± 1 Hz in the anesthetized preparation, unpaired t-test; $p=1.2*10^{-21}$) but this did not change the effectiveness of the Gaussian filter. The classifier performed at an average of 45.0 ± 8.3 % using random groups of 100 sites and a Gaussian filter with a half width of 15% the total number of sites. This accuracy mimics what the anesthetized classifier was able to accomplish with this number of sites. The result that awake neural activity can perform the neural discrimination task with comparable accuracy to anesthetized recordings is similar to what we saw using our earlier classifier (Engineer et al. 2008). The new classifier described here was able to locate and identify speech sounds in isolation or in sequences and performed with surprising accuracy using both anesthetized and awake neural recordings.

Neural responses predict behavioral ability

Our new classifier performed significantly above chance at identifying speech sounds in real time, but may not have performed the task as well as rats could behaviorally. We used behavioral data published in our previous report (Engineer et al. 2008) for comparison with our new classifier. Six rats were to press a lever when a target speech sound was presented and to withhold pressing when a distracter sound was presented (/d/ vs. /s/, /d/ vs. /t/, and /d/ vs. /b/ and /g/). Using groups of 150 recording sites, the classifier performed with accuracy levels comparable to rats' behavioral performance (average classifier performance on these four tasks

was 89.9 ± 6.9% correct vs. 88.3 ± 2.4% correct by the rats; unpaired t-test, p=0.64). This result suggests that our new classifier performs at the same level of accuracy as the rats and may be applicable to a range of speech stimuli and new behavior tasks.

Since the stimulus onset time is not provided to the classifier, a potential advantage of the current classifier is the ability to identify a speech sound from within a sequence. We developed a novel behavioral paradigm to see if our real-time classifier could predict rats' ability to identify a target speech sound in a stream of speech distracters. Rats were trained to initiate trials using an infra-red nose poke, and to withdraw from the nose poke upon presentation of the target sound /dad/ and to withhold responding to four distracter sounds (Figure 5.5; /bad/, /gad/, /sad/, and /tad/). This task required a longer learning period than previous studies of speech sound discrimination. Our rats required 38.2 ± 1.7 days to reach performance of d'≥1.5 compared to 17.4 ± 2.3 days for isolated speech tasks (Engineer et al. 2008). After ~40 days of training, rats were able to consistently respond to the target (/dad/) and withhold responding to distracters (/sad/,/bad/,/gad/ or /tad/). The average percent correct (hits-false alarms) of rats was 69 ± 5.2%, which was the same as the average classifier performance using neural responses from untrained rats (70.0 ± 0.5%; Figure 5.6A). The new real time classifier we report here can identify stimuli with accuracy levels that are comparable to behavioral ability.

Since our awake recordings (classifier performance: 45.0 ± 8.3% correct) were acquired from trained rats (though they were not performing the task when recordings were acquired), we were able to evaluate if the classifier's ability directly related to the behavior in the same animals. We noticed that the classifier was less accurate on two of the four sequences (/gad gad tad bad sad dad/ and /sad sad bad tad gad dad/). To investigate whether or not these sequences

were also more challenging for the animal, we looked for trials in which these exact sequences were presented. Since the stimuli were presented randomly during the behavior task, these exact sequences were only played a few times. Comparing the average percent correct across all rats on these trials (sequence 1; n=20 trials, sequence 2; n=8 trials, sequence 3; n=9 trials, sequence 4; n=12 trials), the classifier performance was strongly correlated to behavioral ability (Figure 5.6C; R^2= 0.90, p=0.05). Sequences that the classifier was less accurate on were the same sequences that rats had trouble with behaviorally. Neural activity recorded from trained, awake rats predicted the relative difficulty of the same sequences in the behavioral task. This result suggests that our classifier may be able to predict performance in real time using neural recordings acquired from awake and behaving animals.

Behavioral performance and neural responses were robust up to 10 sps

Behavioral discrimination accuracy gradually decreased as the presentation rate increased using a speech vocoder. Performance remained well above chance (0%) up to 10 sps (2 sps: 69.2 ± 5.2%, 4 sps: 62.4 ± 8.7%, 5 sps: 56.5 ± 10.9%, 6.67 sps: 59.0 ± 12.7%, 10 sps: 46.1 ± 9.2%), though performance at this rate was significantly worse than performance at 2 sps (46.1 ± 9.2% vs. 69.2 ± 5.2%, 10 sps vs 2 sps respectively; paired t-test; p=0.007). Poor performance at 20 sps (6.1 ± 2.0% correct) was consistent with performance in humans at the same rate (Figure 5.6A; Ahissar et al. 2001; Ghitza and Greenberg 2009; Poldrack et al. 2001). At presentation rates faster than 2 sps, false alarm rates did not differ between distracters (two-way analysis of variance; F (5, 3) = 2.11; p=0.07). The classifier was able to perform the neural discrimination task as well as the rats were able to behaviorally at every speed tested (classifier performance at 2 sps: 70.0 ± 0.9%; p=0.93, 4 sps: 57.9 ± 1.1%; p=0.72, 5 sps: 72.8 ± 0.7%; p=0.37, 6.67 sps:

71.6 ± 0.7%; p=0.54, 10 sps: 75.9 ± 0.5%; p=0.15, 20 sps: 24.9 ± 0.9%; p=0.17; unpaired t-tests; Figure 5.6B). The classifier performance was robust up until 20 sps, and then performed significantly worse than at 2 sps (Figure 5.6B). Similarly, neural activity patterns were strong and distinguishable at rates up to 10 sps (Figure 5.7). The significant reduction in neural firing strength at 20 sps as well as the impaired performance of the animals and the classifier at this speech suggests that as long as neural response patterns are unique and are distinguishable from spontaneous firing, A1 activity can predict behavioral ability of rats to identify a target sound in a rapid sequence.

DISCUSSION

Calculation of decision thresholds

In our study, we designed a real time classifier that sweeps neural activity for a pattern of activity evoked by a speech sound and decides which sound caused that activity using predetermined decision thresholds. Our classifier used neural activity recorded in primary auditory cortex and supports the idea that the information needed to perform speech sound identification is located in A1 (Engineer et al. 2008; Perez et al. 2012; Ranasinghe et al. 2012; Shetake et al. 2011). However, we found no evidence of a target specific response in A1. Previous studies showed that removing A1 does not impair the ability of animals to perform speech sound discrimination tasks or to learn new speech sound targets (Floody et al. 2010; Porter et al. 2011). In order to predict behavioral accuracy, we needed to transform the data by highlighting simultaneously occurring evoked activity. We smoothed across the spectral dimension, to mimic a biologically plausible way in which the brain weights excitatory synaptic

inputs (Bear, Cooper, Ebner 1987; Poirazi, Brannon, Mel 2003a; Poirazi, Brannon, Mel 2003b). The need for such smoothing suggests that the ability to use A1 activity to identify a speech stimulus requires integration across synapses. It is therefore unlikely that these thresholds are calculated or even exist in this core auditory region.

Brain regions even one synapse higher than the core sensory area may code the relationship between two stimuli. For example, when monkeys were asked to identify whether two tactile stimuli were the same or different, primary somatosensory cortex encoded only the current stimulus, while secondary somatosensory cortex was already beginning to compare the two stimuli (Romo and Salinas 2003). It is likely that higher level brain regions contain integrator neurons that recognize patterns of activity occurring in lower level areas. Neural networks designed to mimic sensory neurons can be trained to integrate basic sensory information into categorical decisions (Buonomano and Merzenich 1995; Mazurek et al. 2003). Single neurons recorded in premotor cortex of monkeys can also predict the intended motor sequence when a maximum-likelihood decoder analyzes the firing rate (Shanechi et al. 2012). Our classifier does not propose a mechanism for how this threshold is created or where in the brain it is stored, but it is the first to show that a real time classifier can predict the location and identity of speech stimuli without being forced to choose between a set list of options. As in behavioral tasks, if the decision threshold is not met, the classifier is not required to guess. In addition, if multiple thresholds are met, our classifier is designed to choose the template which is most like the single trial.

In the current study, we used Euclidean distance to determine the location of maximum sensitivity; the point at which most hits would be captured while minimizing false alarms. Once

calculated, these thresholds did not change as a result of testing, and our classifier used these thresholds to determine which stimulus evoked a single pattern of neural activity. It is unlikely that the brain does not adapt to real-time feedback during testing. If thresholds never changed, the brain would be inept at tasks of generalization. For example, the same word spoken with small changes in pitch, pronunciation and/or context may cause the brain to categorize these as two different words. It is well known that synapses change as a result of real-time feedback (Buonomano and Merzenich 1998; Cohen-Cory 2002; Malenka and Nicoll 1993; Malinow and Malenka 2002), but the question of how the brain monitors these changes and how drastic the adjustments are remains to be answered. A classifier that could adjust its thresholds in relation to real time feedback would provide a more biologically accurate model and may be able to explain models of learning impairments.

Evaluation of the data set and classifier

The data reported in our study was acquired from many animals and analyzed post hoc. In the anesthetized recordings, four electrodes were recorded simultaneously. In the awake preparation, up to seven electrodes were viable at any given time point. Single neuron recordings in premotor cortex can predict motor sequences in real time using spike rate information (Shanechi et al. 2012). Since consonant identification requires precise spike timing information, we were only able to achieve above chance performance with our classifier using more than 60 sites. The development of multi-channel electrode arrays with at least this many channels will allow us to record enough neurons simultaneously to locate and identify an evoking speech stimulus in an awake-behaving animal.

The classifier uses a fixed window (80 ms) to scan a single trial of neural activity for evoked responses. There is sufficient information present in this window for consonant identification to take place (Engineer et al. 2008; Kuhl and Miller 1975; Miller and Nicely 1955). However, it is likely that rats and humans also use information occurring in larger integration windows, especially in difficult hearing environments (Shetake et al. 2011). Our classifier attempts to account for this by analyzing the normalized metric values within 5 ms of the initial guess. This allows the classifier some flexibility to wait until all similar templates are considered and then make a decision using the strongest signal. This time period of flexibility is biologically plausible as it is well within the minimum amount of time in which the brain can make a decision (Stanford et al. 2010).

Future applications for the classifier

In the current study, we demonstrate that a classifier can locate and identify speech sound stimuli in real time using single repeats of A1 neural activity. Real time classifiers have been developed in the motor system to identify and read out sequences of intended motor movements (Shanechi et al. 2012). Such work will be invaluable for the development of prosthetic limbs that carry out intended movements. Neural activity in other sensory systems, such as vision, has also been used in successful classifiers to identify objects presented to human and non-human primate participants. When participants are shown or asked to imagine varying images, the pattern of active voxels acquired by fMRI differs between categories of objects (Haxby et al. 2001; Norman et al. 2006; Stokes et al. 2009). Multiple voxel pattern analysis (MVPA) can be used to identify lines of varying orientation, the direction of movement of an image, or whether the visual object is a picture or a sentence. In a task where two competing images are presented,

MVPA can also predict when one image is perceived over the other, and which image is dominant on a second-to-second timescale (Haynes and Rees 2005; Polonsky et al. 2000). In addition, the use of pattern classification for olfactory information has been successfully integrated into electronic devices that identify odors by mimicking the human olfactory bulbs (Gutierrez-Osuna 2002). Such devices also contain feedback loops for optimization as well as several internal controls to account for differences in sensor settings and other sources of noise. The ability to integrate such controls into a speech sound classifier will greatly improve the performance of the classifier on tasks involving generalization or distortion.

Conclusion

We developed a classifier that can locate and identify a speech stimulus using single trial neural responses. Unlike previous models, the current classifier is not forced to make a guess if the decision criterion is not met and is a good computational model for a possible mechanism of speech sound processing. The current study showed that such a classifier can predict rats' speech sound discrimination ability in a previously described task as well as a novel task in which rats are trained to locate a target sound in a stream of speech sounds. These results indicate that the rat is a good animal model of human speech sound processing and will be valuable in evaluating the neural mechanisms responsible for many human speech sound processing disorders. Neural activity recorded from the awake and anesthetized rat can predict behavioral ability on a variety of tasks using single repeats in real-time.

Acknowledgments

We would like to thank N. Lengnick, H. Shepard, N. Moreno, R. Cheung, C. Im, S. Mahioddin, C. Rohloff and A. Malik for their help with behavioral training, as well as A. Reed, D. Gunter, C. Mains, M. Borland, E. Hancik and Z. Abdulali for help in acquiring neural recordings. We would also like to thank K. Ranasinghe and C.T. Engineer for their suggestions on earlier versions of this manuscript. This work was supported by the National Institute for Deafness and Communication Disorders at the National Institutes of Health (R01DC010433).

APPENDIX

CHAPTER 5 FIGURES

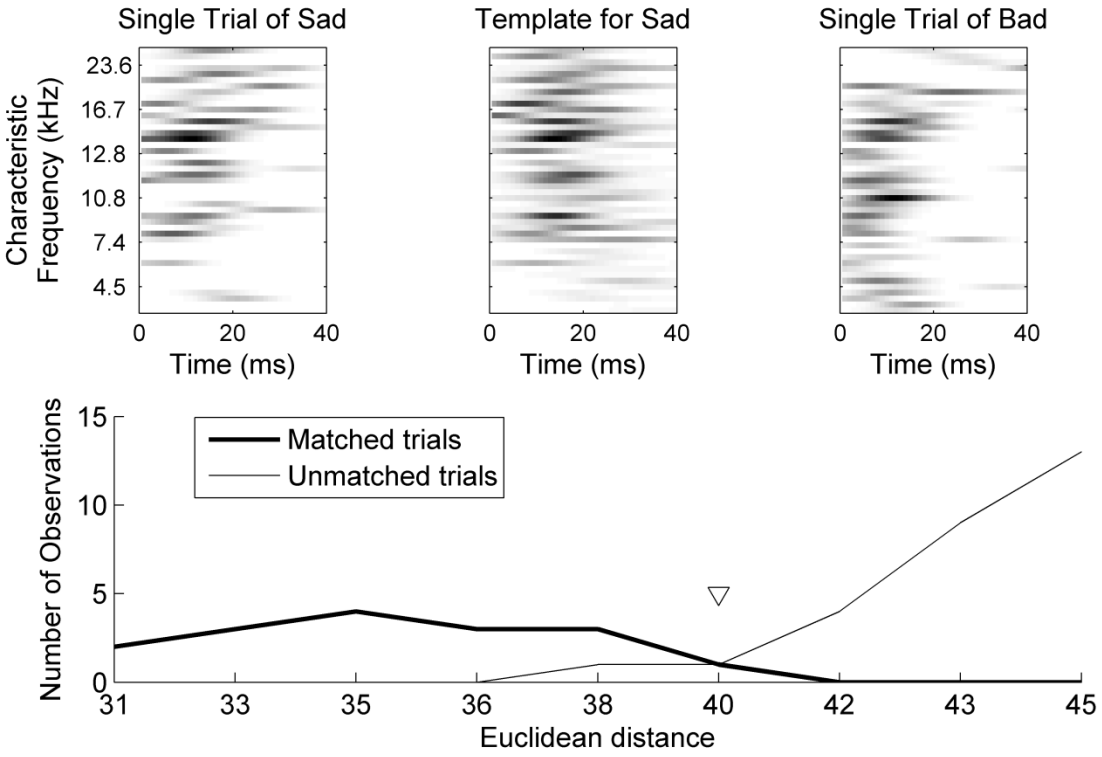

Figure 5.1. Explanation of decision threshold calculation. Decision thresholds were calculated by comparing single trial neural responses to the average evoked response to each consonant sound. For example, to create the decision threshold for the sound /sad/, the average response to this sound (over 19 repeats) was compared to all single trial responses to every sound. The similarity of the single trials to the template was calculated using Euclidean distance. We then plotted the Euclidean distance values generated when the single trials were evoked by the template sound (e.g. when template and single trial were both evoked by /sad/) versus the Euclidean distance values when the template did not match the single trial (e.g. when the template was evoked by /bad/ while the template was evoked by /sad/). The decision threshold was then set at the intersection point between these two distributions, as marked by a * in the bottom half of the figure. This maximized the sensitivity index so that the most correct answers were preserved while excluding the maximum number of false alarms.

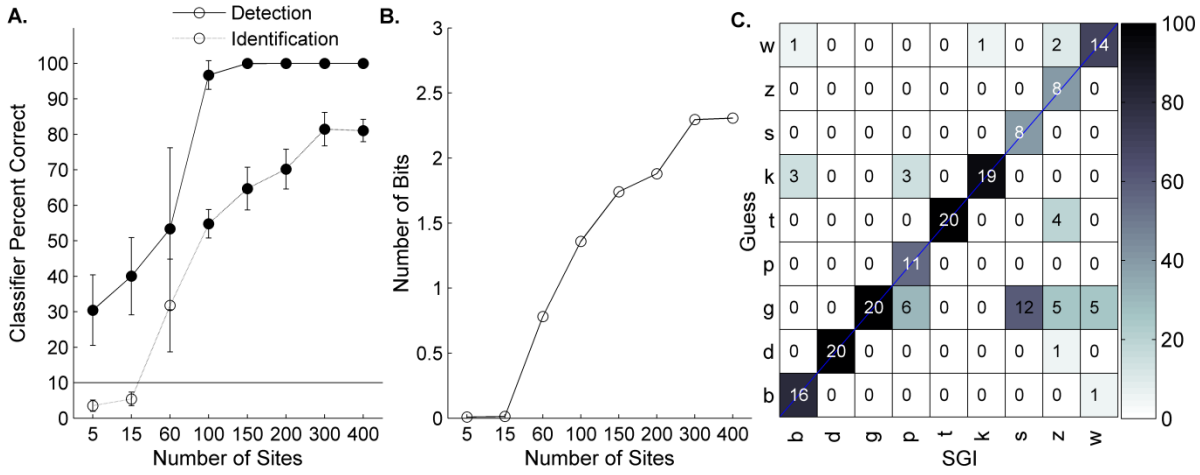

Figure 5.2. A real time classifier could locate and identify nine consonant speech sounds with high levels of accuracy. **A.** The real time classifier was able to locate the onset of a speech stimulus with high levels of accuracy, but required a larger number of sites to accurately identify the speech sound. This is likely due to the limited frequency range included in small groups of sites. Previous classifiers provided the stimulus onset time and were able to achieve high levels of accuracy using single sites of neural activity. Points marked by black circles represent data sets that performed significantly above chance level (8% chance for detection, 10% chance for identification). **B.** Number of bits encoded in various subgroups of sites. 60 sites were able to locate the sound onset, but could not identify the sound, as this number of sites contained less than 0.8 bits of information. Larger groups of sites contained up to 3 bits of information and were better able to perform the task. **C.** Confusion matrix of classifier performance on nine English consonant sounds. The classifier performed the task with high levels of accuracy at every sound presented.

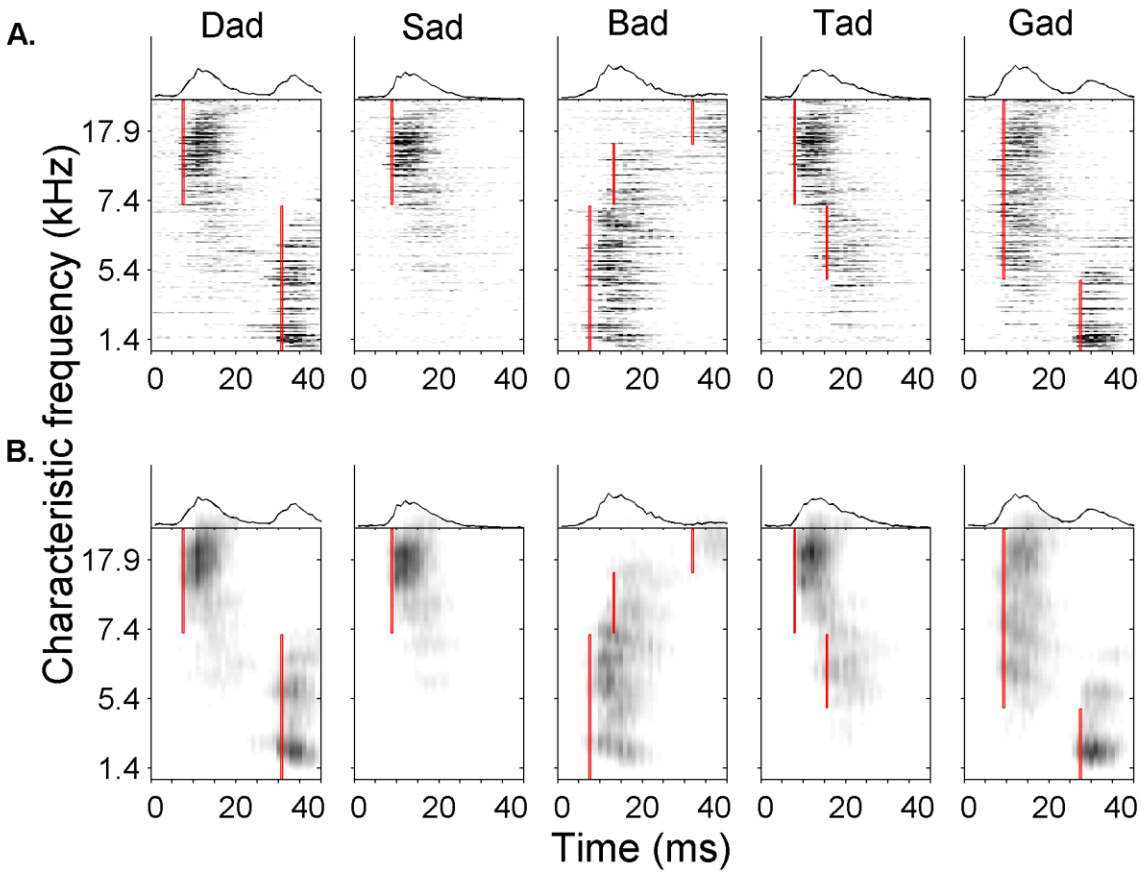

Figure 5.3. A Gaussian filter was necessary for highlighting evoked activity. **A.** Neural activity patterns in A1 without any smoothing. The first 40 ms of average evoked activity from each site is organized by characteristic frequency. Each consonant evoked a unique pattern of activity such that each group of neurons fire at a different latency depending on the characteristic frequency of the group. Red lines mark the onset response of each frequency group. **B.** The same neural activity plotted in A. after a Gaussian filter has been applied to the spectral dimension. We used a filter with a half width of 15% of the total number of sites. This ensured that spontaneous activity is not as influential on the classifier as evoked activity.

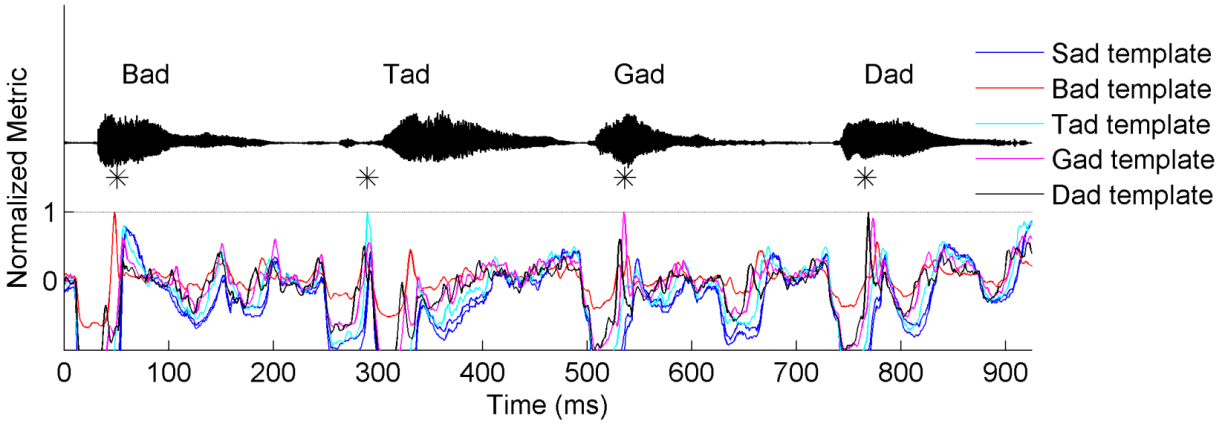

Figure 5.4. The classifier was able to locate and identify multiple speech sounds in a sequence. A single trial example of the classifier's performance on a speech sequence. The classifier analyzed a single trial neural response to the sequence 'bad tad gad dad' by comparing the response to each of five templates. The Euclidean distance responses were converted to the normalized metric (NM) to that responses were between 0 and 1 (see Methods). If the NM value reached 1, the classifier signaled that a speech sound occurred at that time point and guesses that the corresponding template sound was presented. Each colored line represents the values yielded from comparison with each template, and *'s represent the location of a guess. Note that the template /sad/ never registered a guess during this sequence, signaling that the classifier correctly registered that the sound /sad/ was never presented.

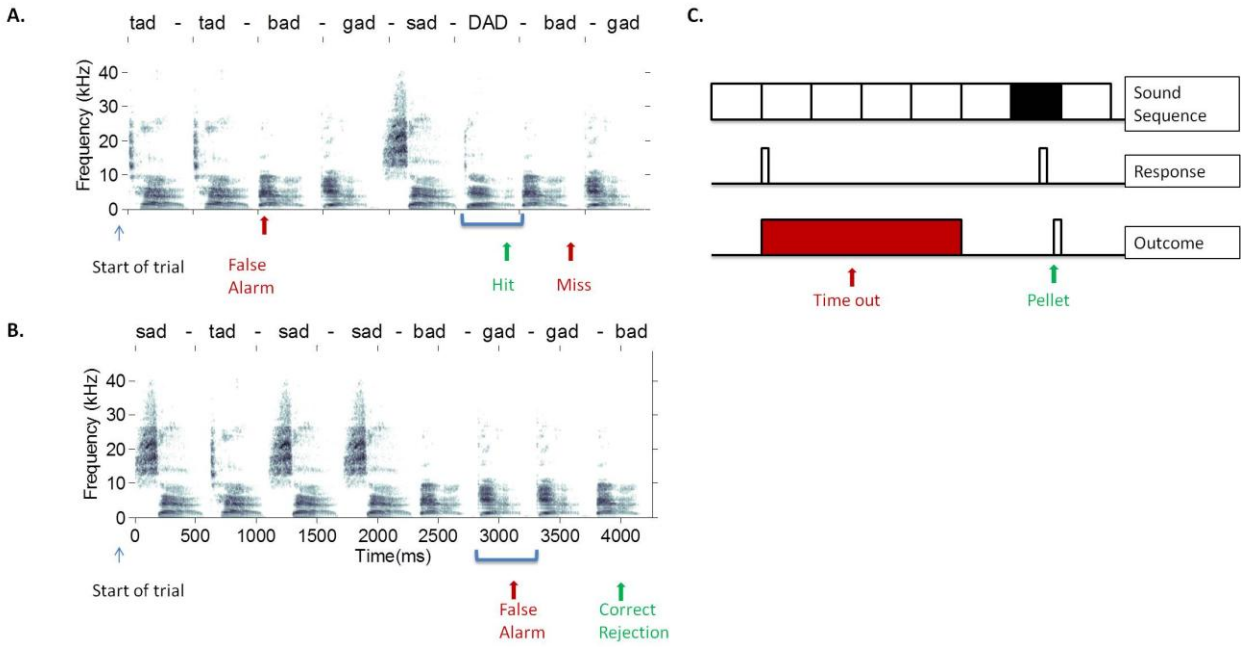

Figure 5.5. Schematic of the behavioral task. Speech sounds are presented in random order beginning when a rat breaks the infra-red (IR) beam. Target sound (/dad/) was presented in a single random location anywhere from the third sound of the sequence until the end of the 2-7 second trial. **A.** Example of a possible target trial. From the onset of the target sound, rats had 500 ms to respond by withdrawing from the IR beam. If the target sound was less than 500 ms long, additional distracters were added afterwards to avoid the use of silence as a cue. Correct responses to the target were rewarded with a 45 mg sugar pellet. Incorrect responses to distracter sounds or missed responses to the target were punished by a 5 second timeout in which the booth lights were extinguished and the IR beam was disabled. **B.** Example of a possible catch trial. Twenty percent of trials were catch trials in which no target was presented. A false alarm to any of the stimuli triggered a time-out period. Correct rejections were un-rewarded and the next trial began after a brief delay. **C.** Simplified schematic of the behavior task. Top row shows a sound sequence, each box represents a speech sound. The colored box represents a possible target location. The middle row represents possible responses by the rat. The bottom row represents the outcomes of each response; a premature response yielded a time out in which the cage lights were extinguished and the program was paused. A correct response triggered a sugar pellet reward.

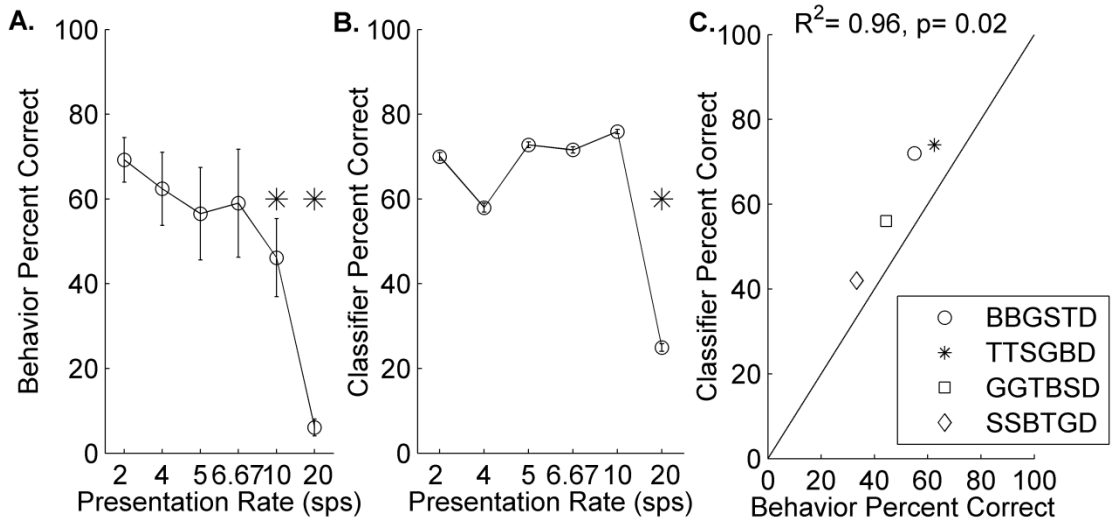

Figure 5.6. Average performance of rats and the classifier on the speech sequence task. **A.** Average behavioral performance by rats was measured by hits-false alarms for each of six presentation rates tested. Performance was plotted across a minimum of 10 sessions per rat of the testing stage in which all presentation rates were randomly interleaved in blocks of 20 trials per block (see Methods). Performance was robust until 10 and 20 sps (compared to performance at 2 sps; *p<.01). The task was almost impossible for rats when sounds were presented at 20 sps (** p<.001 as compared to 2 sps). **B.** Average classifier performance at each of the six presentation rates. Performance was calculated by counting the number of correct responses per sequence over 20 repeats of each sequence. This process was repeated 30 times with random groups of sites and average performance across the 30 runs is plotted. The classifier performed with accuracy levels that did not differ from rats' behavioral ability (unpaired t-tests, p=0.93, p=0.72, p=0.37, p=0.54, p=0.15, and p=0.17 at each presentation rate, respectively). **C.** Awake neural activity was able to predict the order of difficulty for rats to perform the sequence task for four specific speech sound sequences (R^2=0.90, p=0.05). Both rats and the classifier performed best on the sequence /bad bad gad sad tad dad/ and worst on the sequence /sad sad bad tad gad dad/.

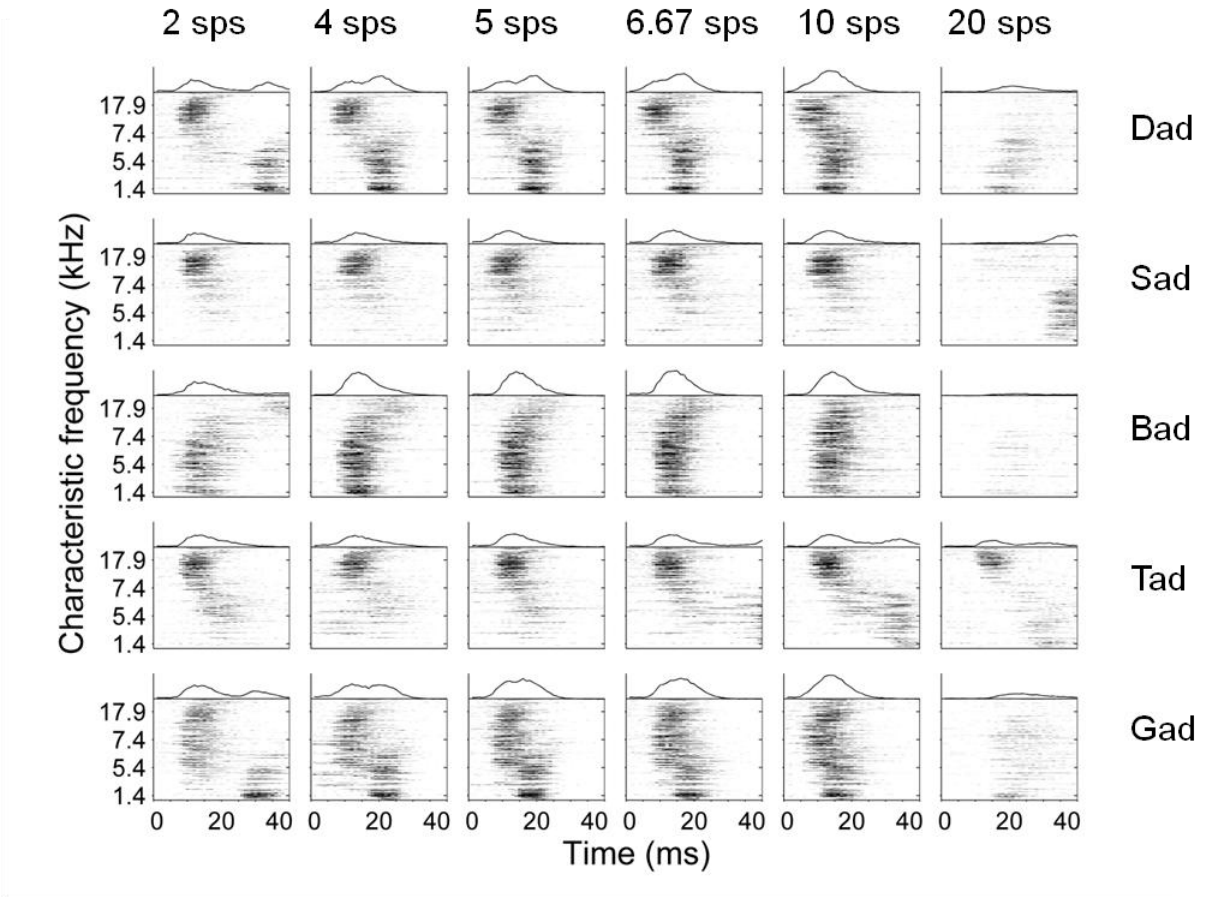

Figure 5.7. Cortical speech-evoked activity patterns were robust up to 10 sps. Neural responses were averaged for each site and plotted organized by characteristic frequency. Each consonant speech sound (by row) evoked a unique pattern of activity at 2 sps (first column). The response of these patterns was robust through the 10 sps presentation rate. At 20 sps, responses were visibly weaker and were less distinct that at the previous presentation rates. This drastic change in neural responses may be the reason that both behavior and classifier performance fall at this speed.

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CHAPTER 6

CONCLUSION

The ability of the auditory cortex to encode natural sounds, specifically speech sounds, is a critical component to a high quality of life of all humans. Many disorders, such as dyslexia and autism, cause abnormal speech sound processing and can lead to poor school performance, low self esteem, and delinquency in adult life. Dyslexia is known to be caused by genetic variants, though the specific genes and severity of the impairment varies widely across individuals. In this dissertation, we provide the first evidence that *in utero* suppression of the dyslexia candidate gene *Kiaa0319* causes a significant increase in the trial-by-trial variability of the neural response to auditory stimuli and that these impairments can be ameliorated with extensive behavioral training.

The role of each associated gene on causing the neural and behavioral symptoms of dyslexia has only recently been explored. Humans with variants in *KIAA0319* have reduced activation in left auditory cortex in response to auditory stimuli, and this corresponds to a significant behavioral impairment (Pinel et al. 2012). Our results show that the reduced activation in human EEG studies is likely due to the increased trial-by-trial variability in neural responses. We show in Chapters 2 and 4 that at least 2 auditory fields (primary and posterior auditory cortices) have neural firing impairments and these deficits improve following training. Variants in another candidate dyslexia gene, *DCDC2,* are relatively common in humans and have been studied in rats as well (Lind et al. 2010; Scerri et al. 2011; Schumacher et al. 2006).

Humans with variants in this gene are known to have reading problems, but whether this gene also causes auditory processing impairments is unknown (Lind et al. 2010; Scerri et al. 2011). *Dcdc2* knockout mice are impaired at rapid auditory processing and spatial maze tasks (Truong 2009; Wang et al. 2011). The role of this gene in causing the behavioral speech processing impairments seen in the KIA- rats is unknown. *DYX1C1* is a candidate-dyslexia gene whose role has been debated in the literature. It seems to cause visual and spatial impairments in rat models (Marino et al. 2007; Threlkeld et al. 2007), but its exact role in auditory processing is still unknown. In addition, some researchers deny that this gene is a risk factor for dyslexia at all (Marino et al. 2005; Scerri et al. 2004). Humans with *ROBO1* variants have phonemic processing problems, but these mutations are rare and have never been studied in an animal model (Bates et al. 2011). Dyslexia is a complex disorder with a variety of symptoms that seem to affect individuals differently. The variability in symptoms is likely due to the differences in genetic variants across individuals. Some genes may be responsible for the visual and spatial symptoms commonly seen in this disorder, while other genes seem to cause the auditory temporal processing deficits. All of these symptoms together likely cause the impaired reading scores which are the hallmark diagnostic criterion of this disorder.

The auditory phoneme processing impairments discussed in Chapters 2 and 4 of this dissertation may be caused by the high trial-by-trial variability we saw in the onset latency of neural responses. Extensive training significantly reduced this variability, and returned neural firing precision of rats with *in utero* RNAi of *Kiaa0319* to control levels. Future experiments are needed to determine whether this reduced variability was the driving mechanism behind the improved behavioral performance or whether this is a non-functional consequence of the

training. In Chapter 3, we described normal neural firing patterns in multiple auditory areas and suggested that non-primary areas may be functioning not only as a stimulus encoder, but also areas like the posterior auditory area (PAF) may be the first auditory area in which integration may be taking place. In primary, anterior, and ventral auditory fields, consonant sounds are more accurately encoded when spike timing information is preserved, while PAF can encode such stimuli with or without spike timing information (Chapter 3). The classifier performance was correlated to behavior using activity from each of the four auditory fields. It is possible that the comparable classifier performance serves as a way to help the brain process sound in adverse listening conditions. For example, in noisy conditions, speech-evoked responses in primary auditory cortex are significantly degraded (Shetake et al. 2011). Other auditory fields may not be similarly affected by background noise, allowing the brain access to necessary components of the speech signal (Figure 6.1). Additional studies are needed to address the speech-evoked responses in other auditory areas and inactivation studies are needed to address the hypothesis that the multiple auditory fields function cooperatively in adverse listening conditions.

People with other communication disorders, such as autism, have a greater impairment in non-primary auditory areas. Neural recordings in our KIA- rats did not provide evidence of this in the two fields we investigated (A1 and PAF), but future studies are needed to evaluate other auditory areas as well as other brain regions. We hypothesize that variants in the dyslexia-associated gene *KIAA0319* will cause high trial-by-trial neural variability in other auditory areas, as well as other brain regions, such as visual and somatosensory cortices. This increased variability in other sensory systems may contribute to other dyslexia symptoms, such as impaired vision and spatial awareness. Additional studies are needed to evaluate whether other sensory

systems are similarly affected by suppression of the gene *KIAA0319.* Studies on visual acuity would also be beneficial for understanding whether *KIAA0319* also causes impairment in this system. Genetic knockout rat models are beginning to be widely available and will provide an ideal system for answering some of these questions. Additional studies are also needed to evaluate the contribution of the other candidate dyslexia genes described above. It is rare for a human with dyslexia to acquire the disorder due to a variant in only one of the dyslexia-associated genes (or one of a number of other currently undocumented dyslexia-associated genes). Rat models will allow researchers to evaluate the consequence of *in utero* RNAi of combinations of genes on neural function in multiple brain areas and the behavioral consequence of such interference.

The real time neural classifier described in Chapter 5 of this dissertation can use primary auditory cortex activity to locate and identify a speech sound stimulus with levels of accuracy comparable to the behavioral ability of rats. This classifier is likely able to perform the same task using neural activity from other sensory systems. Such a classifier would be valuable in evaluating other sensory system processing in not only dyslexia, but in disorders that can be more difficult to study, such as autism or schizophrenia.

APPENDIX

CHAPTER 6 FIGURES

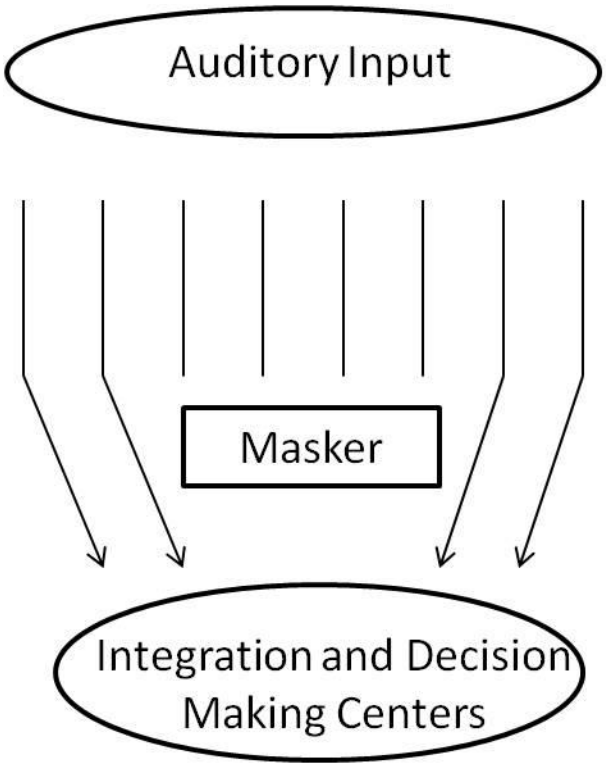

Figure 6.1 Schematic of the possible role of multiple auditory fields for robust speech perception in adverse listening conditions. The auditory pathway consists of many parallel connections, depicted by the series of vertical lines. Since speech perception is robust in many adverse listening conditions, I hypothesize that these parallel connections may facilitate this process. If a particular masker, for example, a certain type of background noise, interferes with the transmission of information in a few of these pathways, the redundancy in other pathways may allow for the transmission of necessary information to integration and decision centers of the brain.

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VITA

Tracy Michelle Centanni was born in Oakland, California to Daniel and Beverly Rosen. Her younger sister, Hannah Rosen, is currently a doctoral student in the Biology Department at Stanford's Hopkins Marine Station. Tracy grew up in Pocono Pines, Pennsylvania and was a member of the championship Pocono Mountain High School marching band for four years. In addition to her musical interests, Tracy began horseback riding and showing in 4-H at the age of eight, and earned 3rd place in the Pennsylvania State Horse Show with a young horse she helped train. Tracy has been an avid musician and horse woman for the majority of her life and hopes to continue both of these pursuits in her personal life outside the lab.

She received her Bachelor's of Science degree in Psychology from The Pennsylvania State University in 2008. While attending Penn State, she was a member of the marching Blue Band for four years. In addition, Tracy marched in the Reading Buccaneers Drum and Bugle Corps of Reading, PA during their first undefeated season in 2005, and marched in the Santa Clara Vanguard Drum and Bugle Corps of Santa Clara, CA for the 2006, 2007 and 2008 seasons. She received two awards for high visual and one award for high musicianship during her time at Santa Clara. While at Penn State, she also volunteered as a research assistant in the Vision, Memory and Cognitive Neuroscience lab headed by Dr. Michael Wenger. During her two and a half years in the lab, Tracy assisted in the design and data acquisition of a study by Dr. Andrea Halpern at Bucknell University investigating the neural response of musicians and non-musicians to major and minor scales. Following her Bachelor's degree, Tracy worked for four

months as a technical writer for Sanofi Pasteur, where she met her husband, Matthew Centanni. They married in September of 2012.

She completed her Master's of Science in Applied Cognition and Neuroscience at The University of Texas at Dallas in 2011 and her Doctorate of Philosophy in Cognition and Neuroscience in 2013. Following completion of her doctoral degree, Tracy plans to move to Boston, Massachusetts to pursue a career in research and live with her husband, Matt and their English Bulldog, Chomper.