INTRACORTICAL MICROSTIMULATION FOR SENSORY INPUTS IN BRAIN-MACHINE INTERFACES

by

Kevin John Otto

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May 2003

APPROVED:

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______________________________, Co-Chair

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Supervisory Committee

ACCEPTED:

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Department Chair

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Dean, Graduate College

ABSTRACT

Disorders of the human nervous system typically result in disabilities and ailments that significantly lower the quality of life for the afflicted individuals. Neural prostheses have emerged in an effort to alleviate some or all of these ailments. One branch of neural prosthetic research aims to restore sensory function due to congenital defects, disease, or injury. Specifically related to this project, cortical stimulation of the primary auditory cortex has been postulated to be a potential mode of operation for an auditory prosthesis. Several historical studies have shown that auditory cortex surface stimulation is inadequate due to the dangerously high threshold currents needed and poor stimulation specificity. Recent advances in neural interface technology, combined with pilot studies conducted in the visual cortex of primates and humans, validate investigations of intracortical microstimulation in auditory cortex as a mode of operation for an auditory prosthesis.

The specific aims of this research were to develop and validate an animal model for an intracortical auditory prosthesis and to evaluate the capacity of this technique to provide multi-channel information transfer through a chronically implanted brain-machine interface. First, a rat-based animal model was developed utilizing a chronic, penetrating microelectrode array. This animal model was successfully validated via behavioral measurements of the ability of the subject to detect single-channel electrical microstimulation. Second, in an effort to further elucidate the sensory parameters of the microstimulation, behavioral results were evaluated in an auditory frequency discrimination task. The results suggest that parameters of the sensation elicited by microstimulation are related to electrophysiological properties of the tissue proximal to

the implanted electrode. Lastly, the behavioral salience of the microstimulation-induced sensation was evaluated relative to natural, auditory-induced sensations. In both detection- and discrimination-based behavioral settings, microstimulation cues resulted in better performance of the task. Further, microstimulation as closely spaced as 250 microns produced significantly different behavior. These results are consistent with recent reports that sensory intracortical microstimulation provides robust, salient cues in a behavioral setting. Further, these results have implications for the feasibility and development of a cortical sensory prosthesis.

DEDICATION

To my parents, who have always led first by example and then by love. To my family and friends, who have understood and maintained regardless. To Elizabeth, for her support, patience, and love.

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CHAPTER 1

INTRODUCTION

Malfunction of the human nervous system can result in disabilities and ailments that significantly lower the quality of life for the afflicted individuals. Diseases affecting the nervous system such as Parkinson's disease, Alzheimer's disease, or Amyotrophic Lateral Sclerosis (Lou Gehrig's disease) are among the most feared and debilitating diseases. Injury to the brain or spinal cord can leave patients paralyzed or result in seizure causing tissue damage. Some neural based congenital defects or autoimmune diseases result in blindness or deafness. The focus of neural engineering is to provide engineering solutions, designed for and tested in biological environments, in order to help people suffering with these and other neurological ailments.

The most successful developments in the field of neural engineering are arguably related to the invention and implementation of neural prostheses. A neural prosthesis can be defined as an artificial device used to replace a missing or damaged part of the nervous system. Recent neural prosthetic development has been fueled by advancements in microelectronics, microcomputing, and materials research among others. Many gaps exist in the orchestration of these disciplines from an initial need assessment through development and implementation. Bioengineers trained in neural engineering principles and techniques offer a unique blend of education and experience to be successful in this endeavor.

One branch of neural prosthetic research aims to restore sensory deprivation due to congenital defects, disease, or injury. Specifically, cortical stimulation of primary auditory cortex has been postulated to be a potential mode of operation for an auditory prosthesis. Several historical studies have shown that surface stimulation is inadequate due to the dangerously high threshold currents needed and poor stimulation specificity. Recent advances in neural interface technology, combined with pilot studies conducted in the visual cortex of primates and humans, validate investigations of penetrating auditory cortex microstimulation as an information transfer mechanism. An effective, high capacity auditory cortical prosthesis could theoretically treat all forms of deafness that are currently known. Additionally, this high capacity information channel to the brain is potentially the first step in “science-fiction” type communication mechanisms and brain-machine interfaces.

The specific aims of the research presented in this document were to investigate the behavioral effects of penetrating auditory cortical microstimulation in an animal model, and to evaluate the capacity of this technique to provide multi-channel information transfer through a chronically implanted brain-machine interface.

BACKGROUND

The basis for a cortical stimulation-based sensory neuroprosthesis derives from neurophysiological mapping studies of the functional architecture of the human cortex. In order to identify indispensable regions of patient’s brains suffering from clinical epilepsy, Wilder Penfield made careful observations of behavior induced by surface electrical stimulation of human cortex (Penfield and Rasmussen 1950). His work showed that surface stimulation of primary sensory areas evoked sensations of varying modes and characteristics. Stimulation in the anterior parietal lobe, specifically in the postcentral gyrus, evoked somatosensory responses. The mapping of the somatosensory sensation was dependent on the medial-lateral position within the postcentral gyrus, and Penfield and Rasmussen reported this map as the sensory homunculus. Stimulation at the pole of the occipital lobe produced visual sensations termed “phosphenes”. The phosphenes were generally in the field of vision on the contralateral side of the stimulation, and were elementary in nature, e.g. made up of lights, shadows, and colors. These findings supported earlier studies stimulating the occipital pole conducted by Foerster (Foerster 1929) and Krause and Schum (Krause and Schum 1931). Most relevant to the work of this dissertation, Penfield found that stimulation in the Sylvian margin of the temporal lobe produced auditory sensations, later termed “audenes” by Dobelle et. al (Dobelle, Stensaas et al. 1973). These audenes were perceived by the subjects as sounding like crickets, ringing, buzzing, etc. Similar to the visual cortical stimulation findings, the sounds were of an elementary nature.

The first implication that these sensations could be the basis of a sensory neural prostheses was reported by Brindley and Lewin (Brindley and Lewin 1968). Brindley aspired to produce a visual cortical prosthesis to remedy blindness. Using relatively large (~0.64 mm^2) platinum electrodes, he examined phosphene production as a result of cortical surface stimulation in two patients (Brindley and Lewin 1968; Brindley, Donaldson et al. 1972). Stimulation in the milliampere range was required to evoke phosphene sensations. Further, overlapping sensations with highly non-linear interactions were produced by simultaneous stimulation of electrodes spaced on 2.4 mm centers. These findings were confirmed in a study by Dobelle and Mladejovsky (Dobelle and Mladejovsky 1974), who reported data from sixteen subjects. The Dobelle and Mladejovsky study reported milliamp range thresholds and a 3 mm resolution with “a variety of complex interactions” from simultaneously excited electrodes. In a separate study, Dobelle et. al reported nearly identical parameters for surface auditory cortical stimulation in a study of eight patients (Dobelle, Stensaas et al. 1973). These results were later confirmed by Howard et. al (Howard, Volkov et al. 2000). For a permanent, multi-channel neuroprosthesis, these stimulus parameters are limiting and dangerous. For independent stimulating channels in a surface stimulation paradigm, the limiting spatial resolution is electrodes separated by 4 mm (Ronner, Foote et al. 1980). This electrode configuration significantly limits the number of channels available for a multi-channel auditory cortical surface prosthesis. Additionally, due to the relatively high thresholds, the power consumption requirements of a permanently implanted, multi-channel auditory cortical surface prosthesis were determined to be a limiting factor.

At this point, research focus turned to investigation of more effective devices for a sensory neuroprosthesis. Several investigators had already begun developing a chronic intracortical device for recording and stimulation of neural tissue. The basic device for many preparations involved one or more insulated microwires (Marg and Adams 1967; Olds, Disterhoft et al. 1972; Burns, Stean et al. 1973). The suggestion of chronic implantation of silicon substrate photoengraved microelectrodes was also introduced (Wise, Angell et al. 1970).

Confident in the fidelity of these developing devices, researchers began to address the potential of these penetrating devices in a chronic, biological setting. In 1980 Bartlett and Doty demonstrated that a macaque monkey could detect microstimulation of visual cortex in the microampere range (Bartlett and Doty 1980). They used permanently implanted 130-200 µm diameter electrodes, and reported very stable, 15-25 µA behavioral thresholds. These stimulation levels are several orders of magnitude below the previously reported surface stimulation findings. Based on these findings Bak et. al (Bak, Girvin et al. 1990), and subsequently Schmidt et. al (Schmidt, Bak et al. 1996), conducted human studies addressing the feasibility of a cortical penetrating visual cortex neural prosthesis. In the first study of three normally sighted patients, Bak et. al reported thresholds in the tens of microamperes and ~1.0 mm spatial resolution. In the study reported by Schmidt et. al, they reported findings from a single blind individual without sight for 22 years. They confirmed the Bak et. al tens of microamperes threshold, and reported separate phosphene discrimination by stimulation margins of 700 µm between stimulating electrodes.

It follows from the research path emblazed by these investigators that future penetrating cortical sensory neural prosthetic solutions should be explored. Chronic penetrating stimulation devices are currently a hot topic for research, and are ready to move into the implementation phase. Recently, Normann et. al (Normann, Maynard et al. 1999) suggested a proof-of-concept device design for a penetrating cortical vision prosthesis. Other investigators have also developed prototypes for chronic intracortical stimulation (Kim and Wise 1996). These devices need behavioral animal models for further development. The focus of the first phase of the research here was to develop a penetrating cortical neuroprosthetic model in the adult rat.

Rats are an ideal animal model for further cortical prosthesis development. Historically, rats are the behavioral model of choice for investigations of classical and operant conditioning (Skinner 1938). Rats are easier to obtain, and financially less-burdensome than higher level organisms. Additionally, several recent literature reports have utilized the auditory system of the rat in order to elucidate mechanisms of auditory processing and nervous system plasticity (Sally and Kelly 1988; Kilgard and Merzenich 1998).

Due to the recent focus on the rat auditory system, it behooves this research to begin with stimulation of the auditory cortex for development of an auditory prosthesis. Additionally, the findings from cochlear implant research can be used to guide research on auditory cortical prosthesis development. However, the anatomical and physiological organization of primary auditory and visual corticies is similar. Both are organized in layers, receiving incoming information in layer IV, and distributing the information both to other cortical areas and back to lower brain centers. Due to this similar organization, results from research conducted in either arena have implications for the other.

It remains to be seen whether spatio-temporal stimulation of primary sensory cortex can provide enough information to serve as a sensory prosthesis. Several topics need to be addressed before multi-channel sensory prostheses are ready for human implementation. The second phase of this research addresses whether stimulation location within the primary auditory cortex can be used as a parameter to increase information transfer. It is intuitive that the more independent information channels that can be used in a sensory neuroprosthesis, the greater the total information that can be transferred. The final phase of this research investigates the resolution of spatial location that can be used for independent stimulation, as well as the behavioral saliency of the cortical stimulation compared with pure-tone auditory stimulation. The investigations reported in this dissertation quantify the behavioral saliency of penetrating auditory cortical microstimulation in an animal model and are on the critical path for development of an auditory cortical neuroprosthetic device.

ORGANIZATION

This dissertation is divided into three main sections. Chapter 2 begins by examination of the behavioral thresholds of penetrating auditory cortical stimulation through chronic microwire arrays. Rats were trained to detect auditory stimulation, and subsequently tested on their ability to report varying intensities of single channel auditory cortical microstimulation. The results show that the rats consistently responded to stimulations as low as 10 μA and that the dynamic range of stimulation, previously unexplored, is a rich mode of information transfer. These results have positive implications for stimulation intensity as a substantial channel of information transfer in a penetrating cortical neuroprosthetic setting. The material comprising Chapter 2 is in press, accepted by *Hearing Research.*

Chapter 3 examines the location of penetrating cortical stimulation within the auditory cortex, and whether this parameter conveys pitch information. Rats were trained to discriminate auditory tones separated by 4 octaves, and tested on the pitch-based sensation that penetrating auditory cortical stimulation produced within this spectrum. The results show that the rats' pitch sensation was significantly affected by different stimulation locations. These results support the implication that location within auditory cortex exhibits a potential information channel for a penetrating auditory cortex implant. Chapter 3 has been submitted to the *Journal of Neurophysiology*.

Chapter 4 addresses the capacity of and how many information channels are available in a penetrating auditory cortical implant. Rats were trained to discriminate between penetrating stimulation of auditory cortex at points located 1.75 mm apart. The rats were subsequently tested on the sensation of eight stimulation points between the bordering 1.75 mm stimulation points. The results show that the rats discriminated the 1.75 mm separated penetrating cortical stimulation more accurately than any of the natural auditory discrimination stimuli. Further, the rats responded to penetrating auditory cortical electrical stimuli presented at eight locations between the trained electrodes in a monotonic, statistically repeatable fashion. These results suggest that stimulation sites separated by 250 μm may be useful for a sensory cortical neuroprosthesis. This work is in preparation for submission to *Science*.

Finally, Chapter 5 summarizes these findings in a neural engineering setting as they relate to cortical sensory neuroprostheses. The results are summarized and discussed, and further studies are recommended.

The technologies and methodologies presented in this dissertation demonstrate the potential of direct cortical stimulation for sensory information transfer. The outcomes of this project provide a foundation for further research on a penetrating cortical auditory prosthesis. Neuroprosthetic research and development represents a very complex problem. This research study approaches this problem in a unique, but appropriate fashion: using a synergy of engineering, biology, and neuroscience knowledge and techniques.

CHAPTER 2

THE DYNAMIC RANGE OF STIMULUS AMPLITUDE IN RAT AUDITORY CORTEX MICROSTIMULATION

ABSTRACT

A combination of electrophysiological mapping, behavioral analysis and cortical microstimulation was used to explore the inter-relation between auditory cortex and behavior in the adult rat. Auditory detection was evaluated in eight rats trained to discriminate the presence or absence of a 75 dB pure tone stimulus. A probe trial technique was used to obtain intensity generalization gradients that described response probabilities to mid-level tones between 0 – 75 dB. The same rats were then chronically implanted in auditory cortex with a 16 or 32-channel tungsten microwire electrode array. Implanted animals were then trained to discriminate the presence of single electrode microstimulation of magnitude 90 μA (22.5 nC/phase). Intensity generalization gradients were created to obtain the response probabilities to mid-level current magnitudes ranging from 0 - 90 μA on 36 different electrodes in six of the eight rats. The 50% point (current level resulting in 50% detections) varied from 16.7 to 69.2 μA, with an overall mean of 42.4 (+/- 8.1) μA across all single electrodes. Cortical microstimulation induced sensory-evoked behavior with similar characteristics as normal auditory stimuli. The results highlight the importance of auditory cortex in a detection task and suggest that microstimulation of auditory cortex might be an effective means for graded information

transfer of auditory information directly to the brain as part of a cortical auditory prosthesis.

INTRODUCTION

The participation of the primary auditory cortex in hearing and auditory processing has been a debated topic in the auditory physiology literature for several decades. Using lesions of the auditory cortex, several studies concluded that the auditory cortex was not necessary for complex auditory behaviors such as frequency discrimination (Birt, Nienhuis et al. 1978; Kelly and Kavanagh 1986). In light of findings in the past few decades concerning the rapid plasticity of sensory neural responses (for a review, see (Buonomano and Merzenich 1998), Talwar et. al conducted a study describing the effect of *rapid, temporary* inactivation of auditory cortex on tone detection and discrimination (Talwar, Musial et al. 2001). Talwar et. al demonstrated that application of muscimol (a GABAergic receptor agonist) directly to the auditory cortex of the rat resulted in temporary inactivation of auditory processing within the auditory cortex, validated by measurements of auditory evoked potential recordings. Further, Talwar et. al theorized that the rapid and temporary nature of this deactivation would not invoke plasticity of the cortex that could obscure the actual role of the primary auditory cortex in tone detection and discrimination. This theory was supported, and it was found that the temporary inactivation of the auditory cortex completely eliminated tone detection behavior for approximately 5 hours post-drug delivery, and significantly affected tone discrimination behavior for approximately 15 hours post-drug delivery. From these results they concluded that the auditory cortex was necessary in some capacity for normal hearing, and attributed the results from the lesioning experiments to post-ablation auditory cortical plasticity. To further expand on the literature of the role of the auditory cortex in auditory behaviors, this chapter describes results from a study designed in the reverse fashion of the experiment conducted by Talwar et. al. We sought to determine if neural activity in the auditory cortex evoked by direct electrical stimulation is sufficient to elicit auditory detection behavior in the rat.

The topic addressed by this experiment has implications for the field of basic neuroscience as well as the field of neural engineering, specifically in the research and development of an auditory cortical neuroprosthesis. The role of the cortex in sensation and perception has long been the subject of neuroscience research. The systems-level approach and the behavioral nature of the results allow discussion of the auditory cortex and its role in the perception of sounds within the higher level processing stages of the auditory pathway. Elucidation of the role of the auditory cortex in the sensation and perception of sound has direct implications for the feasibility of a multi-channel auditory neuroprosthesis. Furthermore, the outcomes of this experiment will provide the proof-of-concept for an information channel via a brain-machine interface in the auditory cortex of the rat.

Electrical stimulation of neural tissue has been employed throughout the history of neurophysiological research as one of the basic tools to study neural organization, functional connectivity, and behavior. In the sensory systems, electrical stimulation combined with careful behavioral observation has been shown to be a particularly effective method to study the functionality of various processing centers within their respective sensory modality. The groundbreaking studies in this field were conducted by Penfield and colleagues on humans using brain surface stimulation mapping studies to determine the location and extent of epileptic tissue (Penfield and Boldrey 1937; Penfield 1938; Penfield and Rasmussen 1950; Penfield and Jasper 1954; Penfield 1958; Penfield and Perot 1963). Penfield reported that the subjects perceived basic sensations upon stimulation of various locations on the brain surface. The modality of the sensation was dependent on the location of the stimulation, with somatosensory sensations evoked from anterior parietal lobe stimulation, visual sensations from stimulation of the occipital pole, and auditory sensations from the medial edge of the temporal lobe.

Several investigators have expanded on the Penfield findings. Romo and colleagues stimulated in the hand region of primate somatosensory cortex. Using a psychophysical comparison test they reported data suggesting that the mean stimulation pulse rate is perceived as a flutter sensation. As mentioned in Chapter 1, there have been various reports of the perceptions elicited by stimulation of the visual cortex. Briefly, subjects typically perceive phosphenes, or spots of light. The phosphenes are generally in the field of vision on the contralateral side of the stimulation, and are elementary in nature, e.g. made up of lights, shadows, and colors (Foerster 1929; Krause and Schum 1931; Penfield and Rasmussen 1950; Brindley and Lewin 1968; Dobelle, Quest et al. 1979; Bak, Girvin et al. 1990; Schmidt, Bak et al. 1996).

There have been far fewer reports of the perceptions generated by auditory cortical stimulation. As mentioned in Chapter 1, the subjects in the Penfield study reported perceptions such as tones, ringing, or buzzing (Penfield and Rasmussen 1950).

Occasionally, patients reported more abstract auditory sensations, such as, “rushing sound like a bird flying,” or, “I hear funny things.” There were also reports of other auditory effects such as, “I am deaf a little,” and, “Key of your voice changed.” Penfield noted that the stimulations that were close to the primary auditory cortical area located in Heschl’s gyrus were more likely to produce elementary sounds. With the purpose of investigating the feasibility of an auditory cortical prosthesis, Dobelle et. al conducted a study on eight human subjects (Dobelle, Stensaas et al. 1973). Using surface stimulation on the lower lip of the Sylvian fissure, they reported stable, repeatable, perceptions similar to the elementary sensations reported by Penfield and Rasmussen, with typical thresholds of about 6 mA. These results were later confirmed by Howard et. al (Howard, Volkov et al. 2000). It was subsequently shown by Ronner et. al that stimulation of this magnitude can excite neurons up to 2 mm from the stimulation site (Ronner, Foote et al. 1980). Thus, for independent stimulating channels in a surface stimulation paradigm, the limiting spatial resolution is electrodes spaced on 4 mm centers. This electrode configuration significantly limits the number of channels available for a multi-channel auditory cortical surface prosthesis. Additionally, due to the relatively high thresholds, the power consumption requirements of a multi-channel auditory cortical surface prosthesis were determined to be a limiting factor.

Several investigators began research using penetrating microelectrodes with the hypothesis that they could produce similar sensations to the surface stimulation, but with significantly lower thresholds. In 1980, Bartlett and Doty reported a study in which rhesus macaque monkeys could reliably detect penetrating stimulation of the visual cortex with stimulation amplitudes of 2-4 µA. This was an exciting result indeed, and using a technique of simultaneous recording and stimulation, Ronner et. al confirmed that these lower stimulation amplitudes resulted in an over 20 fold smaller excitation radius (Ronner, Foote et al. 1981; Ronner 1982; Ronner and Lee 1983). Bak et. al, and subsequently Schmidt et. al conducted studies on human subjects to determine the perception of the penetrating microstimulation (Bak, Girvin et al. 1990; Schmidt, Bak et al. 1996). They confirmed that phosphenes could be generated by penetrating microeletrodes with 2-3 orders of magnitude lower thresholds, and were basically similar to the surface generated phosphenes. Further, they found that penetrating electrode generated phosphenes were generally more stable than surface generated phosphenes, with no flicker, or cloud sensations as reported occasionally by Brindley and Lewin or Dobelle et. al. In general, penetrating stimulation of sensory cortex has resulted in drastically lower thresholds, and perceptions more amenable to application in a sensory neuroprosthesis. These findings give credence to research addressing penetrating stimulation of auditory cortex.

There have been two studies reported assessing the behavioral responses associated with penetrating stimulation of auditory cortex. In 1999 Rousche and Normann described a study on the ability of cats to detect trains of stimulation delivered through a chronically implanted microelectrode array, the Utah Intracortical Electrode Array (Rousche and Normann 1999). They reported minimum and maximum detection behavioral thresholds of 2 µA and 43 µA across 71 sessions in three cats with an overall average of 10 µA. In 2002 Scheich and Breindl published a study on the ability of

Mongolian gerbils to discriminate various patterns of auditory cortical microstimulation (Scheich and Breindl 2002). They reported that gerbils successfully learned to discriminate spatial (n=7), temporal (n=8), and spatio-temporal (n=7) patterns of penetrating cortical microstimulation delivered through two chronically implanted microwires in auditory cortex. These studies demonstrate the plausibility of a penetrating auditory cortical prosthesis, and validate further research in animal models to elucidate the parameters of electrical microstimulation that will maximize information transfer through the chronic brain-machine interface.

The rat auditory system and vocalization behavior suggest that the rat is a valid model for this study. Rats exhibit a substantial range of complex natural auditory behaviors (Blumberg 1992; Blumberg, Sokoloff et al. 2000; Sachs and Bialy 2000), including behaviors that can be operantly trained (Skinner 1938; Harrison 1990). Auditory neurophysiology experiments conducted by Kelly et. al have described a reliable tonotopic organization in the rat primary auditory cortex (Kelly and Masterton 1977; Kelly and Sally 1988; Sally and Kelly 1988). From these experiments, the rat auditory cortex is easy to identify and accessible for implementation of a chronic electrophysiological device interface (Williams, Rennaker et al. 1999). Recently, studies by Kilgard and Merzenich have demonstrated that the rat auditory cortex is a valid preparation for the study of cortical plasticity (Kilgard and Merzenich 1998; Kilgard and Merzenich 1998; Kilgard and Merzenich 1999). The combination of the results from the auditory physiology literature and the foundation of behavioral research in the rat model implicate that rats are an appropriate choice as the experimental model utilized in this study.

There are common questions to which users of penetrating electrical microstimulation in the auditory cortex seek answers: What are the behaviorally relevant electrical stimulation parameters? What are the effects of variations in stimulation amplitude? What are the effects of stimulus location within the auditory cortex? What is the perception of the electrically-induced sensation? In order to address these and related questions, we conducted a study of the behavioral effects of penetrating auditory cortical stimulation in the rat. The objective of this study was to assess the effectiveness of the rat as a model in this experimental setting. This was realized by investigating the behavioral effects associated with varying electrical microstimulus amplitudes in a detection paradigm.

METHODS

Animals and Apparatus:

Eight male Sprague-Dawley rats (250-350 grams) maintained at 85% of their free-feeding body weights were trained to lever press for food in standard operant-conditioning chambers (Med-Associates, Mount Vernon, Indiana) located within a custom-built sound-dampened enclosure. Auditory stimuli were presented via a calibrated loudspeaker (Yamaha NS-10M Studio, Yamaha Corporation) located in the enclosure ceiling 24 inches above the chamber. Three retractable 1 inch levers positioned 5-6 inches from the chamber floor served as the manipulanda. Single 45 mg pellets (P.J.

Noyes Co., Lancaster, NH) were used as reinforcers. They were delivered into a ~5.0 cm by ~5.0 cm food tray located ~10 cm below the center lever. A 24-volt bulb in the upper rear of the chamber provided the only ambient lighting. Rats were housed individually under a reversed 12 hour light/dark schedule.

Behavioral Training

Rats were trained to perform a 3-lever (left, center, right) modified forced choice auditory detection task. Complete training took roughly 4 weeks. Rats were required to depress the center lever two times to begin each trial. For 2.25 s after center lever press, the rats were presented with either a 16 kHz pulsed pure tone sequence (the standard auditory stimulus) or silence (no stimulus). The 16 kHz standard auditory stimulus consisted of 5 tone pips of 250 ms duration (separated by 250 ms) delivered at 75 dB. Four cumulative responses on the left lever following tone burst presentation (hit) or four cumulative responses on the right lever following tone absence (correct rejection) resulted in food reward. Incorrect responses, i.e., four right lever presses following tone presence (miss) or four left lever presses following tone absence (false alarm), resulted in a 15-30 s time out in which the chamber was darkened. A non-dark intertrial interval of 10 s was employed between all trials. Sessions occurred daily and were limited to 150 reinforcers to avoid satiation.

Rats performing above a criterion of >90% correct were implanted with 16 or 32 channel microwire electrode arrays (as described below). During subsequent electrical stimulation sessions, the usual present-or-absent auditory stimulus was replaced with a present-or-absent constant current electrical stimulation of a single implanted electrode in the auditory cortex. To closely mimic the temporal envelope of the learned auditory stimuli, the electrical stimuli consisted of 5 bursts of electrical stimulation (250 ms per burst) using biphasic constant current pairs (cathodic first, pulse width = 250 μsec) delivered at 150 Hz separated by 250 ms. An initial current level of 90 μA (22.5 nC/ph) was chosen to ensure suprathreshold neural activation. A return current pathway was provided via a cranial bone screw or a fully de-insulated 50 μm diameter microwire with large exposed area implanted as part of the array.

Generalization Gradient Procedure:

Auditory Stimuli:

To characterize the auditory capabilities of each rat and the resultant discriminative properties of the auditory stimuli, generalization gradients were obtained. Unrewarded probe trials were randomly presented on 30% of all trials. On probe trials, the tone frequency was held constant at 16 kHz with the intensity lowered to one of five randomly-chosen discrete values (70, 65, 55, 35, or 20 dB). Each discrete probe level was repeated at least 10 times per session. Response data was used to construct intensity generalization gradients for each session. Detection rates fell predictably with stimulus intensity. From the ogive gradient curves, interpolation was used to calculate the stimulus magnitude (dB) which resulted in a 50% detection rate (hereafter referred to as the 50% point).

Electrical Stimuli:

Intensity generalization gradients were also obtained for rats trained to discriminate single-electrode electrical stimulation. After recovery from implant, rats were re-trained to discriminate the presence or absence of electrical stimulation on a single electrode using a fixed current amplitude of 90 μA (the standard (or reference) electrical stimulus). To obtain intensity generalization gradients, discrete current levels of 72, 54, 36, and 18 μA were randomly delivered during unrewarded probe trials (30% of all trials, at least 10 presentations per level per session). The resulting curves showed that response probabilities decreased predictably with stimulus intensity. The gradients obtained with electrical microstimulation were similar to those generated using auditory stimuli.

Electrode Arrays, Surgical Procedure, and Neural Recordings

Details of multi-electrode construction, implant procedures and recording performance are fully described in detail in another publication (Williams et al., 1999). Briefly, 16 or 32 channel electrode arrays were fabricated in-house using 50 μm polyimide-insulated tungsten wire aligned in rows of 8 wires each terminating in a small connector (GF-10, Microtech Inc., Boothwyn, PA) (inter-row spacing = 250 μm, inter-electrode spacing = 250 μm). Ethylene-oxide sterilized arrays were implanted using a micromanipulator under aseptic surgical conditions. Vascular landmarks and/or stereotaxic coordinates were used to identify the primary auditory cortex (Sally and Kelly, 1988). In addition, 2 animals also received identical microwire implants (but with only 4 electrodes) in visual cortex (as determined via stereotaxic coordinates). Neural recordings from the auditory and visual implants were used to assess electrode response to pure tone, click or light flash stimuli for several weeks following recovery. Recordings were performed in awake animals (signals were simultaneously amplified, bandpass filtered (500 - 7000 Hz), and displayed with a commercial multi-channel neural recording system (Plexon, Inc., Dallas, TX, see Appendix A). The visual cortex implants were tested with a simple 4-LED flashing stimulus. For the auditory implants, a 450 element set of short-duration tone pips (200 ms with 5 ms rise and fall, 300 ms inter-tone interval) spanning 30 frequencies (0.5 - 32 kHz) and 15 intensities (20 - 90 dB SPL) were delivered and frequency response areas relating firing rate to tone frequency and intensity were created with custom-built software using Mathematica™. Peri-stimulus time histograms (Nex software, Plexon, Inc., Dallas, TX) were also used to characterize auditory neural activity in response to 50 μsec clicks (100 dB). All experimentation was performed under the guidance of the Institutional Animal Care and Use Committee of Arizona State University.

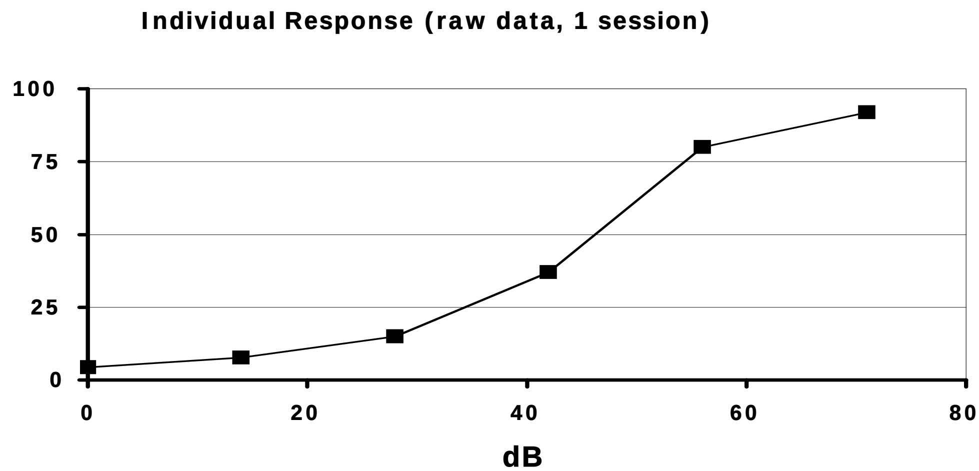
RESULTS

To investigate the relationship between auditory cortex, electrical stimulation and behavior, a combination of electrophysiological mapping, pure tone behavioral analysis and cortical microstimulation behavioral analysis was performed in a series of 8 trained and implanted rats. Intensity generalization gradient curves relating stimulus strength to probability of behavior were obtained for both pure tone stimuli and for electrical stimuli in all subjects.

Pure Tone Behavioral Responses:

To establish baseline performance in the auditory detection task, rats were first trained to detect the presence or absence of a 5-pip 16 kHz pure tone stimulus. Following criterion behavior in discriminating these stimuli (>90% performance), intensity generalization gradient curves were obtained. Gradient curves describe response probabilities for tone intensities lower than the standard auditory stimuli used in training. Figure 2.1A shows an intensity gradient curve for pure tone detection in a single animal recorded in a single session. Each data point is marked with the total number of times it was presented during the course of the trial. A typical gradient relates increases in detection percentages with increases in stimulus magnitude. In Figure 2.1B, the mean auditory response curves obtained from 23 different sessions in 6 different rats is presented. As in the individual case, the mean curve clearly demonstrates that rats achieve higher discrimination percentages for stimulus magnitudes closest to the level of the training stimulus. This curve shows profound overall auditory intensity generalization among the subjects.

A



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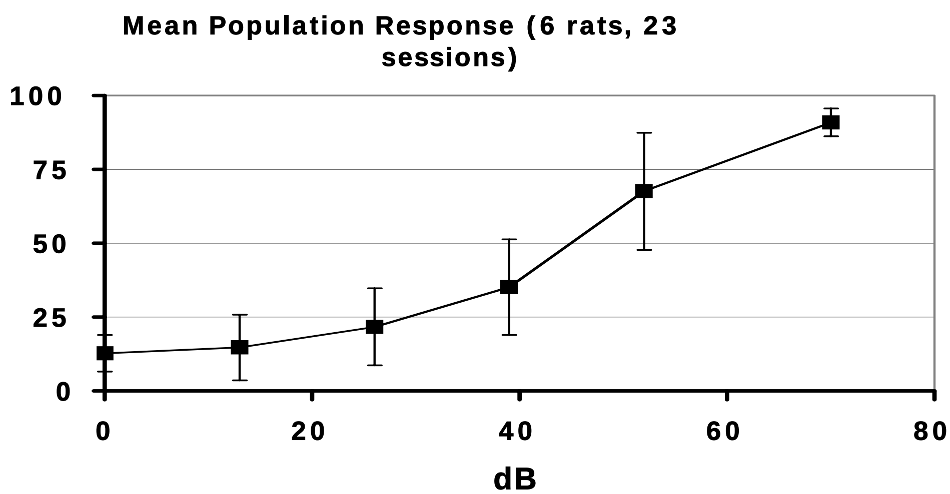

Figure 2.1 Pure tone intensity generalization gradient curves

Neural Recording:

Following pure tone behavioral characterization, all animals received a 16- or 32-channel microwire array implant into auditory cortex. Two of the 8 animals also received 4-channel microwire implants into the visual cortex. Implanted auditory electrodes in each subject were tested periodically following implant to determine the neural response properties. In Figure 2.2A simultaneously recorded peri-stimulus time histograms in response to 100 clicks are shown. Vigorous onset firing is evident on every implanted electrode. All animals except one exhibited similar robust click responses. Figure 2.2B shows frequency response areas for the same electrodes. A gray scale shows interpolated average neural firing rates (white highest) in response to a set of 450 pure tones of varying intensity and frequency (30 frequencies, 15 intensities). Excitatory frequency specificity is revealed via the appearance of clustered white regions within each plot (highlighted in one plot with a black dotted line). Inhibitory frequency specificity is revealed via the appearance of clustered dark regions within each plot (highlighted in a different plot with a white dotted line). Gray regions outline those frequency-intentisty stimuli that cause no change in firing rate.   Nearly every electrode displays some type of frequency tuning. In this recording session, the excitatory best frequency (that pure tone preferred by each electrode) ranged from 922 to 27,918 Hz as calculated according to the interpolated firing rates.

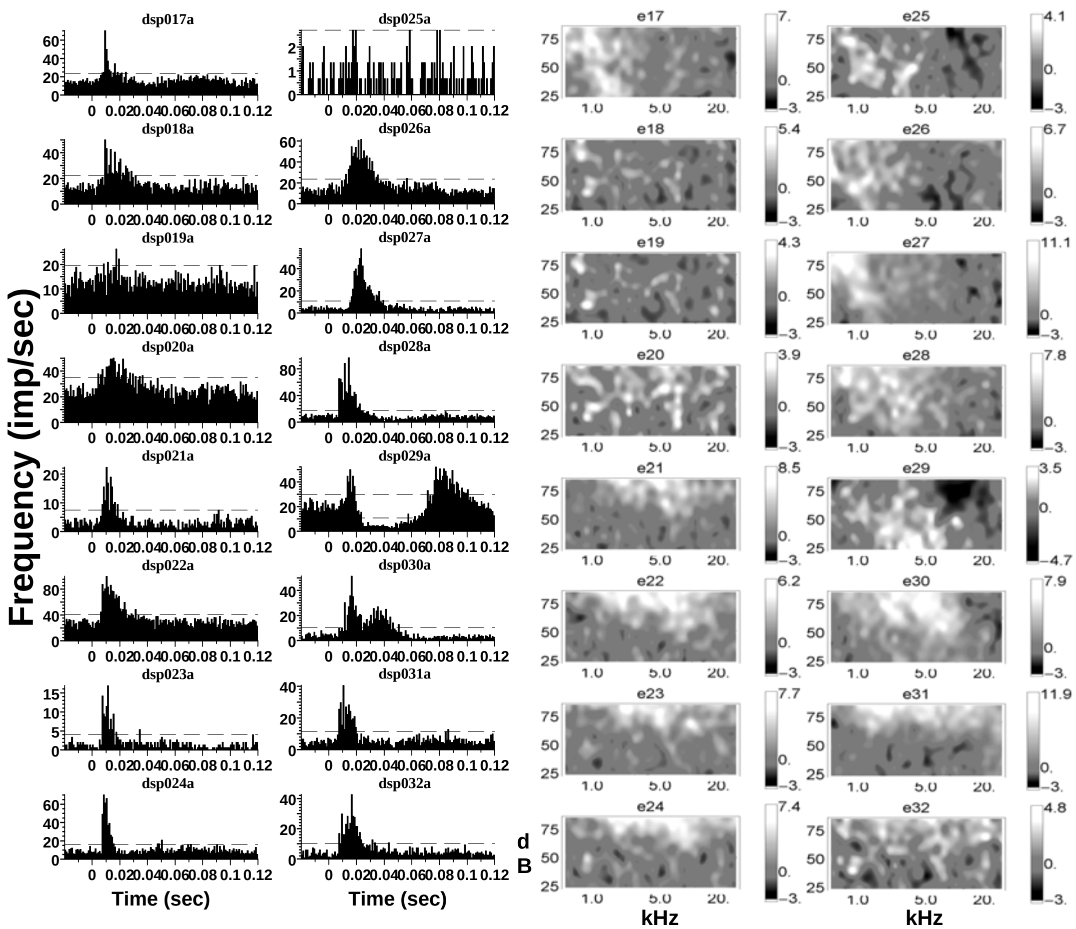

Figure 2.2 Multi-unit neural recording. Both histograms (left) and frequency response areas (right) are shown.

Cortical Microstimulation -- Auditory Cortex

Up to 8 (out of 16 or 32) electrodes with measurable neural activity from each array were tested for each subject in electrical stimulation sessions. After preliminary training on a single electrode to learn how to detect a standard stimulus of magnitude 90 μA, intensity generalization gradient curves were obtained for a variety of electrodes to characterize behavioral responses to electrical microstimulation of the auditory cortex.

Figure 2.3A shows a single gradient curve obtained from a single cortical microstimulation session performed on one implanted electrode. This subject is the same subject whose neural response properties are shown in Figure 2.2. Figure 2.3B shows 8 electrical stimulation intensity gradient curves collected in different sessions over a 10 day period from this same subject. Each curve shows intensity generalization in response to electrical stimulation of a different electrode. A 9th curve on this plot demonstrates very poor detection performance in a control situation whereby the constant current stimulator is triggered for the appropriate current level, but was in fact disconnected from the subject for the entire session. The 50% point (interpolated) for the individual experimental curves in this subject ranged from 39.2 to 59.1 μA with a mean of 50.6 μA. In Figure 2.3C, the mean of 36 intensity gradient curves collected from cortical microstimulation of 36 different electrodes in 6 different subjects is displayed. Minimum and maximum 50% points obtained via a 90 μA reference current differed by ~50 μA and ranged from 16.7 to 69.2 μA, with a mean 50% point of 42.4 μA. Overall, electrical stimulation of 36 electrodes in 6 rats using a 90 μA reference stimulus was effective in its ability to elicit *graded and typical* behavioral responses as revealed by the intensity generalization gradients.

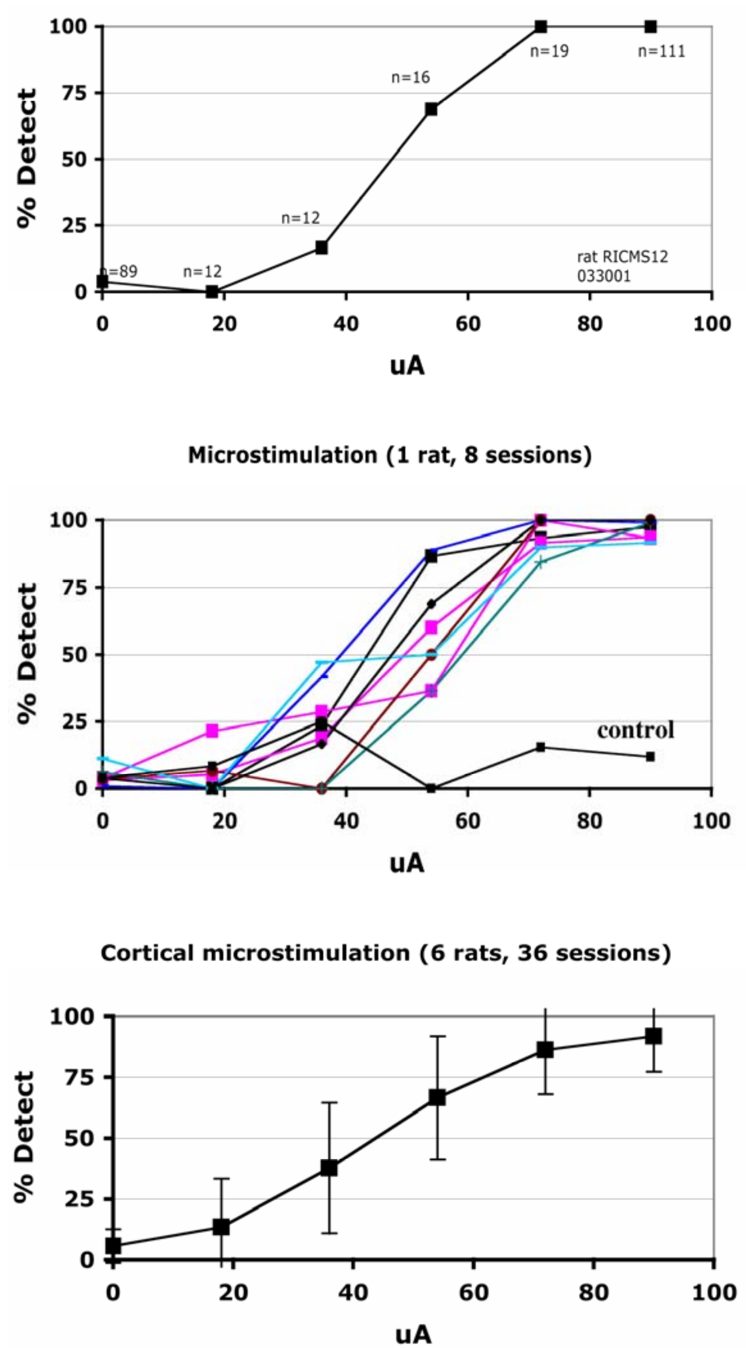

Figure 2.3 Auditory cortex microstimulation intensity generalization gradient curves

It is important to note that behavioral response probabilities depend on the magnitude of the standard reference stimulus. To demonstrate this, we obtained intensity gradient curves using a variety of reference stimuli spanning from 22.5 μA up to the standard 90 μA in several different animals. Figure 2.4A reveals 2 intensity generalization curves from stimulation of AI using a reference current of only 22.5 μA. The curves show stimulus results for the same subject on the same electrode in two different sessions. Despite the low reference current, a typical behavioral response relationship between decreasing stimulus intensity and detection probability is still present. The mean 50% point for these two cases was 13.9 μA. The data suggest that the detection behavior is stable between sessions on different days. In Figure 2.4B, a plot of curves obtained from multiple subjects tested with multiple reference currents is presented. Note that despite the level of the individual reference current (22.5, 36, 55, 78 or 90 μA) response probabilities decay accordingly with current magnitude in every case, suggesting that intermediate current levels are able to be discriminated. In some cases, current levels that induce discrimination behavior with a high probability in one session induce practically no detection when employed in another session (compare the range of probabilities for current levels of 20 and 40 μA).

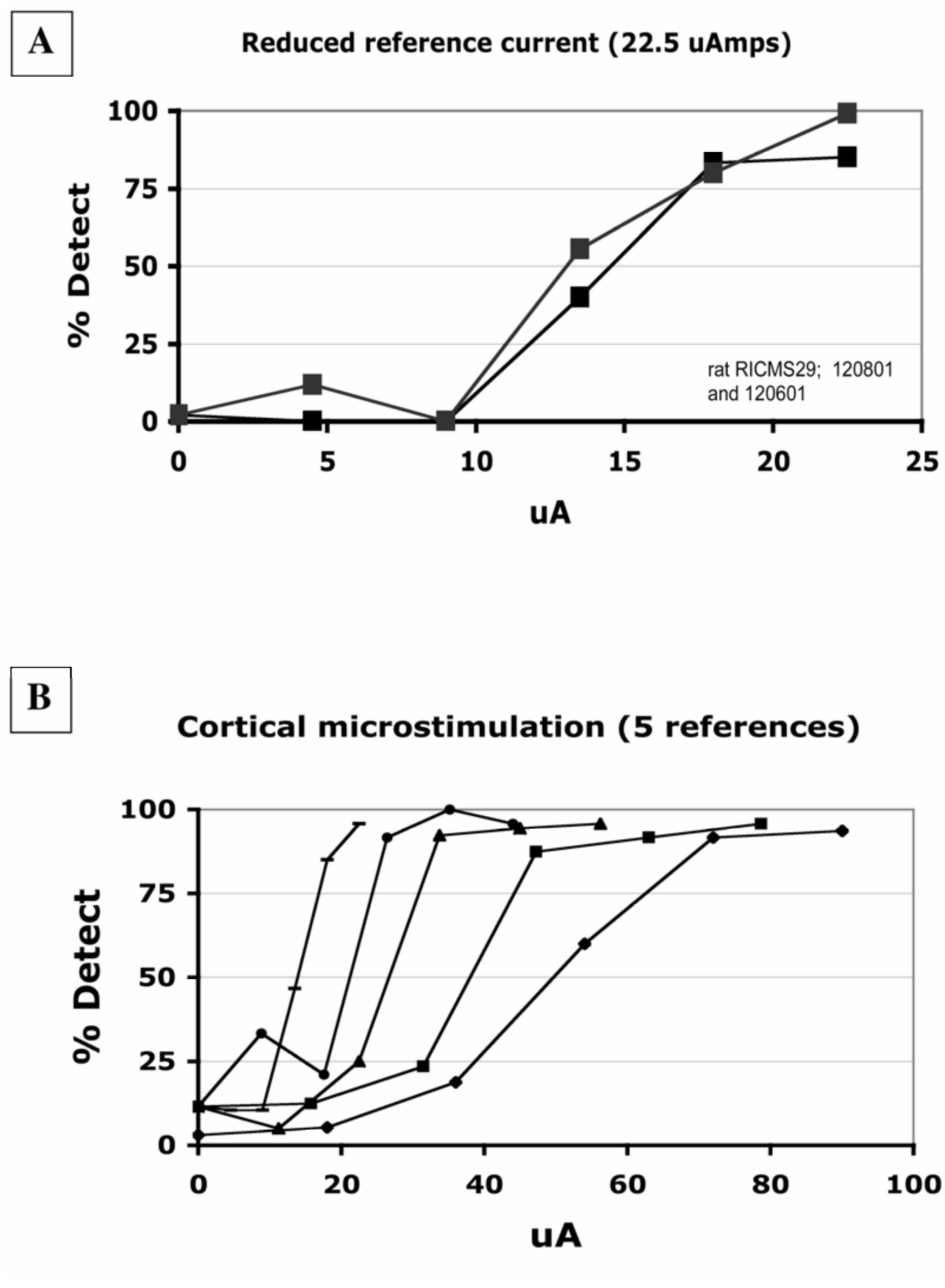

Figure 2.4 Reduced reference microstimulation intensity generalization gradient curves

Cortical Microstimulation -- Visual Cortex

As a control, 2 of the 8 rats also had additional electrode arrays (4-electrodes each) implanted in the visual cortex. Animals were anaesthetized and each of these eight electrodes was tested for visual-evoked multi-unit neural responses via a flashing LED system. These electrodes were subsequently tested for the presence of any auditory click-evoked neural activity. Significant neural activity occurred only due to the LED flash stimulus and did not occur for the auditory click stimulus. A day later these same animals (also originally trained on an auditory 0 or 75 dB, 16 kHz tone pip stimulus) were put into the training box and trained to detect the presence or absence of a 90 μA reference stimulus on a single auditory cortex electrode. Following criterion performance on this task, intensity generalization gradient curves were again obtained. However, in this control case, for each *probe* trial we substituted microstimulation of a single electrode in visual cortex (the same electrode was stimulated for each probe trial). Animals responded robustly to the 90 μA reference stimulus on the auditory cortex electrode (detection percentage close to 100%). However, stimulation of the visual cortex at *any* of the probe trial current levels did not induce any discrimination percentages significantly above zero. The two dark lines (marked ***Pre***) in Figure 2.5 show the same results for 2 different visual cortex electrodes in the 2 different subjects. Clearly, the resultant sensation from visual cortex stimulation was not similar enough to the auditory cortex sensations to be effectively generalized.

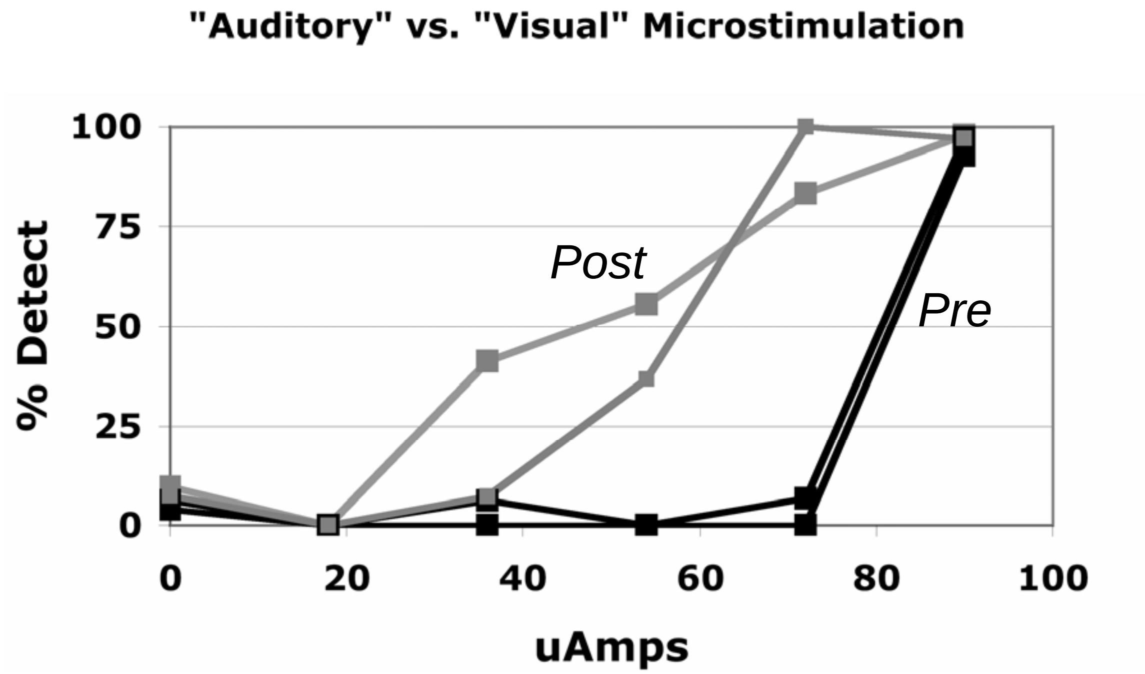

Figure 2.5 Visual cortex microstimulation intensity generalization gradient curves

A follow-up experiment was performed to insure that the lack of discriminable behavior when the visual cortex was electrically activated was not due to a sudden (if improbable) failure of the stimulated visual cortex electrode on the testing day. Animals were re-trained to detect electrical microstimulation of a 90 μA reference stimulus delivered to the *visual* cortex through the same electrode that previously was ineffective as a salient cue. After re-training to achieve criterion performance (>90%) in response to visual cortex micro-simulation, we then again used the probe trial procedure to obtain intensity generalization curves for single electrode microstimulation of visual cortex alone. As shown in Figure 2.5, the gray lines (marked ***Post***) now reveal a standard behavioral response relationship between current magnitude and detection percentage for the same electrodes that were previously ineffective in generating a behavioral response.

The gray and black lines represent results of microstimulation sessions for the *same single visual cortex electrodes* in two different subjects. The quantitative difference in detection comes only when 1) the animals are re-trained to understand the saliency of the visual cortex microstimulation, and 2) the resultant probe trial sensations are similar enough to that evoked by the reference stimulus that they can be effectively discriminated.

DISCUSSION

The rat as an animal model of a penetrating auditory cortical prosthesis was assessed through generalization testing of single-electrode microstimulation intensity in a detection paradigm. Microstimulation of the auditory cortex was found to provide a salient and reliable sensory cue in the behavioral setting. The microstimulation detection behavior varied monotonically with intensity, exhibiting a surprisingly large dynamic range. These results have broad implications in the areas of auditory neurophysiology and neuroprostheses.

An Information Channel in Auditory Cortex

Electrical microstimulation of a single channel in auditory cortex is a robust information channel in a behavioral context. The results in figure 2.3 clearly demonstrate that rats can easily discriminate 90 μA stimulation from null stimulation. The monotonic relationship between behavior and stimulation intensity imply that a continuum may exist between the amplitude of a microstimulation train and the sensation it evokes. Dobelle reported that loudness could be “repeatedly controlled” in the sensations evoked by surface stimulation of human auditory cortex (Dobelle, Stensaas et al. 1973). In our experimental paradigm, it is likely that increasing stimulation amplitude results in a stronger auditory sensation. This is supported by the comparison of the increasing probability of detection with loudness in the auditory evoked responses as shown in Figure 2.1.

The dynamic range of the continuum between stimulation amplitude and sensation in this study was ~60 µA for the behavioral case of the 90 µA reference stimulus (as in Fig 2.3C). This result is both surprising and appealing when compared with the 5-20 µA penetrating microstimulation thresholds reported in the literature (Bartlett and Doty 1980; Bak, Girvin et al. 1990; Schmidt, Bak et al. 1996; Rousche and Normann 1999). The previous studies focused on the sensory threshold of microstimulation; consequently, the available bandwidth of information through super-threshold penetrating microstimulation has not been previously reported. Cochlear implant speech processors employ compression schemes utilizing amplitude modulation to compensate for a lack of frequency resolution (Loizou, Dorman et al. 2000). In general, a trade-off exists, whereby a lower frequency resolution, e.g. number of channels, can be compensated for by a higher intensity dynamic range. The dynamic range results demonstrated in this study suggest a high bandwidth of a single channel of auditory cortical microstimulation, and an amplitude modulated information processing scheme may be fruitful in an auditory cortical prosthesis comprised of only a few channels.

Electrical Stimulation to Convey Information

Optical images of global auditory cortex activity during suprathreshold tone (25-80 dB) show regions of cortical area as large as 1000-1500 µm are activated (Bakin, Kwon et al. 1996). In our studies, as shown in Figure 2.3C, the mean 50% point for electrical stimulation was 42.4 µA, or a charge of 10.25 nC/phase. This is slightly larger than a charge per phase of 8 nC that has been estimated to activate neurons within a spherical diameter of 340 µm during stimulation of sensory cortex of the cat (Ronner and Lee 1983). Although the effect of the direct spread of current remains difficult to quantify, it is reasonable to postulate that electrical stimulation of primary auditory cortex using microelectrodes in our experiment activated significantly fewer neurons per stimuli than did the pure tone stimuli. Electrical stimulation is generally considered to be excitatory, but it is probable that microstimulation results in some activation of inhibitory neurons. Thus the net behavioral effect of microstimulation depends on the specific electrode position with respect to both excitatory and inhibitory neurons and the extent of the stimulus field.

Auditory Specification

Our results indicated that stimulation of different sensory corticies provided different sensory cues and did not generalize. Fig 2.5 indicates that intensity generalization curves could only be produced from stimulation of the same sensory cortex as the reference stimulus. Visual cortex stimulation was not generalized to auditory cortex stimulation. Results from surface stimulation of human auditory cortex indicate that the perception elicited is an auditory sensation (Penfield and Rasmussen 1950; Dobelle, Stensaas et al.

1973; Howard, Volkov et al. 2000). Additionally, surface stimulation of human visual cortex elicits visual percepts (Penfield and Rasmussen 1950; Brindley and Lewin 1968; Dobelle and Mladejovsky 1974). Our results in the rat support the findings of the human studies, indicating that the sensations evoked by visual cortex stimulation are significantly different from those evoked by auditory cortex stimulation. However, upon visual cortex training, the resultant behavior evoked is a monotonic generalization with respect to stimulus intensity. This result is consistent with the sensory-evoked behavior of the natural and electrical auditory-evoked behavior.

Advantages of Penetrating Stimulation

The mean 50% point, or point of subjective equality, was 42.4 μA with a standard deviation of 8.1 μA. This measure is important to quantify behavior in this task, but is not necessarily equivalent to 'true' threshold, or a level of current that induces a just noticeable sensory event. This measure is dependent on the behavioral task parameters as indicated by the decreasing 50% point in Figure 2.5. It is likely that actual *sensory* thresholds are quite lower than the current values which induce a 50% point in the 90 μA generalization task. Of interest, results in Figure 2.5 show that rats can consistently detect a stimulus of 20 μA. From this result, it follows that the actual psychophysical sensory activation threshold for rats with this microstimulation paradigm are lower than this level.

An Animal Model of Auditory Cortex Prostheses

These experiments suggest that the basic components necessary to develop and test a penetrating auditory cortical prosthesis can be implemented in an awake behaving rat. The behavior in this paradigm is consistent, as shown by the stability of the behavior over two separate sessions in Figure 2.4A. Additionally, the auditory cortex of the rat is easily located and exhibits robust neurophysiological responses as shown in Figure 2. The rat as an animal developmental model of a penetrating auditory cortical prosthesis has demonstrated initial viability and deserves further exploration.

Significance

The long term goal of this research is to develop auditory prostheses that can effectively transmit at a sufficient rate to demonstrate marginal efficacy, particular over nonsurgical techniques involving a remapping of auditory sensation to other sensory modalities. The animal model described here is shown to be a reliable testbed for stimulation parameter testing and hardware development. Additionally, single-electrode penetrating microstimulation of the auditory cortex is shown to provide a robust, stable, and salient sensory cue in a behavioral context. While this is a significant accomplishment from an experimental standpoint, the device and techniques need further testing concerning lifespan of the interface and information transmission. Although single channel stimulation is adequate for this sensory detection paradigm, it is far from demonstrating effectiveness of a chronic clinical device.

One issue that has not been addressed in this study is the underlying differences in sensation that surely occur from different stimulation sites within auditory cortex. It is hypothesized that some variability of the behavioral responses can be attributed to these sensation differences. Additionally, the different sensory cues related to stimulus location within the auditory cortex may provide another dimension for information transfer. In the next chapter experimental studies are presented that investigate the relationship between the electrophysiological properties of the local tissue and the behavior induced.

CONCLUSION

This chapter details the development of a rat-based animal model for behavioral evaluation of a penetrating auditory cortical prosthesis. We provide a quantitative description of the generalization behavior from 6 rats elicited by electrical microstimulation of stimulus amplitudes varying between 0 and 90 µA. We report a remarkable dynamic range of ~60 µA for single-electrode microstimulation. These results are significant for the amount of information transmission possible in a penetrating auditory cortical prosthesis. Further, due to similar cytoarchitecture and processing schemes, these results also have implications for development of a penetrating visual cortical prosthesis. This report is unique in its application of the field of psychophysics to sensory neuroprosthetic development in an attempt to quantify device and stimulation parameters and operating characteristics.

CHAPTER 3

RECEPTIVE-FIELD DEPENDENCY OF BEHAVIOR INDUCED BY AUDITORY CORTICAL MICROSTIMULATION

ABSTRACT

Electrical activation of the auditory cortex with surface electrodes has been shown to elicit an auditory perception. Penetrating electrical stimulation of visual cortex has been proven to have lower stimulation thresholds than surface stimulation, which is preferred for a chronic auditory cortical prosthesis; however, the exact perceptual effects of auditory cortical microstimulation delivered through penetrating electrodes have not been clearly elucidated. This study examines the relationship between penetrating electrical microstimulus location within the adult rat auditory cortex and the subsequent behavior induced. Four rats were trained on an auditory frequency discrimination task. After training, frequency-dependent auditory behavior was quantified by intermixing the standard discrimination trials with trials of intermediate auditory frequencies. Each trained rat was then implanted with a chronic microwire array in the auditory cortex of the left hemisphere. Best frequencies (BF) of each electrode in the array were determined by analyzing maximum local field potential variations to pure tone stimuli. In order to behaviorally evaluate cortical microstimulation, a cross-dimensional psychophysical generalization paradigm was used. This paradigm intermixed auditory discrimination trials with single-electrode microstimulation trials. Microstimulation-induced behavior was dependent on the BF of the electrode used for stimulation (R=0.24,

$p<0.05$). These results are consistent with recent reports indicating that electrophysiological recordings of neural responses to sensory stimuli may provide insight into the sensation generated by electrical stimulation of the same sensory neural tissue.

INTRODUCTION

Several studies have reported on the perceptions induced by surface electrical stimulation of human sensory cortex (Penfield and Rasmussen 1950; Brindley and Lewin 1968; Dobelle, Stensaas et al. 1973; Dobelle, Mladejovsky et al. 1974). However, these cortical surface stimulation studies produced unreliable percepts, usually involving a "chirping" quality, and required high stimulation currents. The benefits of using penetrating electrodes for cortical stimulation, including more confined stimulus volumes and lower thresholds, have been demonstrated for several decades (Bartlett and Doty 1980; Ronner, Foote et al. 1981; Ronner and Lee 1983). Further, several recent studies have shown penetrating stimulation of auditory cortex to provide salient sensory cues to a behaving animal (Rousche and Normann 1999; Scheich and Breindl 2002; Rousche, Otto et al. 2003). However, due to the nature of these studies, the exact percept of the penetrating auditory cortex electrical stimulation was not clear.

Cortical microstimulation has been shown to activate a local region of neurons (Stoney, Thompson et al. 1968), and is used in several research and development aspects. Cortical microstimulation delivered through a brain-machine interface is a valuable tool in studying neural coding. It is also a potential mode of operation for neuroprosthetic systems.

In terms of neural coding, cortical microstimulation has been shown to provide a "virtual" signal that subjects use in the processing of local information. Through a series of now classic experiments, Newsome and colleagues have shown that a primate's perceptual decisions can be biased by microstimulation of the middle temporal visual cortical area (Salzman, Britten et al. 1990; Salzman, Murasugi et al. 1992; Groh, Born et al. 1997; Seidemann, Zohary et al. 1998). Recent studies by Romo and colleagues have shown that microstimulation of the primate somatosensory cortex using varying temporal stimulation parameters provides sensory information to behaving primates (Romo, Hernandez et al. 1998; Romo, Hernandez et al. 2000).

In the field of neuroprosthetic systems, cochlear implants, as well as the recently developed auditory brainstem implants, have shown that electrical activation of neural tissue is sufficient to provide a damaged neural system sensory cues adequate to replace auditory function (Otto, Shannon et al. 1998; Rauschecker and Shannon 2002). Animal studies have shown that electrical stimulation of the inferior colliculus can be used as a conditioned stimulus in a classical conditioning paradigm (Patterson 1970; Patterson 1971; Brandao, Troncoso et al. 1997). Several experimenters have suggested that stimulation in further downstream processing areas, such as the primary sensory cortex, could serve as a similar sensory substitute in the case of sensorineural hearing loss or blindness (Brindley and Lewin 1968; Dobelle, Stensaas et al. 1973; Bak, Girvin et al. 1990; Schmidt, Bak et al. 1996; Normann, Maynard et al. 1999; Rousche and Normann 1999; Rauschecker and Shannon 2002; Scheich and Breindl 2002). In order to further understand neural coding and also apply this technology in a neuroprosthesis, further optimization of the electrical stimulus parameters and encoding strategies to increase the information capacity of cortical microstimulation are required. The most intuitive way to create independent information channels in a cortical sensory neuroprosthesis is utilization of multi-electrode arrays of stimulation. However, this technique requires more investigation before it can be implemented.

The previous chapter reports that rats are able to detect microstimulation of auditory cortex (Rousche, Otto et al. 2003). Furthermore, cortical microstimulation intensity generalization paralleled auditory tone intensity generalization behavior. Results were identical regardless of the stimulus location within the auditory cortex; however, since a stimulus-detection paradigm was implemented, it is unclear from those results what differences in electrical stimulus sensation exist due to varying the stimulus location within auditory cortex. In two separate studies, Penfield and Perot and Dobelle et. al reported that stimulation of human primary auditory cortex created perceptions that contained a pitch (Penfield and Perot 1963; Dobelle, Stensaas et al. 1973). The pitch seemed to depend on electrode location within the primary auditory field. Dobelle theorized that this phenomenon may be attributed to the arrangement of iso-frequency bands into a tonotopic map. Iso-frequency response bands also exist in the primary auditory cortex of the white rat (Sally and Kelly 1988). Due to this arrangement, cortical microstimulation of neurons from a single iso-frequency band may be expected to excite similar auditory cortical neurons as those excited by pure-tone natural auditory stimuli of the same frequency. The objective of this study is to determine the relationship of the auditory sensation induced by electrical activation of these neurons and the auditory-evoked electrophysiological properties of this tissue. This objective will elucidate the capacity of electrical stimulation in differing locations within the auditory cortex to provide “virtual” auditory signals that differ in spectral information.

To accomplish this objective, a forced-choice behavioral paradigm has been developed in the adult white rat. In this paradigm, a cross-dimensional psychophysical task is used to determine the relative sensations of induced by auditory and cortical stimulation. This paradigm requires the subject to behaviorally respond to either auditory stimulation or cortical microstimulation delivered through a single channel in an array of chronically implanted electrodes in the auditory cortex.

METHODS

Behavioral Training

Four male, naïve Sprague-Dawley rats (250 g - 300 g) were trained in an auditory discrimination task. Initially, the rats were food deprived to 80% of their free-feeding weight. Subjects responded in standard operant conditioning behavioral boxes (Med Associates, St. Albans, VT) located within a semi-anechoic room. The response wall of the test box included three side-by-side retractable response levers approximately 4” above the cage floor. A house light at the rear of the box was utilized for both illumination and negative reinforcement. The behavioral apparatus was controlled and monitored by software developed in-house, running on a PC interfaced with digital input-output hardware (System II, Tucker-Davis Technologies, Gainesville, FL). This equipment was also used to generate all auditory stimuli used in the experiment. The auditory stimuli were delivered via a speaker (Yamaha NS-10M Studio, Yamaha Corporation, Buena Park, CA) located 1 m directly above the test box. The system delivered a near-flat frequency response between 500 Hz and 32 kHz. The system was calibrated to a position directly above the center lever, although calibration measurements indicated that intensity variations within the test box did not exceed 5 dB.

A discrimination task in a forced-choice psychophysical paradigm was used to assess stimulus generalization (Figure 3.1). Subjects were positively reinforced via single food pellets (P.J. Noyes, 45 mg rodent diet I, Lancaster, NH) for correct responses to trains of auditory stimuli. Initially, all three levers were retracted, and the house light was illuminated. Extension of the center lever signaled the start of a trial. Trials were subject-initiated by two recorded presses of this center response lever. Subsequently, the center lever was retracted, and a train (250 ms on, 250 ms off) of five pure-tone bursts was delivered. Auditory training stimuli trains at either 1 kHz, or 16 kHz were delivered at 70 dB SPL. Upon completion of the auditory stimulus presentation, the two outer levers were extended. A fixed-ratio (FR4) response paradigm was utilized, and subjects were reinforced after four responses on a given lever within 7 s of outer lever presentation. Most correct responses occurred within 1.5 s. Responses were designated correct and positively reinforced for a left lever response to the 1 kHz stimulus or a right lever response to the 16 kHz stimulus. Left responses to the 16 kHz stimulus or right responses to the 1 kHz stimulus were designated incorrect and punishment was given in the form of a 30 s dark timeout. A response was considered null, and punished if the subject did not respond within the 7 s response window. Null response trials in all of the training or testing were rare (zero for > 95 % of the sessions) and were not used in the behavioral data analysis.

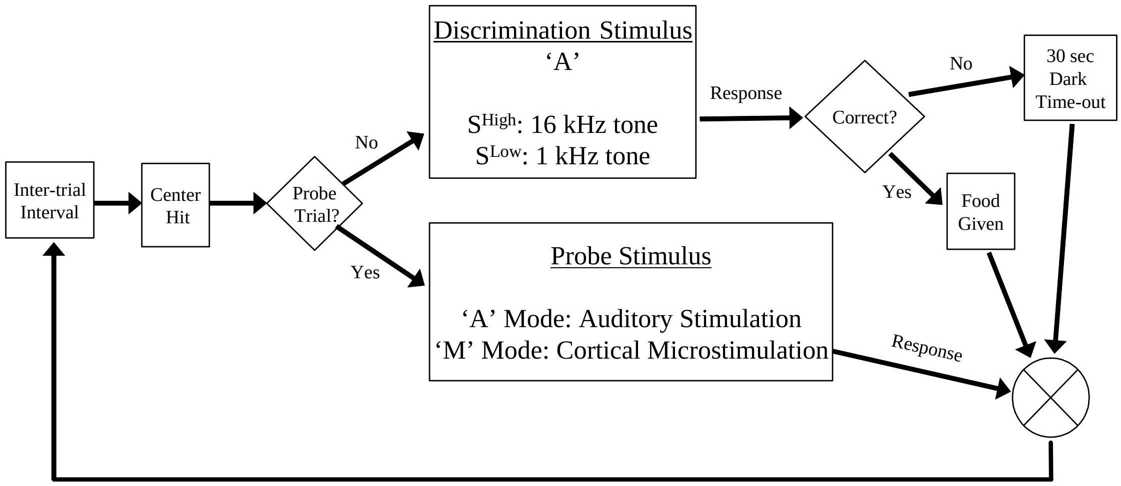

Figure 3.1 Trial behavioral flowchart

Auditory Generalization Behavioral Testing

Upon criterion performance of the auditory training paradigm (above 90% for three consecutive days), psychophysical curves were created to assess auditory generalization behavioral performance. The standard auditory 1 kHz or 16 kHz reinforced stimuli were delivered, indicating left or right behavioral cues respectively; however, in the generalization testing paradigm, approximately 25% of the trials were randomly chosen as "probe" trials in which stimuli of intermediate auditory frequencies were presented, and the subsequent behavior recorded. The probe trials were never reinforced. The rats' generalization behavior in response to four auditory probe frequencies was tested. The auditory probe frequencies were chosen as tones spaced evenly on a logarithmic scale (1740 Hz, 3030 Hz, 5280 Hz, and 9190 Hz). Hereafter, this test is referred to as “A-A” (the standard auditory discrimination task, ‘A’, coupled with auditory probe testing, ‘A’). Daily testing sessions continued until each subject received 200 positive rewards. Due to the random trial nature of the probe presentation in the experiment, the exact number of daily trials varied, but was on average approximately 275. Daily testing sessions lasted approximately 80 min.

Surgical Implantation

After three consecutive successful auditory generalization testing sessions, each rat was chronically implanted with an array of microelectrodes. Electrode array construction and surgical implantation have been described in a previous publication (Williams, Rennaker et al. 1999). Briefly, the electrode arrays consisted of two rows of eight, 50µm diameter, polyimide coated tungsten microwires (Figure 3.2*A* and 3.2*B*). Electrode spacing in a given row and between rows was 250 µm. Animals were anesthetized with a combination of Ketamine 75.0 mg/kg, Xylazine 7.5 mg/kg, and Acepromazine 1.5 mg/kg. The scalp was removed over the left hemisphere, and a 4 mm x 4 mm crainiotomy was performed at 4 mm lateral and 5 mm posterior to bregma. The dura mater was removed and the tissue moistened with sterile saline. The auditory cortex was located stereotaxically and from vascular landmarks as identified in previous studies (Sally and Kelly 1988). Viewed through a microscope, the electrode array was positioned at the surface of the brain between the large anterior and posterior dorsoventral vessels that have been shown in literature to delineate the auditory cortex in the rat (Figure 3.2*C*). The array was rapidly inserted into cortex until visual pia mater penetration was confirmed. The electrode array was then retracted to the previously defined cortical surface and subsequently lowered to 600 µm. Gelfoam© was positioned on the brain around the electrodes to serve as a protective barrier, and the array was affixed to stainless steel bone screws in the cranium with polymethyl-methacralate. All procedures complied with the United States Department of Agriculture guidelines for the care and use of laboratory animals and were approved by the Arizona State University Animal Care and Use Committee.

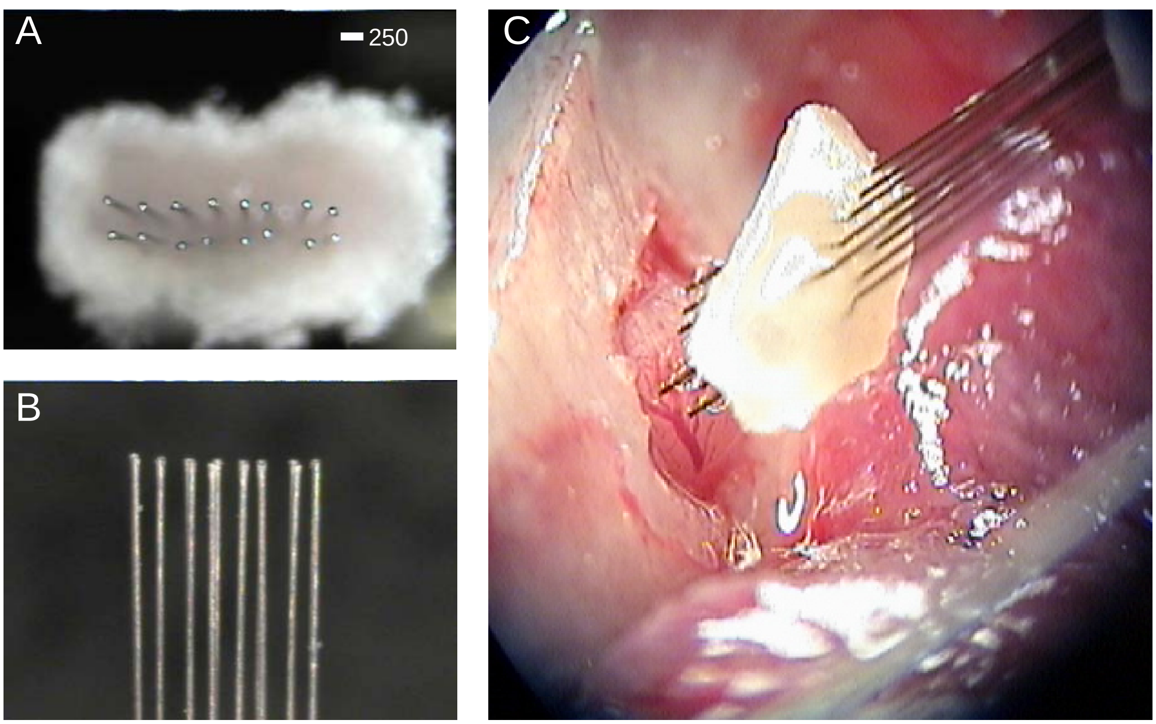

Figure 3.2 Chronic microwire rat auditory cortex preparation

Cortical Microstimulation Behavioral Testing

After surgical recovery (approx 1.5 weeks), the rats were tested to ensure that their behavior to the auditory task was unchanged. In all cases, subject behavior returned to pre-surgical level in less than 2 sessions.

In order to assess cortical microstimulation, the method of intermixing probe trials with the standard reinforced auditory discrimination trials was again employed. Daily testing sessions were conducted in an identical manner as the A-A testing sessions. The trials were subject initiated by center lever presses, and the trial stimulation was presented after center lever retraction. Approximately 75% of the trials were the normal auditory task 1 kHz or 16 kHz cues (‘A’), and were reinforced appropriately. The resultant 25% of the trials were microstimulation probe trials, where the cue was cortical microstimulation delivered through a single electrode (‘M’). This data is hereafter referred to as “A-M”. Daily A-M testing sessions were conducted within a single row of the electrode array.

Electrodes within an array were labeled such that electrodes 1-8 were the anterior to posterior electrodes on the dorsal row, and 9-16 on the ventral row. Four cortical microstimulation electrodes were tested on a given row in order to maintain consistency between the A-M and A-A testing sessions. On A-M1 sessions the four different probe stimuli were microstimulation delivered through electrode 1, 3, 5, or 7. A-M2 probe stimuli were microstimulation delivered through electrode 9, 11, 13, or 15.

Microstimulation pulse trains consisted of cathodic first, charge-balanced, biphasic square-wave pulses (250 μs pulse width) delivered at 200 Hz and 68 μA. This stimulus intensity was chosen for two reasons. First, a calculated estimation of current spread based on parameters reported in the literature led to minimal effective stimulation radii (100 μm) between neighboring electrodes at 68 μA (Stoney, Thompson et al. 1968; Nunez 1981). Second, in several literature references, 68 μA was a sufficient microstimulation level to ensure that the stimulus was behaviorally robust (Tehovnik 1996; Rousche, Otto et al. 2003). A waveform generator (WaveTek, Everett, WA) was used to generate the pulse train, which was delivered through an optical stimulus isolator (A-M Systems, Carlsborg, WA) in constant-current stimulation mode. The cortical microstimulation stimulus intensity was confirmed using a 1 kΩ resistor circuit prior to testing. The cranial stainless-steel screws served as the stimulation return pathway.

The temporal parameters of the microstimulation were chosen to mimic the temporal envelope of the auditory stimuli. Microstimulation pulse trains were delivered in five bursts (250 ms on, 250 ms off). The behavioral apparatus software recorded responses to both the task stimuli and the probe stimuli. The microstimulation-evoked behavior was evaluated based on BFs of the electrodes as discussed below.

Cortical Microstimulation Control

A control experiment was conducted to ensure that no environmental behavioral cues were affecting behavioral discrimination. In the control experiment the subject was reinforced for correct discrimination of the standard auditory task. Session probe stimuli were constructed with the same parameters and probability as the previous A-M sessions; however, in this experiment the stimulus isolator was turned off, ensuring that the animal could not receive actual cortical microstimulation. Behavioral responses to both the normal auditory discrimination trials and the control probe trials were tabulated and analyzed.

Electrophysiological Recording and Analysis

Electrophysiological recordings in response to auditory stimulation were conducted under anesthesia using the previously described Ketamine/Xylazine/Acepromazine mixture. Anesthetized subjects were positioned on the cage floor in the center of the calibrated environment. Frequency response characteristics of LFPs were determined from 15 logarithmically spaced pure tones ranging in frequency from 1 kHz to 32 kHz delivered at 70 dB SPL. Tone intervals were 100 ms on, 900 ms off. Each frequency was randomly repeated 48 times. All tones had a 5 ms cosine gated rise and fall time.

Electrophysiological data were recorded on a Multichannel Acquisition Processor (MAP) simultaneously for the 16 channels at 40 kHz (Plexon Inc, Denison, TX). The data were filtered and amplified for LFP recording (1-300 Hz, gain = 10000). The responses were analyzed based on a previous study showing that the N1 peak of the LFP responses of the primary auditory cortex of guinea pig are tuned in the same way as single unit responses and exhibit this tuning over long-time periods (Galvan, Chen et al. 2001). The data from the first 50 ms after each stimulus onset were examined for auditory tuning. The LFP data were averaged for each of the fifteen tones and the frequency with the largest negative deflection from the 50 ms mean was determined to be the BF.

RESULTS

In order to quantify microstimulation-induced behavior, both auditory-induced electrophysiological responses and auditory-induced behavior were characterized in four rats over 32 electrodes. The BFs across the electrode array were determined via analysis of auditory evoked LFP fluctuations. Microstimulation-induced behavior was evaluated relative to the BFs of the stimulating electrode and compared to auditory-induced behavior of the same frequency.

Auditory Response Electrophysiology

Recording data consisted of LFP activity evoked by auditory stimuli across four, 16-channel microelectrode arrays implanted in the auditory cortex of the four behaviorally trained rats. An example of stimulus-triggered and averaged data from a single electrode is shown in Figure 3.3. In this example, 1640 Hz produced the largest N1 peak deflection from the mean, and was determined to be the BF. The negative wave exhibits systematic tuning around 1640 Hz. BFs were found spanning nearly the entire spectrum used for auditory stimulation, from 999 Hz to 24983 Hz.

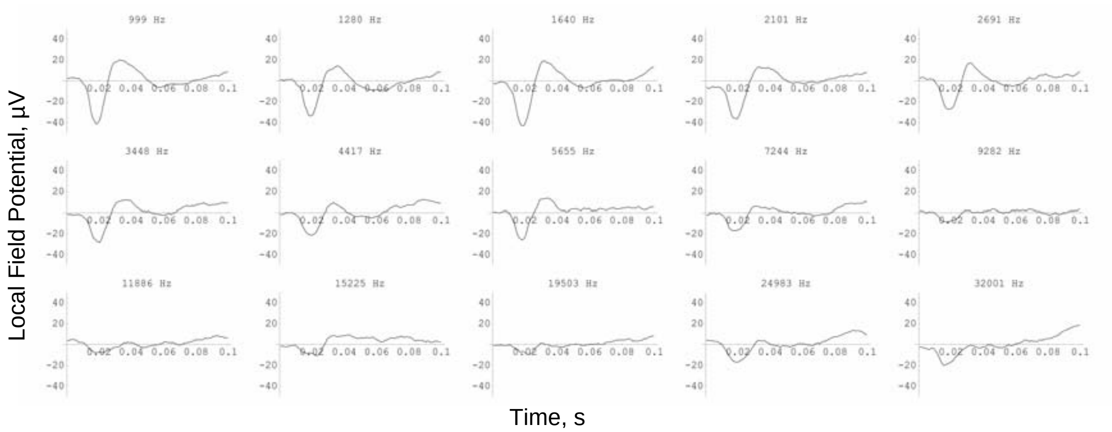

Figure 3.3 Single-channel example of auditory evoked local field potentials

A-A Behavior Results

The rats responded to auditory frequencies intermediate of the 1 kHz and 16 kHz reinforced frequencies in a sigmoidal fashion. For every animal, all response curves increase in an overall monotonic fashion as shown in Figure 3.4, *A-D*.

There is a marked behavioral difference between subjects; however, the behavior within a subject is consistent between testing sessions. This is evident in subject R18, who exhibited frequency-specific behavior over 30 days. Specifically, note the first three post-surgery behavioral sessions (d10, d11, and d12). The subject then completed 16 days of A-M testing. Following the A-M testing sessions, the subject was tested to ensure that the A-A behavior persisted (d28). After another extended period involving A-

M testing, the subject's A-A behavior was again tested (d47) and behavior was consistent with the 4 other testing sessions.

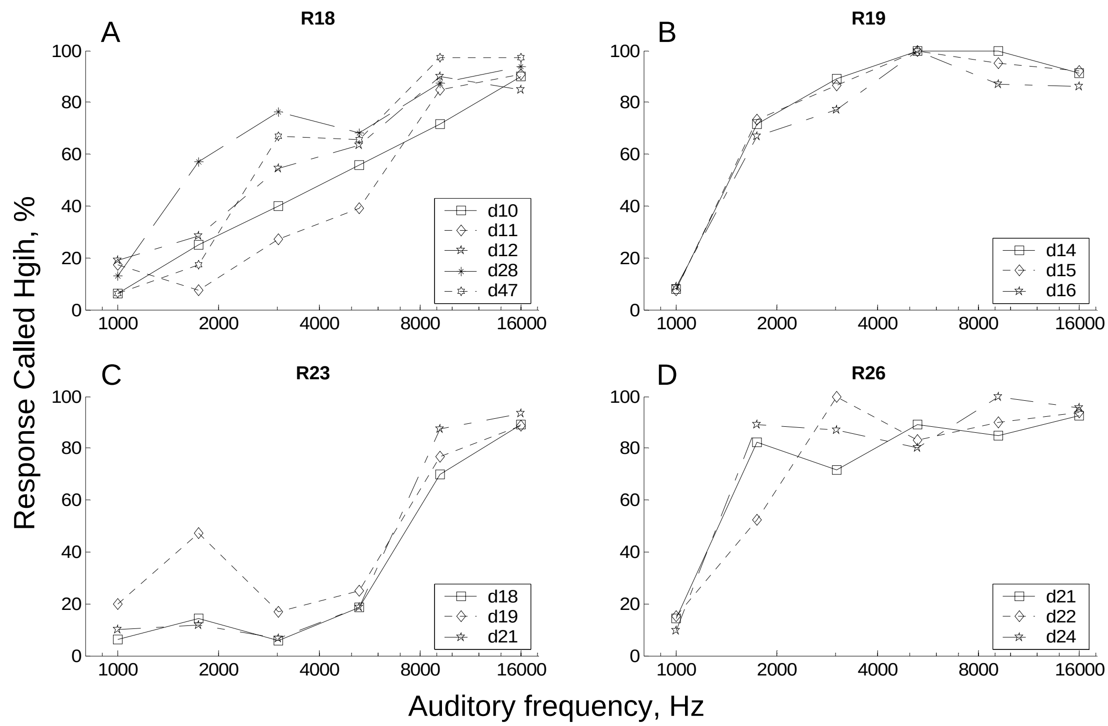

Figure 3.4 Daily auditory probe generalization gradients in each of four rats

A-M Behavior Results

Figure 3.5, *A-D*, shows the A-M results plotted vs. the BF of the electrode used for stimulation. Microstimulation of the different electrodes resulted in behavior that was dependent on the BF of the stimulated electrode. For each subject, averaged results for two or three A-M testing sessions for each electrode array row are plotted. A-M1 and A-M2 refers to stimulation of four electrodes in the dorsal and ventral rows respectively. The A-A results were averaged and plotted for reference to individual rat behavior. In both cases, the error bars indicate the standard error of the measurements.

Microstimulation-induced behavior tended to exhibit more variability between testing sessions relative to the auditory-induced behavior. This is not completely surprising given that the auditory behavior is a result of months of repetition of the same task.

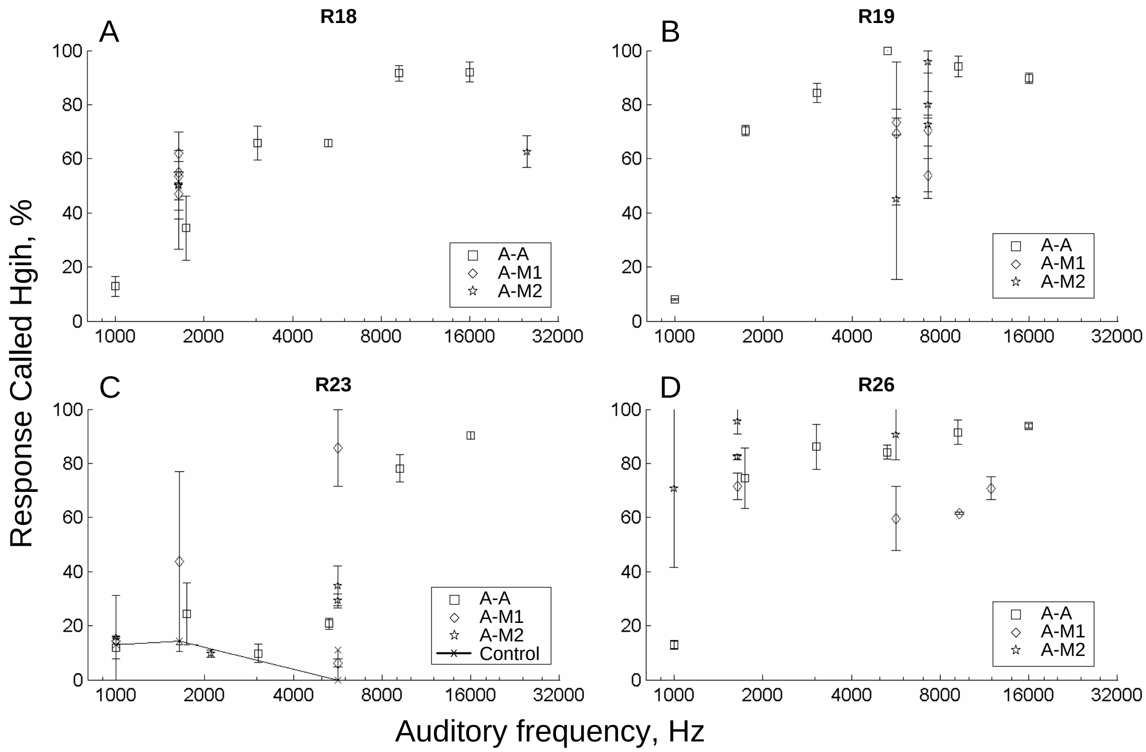

Figure 3.5 Auditory and microstimulation probe response curves for each rat

A control experiment was conducted using subject R23 to ensure that only the electrical stimulation provided behavioral cues. In this experiment, the session was conducted as a normal A-M session, except that the electrical stimulus isolator was turned off, ensuring no microstimulation cues were delivered to the subject. The data are shown as the solid line in Figure 3.5 *C*. The subject responses to any of the control stimuli were not significantly different from each other (t-test, $p<0.05$). This result suggests that there were no behavioral cues besides microstimulation location.

To further evaluate the microstimulation-BF relationship, the data for all four rats were evaluated cumulatively, as shown in Figure 3.6. The open triangles represent microstimulation-induced behavioral data for all electrodes with BFs between 1 kHz and 16 kHz. The data are plotted on a semi-log scale and a linear regression is shown as the dotted line ($R=0.24$, $p<0.05$). The regression shows a positive correlation between microstimulation behavior and BF of the electrode used for stimulation. For comparison, the auditory data for all rats are plotted cumulatively as the solid diamonds. These data show the trained positive relationship in the regression between auditory behavior and frequency ($R=0.74$, $p<10^{-14}$).

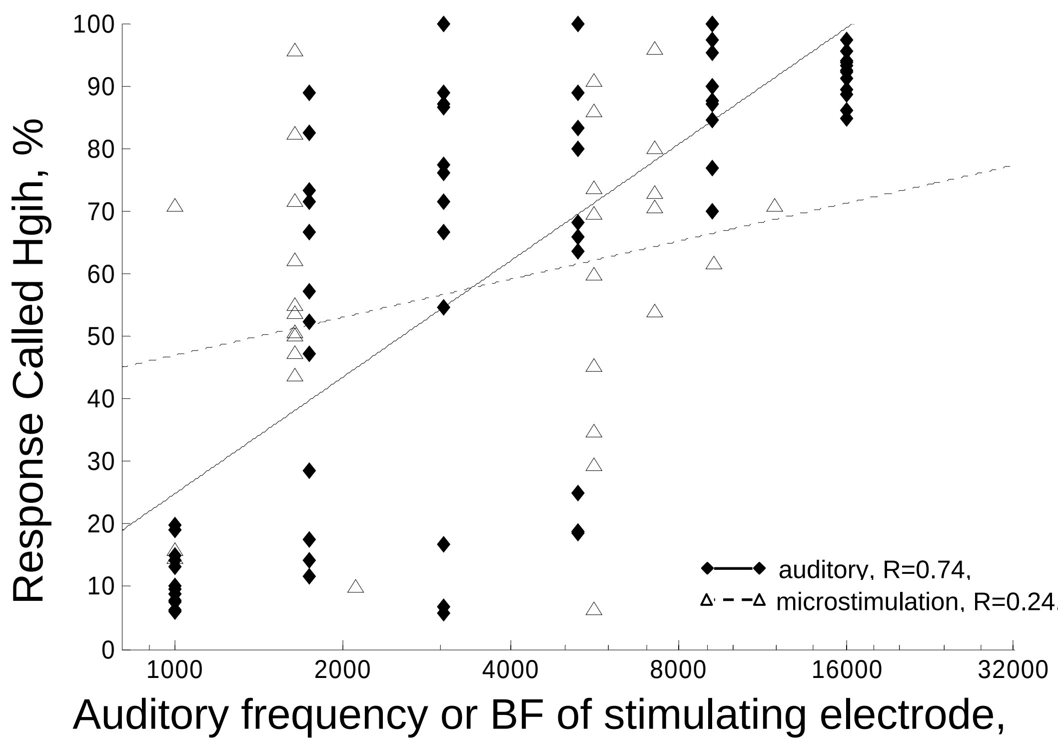

Figure 3.6 The relationship between the auditory and stimulation evoked responses

DISCUSSION

The objective of this study was to investigate the behavioral effects associated with penetrating electrical activation of the auditory cortex in the adult rat model. Subjects were required to behaviorally respond to cortical microstimulation probe trials randomly inserted between trials of an auditory frequency discrimination task. The microstimulation-induced responses were analyzed relative to each microelectrode's auditory electrophysiology characteristics determined via LFP recording.

The Sensation Basis of Auditory Cortex Stimulation

Animal stimuli-detection behavior in response to penetrating visual cortical stimulation was first evaluated by Bartlett and Doty in the primate (Bartlett and Doty 1980). Subsequently, Rousche assessed stimuli-detection behavior in response to auditory cortical stimulation first in the cat model (Rousche and Normann 1999) and later in the rat model (Rousche, Otto et al. 2003). These studies show that subjects readily respond to single electrode stimulation in a sensory detection paradigm. Recently, Scheich and Briendl (Scheich and Breindl 2002) evaluated penetrating auditory cortex stimulation in a discrimination paradigm in the Mongolian gerbil. The gerbils were able to discriminate spatial, temporal, and spatio-temporal patterns of two electrode stimulation. In the study reported here, an evaluation of the spectral dependence of the auditory sensations evoked by single electrode microstimulation in different spatial locations within auditory cortex has been determined.

The results in Figure 3.6 show that a component of the elicited sensations was positively correlated with increasing BFs in the auditory cortex. This is consistent of analogous visual cortex studies that show systematic variation of sensation with microstimulation location. Both Brindley and Lewin and Dobelle et. al reported that surface visual cortex stimulation produced phosphenes that roughly corresponded to classical map expectations (Brindley and Lewin 1968; Dobelle and Mladejovsky 1974).

Temporal Stimulation Parameters

The electrical stimulus pulse train implicated in this study was designed to match the temporal envelope of the trained auditory stimulus train. Using surface stimulation of visual cortex, Dobelle reported 10-15 s of continuous stimulation before the elicited phosphenes faded (Dobelle and Mladejovsky 1974). Using penetrating stimulation, Schmidt et. al reported a phosphene duration of 930 ms before the sensation faded (Schmidt, Bak et al. 1996). Dobelle employed 1 s pulse trains for surface auditory cortex stimulation, and did not publish any subject reports of the auditory sensation fading (Dobelle, Stensaas et al. 1973). The electrical stimulation used in study consisted of five 250 ms pulse trains (with 250 ms inter-train intervals). The pulse trains used here are well below the train lengths that led to fading sensation in the human studies and it is probable that the sensation persisted for the full 250 ms. Schmidt et al. noted an accommodation to repeated stimulation that decreased the brightness of the phosphenes produced (Schmidt, Bak et al. 1996). Over 5 repetitions the intensity dropped 20-30%. Our stimulus trains may have resulted in accommodation, which likely would have decreased the loudness of the perceived sensation.

It is well accepted that auditory cortex neurons typically exhibit “on” responses to stimuli; however, whether this feature can be captured via microstimulation using a modulated pulse rate has yet to be investigated. In primate somatosensory cortex, Romo et. al reported identical behavioral responses to periodic and aperiodic cortical microstimulus trains of the same mean frequency (Romo, Hernandez et al. 1998). This result suggests, at least for the train durations and stimulation modulation rates investigated in that study, that varying the frequency of stimulation may not convey additional stimulus information.

Spatial and Temporal Pattern of Microstimulation

Sensory stimulus encoding occurs in distributed spatial and temporal patterns (Villa and Abeles 1990; Chapin and Nicolelis 1999; Furukawa, Xu et al. 2000). Given this characteristic, current microstimulation paradigms are not technologically able to produce stimulus equivalency between natural auditory and electrical stimulation. However, for applications to cortical neuroprosthetic design, auditory and electrical stimulus equivalency may not be necessary. Cochlear implant patients show encouraging plasticity of neural representations as the brain adapts to use the alternate auditory information (Rauschecker and Shannon 2002).

Based on tissue conductivity values reported in the literature, the volume of tissue affected by cortical microstimulation at 68 μA will include neurons within 100 μm from the electrode site (Stoney, Thompson et al. 1968; Tehovnik 1996). Due to this volume, LFP recordings were chosen preferentially to single-unit recordings in order to determine BFs of the tissue local to the electrode. Recently, Galvan et al. reported long term frequency of tuning of LFPs in auditory cortex of guinea pig (Galvan, Chen et al. 2001). Our recordings in auditory cortex of the rat produced tuning curves from 999 Hz to

24983 Hz. This is slightly lower than the upper ranges of ~40 kHz found by Sally and Kelly (Sally and Kelly 1988); however, most of the responses > 30 kHz found by Sally and Kelly were proximal, or even anterior to the large anterior dorsoventral vessel in primary auditory cortex. Our electrode arrays were intentionally implanted significantly caudally of this vessel in order to avoid vascular injury. This may have biased our electrode placement in the lower frequency ranges. Additionally, the lower frequency representations of our rats may have been expanded due to training and microstimulation-induced plasticity, as discussed below.

Cortical Reorganization

Due to the increasing probability of failure of the microelectrode array with time, implantation did not occur until after the rats were successfully trained in the 1 kHz/16 kHz discrimination task. It has been shown that behavioral training alters the representation of sensory stimuli in cortex (Recanzone, Merzenich et al. 1992; Recanzone, Schreiner et al. 1993; Bakin, South et al. 1996; Suga, Xiao et al. 2002). Furthermore, cortical microstimulation has been shown to change the functional organization of cortex (Nudo, Jenkins et al. 1990; Dinse, Recanzone et al. 1993; Maldonado and Gerstein 1996; Chowdhury and Suga 2000; Talwar and Gerstein 2001). In the current study training was conducted for several months at 1 kHz and 16 kHz. Additionally, several sessions of microstimulation were conducted before BF measurements were made. Our electrophysiological results show an over-representation of the lower frequencies, with 97 % of the stimulated electrodes having BFs of 16 kHz or lower.

The expanded cortical representations of the lower frequencies may have influenced the results of the microstimulation-induced behavior. The results from Figure 3.6 show a lower correlation of microstimulation-induced behavior with BF, relative to the correlation of the auditory-induced behavior with frequency. If, in fact, the frequency representation within auditory cortex was distorted, this may have tended to "wash-out" the strength of the BF-dependent microstimulation behavior.

Anesthetized vs. Awake Recordings

This study determined auditory best frequencies under anesthesia. Recordings were not conducted in the awake state due to the small amplitudes of LFP recordings, and the recording artifact induced by movement; however, Talwar et. al reported that BFs of single units did not change from awake to anesthetized states in rat auditory cortex (Talwar and Gerstein 2001).

Significance

The long term goal of this research is to develop brain-machine information channels in auditory cortex with a primary application in an auditory cortical prosthesis. Independent parameters available for information transmission need to be investigated for optimal operation of such a device. In this study we investigated the role of auditory cortical location as an information carrying parameter. To be a plausible information coding strategy, different locations within cortex must invoke independent sensations. Further, information coding can be optimized through the elucidation of the relationship between the stimulus location and the sensation evoked. The results here indicate that there are various sensations evoked by penetrating microstimulation in different locations within the auditory cortex. Further, these variations show a positive correlation with the auditory-evoked neurophysiology of the tissue local to the electrodes. These results validate further research on auditory cortical stimulation location based encoding of information in an auditory cortical prosthesis.

One issue that has not been addressed in this study is the resolution achievable for different sensations within the auditory cortex. It is certain that there is a limit to how closely spaced stimulating electrodes can be placed to invoke different sensations. Additionally, it is still not clear how salient the microstimulation cues are in a behavioral context. The degree that these cues are prominent in a sensory context and can be interpreted and utilized by the subject needs more investigation. In the next chapter, experimental studies are presented that investigate both a higher resolution of microstimulation and the saliency of the microstimulation compared with the natural auditory stimulation.

CONCLUSION

In conclusion, the data suggest that cortical microstimulation in different locations in auditory cortex provides a behaviorally relevant auditory sensation. Furthermore, the behavior evoked by this sensation is dependent on the best frequency of the tissue local to the implanted electrode. In order to quantify the information-carrying capacity of chronically implanted electrodes in the auditory cortex for a brain-machine interface, more parameters require investigation. Putatively, more knowledge of the sensations elicited by penetrating electrical stimulation of sensory cortex would allow for better engineering of the stimulus and electrodes to increase the information transfer. In our studies, the subject was not required to respond to other components of the stimulus, (for example, bandwidth, background stimuli, and temporal parameters) the sensation can not be fully described; however, this study validates further exploration of the electrical stimulus perceptual parameters of penetrating auditory cortical stimulation.

CHAPTER 4

THE RESOLUTION AND SALIENCY OF AUDITORY INTRACORTICAL MICROSTIMULATION

ABSTRACT

Intracortical electrical activation of the auditory cortex has been shown to provide a behaviorally salient cue; however, the ability of this cue to replace natural auditory cues is largely not known. Furthermore, behavioral salience is indirectly related to the discernable spatial resolution of intracortical microstimulation in auditory cortex. Thus it has direct implications for design and development of a cortical prosthesis. This study examines the behavior of subjects utilizing penetrating cortical microstimulation to complete behavioral tasks. Two separate behavioral experiments were conducted. The first set consisted of four rats trained on an auditory tone detection task. The second set consisted of five different rats trained on an auditory frequency discrimination task. Both sets of rats were then implanted with chronic microwire arrays in the auditory cortex of the left hemisphere. The two sets of rats were then required to either detect or discriminate cortical microstimulation respectively. Microstimulation resulted in superior performance in both the detection task (32.9%, $p<0.05$), and the discrimination task (38.9%, $p<10^{-9}$). Further, microstimulation as closely spaced as 250 μm produced significantly different behavior. These results are consistent with recent reports that sensory cortical microstimulation cues provide robust, salient cues in a behavioral setting.

Additionally, these results have strong implications for the feasibility and engineering of a cortical sensory prosthesis.

INTRODUCTION

In the past two chapters we have demonstrated the use of penetrating cortical microstimulation in the rat as an effective model for the validation and investigation of an auditory cortical prosthesis. Further, we have demonstrated results that are of interest to the field of neurophysiology, particularly in the areas of sensory physiology and neural coding. These results lead to more physiology and engineering questions that can be addressed with this preparation and animal model: What is the behavioral saliency of single-electrode cortical microstimulation? And, given the behavioral saliency of microstimulation, what is the corresponding trade-off between information transfer and spatial resolution (inter-electrode distance)?

Several investigators have shown that electrical stimulation of the brain can produce extremely potent behavioral effects. In 1954 Olds reported that stimulation of the septal area resulted in positive reinforcement (Olds and Milner 1954). Also in 1954, Delgado reported stimulation of the brain that motivated learning (Delgado, Roberts et al. 1954). These reports, along with several others, demonstrated that stimulation of the brain can have extreme behavioral saliency, as much, or more so than natural behavioral cues and settings. These findings rationalize investigation of the behavioral significance of penetrating sensory cortical microstimulation.

Recently, several investigators have shown that penetrating sensory cortical microstimulation can provide behavioral cues. In 1990, Salzman et. al demonstrated that penetrating cortical microstimulation of the primate visual motion processing cortex biased perceptual decisions in a motion discrimination task (Salzman, Britten et al. 1990). In 1998 Romo et. al showed that penetrating cortical microstimulation of primate somatosensory cortex provided an strikingly robust sensory signal during the performance of a vibrotactile discrimination task (Romo, Hernandez et al. 1998). In 2002 Talwar et. al showed that penetrating cortical microstimulation of the rat somatosensory cortex could provide directional cues in a navigation task (Talwar, Xu et al. 2002). Further, Scheich et al. showed that penetrating auditory cortical microstimulation provided discriminable cues to a gerbil in an avoidance task (Scheich and Breindl 2002). All of these reports attested to the strength and repeatability of the microstimulation-induced behavior. However, none of the experimenters provided data comparing natural and stimulus evoked behavior.

There have been few reports of the spatial resolution that is achievable with cortical microstimulation. Using surface stimulation, requiring much higher current that results in a larger stimulus volume, Brindley and Lewin reported that subjects could distinguish phosphenes produced by electrodes spaced 2.4 mm apart (Brindley and Lewin 1968). Consistent with those findings, Dobelle and Mladejovsky reported that the limit of resolution in their patients was near 3 mm (Dobelle and Mladejovsky 1974). These estimates are consistent with electrophysiological studies in cats showing that the radius of neural activation for stimulation of 0-10 mA is 1-2 mm (Ronner, Foote et al. 1980).

Thus, it is approximated that the subjects reported by Brindley and Dobelle are only able to discern non-overlapping stimulation radii. This is a major design limitation for a cortical neuroprosthesis.

Intracortical microstimulation of sensory cortex allows for super-threshold sensations at much lower current levels than surface stimulation. Several previous studies have reported typical thresholds of sensation for cortical surface stimulation in the mA range (Penfield and Rasmussen 1950; Brindley and Lewin 1968; Dobelle, Stensaas et al. 1973; Dobelle and Mladejovsky 1974). The sensory threshold for penetrating cortical stimulation is 2-3 orders of magnitude lower than surface values, in the µA range (Bartlett and Doty 1980; Bak, Girvin et al. 1990; Schmidt, Bak et al. 1996). These lower stimulation amplitudes result in more compact stimulation volumes, enabling a greater number of non-overlapping channels for a cortical neuroprosthesis. Schmidt et. al reported that subjects could discern penetrating stimulation of visual cortex as close as 700 µm (Schmidt, Bak et al. 1996). This value is surprisingly large, given volume conductor models and other stimulation recording results (Stoney, Thompson et al. 1968; Nunez 1981; Tehovnik 1996). These results indicate that the stimulation used by Schmidt et. al activated a volume of approximately 100-200 µm. The issue of how closely electrodes can be placed to elicit independent sensations needs further exploration in a carefully conducted psychophysical study.

Results from optical imaging studies show that suprathreshold auditory tones activate a large, non-uniform spatial area within auditory cortex (Bakin, Kwon et al. 1996). Areas as large as 2 mm were shown to be activated by 50 dB tones. Electrical microstimulation of 70 µA activates tissue within approximately 100 µm. Thus, stimulation of auditory cortex with intracortical microelectrodes activates a more confined area of cortex compared with natural auditory stimulation. These findings have implications regarding the saliency and resolution that can be achieved in an auditory cortical prosthesis.

The objective of this study was to investigate the behavioral saliency of single-electrode cortical microstimulation of the auditory cortex. To accomplish this objective, we examined the behavior evoked by microstimulation location within a carefully designed psychophysical testing paradigm and compared the performance to that of an auditory behavioral task.

METHODS

Detection and Discrimination Training

Four male, naïve Sprague-Dawley rats (250 g - 300 g) were trained in an auditory detection task. Additionally, five rats were trained in an auditory discrimination task. Initially, the rats were food deprived to 80% of their free-feeding weight. Subjects responded in standard operant conditioning behavioral boxes (Med Associates, St. Albans, VT) located within an anechoic chamber. The response wall of the test box included three side-by-side retractable response levers approximately 4” above the cage floor. A house light at the rear of the box was utilized for both illumination and negative reinforcement. The behavioral apparatus was controlled and monitored by software developed in-house, running on a PC interfaced with digital input-output hardware (System II, Tucker-Davis Technologies, Gainesville, FL). This equipment was also used to generate all auditory stimuli used in the experiment. The auditory stimuli were delivered via a speaker (Yamaha NS-10M Studio, Yamaha Corporation, Buena Park, CA) located 1 m directly above the test box. The system delivered a near-flat frequency response between 500 Hz and 32 kHz.

Detection and discrimination experiments were conducted identically, with respect to all task parameters except the nature of the stimuli. A forced-choice psychophysical paradigm was used to assess task performance and stimulus generalization. Subjects were positively reinforced via single food pellets (P.J. Noyes, 45 mg rodent diet I, Lancaster, NH) for correct responses to trains of auditory stimuli. Initially, all three levers were retracted, and the house light was illuminated. The subjects were signaled to start a single trial by the extension of the center lever. Trials were subject-initiated by two recorded presses of this center response lever. Subsequently, the center lever was retracted, and the trial stimulus was delivered. In the detection task, presence trials consisted of auditory stimuli trains at 16 kHz delivered at 70 dB SPL. In the discrimination task, auditory training stimuli trains at either 1 kHz, or 16 kHz were delivered at 70 dB SPL. Tones were delivered in a train (250 ms on, 250 ms off) of five bursts. Upon completion of the trial stimulus presentation, the two outer levers were extended. A fixed-ratio (FR4) response paradigm was utilized, and subjects were reinforced after four responses on a given lever within 7 s of outer lever presentation. Responses were designated correct and positively reinforced for a left lever response to the null or 1 kHz stimulus in the detection and discrimination tasks respectively, or a right lever response to the 16 kHz stimulus in both tasks. A response was considered null, and a 30 second dark time-out punishment delivered, if the subject did not respond within the 7 s response window. Null response trials in all of the training or testing were rare (zero for > 95 % of the sessions) and were not used in the behavioral data analysis.

Hit rate and false alarm rate were calculated identically for the detection and discrimination experiment. Hit rate in the detection task is calculated as the percent of stimulus present trials answered correctly. The false alarm rate for the detection task is the percent of stimulus absent trials answered incorrectly. Hit rate in the discrimination task is calculated as the percent of 16 kHz trials answered correctly, and false alarm rate as the percent of 1 kHz trials answered incorrectly.

In order to quantify daily subject performance, signal detection theory (Green and Swets 1966) was used to calculate a session discriminability index, d':

$$d' = z(h) - z(f) \qquad (4.1)$$

with z expressing the z-score of the argument, h expressing the hit rate, and f expressing the false alarm rate. The parameter d' is useful in its ability to analyze behavior independent from bias effects. Figure 4.1 shows an idealization of the data and the method to calculate d'.

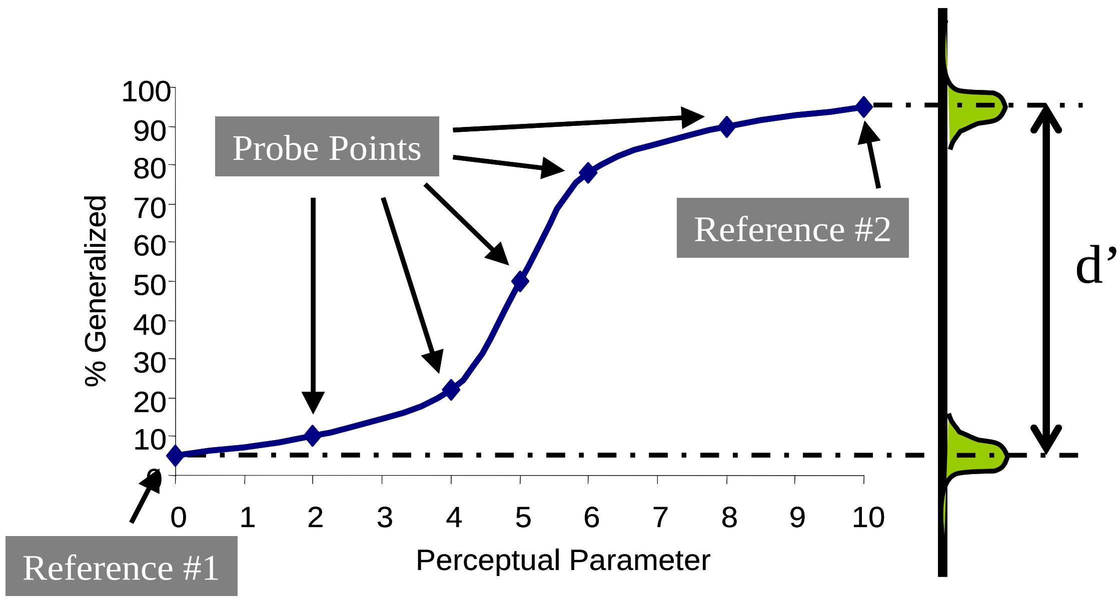

Figure 4.1 The form of generalization data and the schematic calculation of d'

Auditory Generalization Behavioral Testing

The discrimination trained rats underwent auditory frequency generalization testing. After criterion performance of the auditory training paradigm (above 90% for three consecutive days), psychophysical curves were created to assess auditory generalization behavioral performance. The standard auditory cues were delivered for 75% of the trials; however, in approximately 25% of the trials the behavior was assessed for stimuli of intermediate auditory frequencies. These trials are henceforth referred to as probe trials, as shown in figure 4.1. The probe trials were never reinforced. Four intermediate auditory probe frequencies were chosen from tones spaced evenly on a logarithmic scale (1740 Hz, 3030 Hz, 5280 Hz, and 9190 Hz). Daily testing sessions continued until each subject received 200 positive rewards. Approximately 275 daily trials were completed.

Electrode Implantation and Neural Recording

After successful auditory detection and discrimination training, each rat was chronically implanted with an array of microelectrodes. Electrode arrays were identical to the arrays used in Chapters 2 and 3, and details of multi-electrode construction, implant procedures and recording performance are fully described in detail in another publication (Williams et al., 1999). Briefly, 16 channel electrode arrays were fabricated in-house using 50 μm polyimide-insulated tungsten wire aligned in rows of 8 wires each terminating in a small connector (GF-10, Microtech Inc., Boothwyn, PA) (inter-row spacing = 250 μm, inter-electrode spacing = 250 μm). Ethylene-oxide sterilized arrays were implanted using a micromanipulator under aseptic surgical conditions. Vascular landmarks and/or stereotaxic coordinates were used to identify the primary auditory cortex (Sally and Kelly, 1988). Neural recordings from the implants were used to assess electrode response to pure tone, click or light flash stimuli for several weeks following recovery. Recordings were performed in awake animals. Signals were simultaneously amplified, bandpass filtered (500 - 7000 Hz), and displayed with a commercial multi-channel neural recording system (Plexon, Inc., Dallas, TX, see Appendix A). Peri-stimulus time histograms (Nex software, Plexon, Inc., Dallas, TX) were used to characterize auditory neural activity in response to 50 μsec clicks (100 dB). All experimentation was performed under the guidance of the Institutional Animal Care and Use Committee of Arizona State University.

Microstimulation Behavioral Testing

After surgical recovery (approx 1.5 weeks), the rats were then trained to detect microstimulation of a single-electrode or to discriminate microstimulation of electrodes spaced 1.75 mm. Microstimulation pulse trains consisted of cathodic first, charge-balanced, biphasic square-wave pulses (250 μs pulse width) delivered at 200 Hz and 68 μA. This stimulus intensity was chosen based on a calculated estimation of current spread based on parameters, reported in the literature, that led to a minimal effective stimulation radii (100 μm) between neighboring electrodes at 68 μA (Stoney, Thompson et al. 1968; Nunez 1981). The estimated minimal and maximal stimulation radii are displayed in Figure 4.2. A waveform generator (WaveTek, Everett, WA) was used to generate the pulse train, which was delivered through an optical stimulus isolator (A-M Systems, Carlsborg, WA) in constant-current stimulation mode. The cortical microstimulation stimulus intensity was confirmed using a 1 kΩ resistor circuit prior to testing. The cranial stainless-steel screws served as the stimulation return pathway. The temporal parameters of the microstimulation were chosen to mimic the temporal envelope of the auditory stimuli. Microstimulation pulse trains were delivered in five bursts (250 ms on, 250 ms off). The behavioral apparatus software recorded responses to both the task stimuli and the probe stimuli. The signal detection theory based behavioral parameter d’ was calculated according to equation 4.1.

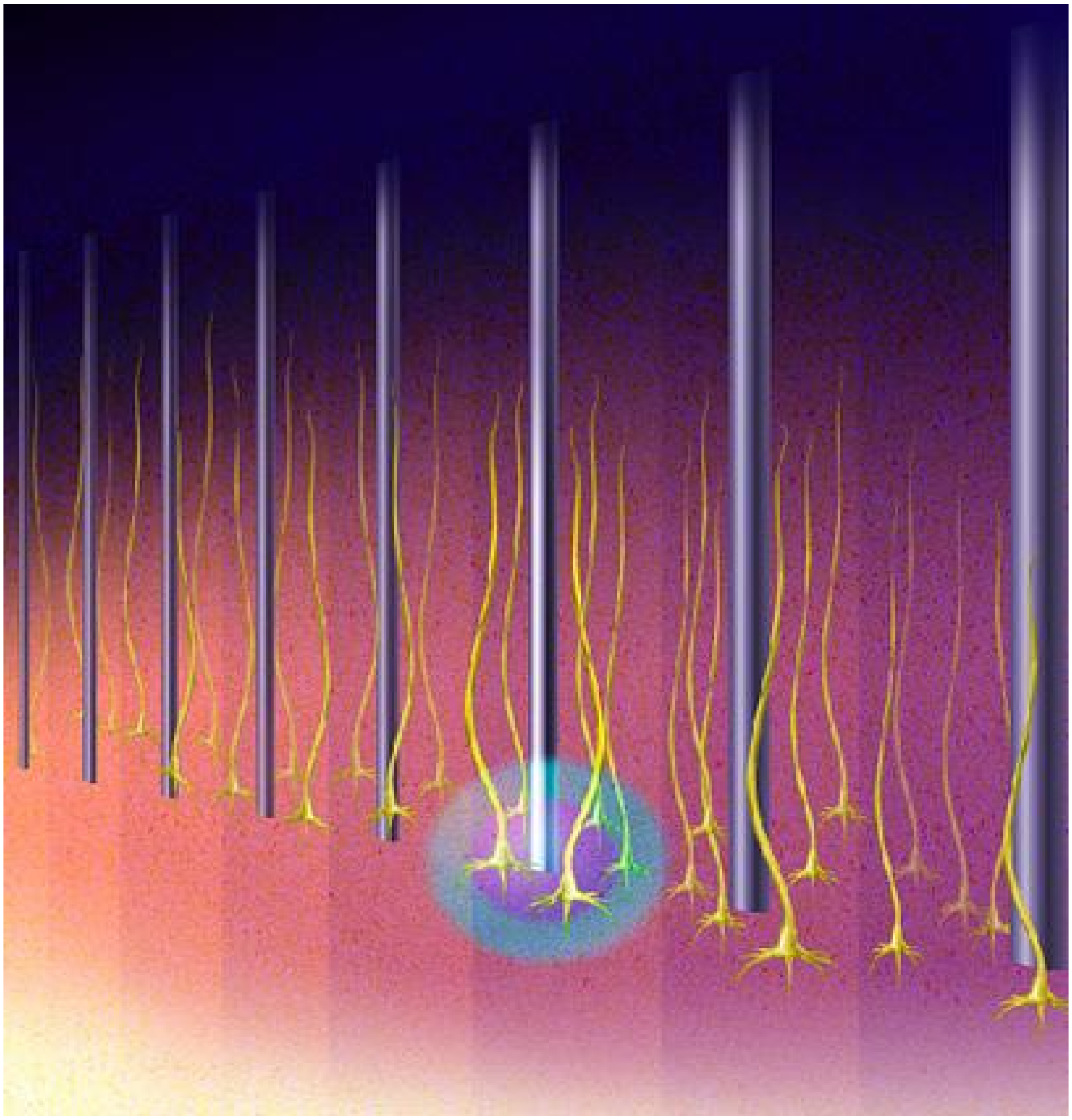

Figure 4.2 A schematic representation of the calculated upper and lower radii of stimulation. The electrodes are separated by 250 microns.

The discrimination set of rats was tested in a psychophysical fashion to elucidate how varying the location of the stimulus on another electrode in the array affected the behavior. In 75% of the trials, microstimulation was delivered on the 1.75 mm separated electrodes, indicating left or right behavioral cues respectively. However, in the generalization testing paradigm, approximately 25% of the trials were randomly chosen as “probe” trials in which stimuli of were presented on one of the intermediate electrodes in the row. The probe trials were never reinforced. The rats’ generalization behavior in response to four probe electrodes was tested daily. Testing sessions continued until each subject received 200 positive rewards. Due to the random trial nature of the probe presentation in the experiment, the exact number of daily trials varied, but was on average approximately 275. Daily testing sessions lasted approximately 80 min.

RESULTS

In order to quantify the saliency of auditory cortical microstimulation in a behavioral setting, psychophysical tests were conducted and performance was evaluated relative to identical auditory-evoked behavior.

Auditory Response Electrophysiology

Recording data consisted of single-unit and multi-unit activity evoked by auditory stimuli across the 16-channel microelectrode arrays implanted in the auditory cortex of the behaviorally trained rats. An example of stimulus-triggered peri-event histograms tabulated from data across the electrode array is shown in Figure 4.3. In this example, 16 out of the 22 units displayed significant responses to the onset of the auditory stimulus. The latencies indicate that the electrode arrays are positioned within primary auditory cortex.

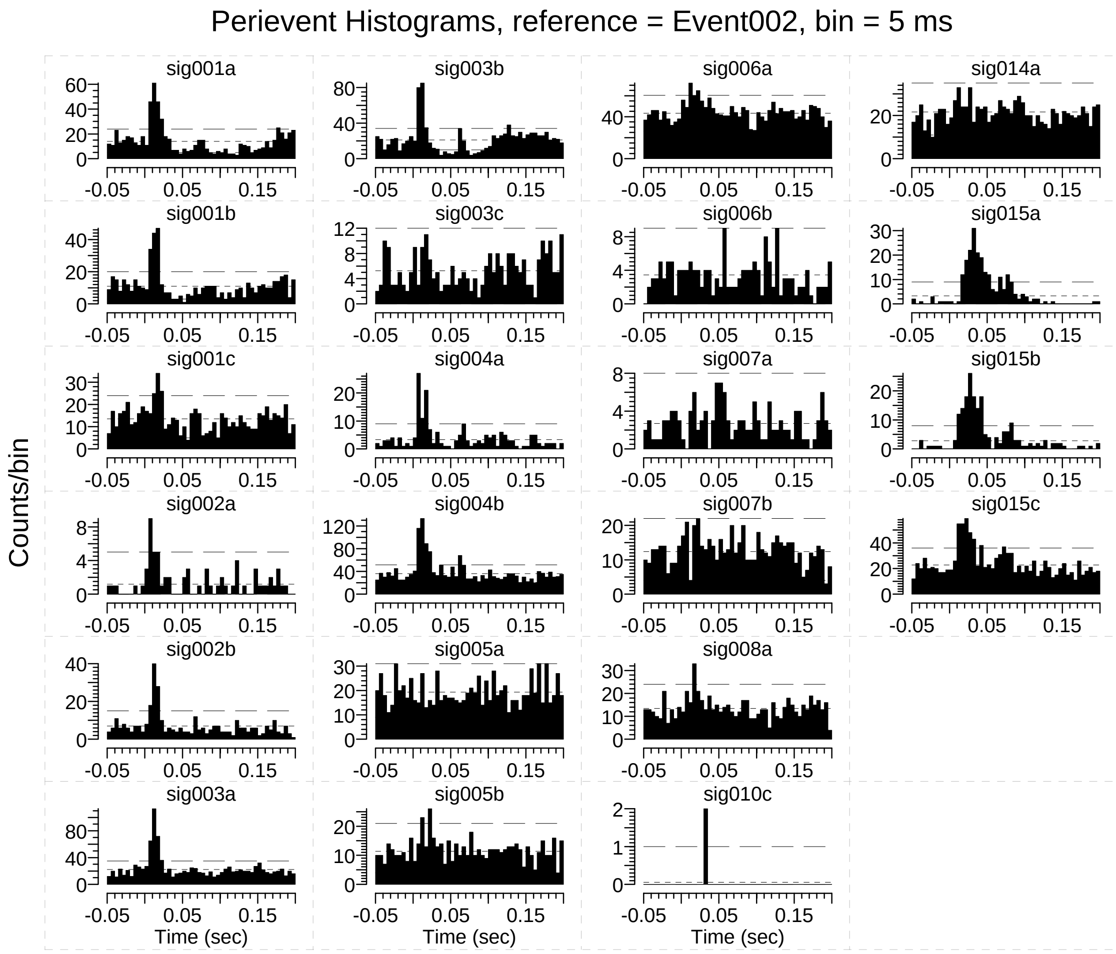

Figure 4.3 Peri-stimulus time histograms simultaneously recorded from one rat

Auditory Frequency Generalization

The discrimination trained rats generalized frequency normally. Figure 4.4 shows a box and whisker plot of the cumulative results from the five discrimination trained rats. Data are taken from three testing sessions for each rat. The percent of trials generalized as the 16 kHz stimulus is plotted relative to the frequency of auditory stimulation. The results show a monotonic increase of generalization with frequency.

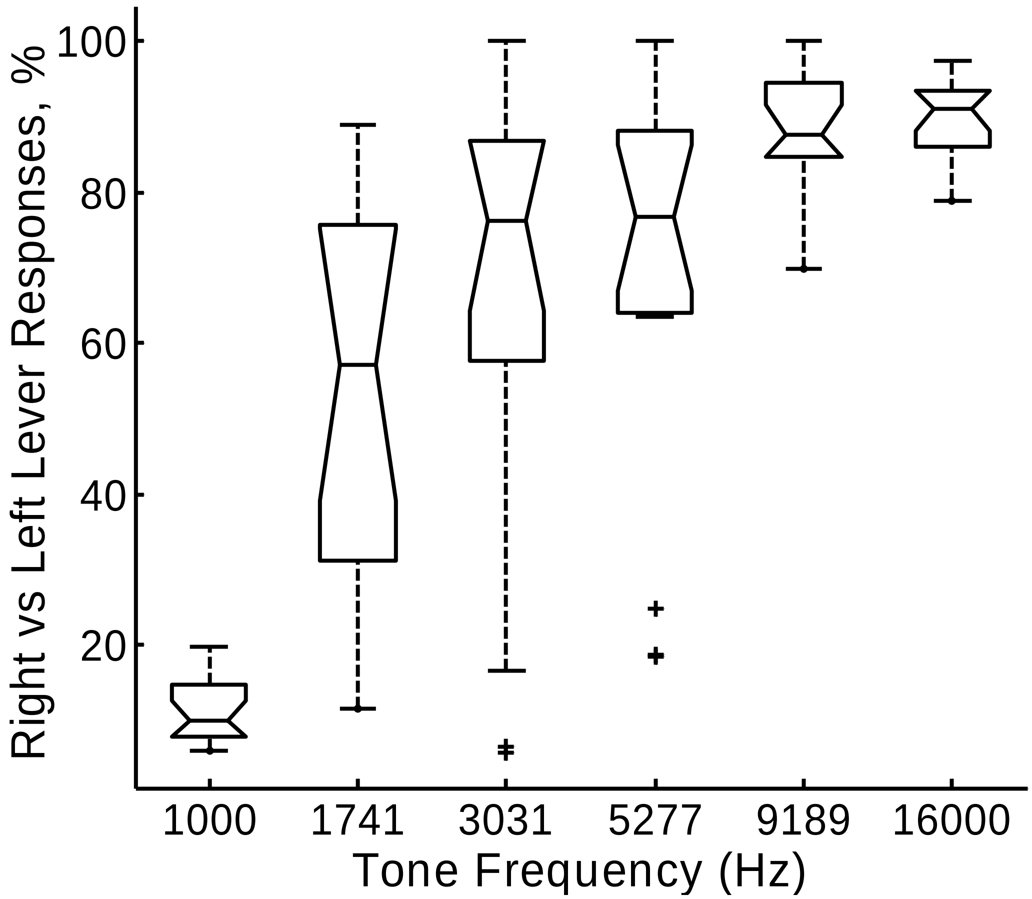

Figure 4.4 Cumulative auditory frequency generalization of five rats

Microstimulation Location Generalization

The discrimination trained rats generalized microstimulation location in a normal psychophysical fashion. Figure 4.5 shows a box and whisker plot of the cumulative results from the five discrimination trained rats. The percent of trials answered on the right vs. the left lever is plotted relative to the location of microstimulation within the electrode array. The results show a monotonic increase of generalization with location of microstimulation within the electrode array.

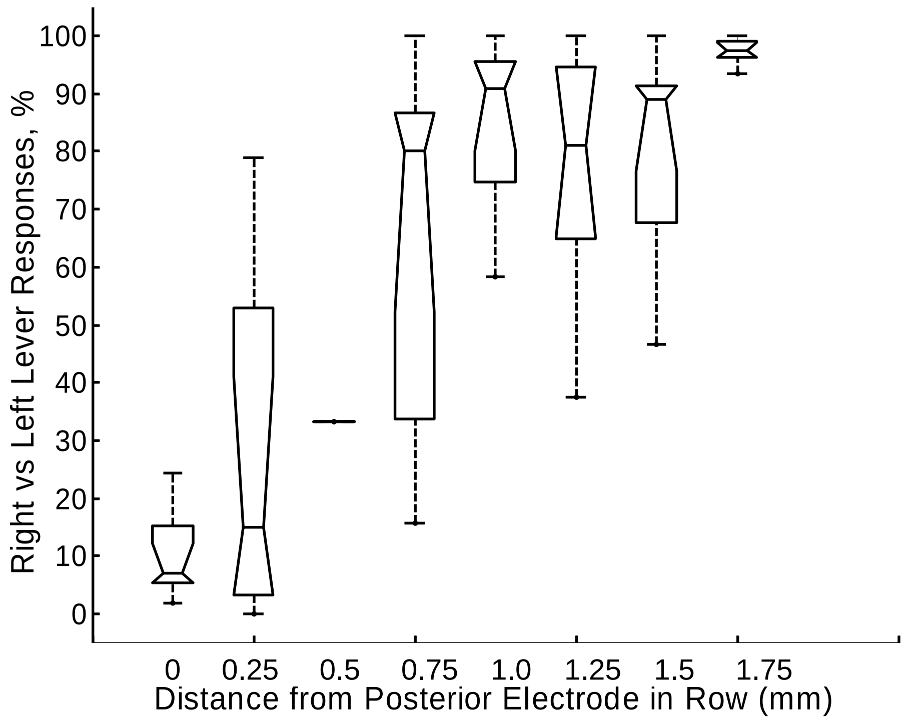

Figure 4.5 Cumulative microstimulation location generalization of five rats

Performance of Auditory and Microstimulation Detection

The psychophysical parameter d' for the auditory detection data and the microstimulation detection data was calculated as the difference of the z scores of the hit rate and false alarm rate as shown in Figure 4.1. The results for four rats in auditory detection vs. microstimulation detection are shown as Figure 4.6. In 3 of the 4 cases the microstimulation d' was significantly higher than the auditory d'. There was a 32.8 % overall increase in performance of the microstimulation relative to the auditory cues (t-test, $p<0.05$).

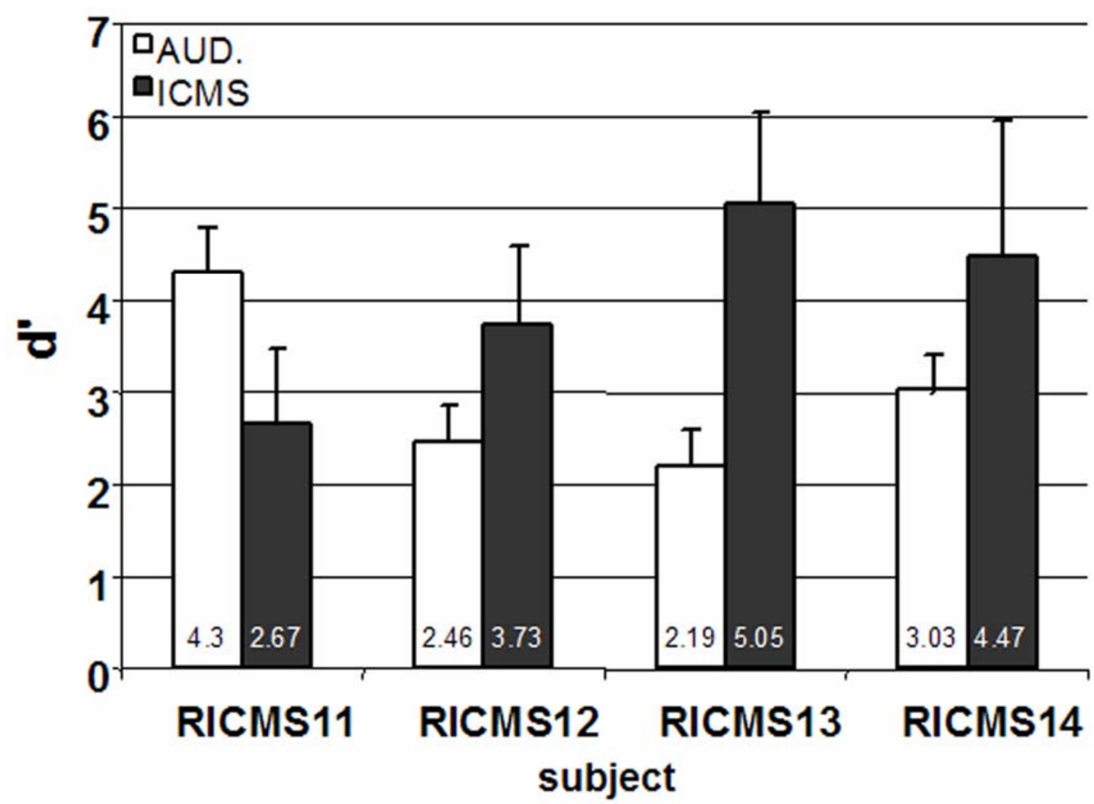

Figure 4.6 Behavioral comparison of detection of auditory and microstimulation cues

Performance of Auditory and Microstimulation Discrimination

The psychophysical parameter d' for the auditory detection data and the microstimulation detection data was calculated as the difference of z scores as shown in Figure 4.2. The results for five rats for auditory discrimination vs. microstimulation location discrimination are shown as Figure 4.7. In all of the cases the microstimulation d' was significantly higher than the auditory d' ($p<0.05$). There was a 38.3 % overall increase in performance of the microstimulation relative to the auditory cues (t-test, $p<10^{-8}$).

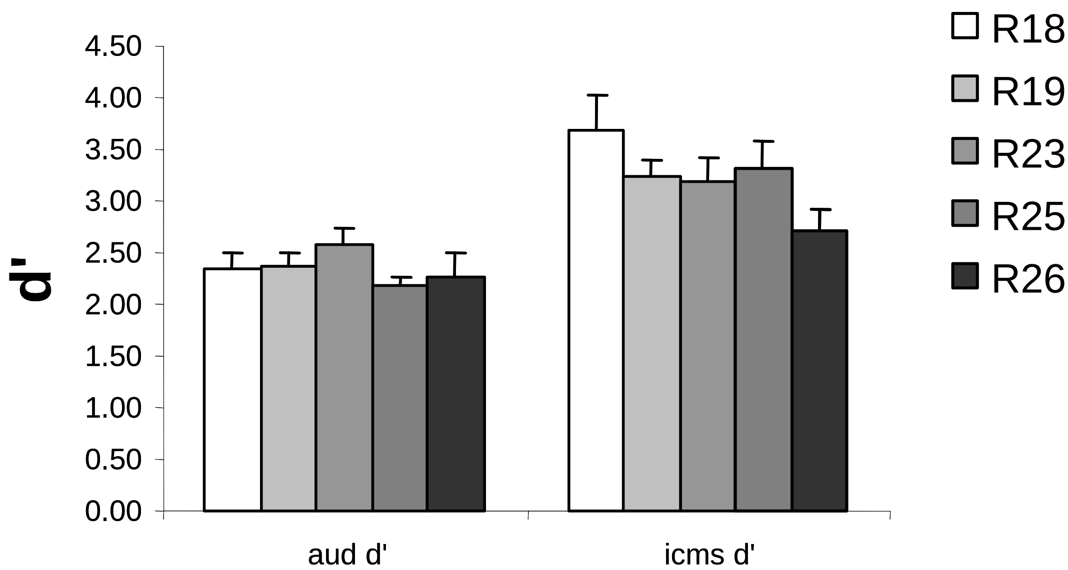

Figure 4.7 Comparison of auditory and microstimulation discrimination

DISCUSSION

The objective of this study was to investigate the behavioral effects associated with penetrating electrical activation of the auditory cortex in the adult rat model. Subjects were first trained in either an auditory detection task, or an auditory discrimination task. These subjects were then required to behaviorally respond to auditory cortical microstimulation instead of the natural auditory cues.

Relative Saliency of Cortical Microstimulation

In both groups of rats, the auditory training required several months to achieve satisfactory performance (~90% overall correct). Thus, the d’ results for the auditory performance for both the detection and the discrimination are “peak” performance levels after daily training conducted for months. For the detection of microstimulation, usually only one or two days of reinforced microstimulation was required to achieve or surpass this level of performance. Subsequent sessions then maintained and exceeded this performance as shown in Figure 4.6.

These results were even more evident for the discrimination trained rats. Interestingly, on the surely more complex discrimination task, all five rats were able to discriminate the microstimulation cues on the first session of microstimulation, resulting in better performance in all 5 rats tested, as shown in Figure 4.7. Basically, no “training” was required for the rats to accomplish the cortical microstimulation task. This was the first indication that the saliency of the microstimulation sensation is greater than the sensation of the natural auditory cues.

It is possible to make the case that the microstimulation conveys more information to the behaving subject than the natural auditory stimulation. In the context that we are providing the behaving rat with information to accomplish a goal, and given a controlled and constant motivation, the performance by the rat is a direct function of the information we are providing. In both the detection and discrimination paradigm the microstimulation-induced d’ was significantly higher than the auditory-induced d’. However, it should be noted that these values are a function of the parameters of the behavioral task, and other behavioral parameters need to be investigated.

Spatial Resolution of Discernable Cortical Stimulation

Electrodes as closely spaced as 250 µm in some cases resulted in significantly different behavior, as shown in Figure 4.5. This is smaller than the 700 µm resolution reported by Schmidt et. al in their visual cortex assessment (Schmidt, Bak et al. 1996). However, Schmidt et. al did not use a psychophysical test to assess the behavior of their subjects. Statistically, significance is thus hard to determine, and their results may be influenced by this fact.

The microwire arrays used in our experiments are not perfectly rigid, and thus are not a clear indication of absolute microstimulus location. Thus, the actual resolution of a penetrating cortical requires further exploration using devices that have more reliable electrode geometries. Such devices include the University of Michigan silicon probe (Wise and Angell 1975), or the Utah Intracortical Electrode Array (Maynard, Nordhausen et al. 1997).

Microstimulation Location Generalization

It is not intuitive that microstimulation location within the primary sensory cortex would be monotonically generalized. Given the vagaries of cortical neurophysiological responses, this result is somewhat surprising. However, this result is more than likely explained by the organization of primary auditory cortex. The rat primary auditory cortex is excited by auditory frequencies arranged in a tonotopic map (Sally and Kelly 1988). Thus, stimulation of locations along a spatial axis within this map will excite neurons that are involved in processing of sounds of gradually changing frequency. This result may not be expected to occur in higher sensory and sensory-motor processing centers where a mapping of some sort has not been found.

Additionally, it is interesting to consider that generalization of the microstimulus location occurred relative to the end electrodes on the array. Although we aspired to implant the electrode arrays in a consistent location, there are inherent differences in the cortical anatomy and physiology across rats. Thus, there was inherent variation of the electrode array placement within the auditory cortex of the rats; however, this did not affect the ability of the rat to detect or discriminate the microstimulation, nor did it affect the generalization of the intermediate stimulus locations.

Auditory Cortical Prosthesis Implications

These results have direct implications for the field of auditory cortical prostheses. To accomplish a task, e.g. understanding speech, an auditory cortical prosthesis user will require a finite amount of information. This information level can be achieved through a cortical prosthesis by encoding information in several methods, including incorporating more channels, and using multiplexing of information on a given channel. The results from this study indicate that single electrode penetrating cortical microstimulation provides a rich information source, implying that they possess a substantial bandwidth available for information encoding. Additionally, based on the fact that electrodes as closely spaced as 250 μm produced different behaviors, the potential of a multi-channel auditory cortical prosthesis is further validated. Human primary auditory cortex is relatively large, on the order of centimeters (Howard, Volkov et al. 1996), and could potentially fit a multi-channel stimulation device with hundreds of electrode sites.

Significance

The long term goal of this research is to develop brain-machine information channels in auditory cortex with a primary application in an auditory cortical prosthesis. It is assumed that, for application in an auditory prosthesis, the highest information transfer rate possible is desirable. Thus, the information capacity of a single electrode and the total number of independent channels available are the primary parameters of interest. In this study we investigated both of these parameters in a chronic rat model. The results indicate that single-electrode microstimulation provides a rich informational source, and that an electrode spacing of 250 μm may be achievable in an auditory cortical prosthesis. Further, these results also imply that primary sensory cortical stimulation may be an optimal communication mode for a brain-machine interface.

CONCLUSION

The behavioral salience of the microstimulation-induced sensation was evaluated relative to natural, auditory-induced sensations. In both a detection and discrimination based behavioral setting, microstimulation cues resulted in better performance of the task. Further, microstimulation as closely spaced as 250 μm produced significantly different behavior. These results are consistent with recent reports that sensory intracortical microstimulation cues provide robust, salient cues in a behavioral setting. These results have implications for the feasibility and engineering of a cortical sensory prosthesis.

CHAPTER 5

FUTURE DIRECTIONS AND FINAL CONCLUSIONS

This dissertation describes techniques for providing sensory information to a behaving animal via a sensory cortical prosthesis. This was accomplished with a combination of electrophysiological recording, auditory psychophysical testing, and microstimulation psychophysical testing.

Chapter 2 began by examining the behavioral thresholds of penetrating auditory cortical stimulation delivered through chronic microwire arrays. Rats were trained to detect auditory stimulation, and subsequently tested on their ability to report varying intensities of single channel auditory cortical microstimulation. The results indicated that the rats could consistently respond to stimulation intensities as low as 10 μA and that the dynamic range of stimulation, previously unexplored, is a rich mode of information transfer. These results have positive implications for stimulation intensity as a substantial mode of information encoding in a penetrating cortical neuroprosthetic setting.

Chapter 3 examined penetrating cortical stimulation location within auditory cortex, and its ability to convey pitch information. Rats were trained to discriminate auditory tones separated by 4 octaves, and tested on the pitch-based sensation that penetrating auditory cortical stimulation produced within this spectrum. The results indicated that the rats' pitch sensation was significantly affected by different stimulation locations. These results support the implication that location within auditory cortex exhibits a potential information channel for a penetrating auditory cortex implant.

Chapter 4 addressed the capacity of a single information channel, and the number of information channels that are available in a penetrating auditory cortical implant. Rats were trained to discriminate between penetrating stimulation of auditory cortex at points located 1.75 mm apart. The rats were subsequently tested on the sensation of eight stimulation points between the bordering 1.75 mm stimulation points. The results indicated that the rats could discriminate the 1.75 mm separated penetrating cortical stimulation more accurately than any of the natural auditory discrimination stimuli. Further, the rats responded to penetrating auditory cortical electrical stimuli presented intermediate of the 1.75 mm electrodes in a monotonic, statistically repeatable fashion. These results suggest that 250 µm centered stimulation sites may be useful for a sensory cortical neuroprosthesis.

One estimate of the relevance and the significance of this project is in the implication it bears for future experiments. To address this, the next section outlines the future directions for this research.

FUTURE DIRECTIONS

The techniques and results described in this document lay down the foundation for a wealth of new experiments that will further our knowledge of the feasibility and capacity of an auditory cortical prosthesis. Some of these experimental efforts are being currently realized, and are briefly described in this section. These future directions illustrate the vast potential of the techniques described in this paper. They also demonstrate how the relationships between the three main chapters of this dissertation are synergistic in achieving the long-term goal of developing a reliable, high capacity cortical prosthesis.

Multichannel Stimulation

Several results from this experiment suggest that multichannel stimulation will result in greater information transfer in a cortical auditory prosthesis; however, this hypothesis deserves further investigation. Additionally, stimulation involving multiple channels invokes more questions that need to be addressed. Specifically, does the behavioral threshold change in the multichannel stimulation setting? Does the dynamic range finding of Chapter 2 hold for super-threshold multichannel stimulation? What is the optimal separation distance for simultaneously activated electrodes? Does the simultaneous activation of neighboring electrodes require complete independency of sensation, or is there a synergy relationship between overlapping stimulus fields? The behaving chronic rat cortical stimulation model presented in this document is an appropriate model to answer these questions. A careful examination of electrode spacing and its effect on threshold and sensation during individual and simultaneous activation of neighboring electrodes is required. This experimental examination is enabled by novel electrode devices, as described below.

Another parameter that was beyond the scope of this work, but may prove important, especially in multichannel stimulation design, is the electrical waveform used for stimulation. The waveform used in this experiment was biphasic and charge-balanced based on a report by Lilly et. al (Lilly, Hughes et al. 1955). However, recent reports have suggested that the stimulation parameters can be tailored to selectively stimulate either cell bodies or cell axons and fibers (McIntyre and Grill 2000). Additionally, several waveforms resulted in more efficient stimulation with lower neural activation thresholds.

These parameters may be available as another method of encoding information, and the sensation elicited by these alternative waveforms deserves further investigation.

Stimulation Patterning

The utility of single-electrode temporal stimulation patterning as a mode of encoding information has resulted in discrepancies in the literature. Romo et. al reported that periodic stimulus trains resulted in the same behavior as aperiodic stimulus trains of the same average pulse rate (Romo, Hernandez et al. 1998). Other investigations support this finding, reporting similar behaviors or sensations for various stimulation train frequencies from 50-2000 Hz (Dobelle, Stensaas et al. 1973; Rousche and Normann 1999). However, recently Scheich and Breindl reported that gerbils could discriminate temporally changing pulse trains (Scheich and Breindl 2002). The discernable pulse trains either increased from 10-1000 pulses per second over the course of 700 ms or decreased in a mirror imaged fashion. Therefore, this parameter requires further elucidation, and can be easily implemented in the rat model developed as part of this work.

Regardless of whether fine temporal patterns via single-electrode stimulation can be used to encode information, upon implementation of multi-electrode stimulation, spatio-temporal patterning across the array will gain relevance. The methods used to encode this information have not been investigated, but may be based on the cochlear implant literature. There are indications that electrophysiological recording conducted at the electrode site may provide information that can be used to encode information (Salzman, Britten et al. 1990; Groh, Born et al. 1997; Romo, Hernandez et al. 2000). The results from Chapter 3 also support this idea. However, the relevant aspects of the electrophysiology require further investigation. Whether local field potential recordings, or extracellular action potential recordings are more representative of the optimal microstimulation encoding mechanism has yet to be elucidated.

Physiological Effects of Cortical Microstimulation

There are several other physiological effects of cortical microstimulation that need to be investigated before a chronic stimulating device could be validated for human implementation. As noted in Chapter 2, the nature and number of neurons excited for a particular microstimulus location, amplitude, etc. determine the overall effect of the stimulation. Thus, whether the microstimulation produces an overall excitatory effect or inhibitory effect, and whether this can be manipulated by varying the stimulation parameters requires investigation. An important processing strategy that neural sensory systems implicate is excitation patterns framed by inhibitory sidebands. It is currently thought that this strategy is effective in stimulus contrast enhancement. This strategy may be effective in a microstimulation setting, using a central electrode for excitation, and either anti-phase stimulation or hyper-polarizing pulses on neighboring electrodes.

Since our preparation to date involves normal hearing rats, several questions arise as to the physiological effects of stimulation vs. the competing natural auditory information. Given the saliency findings presented in Chapter 4, the electrical stimulation may lead to a masking sensation, overriding the natural stimulation. This idea requires further investigation, and with careful design of relative stimulus intensities, may elucidate some mechanisms for greater saliency of microstimulation. This phenomenon also has implications for the implementation of these techniques in the area of human augmentation.

One complicating physiological phenomenon in the arena of chronic cortical stimulation that has been mentioned repeatedly throughout this manuscript is neural plasticity invoked by the repetitive stimulation. It is well accepted in the literature that chronic cortical stimulation causes changes in the neurophysiology of neurons effected by the stimulation (Nudo, Jenkins et al. 1990; Maldonado and Gerstein 1996). It is not clear whether neural plasticity will positively or negatively affect the information transfer rates achievable through cortical microstimulation, and this question requires further investigation.

Alternative Stimulation Devices

As mentioned earlier, there are several advantages associated with alternative stimulation devices. Although microwire arrays are the tried and trusted first generation device of most neurophysiology studies, recently more and more investigators are turning to other device designs for several reasons. Silicon based devices have several advantages over microwire based devices, including: batch fabrication, a lower total volume of tissue displaced by the array, unique electrode site geometries including 3-D arrangements, and the availability to incorporate integrated circuits and MEMS technologies in the device (Hetke, Williams et al. 2003). Additionally, a wireless, high-channel count silicon based stimulating array is currently in development (Ghovanloo, Wise et al. 2003). A collaboration with this research group in order to validate the device using the chronic rat preparation presented in this document has been established.

Additional Behavioral Paradigms

As the information being encoded continues to increase with cortical microstimulation advances in multichannel devices and stimulation patterns, a behavioral paradigm allowing more information from the behaving subject will be needed. This paradigm improvement may be ultimately limited by the behavioral repertoire of the rat. However, recent advances in brain-machine interface strategies show promise for a high information flow from the subject via neural responses (Chapin, Moxon et al. 1999; Wessberg, Stambaugh et al. 2000; Serruya, Hatsopoulos et al. 2002; Taylor, Tillery et al. 2002; Otto, Vetter et al. 2003; Vetter, Otto et al. 2003). These methods have already been implemented by this author, and will continue to be used in further investigations.

CONCLUDING REMARKS

Bioengineers trained in neural engineering are poised in a unique and exciting situation. Using the latest advances in engineering to apply cutting edge devices and techniques, they can address severely debilitating neurological diseases and ailments. The combination of a chronic neural interface preparation, extracellular neural recording, an operantly conditioned, behaving animal, and penetrating cortical stimulation comprise a critical mass of techniques that enable both development of cortical neural prostheses and research of the input-output processing strategies of cortical neural tissue. The results of this study are threefold. First, the rat animal model is an excellent development environment for chronic cortical stimulation. Second, the information content of single electrode stimulation is surprisingly rich, both in bandwidth and behavioral saliency. Lastly, electrodes as closely spaced as 250 µm may be used for neighboring stimulation channels, allowing hundreds of electrodes to be placed in the human auditory cortex. These results validate further animal and eventual human studies towards the development of an auditory cortical prosthesis. These results also have potential extension in the emerging area of human augmentation through application of a brain-machine interface.

REFERENCES

Bak, M., J. P. Girvin, et al. (1990). "Visual sensations produced by intracortical microstimulation of the human occipital cortex." Medical and Biological Engineering and Computing **28**(3): 257-9.

Bakin, J. S., M. C. Kwon, et al. (1996). "Suprathreshold auditory cortex activation visualized by intrinsic signal optical imaging." Cerebral Cortex (New York, N.Y. : 1991) **6**(2): 120-30.

Bakin, J. S., D. A. South, et al. (1996). "Induction of receptive field plasticity in the auditory cortex of the guinea pig during instrumental avoidance conditioning." Behavioral Neuroscience **110**(5): 905-13.

Bartlett, J. R. and R. W. Doty (1980). "An exploration of the ability of macaques to detect microstimulation of striate cortex." Acta Neurobiol Exp (Warsz) **40**(4): 713-27.

Birt, D., R. Nienhuis, et al. (1978). "Effects of bilateral auditory cortex ablation on behavior and unit activity in rat inferior colliculus during differential conditioning." Journal of Neurophysiology **41**(3): 705-15.

Blumberg, M. S. (1992). "Rodent ultrasonic short calls: locomotion, biomechanics, and communication." Journal of Comparative Psychology (Washington, D.C. : 1983) **106**(4): 360-5.

Blumberg, M. S., G. Sokoloff, et al. (2000). "Distress vocalizations in infant rats: what's all the fuss about?" **11**(1): 78-81.

Brandao, M. L., A. C. Troncoso, et al. (1997). "Active avoidance learning using brain stimulation applied to the inferior colliculus as negative reinforcement in rats: evidence for latent inhibition." Neuropsychobiology **35**(1): 30-5.

Brindley, G. S., P. E. Donaldson, et al. (1972). "The extent of the region of occipital cortex that when stimulated gives phosphenes fixed in the visual field." Journal of Physiology **225**(2): 57P-58P.

Brindley, G. S. and W. S. Lewin (1968). "The sensations produced by electrical stimulation of the visual cortex." Journal of Physiology **196**(2): 479-93.

Brindley, G. S. and W. S. Lewin (1968). "The visual sensations produced by electrical stimulation of the medial occipital cortex." Journal of Physiology **194**(2): 54-5P.

Buonomano, D. V. and M. M. Merzenich (1998). "Cortical plasticity: from synapses to maps." Annual Review of Neuroscience **21**: 149-86.

Burns, B. D., J. P. Stean, et al. (1973). "Recording for several days from single cortical neurones in the unrestrained cat." **231**(1): 8P-10P.

Chapin, J. K., K. A. Moxon, et al. (1999). "Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex." Nature Neuroscience **2**(7): 664-70.

Chapin, J. K. and M. A. Nicolelis (1999). "Principal component analysis of neuronal ensemble activity reveals multidimensional somatosensory representations." Journal of Neuroscience Methods **94**(1): 121-40.

Chowdhury, S. A. and N. Suga (2000). "Reorganization of the frequency map of the auditory cortex evoked by cortical electrical stimulation in the big brown bat." Journal of Neurophysiology **83**(4): 1856-63.

Delgado, J. M. R., W. W. Roberts, et al. (1954). "Learning motivated by electrical stimulation of the brain." American Journal of Physiology **179**: 587-593.

Dinse, H. R., G. H. Recanzone, et al. (1993). "Alterations in correlated activity parallel ICMS-induced representational plasticity." Neuroreport **5**(2): 173-6.

Dobelle, W. H. and M. G. Mladejovsky (1974). "Phosphenes produced by electrical stimulation of human occipital cortex, and their application to the development of a prosthesis for the blind." Journal of Physiology **243**(2): 553-76.

Dobelle, W. H., M. G. Mladejovsky, et al. (1974). "Artifical vision for the blind: electrical stimulation of visual cortex offers hope for a functional prosthesis." Science **183**(123): 440-4.

Dobelle, W. H., D. O. Quest, et al. (1979). "Artificial vision for the blind by electrical stimulation of the visual cortex." Neurosurgery **5**(4): 521-7.

Dobelle, W. H., S. S. Stensaas, et al. (1973). "A prosthesis for the deaf based on cortical stimulation." The Annals of Otology, Rhinology, and Laryngology **82**(4): 445-63.

Foerster, O. (1929). "Beitrage zur Pathophysiologie der Sehbahn und der Sehsphare." J Psychol Neurol, Lpz **39**: 463-485.

Furukawa, S., L. Xu, et al. (2000). "Coding of sound-source location by ensembles of cortical neurons." The Journal of Neuroscience : the Official Journal of the Society For Neuroscience **20**(3): 1216-28.

Galvan, V. V., J. Chen, et al. (2001). "Long-term frequency tuning of local field potentials in the auditory cortex of the waking guinea pig." Journal of the Association for Research in Otolaryngology **2**(3): 199-215.

Ghovanloo, M., K. D. Wise, et al. (2003). "Towards a Button-Sized 1024-Site Wireless Cortical Microstimulating Array." Proceedings of the 1st International IEEE EMBS Conference on Neural Engineering: 138-141.

Green, D. M. and J. A. Swets (1966). Signal Detection Theory and Psychophysics. New York, Wiley.

Groh, J. M., R. T. Born, et al. (1997). "How is a sensory map read Out? Effects of microstimulation in visual area MT on saccades and smooth pursuit eye movements." Journal of Neuroscience **17**(11): 4312-30.

Harrison, J. M. (1990). "Simultaneous auditory discrimination." Journal of the Experimental Analysis of Behavior **54**(1): 45-51.

Hetke, J. F., J. C. Williams, et al. (2003). "3-D Silicon Probe Array with Hybrid Polymer Interconnect for Chronic Cortical Recording." Proceedings of the 1st International IEEE EMBS Conference on Neural Engineering: 181-184.

Howard, M. A., I. O. Volkov, et al. (1996). "A chronic microelectrode investigation of the tonotopic organization of human auditory cortex." Brain Research **724**(2): 260-4.

Howard, M. A., I. O. Volkov, et al. (2000). "Auditory cortex on the human posterior superior temporal gyrus." The Journal of Comparative Neurology **416**(1): 79-92.

Kelly, J. B. and G. L. Kavanagh (1986). "Effects of auditory cortical lesions on pure-tone sound localization by the albino rat." Behavioral Neuroscience **100**(4): 569-75.

Kelly, J. B. and B. Masterton (1977). "Auditory sensitivity of the albino rat." Journal of Comparative and Physiological Psychology **91**(4): 930-6.

Kelly, J. B. and S. L. Sally (1988). "Organization of auditory cortex in the albino rat: binaural response properties." Journal of Neurophysiology **59**(6): 1756-69.

Kilgard, M. P. and M. M. Merzenich (1998). "Cortical map reorganization enabled by nucleus basalis activity." Science **279**(5357): 1714-8.

Kilgard, M. P. and M. M. Merzenich (1998). "Plasticity of temporal information processing in the primary auditory cortex." Nature Neuroscience **1**(8): 727-31.

Kilgard, M. P. and M. M. Merzenich (1999). "Distributed representation of spectral and temporal information in rat primary auditory cortex." Hearing Research **134**(1-2): 16-28.

Kim, C. and K. D. Wise (1996). "A 64-Site multishank CMOS low-profile neural stimulating probe." IEEE Transactions On Bio-Medical Engineering.

Krause, F. and H. Schum (1931). Neue Deutsche Chirurgie. H. Kuttner. Stuttgart: Enke. **49a:** 482-486.

Lilly, J. C., J. R. Hughes, et al. (1955). "Brief Non-Injurious Waveforms for Stimulation of the Brain." Science **121**: 468-469.

Loizou, P. C., M. Dorman, et al. (2000). "Speech recognition by normal-hearing and cochlear implant listeners as a function of intensity resolution." The Journal of the Acoustical Society of America **108**(5 Pt 1): 2377-87.

Maldonado, P. E. and G. L. Gerstein (1996). "Reorganization in the auditory cortex of the rat induced by intracortical microstimulation: a multiple single-unit study." Experimental Brain Research **112**(3): 420-30.

Marg, E. and J. E. Adams (1967). "Indwelling multiple micro-electrodes in the brain." Electroencephalography and Clinical Neurophysiology **23**(3): 277-80.

Maynard, E. M., C. T. Nordhausen, et al. (1997). "The Utah intracortical Electrode Array: a recording structure for potential brain-computer interfaces." Electroencephalography and Clinical Neurophysiology **102**(3): 228-39.

McIntyre, C. C. and W. M. Grill (2000). "Selective microstimulation of central nervous system neurons." Annals of Biomedical Engineering **28**(3): 219-33.

Normann, R. A., E. M. Maynard, et al. (1999). "A neural interface for a cortical vision prosthesis." Vision Research **39**(15): 2577-87.

Nudo, R. J., W. M. Jenkins, et al. (1990). "Repetitive microstimulation alters the cortical representation of movements in adult rats." Somatosensory & Motor Research **7**(4): 463-83.

Nunez, P. (1981). Electric Fields of the Brain: the Neurophysics of EEG. New York, Oxford University Press.

Olds, J., J. F. Disterhoft, et al. (1972). "Learning centers of rat brain mapped by measuring latencies of conditioned unit responses." Journal of Neurophysiology **35**(2): 202-19.

Olds, J. and P. Milner (1954). "Positive reinforcement produced by electrical stimulation of the septal area and other regions of the rat brain." Journal of Comparative and Physiological Psychology **47**(419-428).

Otto, K. J., R. J. Vetter, et al. (2003). "Brain-Machine Interfaces in Rat Motor Cortex: Implication of Adaptive Decoding Algorithms." Proceedings of the 1st International IEEE EMBS Conference on Neural Engineering: 100-103.

Otto, S. R., R. V. Shannon, et al. (1998). "The multichannel auditory brain stem implant: performance in twenty patients." Otolaryngology and Head and Neck Surgery **118**(3 Pt 1): 291-303.

Patterson, M. M. (1970). "Classical conditioning of the rabbit's (Oryctolagus cuniculus) nictitating membrane response with fluctuating ISI and intracranial CS." Journal of Comparative and Physiological Psychology **72**(2): 193-202.

Patterson, M. M. (1971). "Inferior collicular CS intensity effect on rabbit nictitating membrane conditioning." Physiology & Behavior **6**(4): 273-8.

Penfield, W. (1938). "The cerebral cortex in man: 1. The cerebral cortex and consciousness." Archives of Neurology and Psychiatry **40**: 417-442.

Penfield, W. (1958). The Excitable Cortex in Conscious Man. Springfield, IL, Charles C. Thomas.

Penfield, W. and E. Boldrey (1937). "Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation." Brain **60**: 389-443.

Penfield, W. and H. Jasper (1954). Epilepsy and the Functional Anatomy of the Human Brain. Boston, Little Brown.

Penfield, W. and P. Perot (1963). "The Brain's Record of Auditory and Visual Experience." Brain **86**: 595-696.

Penfield, W. and T. Rasmussen (1950). The Cerebral Cortex of Man. New York, MacMillan.

Rauschecker, J. P. and R. V. Shannon (2002). "Sending sound to the brain." Science **295**(5557): 1025-9.

Recanzone, G. H., M. M. Merzenich, et al. (1992). "Changes in the distributed temporal response properties of SI cortical neurons reflect improvements in performance on a temporally based tactile discrimination task." Journal of Neurophysiology **67**(5): 1071-91.

Recanzone, G. H., C. E. Schreiner, et al. (1993). "Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys." The Journal of Neuroscience : the Official Journal of the Society For Neuroscience **13**(1): 87-103.

Romo, R., A. Hernandez, et al. (2000). "Sensing without touching: psychophysical performance based on cortical microstimulation." Neuron **26**(1): 273-8.

Romo, R., A. Hernandez, et al. (1998). "Somatosensory discrimination based on cortical microstimulation." Nature **392**(6674): 387-90.

Ronner, S. F. (1982). "Prosthesis-related studies on visual cortex neurons." Applied Neurophysiology **45**(1-2): 18-24.

Ronner, S. F., W. E. Foote, et al. (1980). "Activation of single cells in cat visual cortex by electrical stimulation of the cortical surface." Experimental Neurology **70**(1): 47-64.

Ronner, S. F., W. E. Foote, et al. (1981). "Intracortical microstimulation of neurons in the visual cortex of the cat." Electroencephalography and Clinical Neurophysiology **52**(4): 375-7.

Ronner, S. F. and B. G. Lee (1983). "Excitation of visual cortex neurons by local intracortical microstimulation." Experimental Neurology **81**(2): 376-95.

Rousche, P. J. and R. A. Normann (1999). "Chronic intracortical microstimulation (ICMS) of cat sensory cortex using the Utah Intracortical Electrode Array." IEEE Transactions On Rehabilitation Engineering **7**(1): 56-68.

Rousche, P. J., K. J. Otto, et al. (2003). "Single Electrode Micro-stimulation of Rat Auditory Cortex: an Evaluation of Behavioral Performance." Hearing Research **179**(1-2): 62-71.

Sachs, B. D. and M. Bialy (2000). "Female presence during postejaculatory interval facilitates penile erection and 22-kHz vocalization in male rats." Behavioral Neuroscience **114**(6): 1203-8.

Sally, S. L. and J. B. Kelly (1988). "Organization of auditory cortex in the albino rat: sound frequency." Journal of Neurophysiology **59**(5): 1627-38.

Salzman, C. D., K. H. Britten, et al. (1990). "Cortical microstimulation influences perceptual judgements of motion direction." Nature **346**(6280): 174-7.

Salzman, C. D., C. M. Murasugi, et al. (1992). "Microstimulation in visual area MT: effects on direction discrimination performance." The Journal of Neuroscience : the Official Journal of the Society For Neuroscience **12**(6): 2331-55.

Scheich, H. and A. Breindl (2002). "An animal model of auditory cortex prostheses." Audiology & Neuro-Otology **7**(3): 191-4.

Schmidt, E. M., M. J. Bak, et al. (1996). "Feasibility of a visual prosthesis for the blind based on intracortical microstimulation of the visual cortex." Brain **119 (Pt 2)**: 507-22.

Seidemann, E., E. Zohary, et al. (1998). "Temporal gating of neural signals during performance of a visual discrimination task." Nature **394**(6688): 72-5.

Serruya, M. D., N. G. Hatsopoulos, et al. (2002). "Instant neural control of a movement signal." Nature **416**(6877): 141-2.

Skinner, B. F. (1938). The Behavior of Organisms: An Experimental Analysis. New York, Appleton-century-Crofts.

Stoney, S. D., W. D. Thompson, et al. (1968). "Excitation of pyramidal tract cells by intracortical microstimulation: effective extent of stimulating current." Journal of Neurophysiology **31**(5): 659-69.

Suga, N., Z. Xiao, et al. (2002). "Plasticity and corticofugal modulation for hearing in adult animals." Neuron **36**(1): 9-18.

Talwar, S. K. and G. L. Gerstein (2001). "Reorganization in awake rat auditory cortex by local microstimulation and its effect on frequency-discrimination behavior." Journal of Neurophysiology **86**(4): 1555-72.

Talwar, S. K., P. G. Musial, et al. (2001). "Role of mammalian auditory cortex in the perception of elementary sound properties." Journal of Neurophysiology **85**: 2350-2358.

Talwar, S. K., S. Xu, et al. (2002). "Rat navigation guided by remote control." Nature **417**: 37-38.

Taylor, D. M., S. I. Tillery, et al. (2002). "Direct cortical control of 3D neuroprosthetic devices." Science **296**(5574): 1829-32.

Tehovnik, E. J. (1996). "Electrical stimulation of neural tissue to evoke behavioral responses." Journal of Neuroscience Methods **65**(1): 1-17.

Vetter, R. J., K. J. Otto, et al. (2003). Brain-Machine Interfaces in Rat Motor Cortex: Neuronal Operant Conditioning to Perform a Sensory Detection Task. 1st International IEEE EMBS Conference on Neural Engineering, Capri, Italy.

Villa, A. E. and M. Abeles (1990). "Evidence for spatiotemporal firing patterns within the auditory thalamus of the cat." Brain Research **509**(2): 325-7.

Wessberg, J., C. R. Stambaugh, et al. (2000). "Real-time prediction of hand trajectory by ensembles of cortical neurons in primates." Nature **408**(6810): 361-5.

Williams, J. C., R. L. Rennaker, et al. (1999). "Long-term neural recording characteristics of wire microelectrode arrays implanted in cerebral cortex." Brain Res Protocols **4**(3): 303-13.

Wise, K. D. and J. B. Angell (1975). "A low-capacitance multielectrode probe for use in extracellular neurophysiology." IEEE Transactions On Bio-Medical Engineering **22**(3): 212-9.

Wise, K. D., J. B. Angell, et al. (1970). "An integrated-circuit approach to extracellular microelectrodes." IEEE Transactions On Bio-Medical Engineering **17**(3): 238-47.

APPENDIX A

RECORDING EQUIPMENT SETUP

Appendix A:

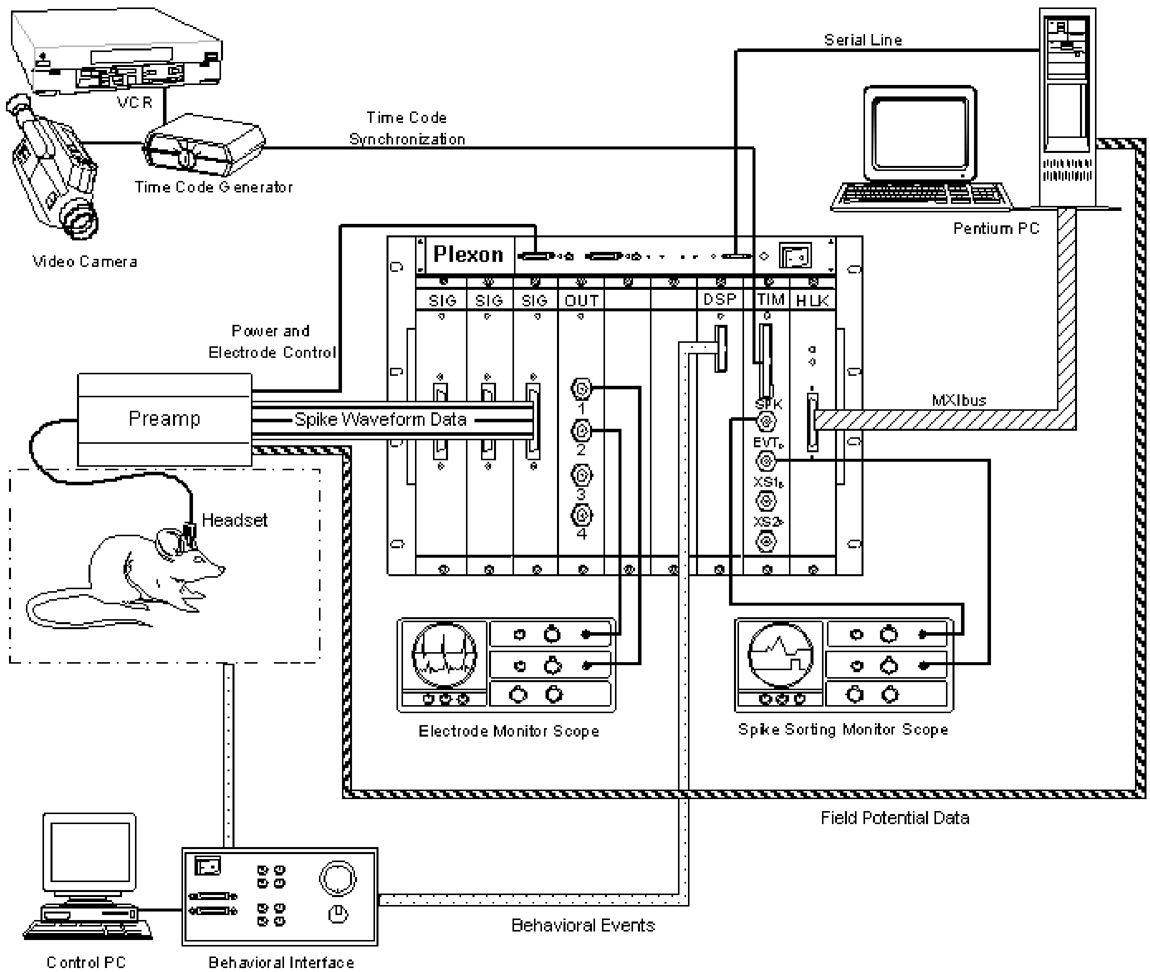

Figure A.1 Example of Plexon hardware setup used for neurophysiological recording.

BIOGRAPHICAL SKETCH

Kevin John Otto was born in Albuquerque, New Mexico, on September 5, 1975. He received his high school education at Delta High School in Delta, Utah. In 1993 Kevin entered Colorado State University in Fort Collins, Colorado. He completed his Bachelor of Science in Chemical Engineering in 1997, graduating with honors. In 1997 he entered the Graduate College at Arizona State University to pursue a doctorate in Bioengineering. He received his Master of Science in Bioengineering in December 2002. He was active as a student member in the local and national Biomedical Engineering Society and as a member and officer in the Biomedical Engineering Honor Society.