Elsevier Editorial System(tm) for Journal of Neuroscience Methods

Manuscript Draft

Manuscript Number: JNEUMETH-D-06-00286R1

Title: Chronically Recording with a Multi-Electrode Array Device in the Auditory Cortex of an Awake Ferret

Article Type: Research Article

Section/Category: Basic Neuroscience

Keywords: chronic; awake; ferret; multi-electrode; cortex

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Abstract: It is known that anesthesia depresses neural activity and inhibits cortico-cortical interactions and cortical output. Hence, it is important to record from awake animals in order to better understand the full dynamic range of neural responses. We have developed a preparation for chronic, multi-electrode physiological recording in the cortex of the awake ferret. This paper discusses several of the advantages and disadvantages of the technique as well as procedures used to overcome potential complications associated with chronic implants in the ferret. Our solutions are well suited to the special species requirements, yet are also easily generalizable to other species.

Title Page

Type of Article: Research Article

(i) Title:

Chronically Recording with a Multi-Electrode Array Device in the Auditory Cortex of an Awake Ferret

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(iv) Pages:

Total Document Length: 33 pages (Table and Figures Submitted separately)

Page Numbers	Section Title	Section Length
1	Title and Abstract	1 page
2-24	Main Body	23 pages
25-26	References	2 pages
27	Table Legend	1 page
28-33	Figure Legends	6 pages

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To confirm your agreement with this, you are required to include the following statement in your cover letter indicating your agreement with these standards: "I have read and have abided by the statement of ethical standards for manuscripts submitted to the Journal of Neuroscience Methods

ETHICAL STANDARDS:

- The authors declare that all experiments on human subjects were conducted in accordance with the Declaration of Helsinki http://www.wma.net and that all procedures were carried out with the adequate understanding and written consent of the subjects.

- The authors also certify that formal approval to conduct the experiments described has been obtained from the human subjects review board of their institution and could be provided upon request.

- If the studies deal with animal experiments, the authors certify that they were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 or the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, or the European Communities Council Directive of 24 November 1986 (86/609/EEC).

- The authors also certify that formal approval to conduct the experiments described has been obtained from the animal subjects review board of their institution and could be provided upon request.

- The authors further attest that all efforts were made to minimize the number of animals used and their suffering.

- If the ethical standard governing the reported research is different from those guidelines indicated above, the authors must provide information in the submission cover letter about which guidelines and oversight procedures were followed.

- The Editors reserve the right to return manuscripts in which there is any question as to the appropriate and ethical use of human or animal subjects.

Author Agreement

This is a statement to certify that all four authors listed below have seen and approved the manuscript being submitted entitled "A Chronic Multi-Electrode Implant for Recording in an Awake Ferret Cortex". This manuscript is the authors' original work, has not received prior publication, and is not under consideration for publication elsewhere. On behalf of all co-authors, the corresponding author, Heather D Dobbins, shall bear full responsibility for the submission.

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Dear Dr. Gerhardt,

Enclosed is the revision of manuscript number JNEUMETH-D-06-00286, submitted to the Journal of Neuroscience Methods, originally titled “A Chronic Multi-Electrode Implant for Recording in an Awake Ferret Cortex”, now titled "Chronically Recording with a Multi-Electrode Array Device in the Auditory Cortex of an Awake Ferret". We would like to thank the reviewers for their time in reviewing this paper, and for their careful and thoughtful comments.

One of the concerns expressed by the reviewers is that the photos in the figures are not in a publication quality resolution. After speaking with the e-submissions help desk, it is our understanding that the reviewers can only access a down sampled, low resolution version of all photos on the website and that they would need to specially request the high resolution figure if desired.

We have summarized all of the reviewers’ comments and the corresponding revisions in the tables below. We hope that you find the revised version to be to your satisfaction.

Sincerely,
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Reviewers' comments:

Reviewer #1:

Comment Summary	**Revision**
Request for a reference regarding the quoted minimum electrode separation required for independent recordings.	Added reference Moffitt MA and McIntyre CC, 2005.
Request for manufacturers of the following: super glue, super glue accelerator, triple antibiotic ointment, bone wax, and iodine	All product specifications and manufacturers have been inserted accordingly.
Suggested word change from “cracks” to “spaces or “gaps”.	Done. (Pg 6)
"Surgery is performed" - should be past tense.	Changed where appropriate.

"5-flurouracil (5-FU, Sigma Chemicals Ltd), is used" and following sentences - should be past tense.	
"keeps excess dural and bone growth to a minimum for the duration of the experimental preparation." - please indicate how long that duration typically is. Some readers might think that you refer to "preparation time" here, others, that you refer to the lifetime of the experiment after implantation.	Wording changed, and the duration specified. (Pg 12)
"so that infection is minimized." - do you mean infection or risk of infection? Was infection common or rare? If it occurred, how big a problem was it and how was it treated?	Wording changed to “risk of infection” (Pg 13), and paragraph added in “Ferret Welfare” section to address the occurrence and treatment of infections (Pg 18).
Check on J Neurosci Methods policy regarding animal photos, and references containing websites and unrefereed abstracts.	Done. We can find nothing in the instructions to authors indicating that this is against the journal’s policy. The website used is to provide a source for the spike sorting method, which is consistent with existing publications using the same method. Also, the abstract listed is published and has been supplemented with another journal reference.
Rephrase “we’ve found” to be less informal.	Done. (Pg 16)
Double check language regarding animal welfare to be sure that phrasing reflects the animals’ treatment in the most possible way. Specifically change the phrase “the guillotine door”.	Phrasing changed (Pg 16). “Handling the Ferrets Day to Day” section renamed to “Ferret Welfare” and expanded to include the requested information (Pg 19).
Request for gross dimensions in Fig. 4b-d.	Scale bars with measurements added in the figure. Parts indicated are named and measurements are given in the Figure 4 caption.
Request to, “elaborate further to indicate how animal welfare concerns are addressed” regarding the ferrets’ depression symptoms.	Paragraph added in “Ferret Welfare” section indicating how often depression has occurred, how it has been treated, how long it typically takes a ferret to recover, and what the protocol is to prevent occurrences of depression (Pg 19).
Request for a table that list all the parts needed for this experimental preparation.	After performing a thorough search of past publications in this journal, there is no indication that this is a common accepted practice. Instead, However, we have been meticulous in making sure that parts are described with manufacturers in the text.

Reviewer #2:

Comment Summary	**Revision**
Request for a table that list all the parts needed for this experimental preparation. (same comment as reviewer #1)	See comment above: We have been meticulous in making sure that parts are described with manufacturers in the text.
"How does your work differ from (and improve upon) the work of Chiu and Weliky? "	Two clarification sentences have been added to the introduction, "Chiu and Weliky (2004) successfully developed a system to record from the visual pathway of ferret neonates using bundles of microwires. In our lab, our experimental preparation consists of electrophysiological recordings with individually moveable electrodes in the central auditory system of awake, adult ferrets (*Mustela putorius furo*)." (Pg 2)
Page 3, near bottom delete the redundant "to able"	Done. (Pg 4)
Page 12, post-surgery list doses of the drugs given	Done. (Pg 13)
Figure 4 provide the dimensions. . (same comment as reviewer #1)	Scale bars with measurements added in the figure. Parts indicated are named and measurements are given in the Figure 4 caption.
Concern that the signal to noise ration is "not very good" in Figure 5.	This figure demonstrates a typical signal to noise ratio in a ferret recording.
I think you need to discuss additional problems (and solutions) with your system.	We are not sure what is meant by this comment. We feel that it is outside the scope of the paper to discuss all of the problems we encountered while the preparation was being developed. And, now that we have the described system, we don't have any significant difficulties.

Reviewer #4:

Comment Summary	**Revision**
I think the authors could diminish the perspective of limited scope by expanding the discussion, and perhaps the introduction, to allow for a consideration of how these techniques could be applied to different types of animals, and different types of neuroscience studies.	The introduction and discussion were reviewed, and the requested information was added where appropriate.
A recommendation that the methods for rotating the MEAD be elaborated and the data incorporated into the paper.	We indicate in the discussion that it is possible to rotate the MEAD and what has to be done differently during surgery in order to be able to perform the rotation. A description past that would require several more pictures and diagrams and the authors believe it to be beyond the scope of the paper. Data collected from a rotated MEAD looks like that presented in the paper, and a sentence was added indicating this in the discussion. (Pg 22)
The 'off the shelf' emphasis appears overplayed	This phrase and concept have been removed from the paper.
It is difficult to ascertain the degree to which the solutions introduced by the authors are cost-effective. I recommend removal of the term, or elaboration of how this parameter was assessed.	This phrase has been removed. Instead, relevant sections were reworded to better illustrate and expand on the intended meaning.
The location information of the vendors is inconsistent - recommend Vendor, city, state.	All vendors have been reviewed and changed to: manufacturer, city, state.
Page 5: it may be useful to cite Figure 1b when referring to the lip of the MEAD base.	Done. (Pg 5)
Page 6: (first paragraph) the direction to Fig. 2a should be replaced with Fig 2c.	Done. (Pg 6)
Page 9: What are the precautions taken when the screws are implanted? A request to be more descriptive when giving instructions for how to insert the mini-screws.	Instructions and description to insert the mini-screws added. (Pg 10)

Page 9: Insert location information for Fine Science Tools, remove it for the later citation on Page 10.	Done. (Pg 10)
Page 11: Details about the headposts should be inserted. Where are they made, what are they made from?	Headpost description added in section "Immobilizing the Head". (Pg 14)
Page 11: The authors mention that the best results were achieved with coral dental cement. Please elaborate on the criteria that were employed.	Dental cement criteria added. (Pg 13)
Page 12: Were the housing conditions of the animals adjusted to maximize implant longevity?	No. The implant modifications have made the MEAD particularly robust with no need for a husbandry modification. (Pg 20)
Page 18 - 'tone' should be plural.	Done. (Pg 21)
The references should be consistent in form: several of the references display commas after the journal title, several do not. Several references have a comma after the last author, several do not.	The format of all references have been reviewed, and some of them have been reformatted in accordance with the "Instructions to Authors" on the J Neurosci Methods webpage. (Pg25)
In the printed versions of Figure 1a, 1c, 3a, 4b, 4d and 6b, diagonal lines are present which appear that they should not be there: it is likely that these lines are artifacts of the drawing program.	We called the e-submissions help line, and they said that there is not a line on the original submitted files.
The dimensions in Fig. 1b are somewhat incomplete, and the dimensions of the figure are not scaled (i.e. the 2mm length is not 1/3rd of the 6mm length).	Several dimensions have been explicitly added, and the scale in Fig 1b had been adjusted to be proportional.
Figure 2, 3d, & 4a: The quality of the pictures is somewhat poor - in the version I have, the pictures are somewhat blurry and out of focus.	We called the e-submissions help line, and they indicated that reviewers only get a downsampled (low quality) copy of the figures, but the resolution of the originals is fine.
Figure 3: The bull's eye is not reproduced as shaded in the figure.	Done. (Fig 3)
The metal ruler in figure 3c is not very helpful. Perhaps two high contrast 1 or 2 cm calibration	The picture was retaken with two rulers in the orientation of the headposts so that they can

bars (one in the horizontal direction, and one parallel to the line of sight) might improve the figure.	better be used for comparison. (Fig 3)
Figure 4 (b-d) is not intuitive. Maybe add different grey scales, add scales, labeling, and/or more specific instructions in the text about how to use the holder.	Grey scales and measurement bars have been added to the figure. Labels with measurements have been added to the figure legend. (Fig 4) Also, instructions have been added to the text. (Pg 15)
Figure 6. Should the horizontal axis in figure 6a be time or sample number?	Sample number and time are interchangeable. A phrase indicating this has been added to the legend of Fig. 6.
Table 1. I'm not sure this table is tremendously useful. I think it is important to cite that the stereotaxic coordinates change with gender, but I believe the authors can do this in the text.	We tried converting the table to text, but because there are six different measurements dependent on sex and location we found it best to leave the information in a table.
Suggestion to remove figure 5 and replaced with a comment about the signal to noise ratio in the text.	We and the other reviewers feel that Fig 5 is helpful in demonstrating the typical raw data, different from the data after amplification in Fig 6. A comment on the signal to noise ratio has been added to the legend of Fig.5.

Reviewer #6:

Comment Summary	**Revision**
"In places the manuscript is presented in a conversational style that does not really befit the journal."	The whole manuscript was reviewed, and sections reworded where possible and appropriate.
The Title does not accurately describe the manuscript. I feel the title should be modified slightly.	The title has been changed to "Chronically Recording with a Multi-Electrode Array Device in the Auditory Cortex of an Awake Ferret".
The manufacturers and/or location information for many of the materials used is absent.	All product specifications, manufacturer names and locations have been added.
Details of the methods used for recording are inadequate. What hardware exactly is used (amplifiers etc., A/D converters etc.)? What is the amplification, sample rate and bit depth?	We do not want to advocate for any particular hardware or software system, and therefore feel it is beyond the scope of the paper to mention these specifics. Settings such as the amplification are dependent upon the recording

	set up and are independent of the main ideas of this paper.
Suggestion to change “bandpassed” to “bandpass filtered”. (Pg 15)	Wording changed. (Pg 17)
Incompatibility between the low pass filter at 6kHz, and sampling the recording at 8kHz.	6kHz was a typo, and was changed to 3kHz. (Pg 17)
The manuscript is vague on the details of the data analysis part of their work. For example: the number of neurons detectable on each electrode, the typical signal to noise ratio, and spike discrimination.	We do not want to advocate any particular method for data analysis or spike sorting. In addition, we do not want to concentrate on data analysis in this methods paper (which is expanded upon in other papers from our lab). Therefore, we feel it sufficient to only briefly mention and describe the method(s) that we use.
Figure 6a axes have no labels.	We tried multiple ways of inserting axis labels, and all of them significantly cluttered the figure. Instead, we have left the description of the axes units in the legend. (Fig 6)
Incompatible vertical scales in figures 5 and 6.	Figure 5 is of the raw signal before amplification, and figure 6 is the processed signal after amplification. Phrases were added to the legends of both figures to indicate this.
Convert dimensions to SI where possible.	Done.
Be consistent with the way that units are reported. (in, inches, or “)	All instances have been changed to “in”.
Quote measurement figures as 0.0089 instead of .0089.	Done. (Pg 6)
It is not entirely clear to me what the Authors are referring to in the last line of page 10.	The wording of the sentence has been changed. (Pg 11, last sentence of “Making the Craniotomy”)
Page 16 lines 17-18 do not quite make sense in their present form.	Wording changed. (Pg 18, line 19)
The authors should define CSF.	Done. Added to beginning abbreviations (Pg 1), and defined in the text. (Pg 8)

Chronically Recording with a Multi-Electrode Array Device in the Auditory Cortex of an Awake Ferret

Heather D Dobbins, Peter Marvit, Yadong Ji, Didier A Depireux

1 Abstract

It is known that anesthesia depresses neural activity and inhibits cortico-cortical interactions and cortical output. Hence, it is important to record from awake animals in order to better understand the full dynamic range of neural responses. We have developed a preparation for chronic, multi-electrode physiological recording in the cortex of the awake ferret. This paper discusses several of the advantages and disadvantages of the technique as well as procedures used to overcome potential complications associated with chronic implants in the ferret. Our solutions are well suited to the special species requirements, yet are also easily generalizable to other species.

Keywords: chronic, awake, ferret, multi-electrode, cortex

Abbreviations: MEAD: multi-electrode array device; 5-FU: 5-flurouracil; CSF: cerebrospinal fluid

2 Introduction

There is clear scientific value in recording from awake animals (Volkov et al., 1985). Recent advances in technology and design have made the chronic multi-electrode array device (MEAD) both practical and popular in a variety of awake preparations (Fee and Leonardo, 2001; Jeantet and Cho, 2004; Wilson et al., 2003; Swadlow et al., 2005; McKown and Schadt, 2006). A few of the advantages of such devices are: long-term stable recordings, increased cell yield per animal, correlative recordings across cells or regions, convenience, and recording during behavior. Most MEADs reported in the literature are custom-made by individual laboratories, range in design from simple to highly sophisticated, and often use many handcrafted components (e.g., tetrodes). While each device may be well-suited to a particular laboratory's needs, the fabrication and adaptation of such devices by other laboratories may increase the start-up cost considerably in both time and money. Companies are now beginning to offer commercial versions of these research tools, which can decrease start-up time and expense considerably. The commercial version of a previously described device (Jog et al., 2002) is the starting point for the work presented here.

We chose to adapt one of the systems available for purchase (Neuralynx, AZ), using as many ready-made components as possible for the entire set-up. This paper presents the successes and caveats of that effort. Chiu and Weliky (2004) successfully developed a system to record from the visual pathway of ferret neonates using bundles of microwires. In our lab, our experimental preparation consists of electrophysiological recordings with individually moveable electrodes in the central auditory system of awake, adult ferrets (*Mustela putorius furo*). The species and age choice has presented some

challenges to a successful implementation of a MEAD system. In particular, ferrets are particularly active and strong for their size, and any device must be robust in order to avoid breakage. For many of our studies, well-calibrated sound field was needed, requiring a ferret-compatible restraint system that can be used for extended recording sessions. Few commercial products have been designed with ferrets in mind. The techniques presented here address: 1) modifications to a commercial MEAD for use on ferrets, 2) flexibility of electrode geometry and ease of loading the MEAD, 3) augmentation of standard surgical techniques, and 4) a robust but comfortable restraint system. These methods enable us to obtain stable recordings for several hours per day up to 5 months, with an excellent signal-to-noise ratio over multiple electrodes. In addition, it should be noted that all of the equipment and methods presented in this paper could easily be adapted to suit the needs of another animal. As demonstrated, the size of the MEAD, craniotomy, and animal holder, as well as the geometry of the electrodes, can be adjusted to accommodate larger or smaller species.

3 Materials and Methods

3.1 The Multi-Electrode Array Device

The original MEAD body design has been described in detail previously (Jog et al., 2002), commercialized by Neuralynx, Inc (Tucson, AZ) and manufactured by Specialty Machining (Wayland, MA). Several versions are available, but we chose the 12-drive H model. Fig. 1a is a diagram of the MEAD configuration illustrating the spatial relationships between all of the elements. In the side view, only two electrode shuttles

are shown for clarity. Designed to be implanted several times and having most of its components repairable, the cost per use is decreased.

REASONS FOR MEAD DESIGN ALTERATION

Two major difficulties had to be overcome before the commercial MEAD was operative in ferrets. Originally designed for rats and mice, the MEAD shell was very thin and light. Unfortunately, ferrets are stronger and more energetic; the first MEADs we used were shattered after only a few days of implantation. We worked with the manufacturer to develop a more robust version of the MEAD with a 1.5mm thick outside shell and cap, which can withstand many months of implantation. Second, the commercially available design for the part of the MEAD that makes contact with the skull, the MEAD base, is not well suited for our applications. We require 1) a flexible electrode geometry to space electrode tips as close or far apart as an experiment needed, 2) an electrode exit that is optimal for a small craniotomy, and 3) compatibility with the headposts used in our animal restraint system. Therefore, we designed a different MEAD base with dimensions better suited for our needs (illustrated in Fig. 1).

[INSERT FIGURE 1 ABOUT HERE.]

CUSTOM MEAD BASE DESIGN

Our MEAD base design is easily fabricated from a rod of Delrin (DuPont, Wilmington, DE) and addresses all three issues. First, in order to preserve a spatial relationship between the electrodes with a design that is easily scaled to cover different surface areas, the polyimide guide tubes are arranged into a honeycomb pattern (See Fig.

2d). Our choice of guide tube diameter and thickness guarantees that the electrodes will be spaced at least 225μm apart (see below for specific diameter and thickness). At this electrode distance the correlation between firing patterns recorded from separate electrodes is negligible, and is therefore the smallest distance at which electrode recordings can be said to be independent of each other (Moffitt and McIntyre, 2005). This distance also keeps the electrodes from physically converging towards each other and forming shared tracks in the neural tissue. The overall size or organization of the electrode configuration can be changed by altering the diameter of the tubing, changing the size of the containing hole through the custom MEAD base, or choosing different tubes in which the electrodes are placed (See Fig. 2d). Similarly, the relative spatial relationship between the electrodes can be scaled to cover a smaller or larger recording area by using more guide tubes in a larger hole in the MEAD base (i.e. using the extra guide tubes as spacers). Second, our custom MEAD base has a small lip that allows the implant to rest on the skull for stability and for precision of placement (See Fig. 1b,c). The diameter of the MEAD base allows for an optimized craniotomy size and shape. The craniotomy is sufficiently large enough to expose at least the primary auditory cortex, and small enough to reduce the risk of infection or swelling. Third, the extended neck insures that the MEAD does not get in the way of the headposts. See Fig. 1b for a blown up diagram of the MEAD base.

3.1.1 Loading the MEAD with electrodes

POLYIMIDE TUBING DIMENSIONS

Getting the MEAD prepared for implantation requires four stages: 1) Creating the exit honeycomb, 2) assembling the inner guide tubing, 3) inserting the metal electrodes

and 4) sealing the MEAD base. The materials required are a standard cyanoacrylic super glue (medium viscosity Gap Filling CA+; Great Planes Pro Adhesives, Champaign, IL) and standard polyimide tubing (Small Parts, Inc., Miami Lakes, FL) in two sizes—outer/honeycomb tubing 0.0089in in diameter (226μm) with a wall thickness of 0.00075in (19 μm), and inner/guide tubing of 0.0056in in diameter (142 μm) with a standard wall thickness of 0.00075in (19 μm) or "triple-walled" thickness of 0.00225in (57μm). The diameter of the inner guide tubes is determined by what will fit around the electrodes, and the outer guide tubes must be wide enough to fit around these inner guide tubes. If more spacing is desired between electrodes so that the complete array of electrodes covers more surface area, the outer guide tubes should have larger diameters. The inner guide tube thickness is chosen so that the tubes do not collapse too easily but are flexible enough to be curved guides. Fig. 2c is a close-up picture of the honeycomb and Fig. 2d is a schematic diagram of a typical exit configuration for the cortical end of the implant.

CONSTRUCTING THE HONEYCOMB

The honeycomb guide has to be assembled to fit into the hole in the MEAD base (Fig. 1b) and will ultimately determine electrode geometry. The hole in the MEAD base is 1.3mm in diameter—approximately five times the diameter of the outer guide tubes, which is just big enough to accommodate all of the tubes that make up the honeycomb. To create the honeycomb, we thread 19 pieces of the outer guide tubes, each 7 to 12cm in length, through the hole in the MEAD base. They self-assemble into the correct shape. A very small dot of super glue is placed on each side of the Delrin MEAD base where the guide tubes exit; there has to be enough glue to wick into the gaps between the tubes. We

have found it beneficial to use super glue accelerator (Pro CA activator; Great Planes® Pro Adhesives, Champaign, IL) which speeds up the curing process preventing the glue from wicking all the way into the tubes and thus obstructing them. When this is dry, a razor or scalpel is used to carefully cut the tubes down to be flush with the MEAD base.

THREADING THE INNER GUIDE TUBES

In the next step, the MEAD base is held in place aligned with the body of the MEAD, as shown in Fig. 2a, and the inner guide tubes are threaded through the honeycomb and back into the shuttles using forceps. It is important not to pinch the inner tubing while threading the MEAD, otherwise kinks in the tubing will prevent the electrodes from being guided smoothly back into the shuttles. Instead, the electrodes will puncture the walls of the tubing at the pinch point, making that guide tube useless. Therefore, each piece of tubing is visually inspected for kinks or pinches, and replaced if necessary before the final gluing. It is also necessary to make a record noting the path of each guide tube (and therefore, later, each electrode) from its location in the honeycomb to its electrode shuttle. This information is referenced later in the experiment and data analysis.

[INSERT FIGURE 2 ABOUT HERE.]

LOADING THE ELECTRODES

When all of the inner guide tubes are threaded and in place, screws are used to attach the MEAD base permanently in place and a small dot of super glue, quickly followed by a drop of curing accelerator, is placed on the outside of the MEAD base to wick into the unused honeycomb tubes. After the glue has cured, the excess guide tubes

are cut to be flush with the outside of the MEAD base (Fig. 2d). Also, the other ends of the guide tubes (sticking out towards the shuttle screws) are cut so that there is only 1-2mm sticking out above the hole. This is so that the guide tube will not block the shuttle from being fully lowered when the MEAD is implanted. The electrodes we use are ordered from Micro Probe, Inc., Gaithersburg, MD. They are 3in long, 0.003in thick, coated with 3micron thick Parylene-C, made of tungsten with a blunted tip profile, and are 3-6MOhm (ordering part # WE3003(3-6)B3). First, all of the shuttles are screwed down approximately 4 turns from the top. This small distance will allow the electrode to be raised into the guide tube honeycomb once it is secured to the shuttle. One electrode at a time, the Parylene-C coating at the connector end is burned off using a butane mini-torch (Weller®/Portasol® All-Purpose Torch KIT; Small Parts Inc., Miami Lakes, FL). The impedance is measured, and the electrode is carefully backed into the honeycomb, blunt end first, then into the main body of the electrode holder. The electrode is secured to a shuttle with a gold electrode attachment pin (Neuralynx, Tucson, AZ) with approximately 4cm sticking out of the top of the shuttle. Next, the shuttle screw is turned to retract the secured electrode until the electrode tip is flush with the end of the MEAD base. Then, two extra turns are added to withdraw the electrode inside the guide tube for protection until surgery. When all of the electrodes are loaded and secured, the top 4cm of the electrodes above the shuttles are bent around and attached to the electrode interface board using small gold pins provided by Neuralynx. The entire assembled MEAD can then be gas sterilized.

SEALING THE MEAD BASE

After sterilization, but before implantation, the base of the loaded MEAD is sealed for two reasons:

- The electrodes need to be protected prior to surgery.
- The ends of the guide tubes need to be filled so that cerebrospinal fluid (CSF) does not wick into the interstitial space between electrode and inner guide tube after implantation. If wicking occurs, the CSF crystallizes within the inner guide tube. The guide tube and electrode would then become cemented to each other and the electrode becomes immovable.

We have used several methods to seal the tips of the guide tubes. One is to just apply triple antibiotic ointment (polymyxin B sulfate, bacitracin, and neomycin sulfate store brand ointment from a local drug store) at room temperature. Another method is to heat the ointment so that it wicks farther into the guide tubes. We have also tried using melted bone wax (Leukens® sterile bone wax; Surgical Specialties Corporation, Reading, PA) or a few drops of mineral oil (Health Pride Mineral Oil; Compass Foods, Montvale, NJ). There has not been a noticeable difference in the longevity of the implant or the quality of the recordings between the different methods.

3.2 Surgery

All experimental procedures were approved by the University of Maryland Animal Care and Use Committee and were in accord with NIH Guidelines on the care and use of laboratory animals.

ANESTHESIA

Originally, surgery was performed under halothane anesthesia (induction: 3.5%, maintenance: typically 1.75 to 2.25%) on domestic ferrets, *Mustela putorius furo*, of

either sex. In the past, halothane was used for surgery because it allowed for quick induction and recovery periods, as well as an effective means to maintain a constant anesthesia level throughout surgery. However, halothane is no longer produced. Currently, we use Aerrane (i.e. isoflurane; Baxter Pharmaceutical Products Inc., Deerfield, IL) at 3.5% for induction and ≤2% for maintenance. Heart rate, blood oxygen saturation (saturation of oxyhemoglobin or SpO2) and body temperature are measured throughout the surgery as indicators of anesthesia level and physiological stress. The anesthesia level is adjusted to keep the heart rate between 210 and 220bpm, and the breathing rate near 30/min.

PRE SURGERY

Just after induction, several injections are given: 0.05ml/kg IM Atropine Sulfate Injection 1/120 Grain (Phoenix Scientific Inc., St. Joseph, MO) to reduce salivation and bronchial secretion; 0.2ml IM dexamethasone sodium phosphate (Phoenix Scientific Inc., St. Joseph, MO) to help prevent brain swelling; and 10mL SC Lactated Ringers Solution (Baxter Healthcare, Deerfield, IL) for metabolism and hydration.

PREPARING THE SKULL

First, the top of the head is shaved and washed with iodine (Betadine® Solution, 10% povidone-iodine solution; The Purdue Frederick Company, Stamford, CT). A few drops of 2% Lidocaine HCl (Abbott Labs, N Chicago, IL) are injected subcutaneously at the midline, to reduce the autonomic reaction to cutting (manifesting itself in a markedly decreased heart rate, from 230bpm to 170bpm for a couple of minutes). A midline incision is made, exposing the skull - specifically the central bony crest. Both temporalis muscles are removed by retracting the scalp laterally, separating the muscles from the

skin and the skull, and clamping the separated muscles for one full minute with hemostatic clamps to cut off the blood supply. The clamps are removed and the muscles are cut with surgical scissors at the site of the clamps. This method prevents nearly all bleeding from the muscle. Then, the skull is very thoroughly cleaned using a Delicate Bone Scraper (Fine Science Tools, Vancouver, Canada). Then, the anchor screws (4.0 mm long, 0.85 mm shaft diameter mini self-tapping bone screws, Fine Science Tools) are put in place. This is done by holding the screw in place with mini forceps (Fine Science Tools) and using a small screwdriver to drive the screw into the skull. Once the screw "catches" (i.e. has begun to tap into the bone) it is rotated 2.5 turns. The number of turns is determined so that the screw is anchored firmly in the skull, but not protruding on the under side of the skull into the meninges. In order for the implant to adhere permanently, the skull must be perfectly and completely clear of any tissue before the next steps. Any tissue remnant on the skull will become a source of tissue regrowth and will eventually lift the implant. Also, the skull must be kept wet with sterile saline right up to the time of cement application; if it remains dry for an extended period of time during surgery, it becomes too porous over the next few days for the screws to anchor properly. See Fig. 3 for a diagram illustrating the spatial relationship of all of the implant components.

[INSERT FIGURE 3 ABOUT HERE.]

MAKING THE CRANIOTOMY

Unless the sulci and gyri can be seen through the skull, the location of the craniotomy is determined as a function of sex and lateral hemisphere (see Table I). For a

picture illustrating this location, see Fig. 3b. Note that adult ferret skulls rarely have identifiable sutures, bregma, or other landmarks, and generally exhibit a large variation in skull size and morphology (He et al., 2002). A 5mm trephine with side opening (Fine Science Tools) is used to cut most of the craniotomy, with a small burr to finish and smooth the edges as necessary, while saline is continuously dripped onto the trephine/skull to prevent heating of the bone and to dampen the noise induced by the trephine which could potentially cause a temporary threshold shift in the neural responses (Evans, 1979).

[INSERT TABLE I ABOUT HERE]

APPLYING 5-FU

If the ferret dura is not contained (by the skull or some other hard surface), then it will quickly grow additional tough fibrous tissue to fill any available space, usually making subsequent electrode penetration difficult or impossible. The mitotic inhibitor, 5-flurouracil (5-FU; Sigma-Aldrich Inc., St. Louis, MO) , is used to prevent growth of the dura (Spinks et al. 2005). The 5-FU solution is made fresh prior to surgery using a 100mg aliquot of the 5-FU powder and 10ml of sterile saline mix stirred with a magnetic stirrer at room temperature for 60 minutes. After the craniotomy is made, 5-FU is applied for 5 minutes on the skull and exposed dura, after which the solution is removed and the craniotomy rinsed once with sterile saline. We have found that the 5-FU application

keeps excess dural and bone growth to a minimum during the subsequent 3 to 5 months while recordings are performed.

IMPLANTING THE MEAD

After exposing the cortex, the MEAD is fit in the craniotomy, and an adjustable collar is tightened around the cap of the MEAD. The collar is attached to a standard stereotaxic micromanipulator (SM-11; Narishige, New York, NY). The headposts (described in more detail in "Immobilizing the Head" below) are then placed in the correct location as shown in Fig. 3b, and dental cement is poured around the apparatus, making sure to cover the anchor screws on the skull and on the MEAD while leaving the ground wire extending out of the cement. We have tried many types of cement to find which particular brand would demonstrate the optimum characteristics for our needs. Some brands significantly degrade the bone structure underneath so that the implant (the MEAD plus the dental cement) easily separates from the skull after a couple of days, two brands cracked after a few days, and several brands got extremely hot regardless of the application method. We have found that Teets Cold Curing Dental Cement (A-M Systems, Carlsborg, WA) does not have any of these problems. Fig. 1c illustrates a cross section of the implant in the skull surrounded by dental cement. The dental cement is applied one thin layer at a time, rather than one thick layer, to minimize the temperature change due to the heat generated in the curing process. In addition to the thin layers, saline is continuously poured over the curing cement to reduce heat build-up. The lateral edges of the cement are shaped over the cut skin to form a small lip under which the skin sits. The purpose of the cement lip is to protect the wound margin so that the risk of infection is minimized. (For more details on how often infections occur and how they are

treated, see "Ferret Welfare" below.) Finally, after the cement has cured, the ground wire is threaded into the implant.

POST SURGERY

The anesthesia mask is removed and several injections are given: 0.05ml/kg at 50mg/ml deep IM Banamine (Schering-Plough Animal Health Corp., Union, NJ) for relief of post-surgery discomfort; 0.2ml/kg IM 2.27% Baytril solution (Bayer Health Care LLC, Animal Health Care Division, Shawnee Mission, KS) for an antibiotic; and 0.4ml/kg at 2mg/ml IM dexamethasone sodium phosphate (Phoenix Scientific Inc., St. Joseph, MO) to reduce brain swelling. In addition, triple antibiotic ointment is applied to the wound margin. Then, the animal is placed inside an animal carrier on an isothermal pad inside the carrier (Deltaphase Isothermal Pad; Braintree Scientific Inc., Braintree, MA) to wake up. Waking usually takes between 3 and 10 minutes. We have found it crucial to lower the electrodes into the dura almost immediately after implantation, as outlined below under "Recording Procedures".

3.3 Animal Restraint

Currently, a custom restraint apparatus is used for 1) adjusting the individual electrodes, and 2) keeping the animal in a calibrated sound field with a free-field speaker during recording sessions. Our restraint apparatus is comprised of a headpost restraint and an adjustable body holder.

IMMOBILIZING THE HEAD

The headposts consist of two cylindrical, stainless steel "horns" attached to a stainless steel base (fabricated in the local departmental machine shop). They are anchored to the animal's skull with dental cement and bone screws. This design has

several advantages. The two horns are more effective than one in preventing the ferret from turning its head while restrained. Placing and orienting the head in the head holder is fast and simple. The surface area held by the dental cement prevents the ferret from dislodging the implant with its strong neck. Finally, the headposts leave the ears unobstructed while the ferret is restrained, which is imperative in auditory research. Fig. 4a is a picture of a ferret with the headposts and MEAD implant.

CONFINING THE BODY

The custom body holder has several advantages. It is easily manufactured in the local departmental machine shop from inexpensive robust materials (in our case, clear acrylic and aluminum). The front dual swinging door design makes it easy to get the ferret in and out of the holder in addition to making sure that the paws can be held back while the MEAD is open. It is highly adjustable in that it can fit a 600g to 2kg ferret easily, which is needed to accommodate males and females of different ages. This adjustability also accommodates the different preferences of each ferret being held. Some of the ferrets will remain stationary only if they fit snugly into the holder, and others strongly prefer to be loosely restrained in the holder. Finally, the holder is portable, which makes it adaptable to many different recording locations and provides an easy means to transport the ferrets within the lab. Fig. 4b is a diagram of the body holder.

To place the ferret in the holder comfortably without damaging the implant, first remove the crossbar from the headpost frame (See Fig. 3c) and the top of the acrylic ferret-body holder. Secure the crossbar onto the implanted headposts, and use it to gently guide the ferret into the holder. Then, secure the crossbar back into place, put the acrylic

top back on, and make adjustments accordingly to fit the size and preference of the animal.

[INSERT FIGURE 4 ABOUT HERE.]

3.4 Recording Procedures

ANCHORING THE ELECTRODES

In our experience, the ferret dura, even when covered by a MEAD implant, will expand to fill in any available space, thereby rendering it much thicker and stiffer. In extreme cases, electrodes cannot penetrate the new growth. The 5-FU virtually alleviates this problem, but penetrating the dura with the electrodes soon after implant surgery is still the most important step for optimizing data collection. Therefore, approximately two hours post-surgery, the ferret is briefly held in the restraint so the electrodes may be lowered to penetrate the meninges. When all of the electrodes are anchored firmly through the meninges (as indicated by a change in electrode impedance, see below), the animal is allowed to recover overnight.

To anchor the electrodes through the meninges, do the following: First, make a small colored mark is made on ½ of the screw head to aid in tracking of the number of shuttle screw turns (and therefore electrode depth). After marking, and during the initial lowering through the dura, the electrodes are lowered by turning the shuttle screws up to one half-turn at a time (78 μm). After each turn, the impedance of each electrode is

measured at 1kHz. When the impedance changes rapidly (typically the value goes from infinite, or unreadable, to 4-6MOhms depending on the electrode), indicating that the electrode has penetrated the dura, we label the depth of the electrode as 0mm. This process is repeated for each electrode. We have tried completing the process turning one electrode at a time so that each electrode is anchored before the next one is turned. We have also tried turning all twelve shuttle screws a half-turn at a time so that the electrodes anchor into the dura at about the same time. There is not a significant difference between the two methods in the quality of the experiment. It is also important to note that when the ferret is in the holder, the cap of the MEAD is removed only when the swinging front doors are secured shut, otherwise electrodes can be pulled out and/or bent by a stray paw.

RECORDING NEURAL ACTIVITY

We have not tested the benefits of different recording systems, and therefore do not want to advocate for particular recording hardware and/or software. In our lab, for neurophysiological recording, we have always used Neuralynx hardware combined with Cheetah software (Neuralynx, Tucson, AZ). Electrode signals are typically bandpass filtered between 300Hz and 3kHz. Recording sessions typically last from 3-4 hours. This is enough time to allow cells to be located on a few of the 12 electrodes and one set of stimuli to be presented: one set of stimuli is typically comprised of a set of tones at different levels to calculate a tuning curve, a set of structured broadband stimuli to calculate a spectro-temporal response function, and a set of stimuli specific for our experimental question. Occasionally, cells are isolated quickly, and therefore, two sets of stimuli are presented with the electrodes in different locations. With a recording session length of 3-4 hours, animals potentially take part in two sessions per day separated by

approximately 1 hour. The length and spacing of the recording sessions were carefully chosen considering several factors. First, if recording sessions are too long, the ferret will start to fidget and/or move vigorously – eventually contaminating the recordings with movement artifacts. Second, if consecutive recording sessions are more than 3-4 hours then, approximately within one week, the ferret will exhibit signs of depression and its health will decline rapidly. The depression and/or declined health is marked by lethargy, weight loss, loss of appetite, dehydration and/or cessation of grooming (In the case that this occurs, see "Ferret Welfare" for a discussion regarding how animal welfare concerns are addressed). Third, the best results are achieved when electrodes are lowered slowly. We have assumed that this is due to dimpling or tissue friction (Kewley et al., 1998; Jensen et al., 2006). It is possible to lower an electrode without initially isolating a cell, but later that day or the next day observing distinct spike activity without moving the electrode shuttle. Generally, we use criterion that an electrode should not be lowered more than 312μm (2 shuttle screw turns at 156μm per turn) per day whether or not a cell is isolated on that electrode. However, the average vertical distance between isolated cells on one electrode is 39μm. Fourth, periodic treats are provided for the ferrets (baby food puréed meats or any ferret vitamin paste, such as Nutri-Cal, manufactured by Tomolyn obtained from any commercial pet supply source) at convenient times between lowering electrodes and presenting stimuli to maintain wakefulness, measured by the EEG on that channel, or monitoring the ferret on closed-circuit television with a camera placed a foot or so from its holder. An equal cell yield can be obtained by lowering all of the electrodes by one-half turn (78μm) at the end of each recording session, and beginning the next recording session without further lowering. This latter method lowers

the daily yield somewhat since the electrodes are not individually adjusted to obtain the best spike isolation. However, there are two major advantages. First, given the finite duration of a recording session, no time is spent finely adjusting electrodes. Second, and more importantly, this removes the natural bias to only record cells that fire strongly and reliably to auditory stimuli. As a result a variety of response patterns have been found that might not have been recorded with the former method. As examples: cells that only fire reliably in response to one frequency; cells that respond with a very long latency; cells that only respond when the same stimulus has been presented multiple times; and cells that respond only to the first presentation of a particular stimulus.

Note that our experiments are typically designed to gather recordings from large numbers of neurons, rather than longitudinal recordings from fewer neurons. Anecdotally, we have seen neural responses with similar or identical response characteristics from a single recording channel over many days (even weeks), after not moving electrodes during that time, suggesting that the setup could be suitable for such longer-term studies.

FERRET WELFARE

As noted above, in the section "Implanting the MEAD", a small lip is formed around the edge of the implant with the dental cement to minimize the risk of infection at the wound margin. In addition to this precaution, triple antibiotic ointment is applied to the wound margin every week day. However, even with these preventative measures, it is not uncommon for the wound margin to become slightly infected over a weekend or when the animal is not examined every day. We have also found that it is not beneficial to scrub the wound margin and remove naturally formed scabs because this increases the

risk of infections. A majority of these infections are minor and do not penetrate below the skin or implant. They are easily cleared up when the triple antibiotic ointment is applied at the beginning of the work week. If the infection does not improve in 24 hours, a small injection of Baytril is used to supplement the ointment.

Early on, our lab tried a longer session length, in which we tried recording for 7 continuous hours at a time. As mentioned above, if the recording sessions are too long, then the animal may become depresses and/or its health may decline as marked by lethargy, weight loss, loss of appetite, dehydration and/or cessation of grooming. Two of our first ferrets exhibited all of these symptoms. When these two specific ferrets dropped below the criterion 80% of their pre-surgery weight, they were immediately removed from the protocol until their health improved. Both ferrets returned to a healthy state in under a week and resumed participation in daily experiments. Since the implementation of the shorter recording sessions (3-4 hours per recording session for no more than 2 sessions per day), we have not observed any symptoms indicating animal distress or any significant weight loss.

Finally, we have developed a day to day handling routine with the ferrets. In our experience, it has been imperative that the ferrets are handled and played with twice daily during the work week. Frequent handling decreases the time for an experimental animal to get used to the recording setup and increases the duration of sessions that they will tolerate comfortably. In addition, we have found that the animals maintain a healthier disposition if they are allowed access to other ferrets. In other words, at night and during the weekend, most of the animals are kept in pairs, and during the day they are all

grouped together in one large, communal cage. This husbandry practice is not changed even when a ferret has an implant.

4 Results

This design has been used, in its final form, on over 20 ferrets, with the implants usable from 4 weeks to over 5 months (3.5 months average). However, in many instances, it seems that even if the ferret stays healthy and infection-free, we are unable to isolate cells easily after 4 months. We suspect that this is because we have lowered the electrode completely through the cortex, and it is generally not possible to isolate spiking activity while reversing the electrode track. This is possibly due to insulating gliosis or other scarring along the track over time. We have noted that the impedance of the electrodes does not vary significantly with time, discounting electrode degradation.

The purpose of this section is to demonstrate the typical quality of daily recordings. Neuronal spikes can be isolated easily and reliably. A sample continuous multi-channel recording is shown in Fig. 5. As is shown, there are many stimulus-evoked spikes that are easily discernable from the background noise on the three channels shown.

[INSERT FIGURE 5 ABOUT HERE.]

After the stimuli are presented, the spikes are isolated and sorted using the standard Matlab *mClust* program developed by Redish et al. (Harris and Redish, 2002). The classification itself uses *KlustaKwik,* which uses a CEM algorithm (*Conditional*

Expectation Maximization) for which we use the Fourier transform, first and second principal components and energy of each event to classify spikes.

Fig. 6a shows four typical waveforms from four different ferrets to demonstrate that the recording technique is successful in many different preparations.

[INSERT FIGURE 6 ABOUT HERE.]

Sorted spikes are used in all subsequent data analyses. A sample raster plot of sorted spikes in response to a set of tones is shown in Fig. 6b.

5 Discussion

Our laboratory encountered a number of challenges in using commercially available products for our experimental paradigm and some unique issues associated with our model species. We have successfully overcome the problems with the experimental setup described here; we can record extracellular neuronal activity from the auditory cortex of the awake domestic ferret. Obtaining the commercial MEAD, loading the electrodes and the implant surgery together typically take 3 days and the implant has been used effectively up to 5 months.

The current design usually uses a fixed-head recording set-up with the animal restrained. It is adaptable to record from a freely moving, behaving animal. Pilot recordings with non-restrained ferrets show virtually no muscle movement artifacts during recordings. However, there is a significant challenge to design a workable tether and commutator system that is robust enough to withstand the energetic movements of

ferrets, their sharp claws and teeth, and their curious, playful nature. We are exploring long-term solutions.

Even with adjustable electrodes, there is a limit to the number of unique penetrations that can be achieved with the current system. We have also developed an extra MEAD feature that allows it to be rotated without an additional surgery. This, along with the off-center honeycomb allows for more than one location to be recorded in the cortex. In order to do this, the outer shell of the MEAD has to be anchored to the cement without anchoring the inner shell and the MEAD base (the upper cone in Fig. 1c). Then, the screws attaching the inner and outer shells can be removed so that the whole inner shell can be removed, rotated, and anchored back in place. In pilot work, we have been able to demonstrate that we can get at minimum three positions, or in other words 36 electrode penetrations. Data collected from the MEAD after such a rotation is identical to that presented in this paper (Figs. 5 and 6).

The commercial MEAD is reusable and repairable. Nonetheless, the lifetime of the MEAD hardware is finite. Possibly due to the repeated physical stress induced by the active ferrets, the shells develop significant fractures and eventually crack over time. Typically, we have used them for 5-6 animals and then must discard them or substantially replace the outer components of the microdrive.

In sum, with certain modifications to a commercially available recording device, we have successfully implemented electrophysiological recordings in a specific model species. The amortized time and resource costs are relatively low for a sophisticated and flexible hardware system that can be re-used. The result of our modifications is a design that lasts easily five months, could be used in a behaving ferret, and can be removed and

cleaned for use in another animal. In addition, there is no evidence that the implant has a negative effect on the ferrets' disposition which would affect the longevity of each experiment. The recording signal to noise ratio allows for spikes to be obtained easily and reliably over the life of the implant. Although our laboratory concentrates on characterizing single units, and uses the multi-electrode system to increase overall yield, the set-up could easily be used for ensemble recording and analysis.

6 Acknowledgements

We are grateful for discussion and advice from Drs Sarah Pallas, Andrew King and Jonathan Fritz. This research was funded in part by a grant from NIDCD, RO1 DC005937 awarded to DAD and an Intramural Bressler Research Grant from the School of Medicine of the University of Maryland. PM also received support from training grant NIH/NINDS 2T32NS007375-11.

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TABLE LEGEND

Table I: Measuring Craniotomy Location

The craniotomy location is dependent on whether the ferret is male or female and whether it is placed over the left or right hemisphere. This table summarizes our measurements to determine the placement of any craniotomy over the primary auditory cortex.

FIGURE LEGENDS

Figure 1: The MEAD

a) A top and side view of the MEAD. The side view shows only two electrode paths and shuttles for clarity. The top view has a square representing the electrode interface board and the twelve microdrives. This thick-wall MEAD weights 12 gm with cover; 34 mm diameter; 53 mm height; 10 mm electrode travel; Electrode adjustment sensitivity is 156.3 microns/turn. b) A close up top and side view of the custom MEAD base. All dimensions optimized for implant in a ferret are noted. The lower neck of the base fits snugly in the craniotomy. The hole is off-center so we can fine-tune which part of cortex will be recorded from by rotating the whole apparatus during surgery. c) Diagram of the MEAD illustrating the relative locations of the dental cement, skull, screws, and brain. The two black "T" shaped objects on either side of the MEAD represent 2 anchoring mini-screws.

Figure 2: Loading the MEAD

a) A picture of the MEAD illustrating how the MEAD base and the main body are held in relationship to one another so that the inner guide tubes can be threaded. b) A close up looking inside the main body of the MEAD illustrating where the inner guide tubes are threaded. c) A close up of the honeycomb made by the outer guide tubes. Note an inner guide tube coming out of one of the outer guide tubes. The hole in the MEAD base is off-center to allow more flexibility in choosing which part of the brain exposed by the craniotomy will be recorded from. d) A diagram of the finished inner- and outer- guide tube configuration. The outer guide tubes make a complete honeycomb, and the inner guide tubes are threaded through them in a snowflake pattern. All of the places shaded in grey are eventually filled with super glue. The white circles are inner guide tubes with electrodes in them.

Figure 3: The surgery layout

a) A front view of a headpost. Several headposts with varying angles are prepared to accommodate for the large variability in skull shape, especially the difference between males and females. b) A schematic demonstrating the layout of all of the components put on the skull during surgery. The circles with crosses represent screws, which are anchors to firmly attach the cement to the skull. Note the ground wire wound around the caudal-most screw. The bulls-eye shaded circle represents the location of the craniotomy. The grey "T" is a top view of the headpost. c) A picture of two headposts. The design on the left is used for males, and the design on the right is used for females. Males typically have a flatter skull than females. d) A picture of a ferret brain with a craniotomy-sized circle over the primary auditory cortex.

Figure 4: An implanted ferret and the holder

a) Ferret with an implanted MEAD and headposts. In this photo the ferret is freely moving and the electrode interface board of the MEAD is attached to a recording tether. b-d) Top, front, and side diagrams of the ferret body and head holder. The body holder is made of clear acrylic, except for the base, which is made of aluminum. There are three shades of grey: dark, medium, and light. The dark grey objects are screws. The medium grey object is the aluminum base. The light grey colors the aluminum "L" braces and the frame which secures the headposts. The objects in white are made of clear acrylic. The detailed dimensions of the holder are not important, but there are several scale bars that illustrate the gross dimensions of the holder. A – length of the aluminum base: 25cm; B – adjustable width of the acrylic ferret-body holder: 5-11cm; C – length of slot for adjustments: 6cm; D – length of the acrylic ferret-body holder: 35cm; E – height of headpost frame: 11cm; F – height of ferret-body holder back: 15cm.

Figure 5: Continuous Data Recording

An example of three simultaneously recorded channels to illustrate the typical signal to noise ratio for awake ferret recordings before amplification. While signal to noise ratios are dependent upon the choice of electrodes and electronics, this demonstrates a typical recording in our lab with the indicated specifications. The vertical axis on each plot is in micro-volts. The bar at the bottom represents the presentation of a 50ms tone at 21160Hz, 45dB SPL.

Figure 6: Sample Data

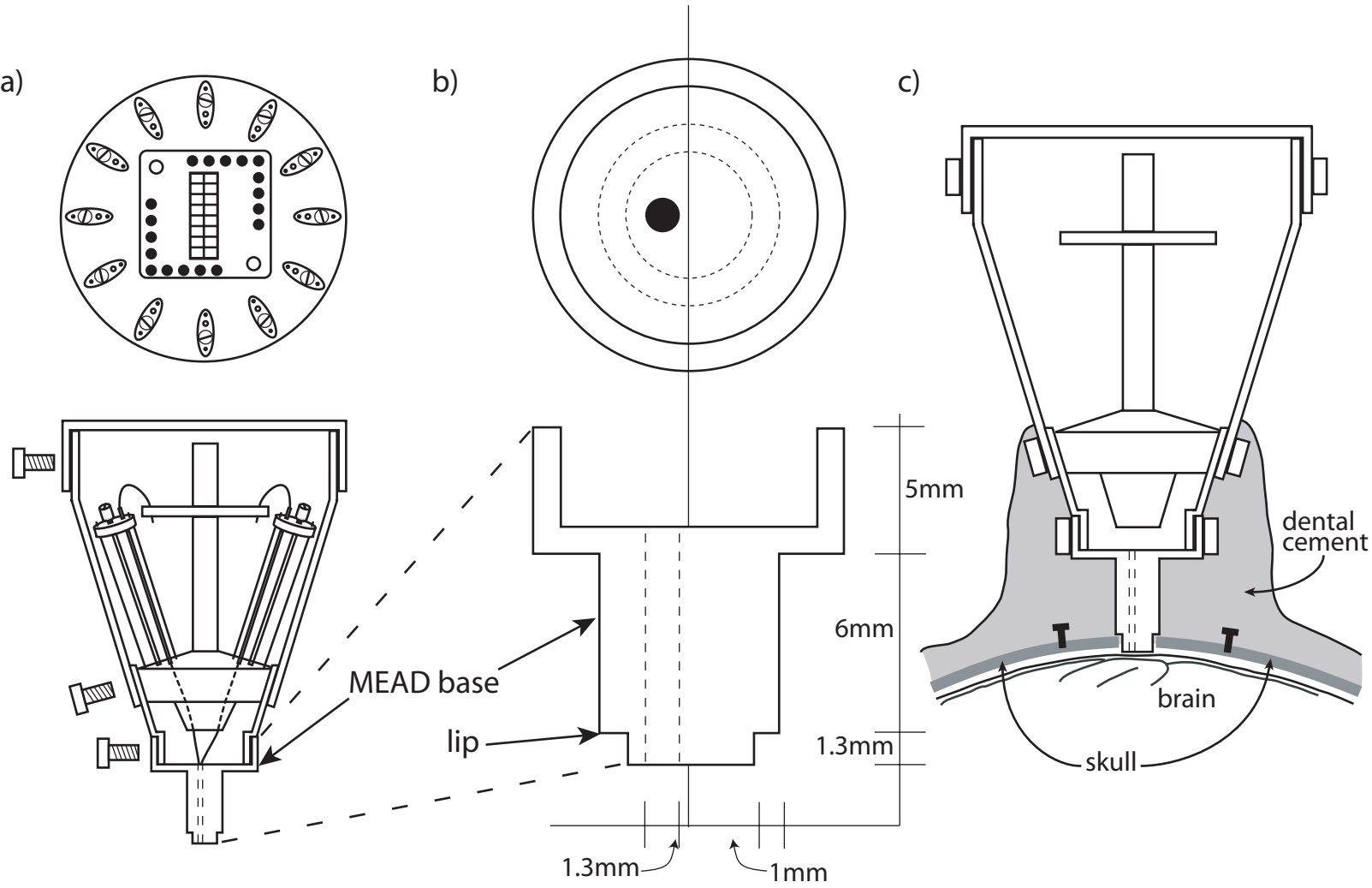
a) Spike waveforms after amplification from four separate experiments are shown to demonstrate the consistency of data quality. For each sample spike, the top graph is 1000 overlaid waveforms and the bottom graph is the mean and standard deviation waveform for the whole recording. The vertical axis is in micro-Volts and the horizontal axis is sample number (or time). Data was sampled at 8000Hz. One time step between two samples is 0.125msec. b) A sample raster plot illustrating the spiking activity in response to tones. Each line represents a presentation of one tone at one decibel level, and each dot represents a spike. The complete set of frequencies was played at three different levels as illustrated on the vertical axis. The horizontal axis is peri-stimulus time.

Table 1

Craniotomy Location

	Male	Female
Distance from left nuchal crest	**13mm**	**12mm**
Distance from right nuchal crest	**11mm**	**10mm**
Distance from central crest	**10mm**	**9mm**

Figure 1


a)
b)
c)
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6mm
1.3mm
MEAD base
lip
1.3mm
1mm
dental
cement
brain
skull

Figure 2

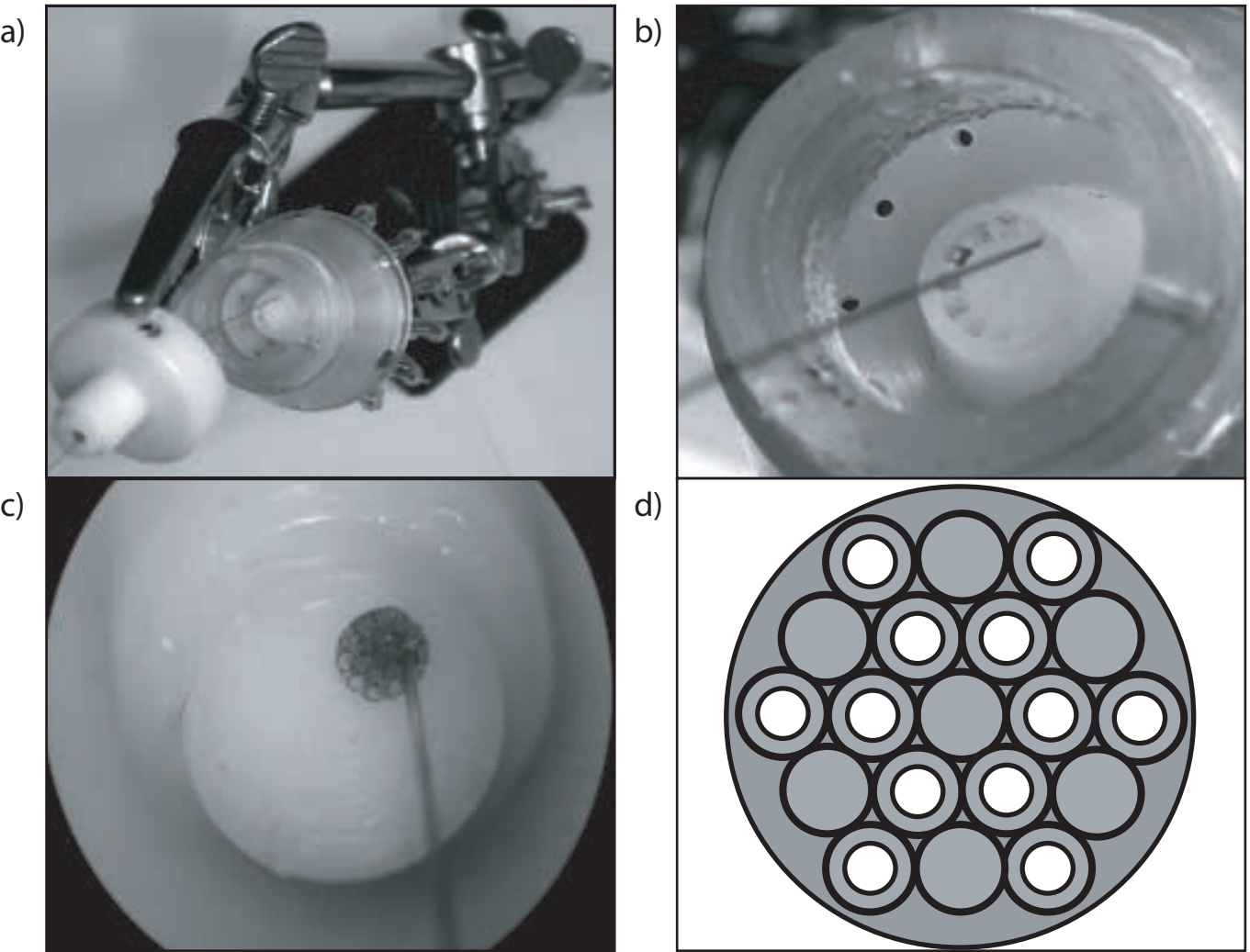


Figure 3

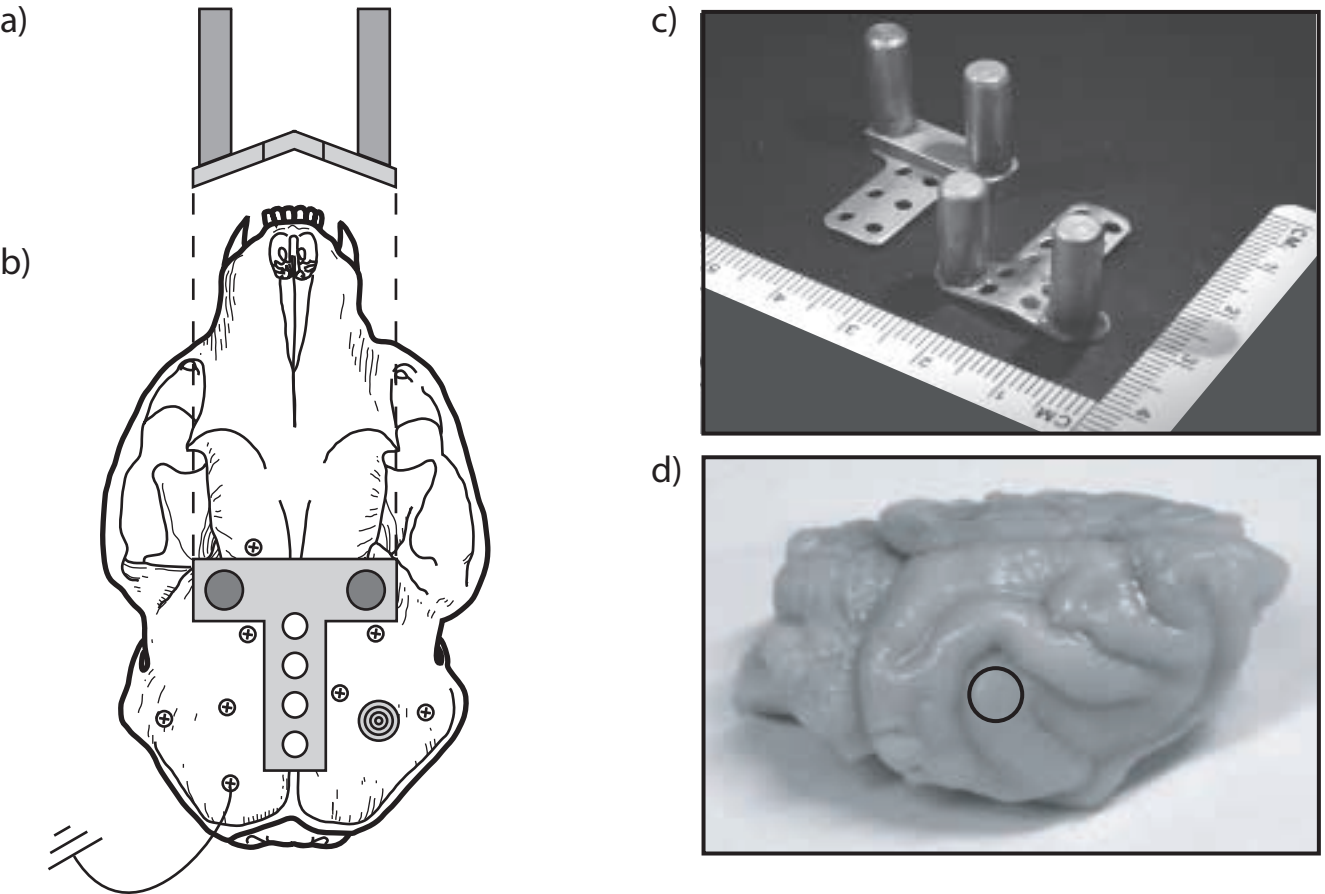


Figure 4

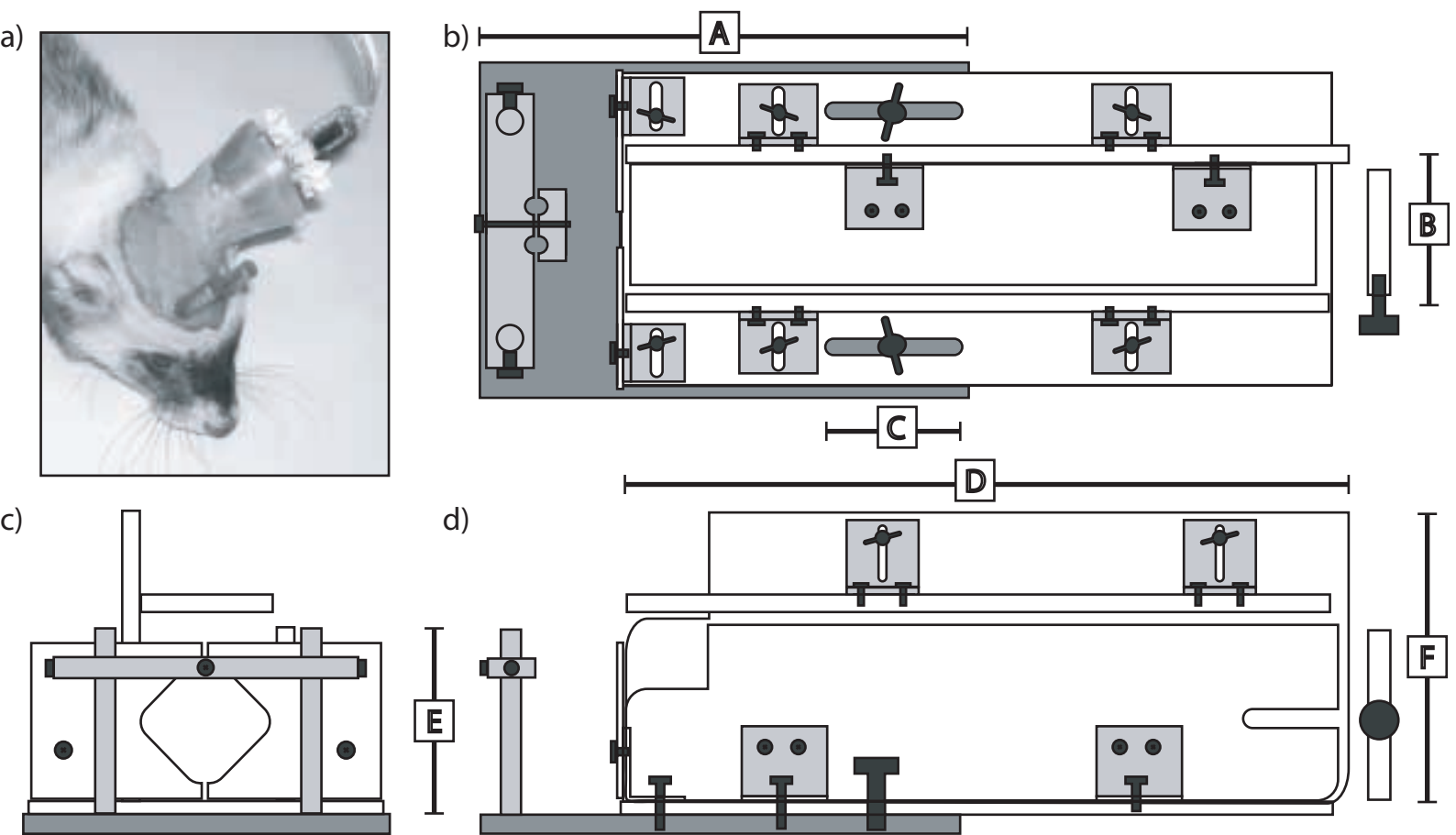


Figure 5

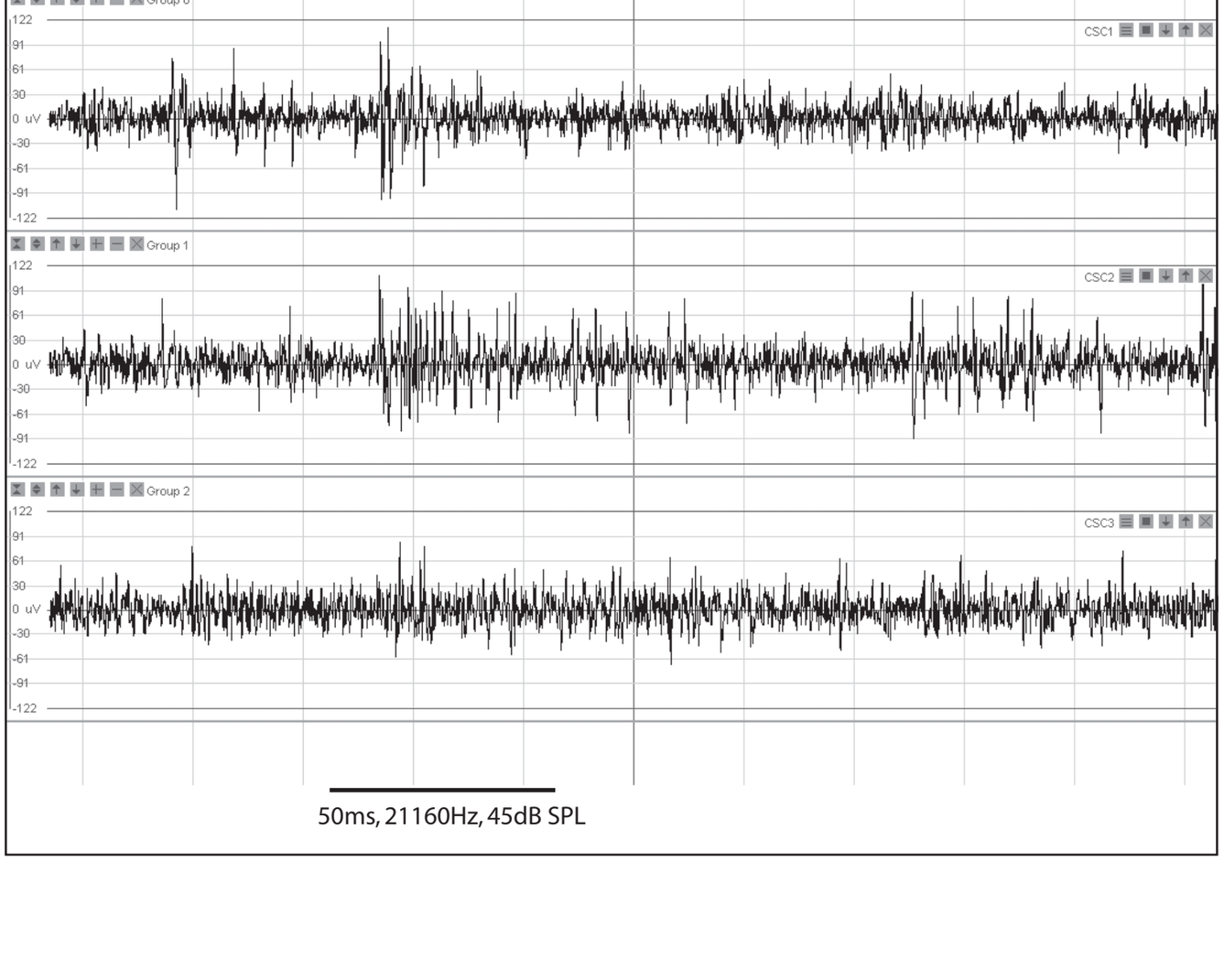
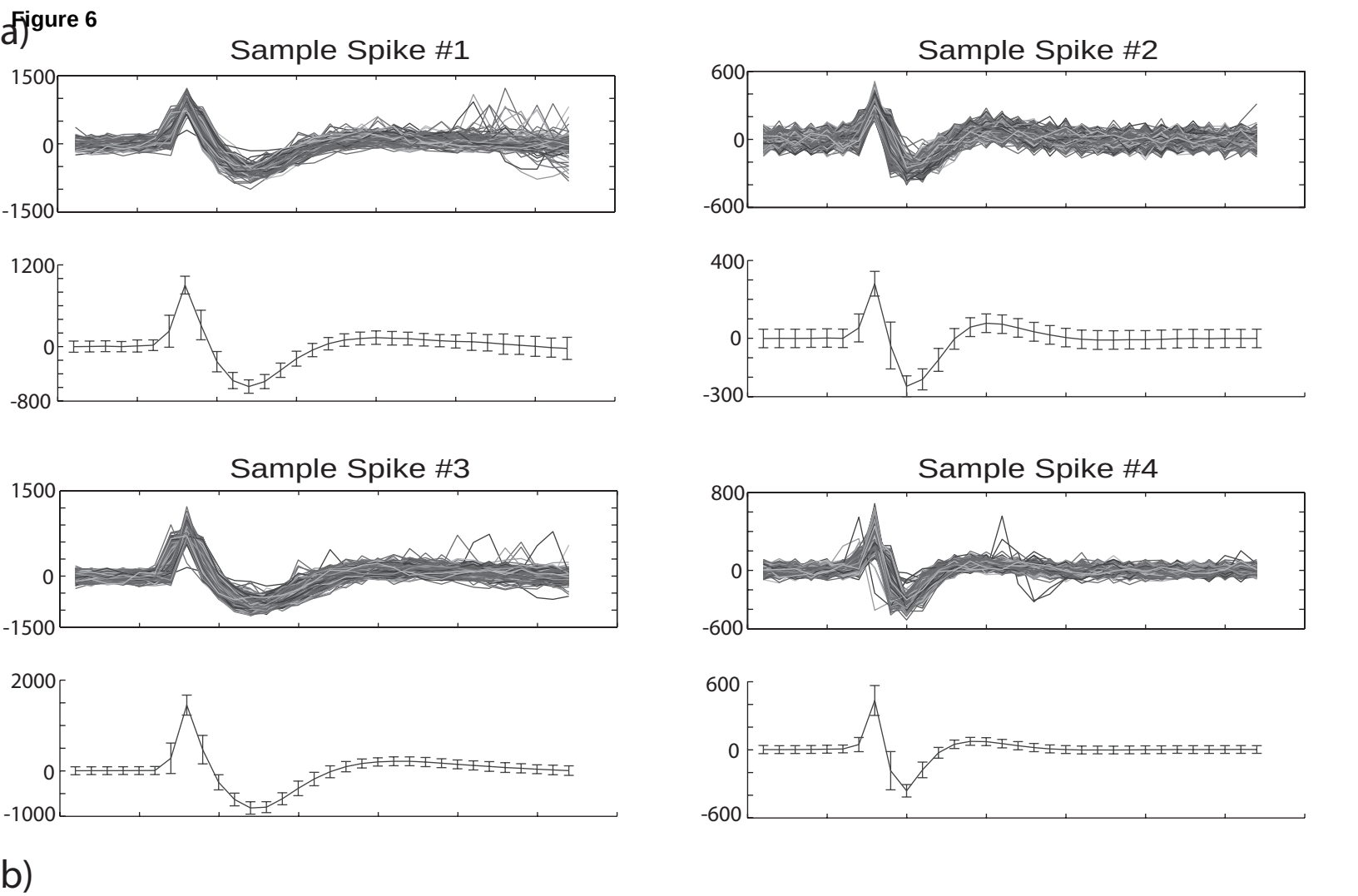
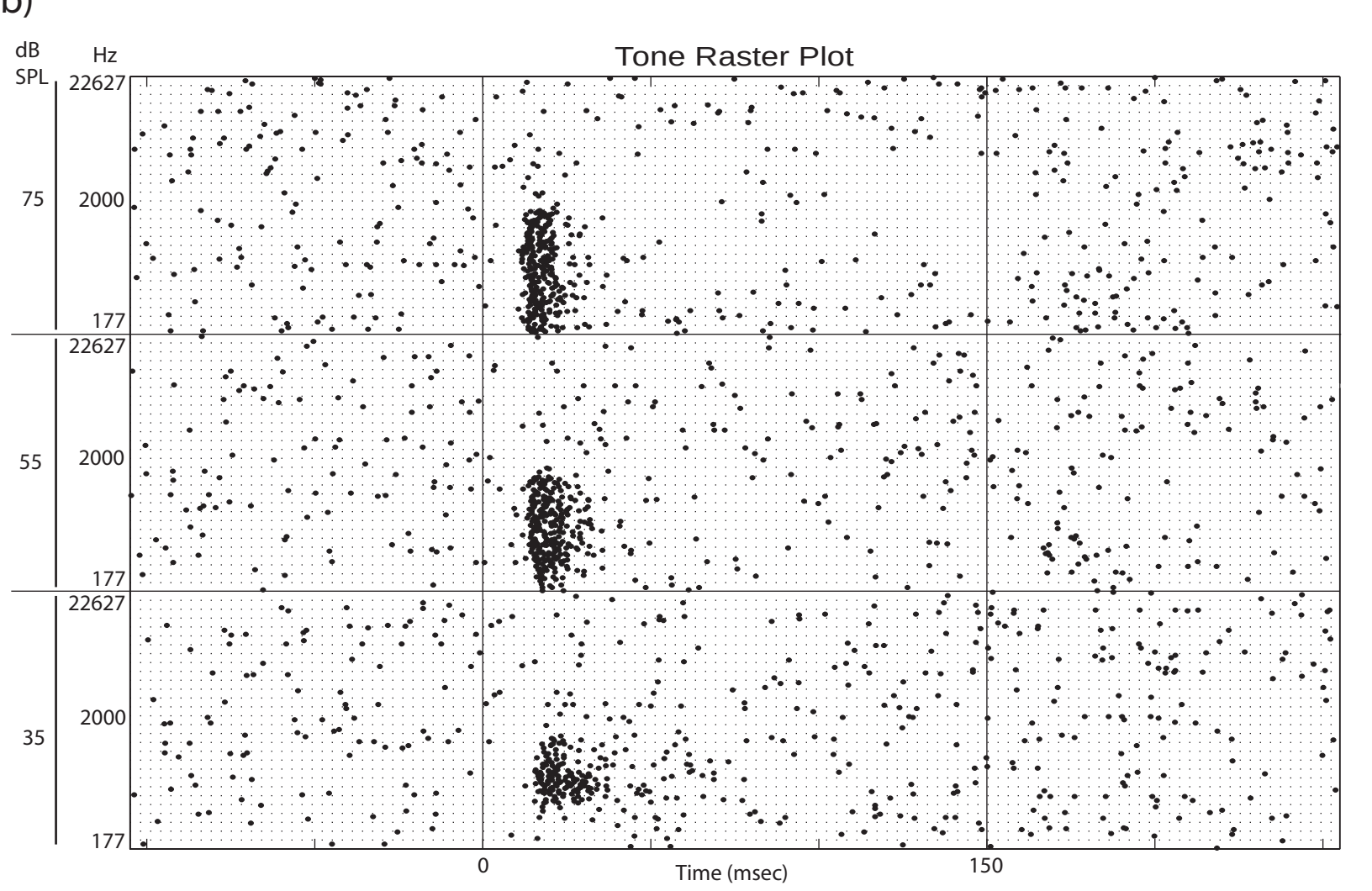

Group 0
CSC1
Group 1
CSC2
Group 2
CSC3
122
91
61
30
0 uV
-30
-61
-91
-122
50ms, 21160Hz, 45dB SPL

Figure 6


a)
Sample Spike #1
1500
0
-1500
1200
0
-800
Sample Spike #2
600
0
-600
400
0
-300
Sample Spike #3
1500
0
-1500
2000
0
-1000
Sample Spike #4
800
0
-600
600
0
-600


b)
Tone Raster Plot
dB SPL
Hz
75
55
35
22627
2000
177
0
150
Time (msec)