Third Quarterly Progress Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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Contents:

1. Introduction

The goal of this contract is to develop methods of protecting the remaining portions of the auditory system from degeneration after loss of hair cells and to improve its effectiveness in extracting information provided by auditory prostheses. We have taken a broad neurobiological approach to this goal in order to study both the short and long-term response of the auditory system to loss of hair cells and the subsequent introduction of afferent input via an auditory prosthesis. Our studies are divided into three major areas of investigation:

(a) The neurophysiological and neuroanatomical response of spiral ganglion neurons (SGNs) and the central auditory system (CAS) following chronic intracochlear electrical stimulation in combination with neurotrophic support of the auditory nerve. This work is designed to investigate whether electrical stimulation and chronic administration of neurotrophins act in synergy to promote auditory nerve (AN) survival in both guinea pig and other mammalian models of sensorineural hearing loss (SNHL). This work will also provide insight into the role of neurotrophins in improving synaptic efficiency in the deafened auditory pathway.

(b) The neurophysiological and neuroanatomical response to prolonged electrical stimulation of the auditory nerve following a neonatal SNHL. This work is designed to provide insight into the protective effects of electrical stimulation on SGNs and the plastic response of the CAS to temporally challenging stimuli presented chronically to one or two sectors of the AN. This work will also examine the ultrastructural changes evident at the AN/cochlear nucleus synapse in response to a neonatal SNHL and to chronic electrical stimulation of the AN.

(c) The application of cell based therapies for rescue and regeneration of SGNs following SNHL. These studies are designed to provide insight into the potential clinical application of cell-based therapies in the severe and profoundly deaf prior to cochlear implantation.

While these studies are designed to provide insight into the plastic response of the deafened auditory pathway to re-activation via an auditory prosthesis, a major objective of this work is to apply our findings to the clinical environment.

2. Summary of activities for the quarter

During the third quarter of this contract the following activities were completed:

- Manuscript preparation: During the quarter one manuscript was published: Shepherd R.K. and Colreavy M.P. Surface microstructure of the perilymphatic space: Implications for cochlear implants and cell or drug based therapies. *Arch. Otolaryngol. HNS*. 130: 518-523, 2004. Two manuscripts were revised in light of reviewer's comments and re-submitted and a fourth manuscript prepared and submitted based on work performed during the present contract.
- The anatomical and functional study of the surgical feasibility of chronic cochlear implantation in the rat was continued. This work has demonstrated that to ensure adequate surgical access to the round window for electrode insertion, the stapedial artery adjacent to the round window must be

cauterized and the opening into the scala tympani from the round window enlarged via a cochleostomy (Fig. 1). We have studied the effects on hearing of cauterizing the stapedial artery by measuring the hearing using frequency specific Auditory Brainstem Responses of five normal hearing adult rats 3 weeks following surgical intervention. There was no statistical evidence of a reduction in hearing across the frequencies tested (0.5 – 32 kHz). The cochleae were then harvested for histological analysis, which will be available during the next quarter.

- We have continued work on the development and testing of our fully implantable small animal stimulators. These devices, designed and built by Rodney Millard, deliver charge balanced biphasic current pulses with a fixed current amplitude of 0.5 mA and a voltage compliance of 6 V. The intensity of the output is adjusted by varying the phase period over a 15 dB dynamic range (50-300 μs). The implant is powered by a pulsed magnetic field generated by coils of wire located outside the animal's enclosure. Two light-emitting diodes are included in the circuit to provide visual conformation through the skin that the stimulator is operating normally. We have completed 56 weeks of *in vitro* testing of two stimulators by continuous stimulation with the devices immersed in saline. One stimulator failed after 48 weeks of testing while the second stimulator continues to function normally.
- During the quarter we implanted a small animal stimulator into an adult rat (Figs. 2-4) and were able to confirm, via x-rays, that the electrode array was successfully implanted into the scala tympani. However, we were unable to successfully record electrically evoked auditory brainstem responses (EABRs) in this animal. The animal made an uneventful recovery from surgery and is currently receiving ~2hr of chronic electrical stimulation/day for a period of 8 weeks. The stimulator will be explanted and evaluated in the following quarter. One outcome from the implant surgery was the need to redesign the electrode assembly, with particular attention given to lengthening the leadwire (Fig. 5) to ensure the stimulator is placed more caudally in order to help reduce the magnetic artifact while recording EABRs. We plan to manufacture and implant additional stimulators in deafened rats during the next two quarters.
- We commenced the assessment of a rat test-box for behavioral studies. This work will evaluate the effects of raising deafened rats with or without simple patterns of electrical stimulation on their ability to perceptually distinguish more complex electrical stimulation patterns. Dr. Paolini and his students at La Trobe University have performed initial assessment, using normal hearing rats and acoustic stimulation. It is expected that minor modifications to the test-box design will be made during the following quarter after which it will be tested for electrical stimulation in deafened/implanted rats.
- We have purchased two acute microelectrode arrays from Cyberkinetics Inc. in preparation for electrophysiology studies of the auditory cortex in cat and rat designed to examine the extent of plastic change in the auditory cortex following deafening and chronic electrical stimulation using a behaviorally relevant stimulation strategy. Each array has 25 electrodes on a substrate of ~2.5x2.5 mm. We also purchased a pneumatic inserter for use with these recording electrodes.
- We have completed the development of a speech processor-based stimulator for use in our feline studies. This work, which was performed in collaboration with Dr. Peter Seligman, a consultant to our contract, uses Nucleus® CI24

cochlear implants in combination with Nucleus® ESPrit 3G behind-the-ear speech processors. The cochlear implants are *not implanted* but are hardwired to connect directly with the animal's percutaneous leadwire system (see Third QPR; Contract NIH-NO1-DC-0-2109). This enables us to have direct electrical access to the electrodes to measure electrode impedance, while removing issues of tissue biocompatibility; i.e. we can use each implant in multiple animals throughout the course of the study. We are currently implanting six active electrodes but plan to extend this to eight in the near future. The present stimulation strategy uses common ground but this can be varied under software control.

- Implanted and commenced stimulation of five young cats previously deafened using multiple neomycin administration (see First QPR; Contract NIH-NO1-DC-3-1005). All animals were successfully deafened and implanted at 7 weeks of age. One kitten has received electrical stimulation constantly for 9 weeks to date (04_929) with no technical problems associated with the stimulation program. Three kittens (04_926, 04_927, 04_928) received between 5-6 weeks of continuous electrical stimulation before their electrode arrays were inadvertently removed from the cochlea. All three animals were extremely active and were encouraged to explore their acoustic environment. We believe that their high levels of activity (including play-fighting) contributed to the displacement of the electrode array. Housing animals individually and minimizing physical contact with other kittens should prevent these problems. However, this incident has also highlighted some flaws in our electrode leadwire fixation technique, which we have addressed during the quarter (see below). Finally, one kitten died from renal failure due to complications associated with the deafening procedure. This is the first death of a cat associated with chronic renal impairment we have had for more than a decade and believe it is related to the revised deafening technique used in this study (see First QPR; Contract NIH-NO1-DC-3-1005). The animal died 11 days following implantation surgery at ~9 weeks of age (04_930). After consultation with our vet, a number of new guidelines for the care of our deafened kittens have been developed. Specifically, we will test renal function throughout the deafening period and prior to implant surgery to ensure each animal has near normal renal function. Deafened kittens will also be placed on a low protein diet.
- We sought to improve our electrode leadwire fixation procedures, which has been previously based on the use of Dacron® mesh ties. We consulted Dr. David Smith from Department of Otolaryngology, Duke University (now at the University of Florida) who kindly provided us with valuable advice regarding the use of Titanium screws to ensure osseointegration and hence excellent skull-based fixation. We have ordered titanium screws together with titanium clips (designed in house) to trial in our next implant surgeries (scheduled for September, 2004).
- Histological evaluation of guinea pig cochleae transplanted with partially differentiated stem cells was completed. This work is now being prepared for publication and will form part of Bryony Coleman's PhD.
- We obtained Animal Research and Ethics Committee approval to commence studies investigating cellular over-expression of BDNF. This work will be led by Dr. Lisa Gillespie. While experiments have not yet commenced on this project, important background work is well underway: (i) in principle

collaboration has been established with Dr. Trevor Kilpatrick of the National Neuroscience Facility and The Howard Florey Institute, Melbourne. Dr. Kilpatrick's group has vast experience in isolating and culturing Schwann cells and is happy to provide us with cultures and/or stocks of Schwann cells together with experimental expertise; (ii) we have sourced both viral and non-viral BDNF cDNA plasmids for use in this work; (iii) we are evaluating the advantages and disadvantages of the various potential experimental options, in particular the use of viral versus non-viral vectors. It is anticipated that experiments will commence on this project within the next quarter.

- Commenced writing a manuscript describing the results from our chronically stimulated cat series performed under our previous contract (see Final QPR; Contract NIH-NO1-DC-0-2109). This work was designed to test the hypothesis that chronic depolarization via electrical stimulation provides trophic support to SGNs following the loss of hair cells. This is a large undertaking and will continue over the following two quarters.
- Continued fabricating electrode arrays and leadwire assemblies for our chronic implantation/stimulation studies. Efforts this quarter included the redesign of the electrode assembly and implantable stimulator for use in the rat (Fig. 5).

3. Plans for Next Quarter

- Continue manuscript preparation and submission including preparation of a manuscript reporting the effects on the deafened guinea pig cochlea following treatment with electrical stimulation (ES) and BDNF for 28 days followed by ES alone for an additional period of period of 2 or 6 weeks. This work tested the hypothesis that ES alone could maintain trophic advantage following initial SGN rescue using BDNF.
- Complete manuscript preparation for our work describing the *in vivo* application of partially differentiated stem cells, and continue *in vitro* studies directed at further differentiating stem cells towards SGNs.
- Complete the development of an electrode array and surgical protocol for chronic cochlear implantation in the rat. Complete histological assessment of normal cochleae following cauterization of the stapedial artery and commence histological studies of acutely implanted cochleae to ensure the surgery results in minimal electrode insertion trauma. Finally we will perform acute electrophysiological studies designed to ensure that we can successfully record EABRs using our fully implantable small animal stimulator. If these studies are successful we will chronically implant a number of deafened rats and commence a chronic stimulation study in this species.
- Complete evaluation of the rat test chamber using acoustic stimulation and begin modifications to allow electrical stimulation in preparation of our rat behavioral studies.
- Commence *in vitro* studies investigating cellular over-expression of BDNF using rat SGNs.
- Deafen a further eight young cats using multiple application of Neomycin. These animals will be implanted at 8 weeks of age and electrically stimulated using a speech processor based stimulation strategy similar to one used

clinically. Their leadwire fixation will be based on the use of Titanium screws and clips.

- Develop a computer-based facility to capture electrode voltage and current waveforms from our chronically implanted cats and guinea pigs. To date we twice daily take measurements manually from an electrically isolated oscilloscope. With the advent of a clinical based stimulator, resulting in a significant increase in the number of stimulus channels, it has become clear that a more automated system was required. Electrode current and voltage waveforms will be captured using a programmable data acquisition device connected to an electrically isolated PC. In-house software will be developed to provide an accurate calculation of electrode impedance for each electrode.
- Commence collaborative studies with Dr. David Ryugo from Johns Hopkins University to study synaptic plasticity in the auditory nerve/cochlear nucleus (end bulb of Held) in our chemically deafened and chemically deafened/chronically stimulated cats.
- Continue to fabricate electrode assemblies for use in our chronic stimulation studies.
- Implant five deafened guinea pigs with electrode arrays incorporating a drug delivery system. These animals will be electrically stimulated and treated with BDNF for 28 days and then be used in acute single unit auditory nerve experiments. This work will be performed in collaboration with A/Prof. S. O'Leary and his colleagues D. Sly and L. Heffer.

4. Personnel

Dr. Lisa Gillespie has commenced her post-doctoral studies as a Research Fellow working full-time with our group. Lisa has expertise in molecular biological techniques and has published several papers relating to the effects of exogenous neurotrophins on SGNs both in tissue culture and in vivo. She will use this expertise to develop intelligent clinically acceptable techniques for exogenous neurotrophin delivery using cell-based therapies as part of our present contract.

5. Acknowledgements

We gratefully acknowledge the important contributions made by our Histologist, Maria Clarke; Veterinarian Dr Sue Peirce; Elisa Borg for management of our animal house; Helen Feng for electrode manufacture; Frank Nielsen for engineering support; Tracey Wasbutzki for human resources expertise; Dr. Tony Paolini, his students and the Mechanical workshop staff at La Trobe University for advice and assistance in manufacturing the rat test chamber; and Jenny Hardman for expert research assistance.

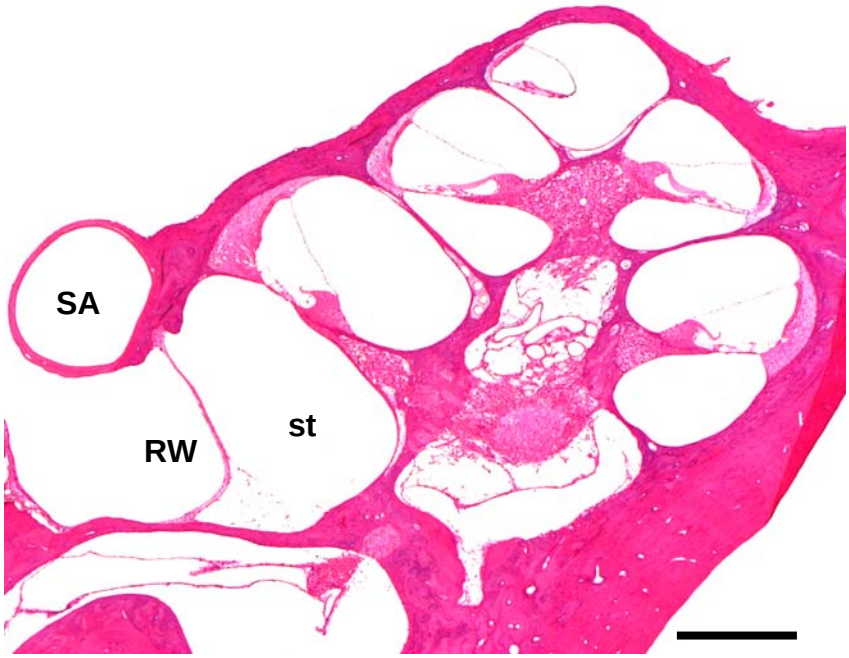

Figure 1. Low power histological image of the rat cochlea illustrating the stapedial artery (SA) in relation to the round window (RW) and scala tympani (st). In order to gain appropriate surgical access for electrode insertion into the scala tympani the stapedial artery must be cauterized and the round window enlarged via a cochleostomy (image courtesy of S. McGuinness). Bar = 500 μm.

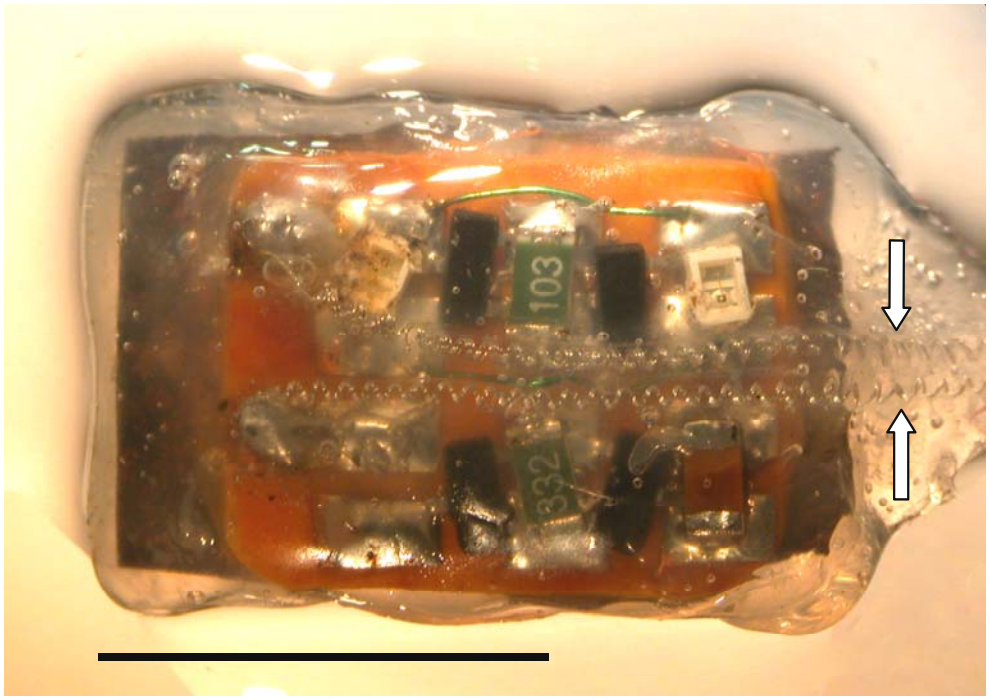

Figure 2. Photograph of a prototype fully implantable small animal stimulator following 2 months of implantation and 1 month of electrical stimulation in a rat. The electrical components are protected from the biological environment by a layer of silicone. Arrow indicates the two helical leadwires that connect to the bipolar scala tympani electrode array (not illustrated). Bar =10 mm.

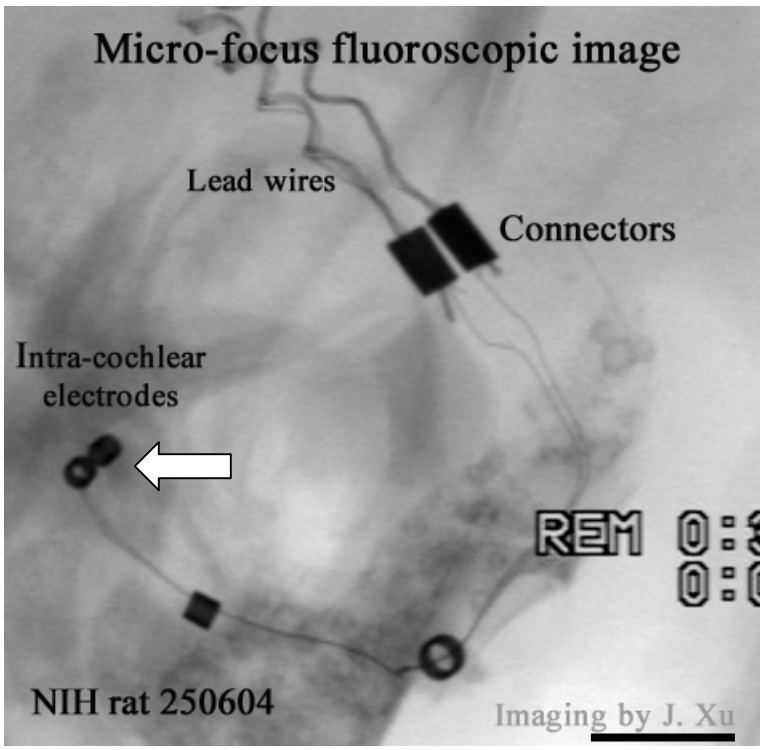

Figure 3. Micro-focus x-ray illustrating the bipolar electrode inserted into the rat scala tympani (arrow). The connectors join each 25 μm diameter platinum wire to a multi-stranded leadwire. Bar = 2 mm.

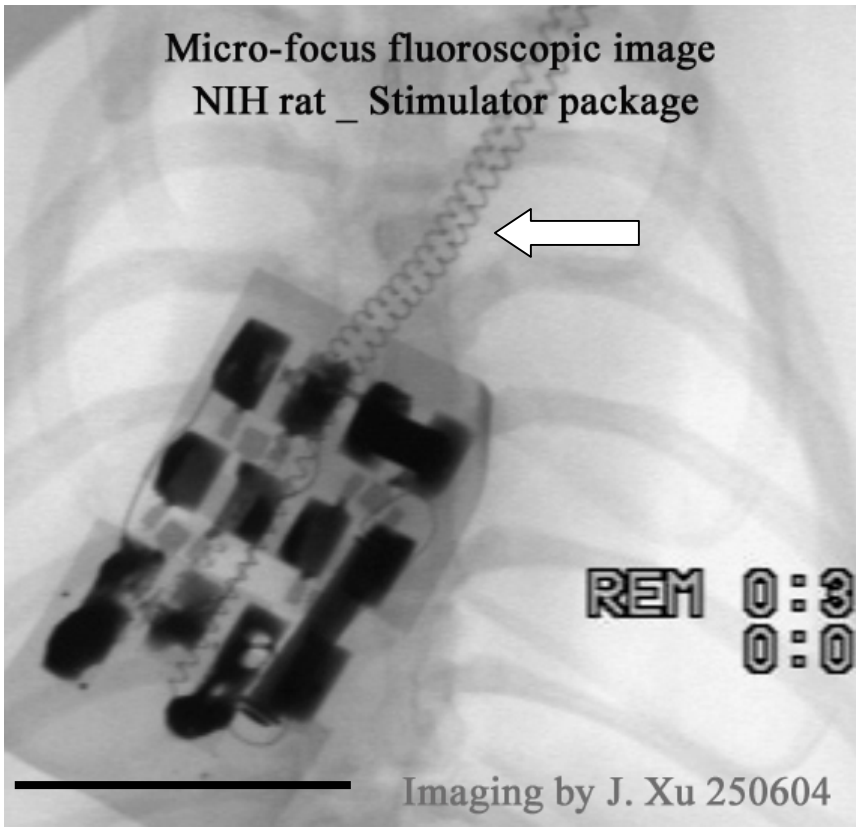

Figure 4. Micro-focus x-ray of the rodent stimulator following chronic implantation in the rat. The stimulator is positioned over the animal's thorax, and the leadwire (arrow) connects to the platinum scala tympani electrodes illustrated in Fig. 3. Bar = 10 mm.

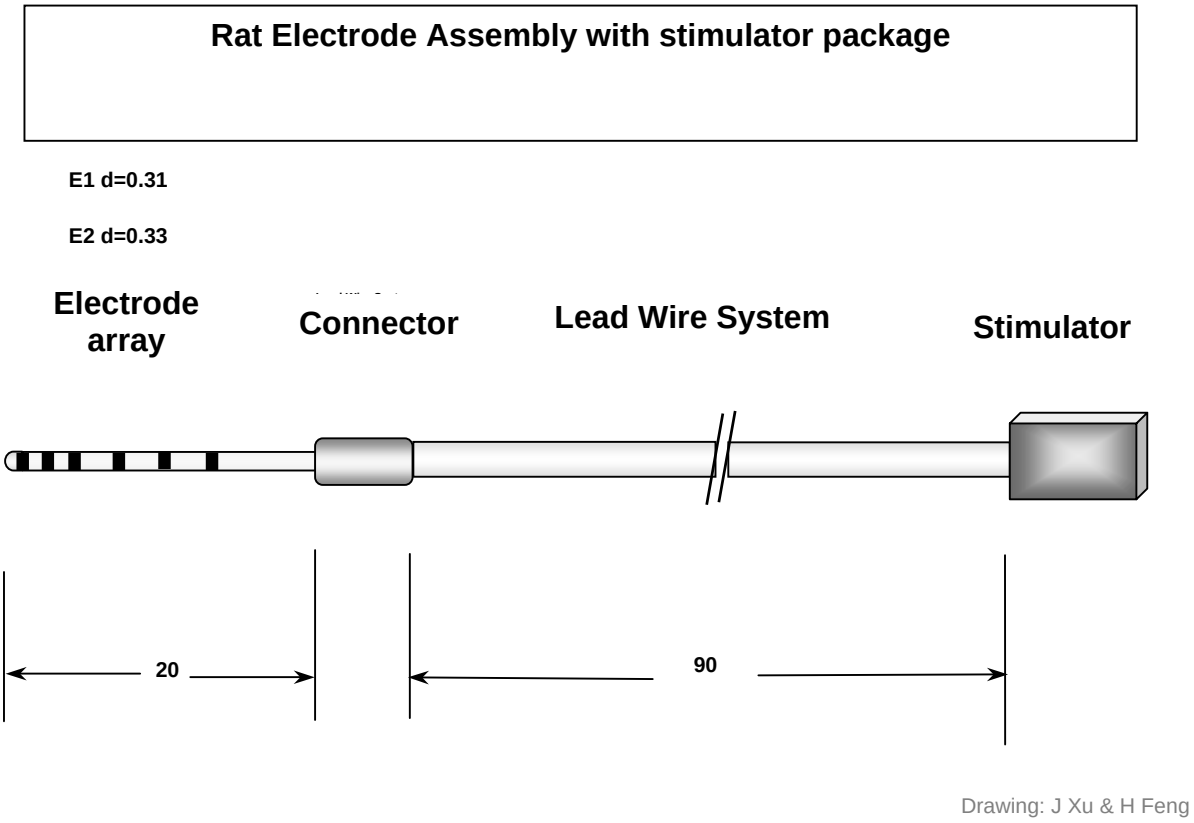

Figure 5. Revised electrode design for chronic implantation in the rat. All dimensions are in mm.