"A Cochlear Nucleus Auditory prosthesis based on microstimulation"

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Progress Report # 1

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INTRODUCTION

The goal of this project is to develop a central auditory prosthesis based on an array of microelectrodes implanted into the ventral cochlear nucleus, in order to restore hearing to patients in whom the auditory nerve has been destroyed bilaterally. The largest such patient population are those afflicted with type II neurofibromatosis (NF2). We will use data and technology developed at HMRI and at HEI to enable Cochlear Ltd. to manufacture the microstimulating arrays at their Engineering and Manufacturing facility in Sydney, Australia, where it will be integrated with a Nucleus 24 acoustic processor and with an array of surface electrodes. The microstimulating array is composed of 8 discrete activated iridium microelectrodes and 2 longer stabilizing pins (Figure 1). HMRI will fabricate and insulate the microelectrodes, expose their tips with an excimer laser, activate the exposed iridium and ship them to Sydney, where Cochlear's personnel will complete the fabrication process using specifications developed at HMRI and HEI. Figure 1 shows a prototype array fabricated in Sydney.

Cochlear's quality assurance protocols dictates that the arrays must be fabricated using materials that are part of their quality control system. Thus, we have agreed that the array's superstructure (button) will be fabricated from EpoTek 301 casting Epoxy and that the electrodes will be insulated with Parylene C. Both of these materials carry USP Category VI certifications. We have previously avoided using Epotek 301 in our animal studies at HMRI because certain of its mechanical properties, including its low viscosity and high surface tension imbue the uncured Epoxy with a marked tendency to wick along the electrodes and leads extending from the casting. Also, we have insulated our electrodes with Epoxylite 6001 varnish rather than with Parylene-C, in part because the Epoxylite can be applied to the electrodes with minimum equipment.

EpoTek 301 has undergone extensive biocompatibility testing pursuant to its certification for chronic human implantation, but to our knowledge it has never been tested in brain. Parylene C has been used extensively as an insulation for chronically-

implanted microelectrodes and for other medical components and the free film carries a USP Category VI classification. However, Cochlear's manufacturing process necessitates placing the electrode shafts under considerable mechanical stress (including bending the shafts at a 90/ angle), and they had observed that Parylene insulation exhibited very poor adhesion to the smooth metal electrodes. However, Specialty Coatings Inc. has developed a procedure for priming metal surfaces with a monolayer of a methyl silane (Silquest A174), which greatly enhances the adhesion of the Parylene to the metal. A174 has undergone toxicity screening and has been found to be quite unreactive. It has been reported that Specialty Coatings performs adhesion-enhanced Parylene coating of medical implants for several manufacturers, although the details are protected by non disclosure agreements between SC and the respective parties. There is no published data on the response of brain tissue to Silquest A174. Although only a minuscule amount of this material is applied to each electrode, and most of that will be isolated from the brain by the over coating of Parylene, the brain parenchyma may be exposed directly to a trace of the silane near the tip of the electrode, at the junction between the laser-ablated Parylene and the exposed iridium metal. We therefore decided to evaluate arrays fabricated with EpoTek 301 and adhesion-enhanced Parylene, in rabbit brain.

METHODS

We elected to implant the electrodes into the cerebral cortex because it and the underlying white matter affords a homogeneous substrate in which to evaluate the implants histologically. Two arrays of 8 electrode shafts extending from superstructures of EpoTek 301 were fabricated at HMRI. The 2 arrays included 6 uninsulated iridium electrodes approximately 50 ：m in diameter and 10 shafts insulated with Parylene C. The latter were cleaned in Ethyl alcohol and sent to Specialty Coatings, Inc. (Ontario, CA), where they were plasma-cleaned, primed with Silquest A174 Saline, and coated with approximately 2.5 ：m of Parylene C. They were returned to HMRI where the electrode tips were exposed to approximately 2,000 ：m² using an excimer laser. Figure 2 shows a scanning electron micrograph of a similar iridium microelectrode (in this case,

with a surface area of approximately 1,000 ：m²). The discrete iridium electrodes were then incorporated into arrays. They extended 1 to 2.5 mm beneath the Epoxy button, so that the tips would reside either in the cerebral cortex or in the underlying white matter. The completed arrays were cleaned using a modified Clemson protocol (Rolands et al., 1986), and sterilized with Ethylene oxide.

The two arrays were implanted into the parietal cortex of a young New Zealand rabbit. The animal was anesthetized with Halothane and Nitrous Oxide, and the arrays were implanted into the subdural space using the custom hand-held inserter tool. The dura and scalp were closed over the array and the animal was given appropriate postoperative care, including medication for relief of pain. Thirty-seven days later, the rabbit was deeply anesthetized and perfused through the ascending aorta with ½ strength Karnovsky's fixative. The electrodes were removed from the fixed brain, the cortex containing the electrode tracks was resected as a single block, embedded into paraffin, sectioned at approximately 6 ：m in a plane perpendicular to the axis of the electrodes and were stained with Toluidine blue (Nissl stain). The tissue surrounding each electrode shaft and the tissue surrounding each of the tip sites was photographed with a digital microscope camera. The tip sites were photographed approximately 20-25 ：m above the bottom of the patent electrode track, near the boundary between the Parylene insulation and the iridium metal. This is the site where we would expect that any tissue response to the silane A174 primer would be most evident.

RESULTS

Figure 3A shows a histologic section perpendicular to the plane of the superstructure (button) of the posterior array and through the shallow depression left in the cortex by the superstructure. The photograph also shows the tenuous remnants of the pia, which are visible as sinuous filaments within the depression. There was remarkably little connective tissue beneath this button, or that of the anterior array. We did note one small proliferation of cells, (arrow) probably leukocytes and fibroblasts which may be the beginning of the formation of a connective tissue capsule. However, the lack of connective tissue at 37 days was quite remarkable. Figure 3B shows the

surrounding molecular layer and the superficial layers of the cerebral cortex at higher magnification. The neuropil appears normal and there is no evidence of edema.

Figure 4 shows a histologic section 400 ：m beneath the posterior array and includes the tracks of 6 of the 8 electrodes. Linear scars extend from most of the tracks indicating some movement of the array during or after implantation. However, the neuropil is not edematous, and the neuronal density within the region encompassed by the array appears to be normal. Figure 5A shows one of the uninsulated electrode shafts from the anterior array. Figure 5B shows a section through an adjacent shaft which was coated with the adhesion-enhanced Parylene C. The thin cellular capsule surrounding the insulated shaft is slightly more prominent, and overall this was typical of all 16 shafts. However, the cellular capsule was less than 5 ：m in thickness, and the surrounding neuropil and neurons appear to be quite normal.

Three of the 6 uninsulated tip sites were in the cortex and the tips of 3 (longer) electrodes were on the underlying white matter. Six of the 10 insulated tips were in the cortex. Figure 6A and 6B show the sites of 2 of the uninsulated tips within the cerebral cortex. Figure 6C and 6D show 2 tip sites of uninsulated electrodes that were in the underlying white matter. As in our previous studies, the gliotic scarring is more pronounced in the white matter than in the cortex. Figure 7A-7D show the tip sites of 4 of the 6 insulated electrodes that were in the cerebral cortex. Overall, there were no discernable differences between the sites of the insulated and uninsulated tips, either in the cortex or in the white matter, except that glial cells were slightly more numerous and prominent in the linear scars trailing from one side of the insulated electrodes.

The spatial distribution of neurons around the insulated and uninsulated electrodes was calculated from the digitized micrographs using a commercial image analysis program (Global Lab Image) and custom editing software written in QuickBASIC. An operator must click with a mouse cursor on each neuron’s nucleolus, and also on the center of the electrode tip. The remainder of the procedure is largely automated. The distribution of neurons around the 6 coated and 4 uncoated sites in the cortex is plotted in Figure 8. Each symbol represents the average number of neurons/ ：m^2 within an annulus that is 25 ：m wide and centered on the center of the tip site and

with mean radius as indicated on the abscissa. The counts were normalized on the area of the annulus so that the neuronal density (the ordinate) is expressed as neurons/ ：m^2. The neuronal density within 50 ：m of the center of the insulated and uninsulated tip sites was reduced (part of this area is encompassed by the patent electrode track and by the surrounding capsule). For distances greater than 100 ：m from the center of the site, the annuli intersect the borders of the photograph and the area normalization procedure yields an erroneously low value for neuronal density. However, for distances up to 100 ：m from the center of the sites, the neuronal density was nearly identical for the insulated and uninsulated electrodes.

In conclusion, at 37 days after implantation on the surface of the rabbit brain, EpoTek 301 induced no detectable connective tissue capsule beneath the array's superstructure, and the underlying neuropil and neurons appeared normal. Electrodes coated with 2.5 ：m of Parylene C that was adhesion-enhanced by a monolayer of Silquest A174 induced tissue responses that were very similar to those of uncoated electrodes.

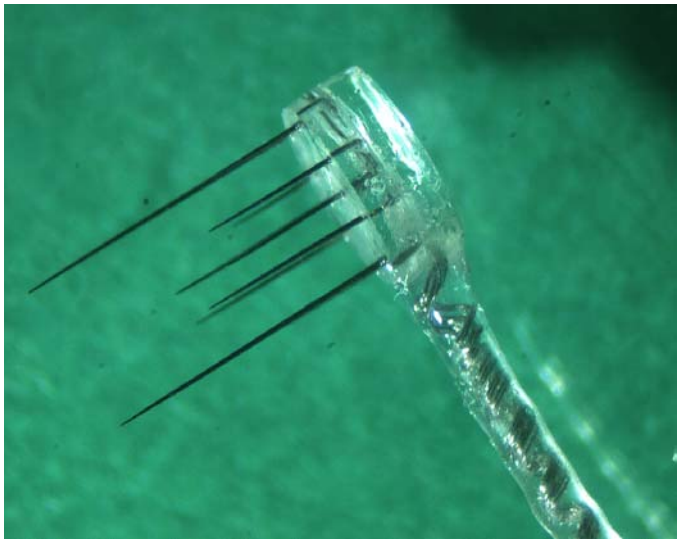


Figure 1.Prototype human array

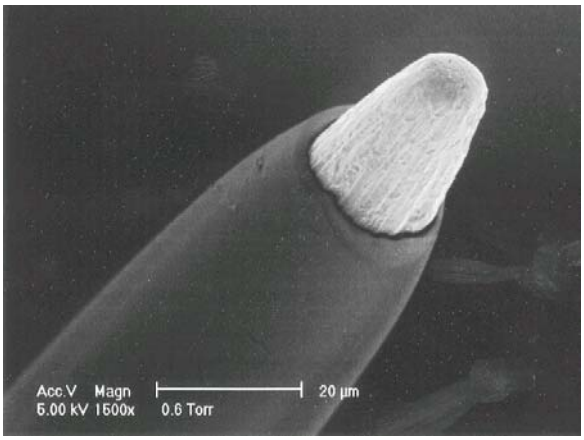


Figure 2. SEM of insulated electrode

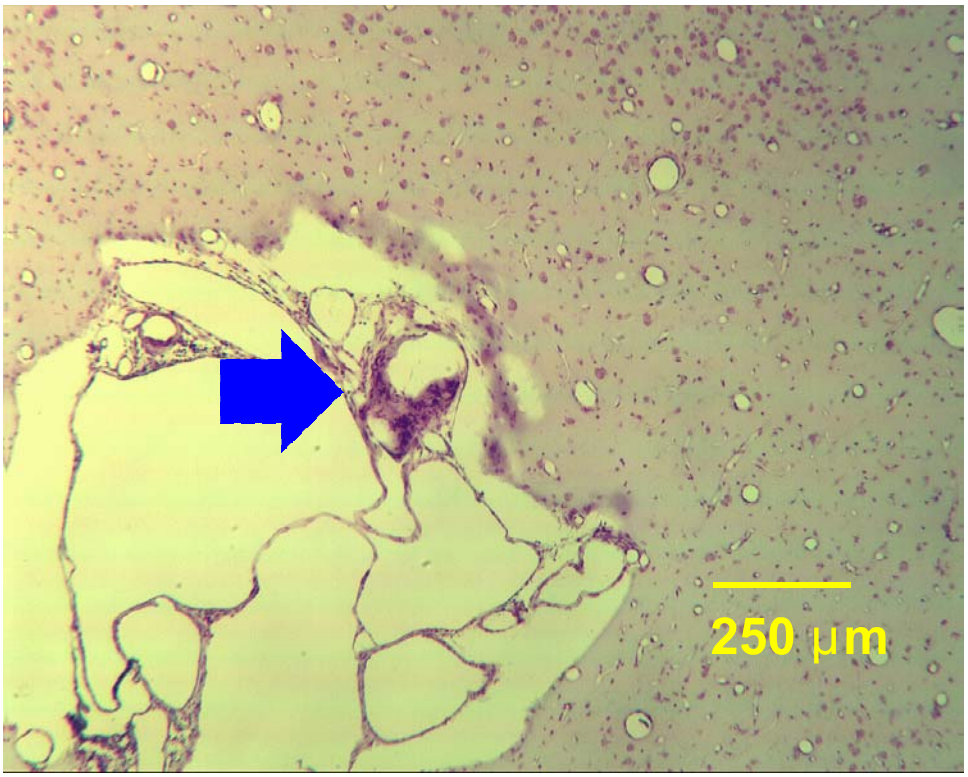


Figure 3A. Depression in cortex under posterior array

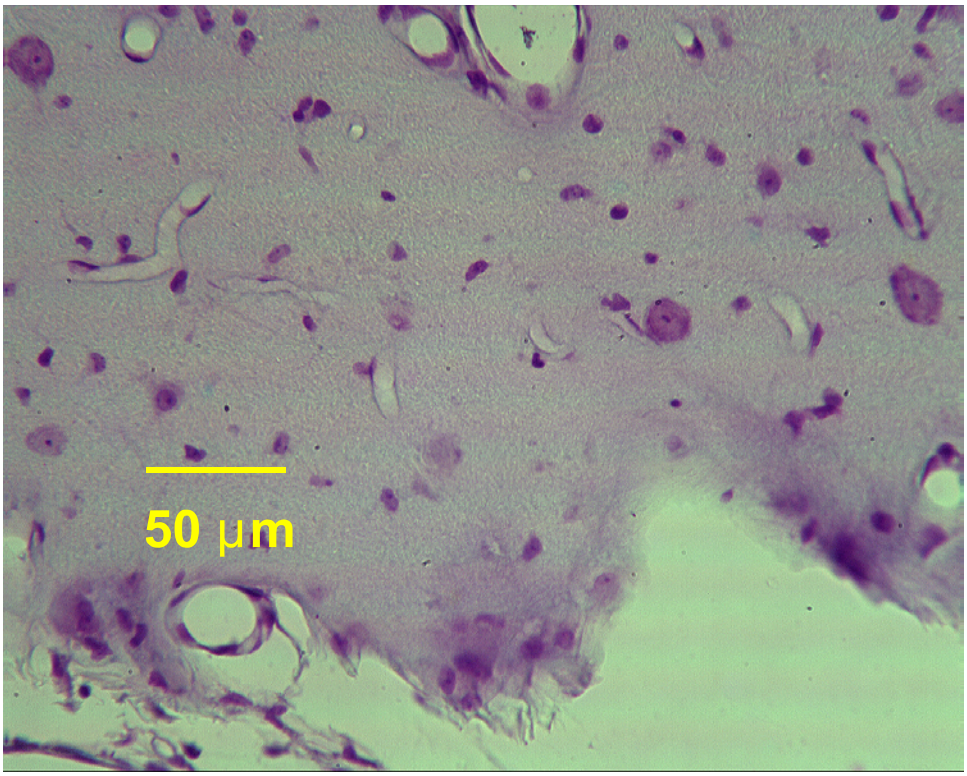


Figure 3B. Superficial cortex adjacent to depression

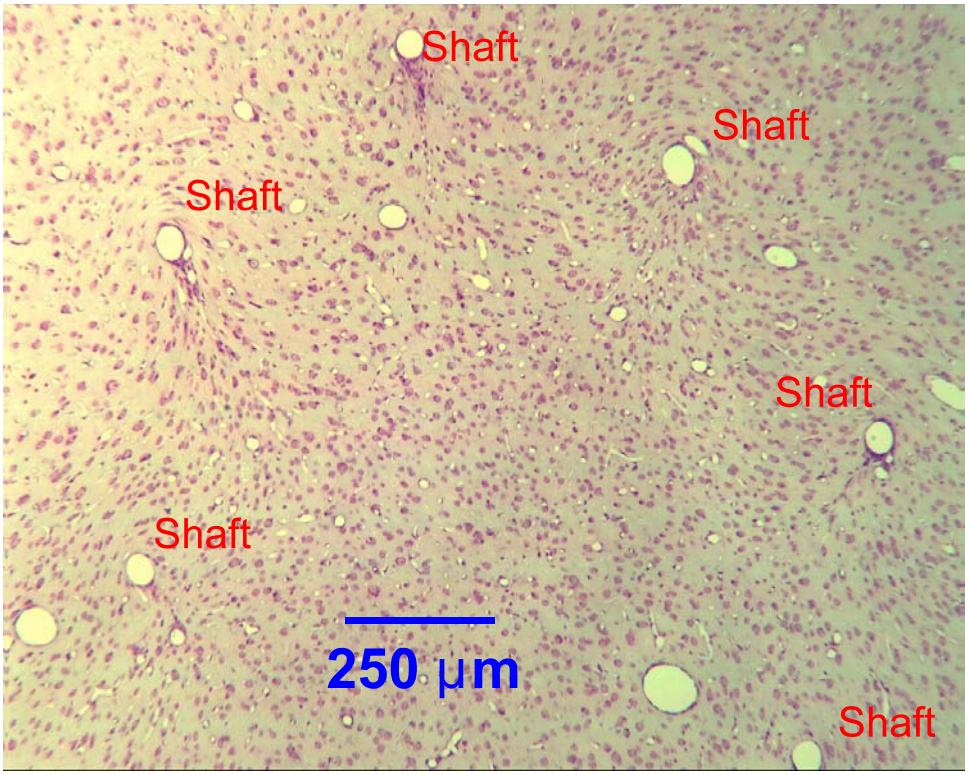


Figure 4. Cortex 400 ：m beneath posterior array

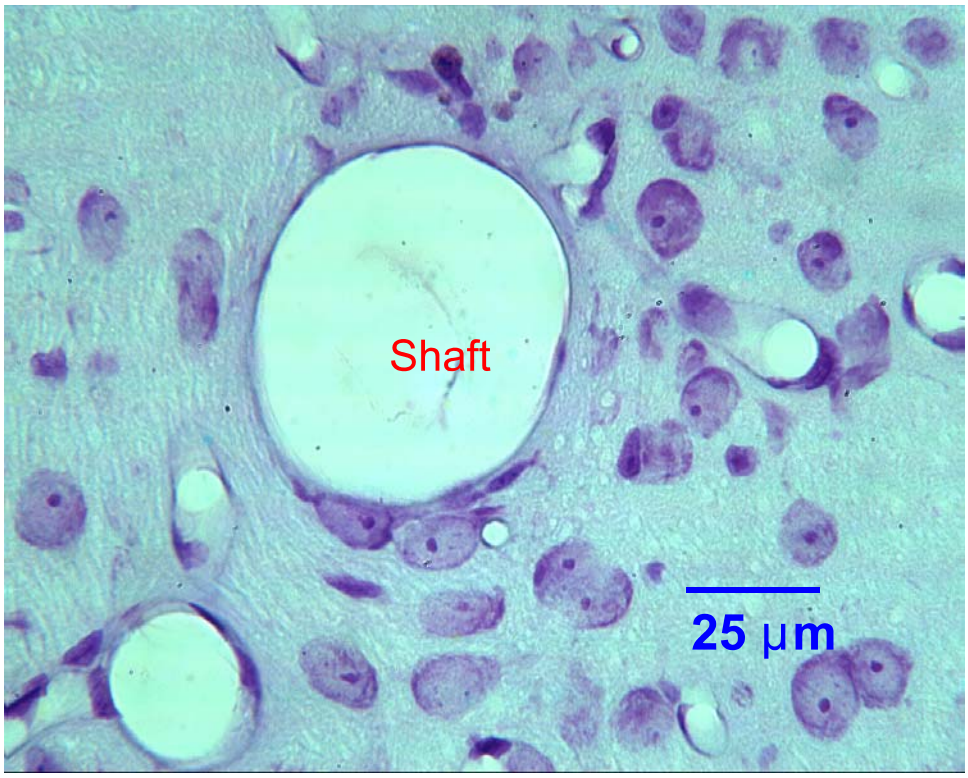


Figure 5A. shaft of uninsulated electrode a7

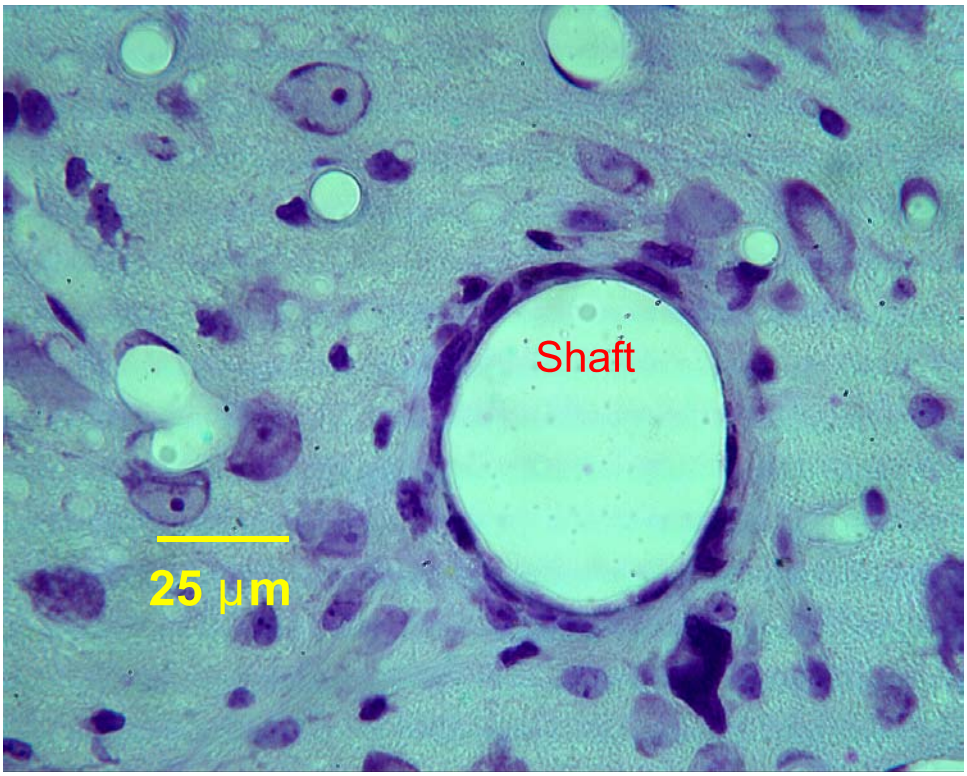


Figure 5B. Shaft of insulated electrode a6

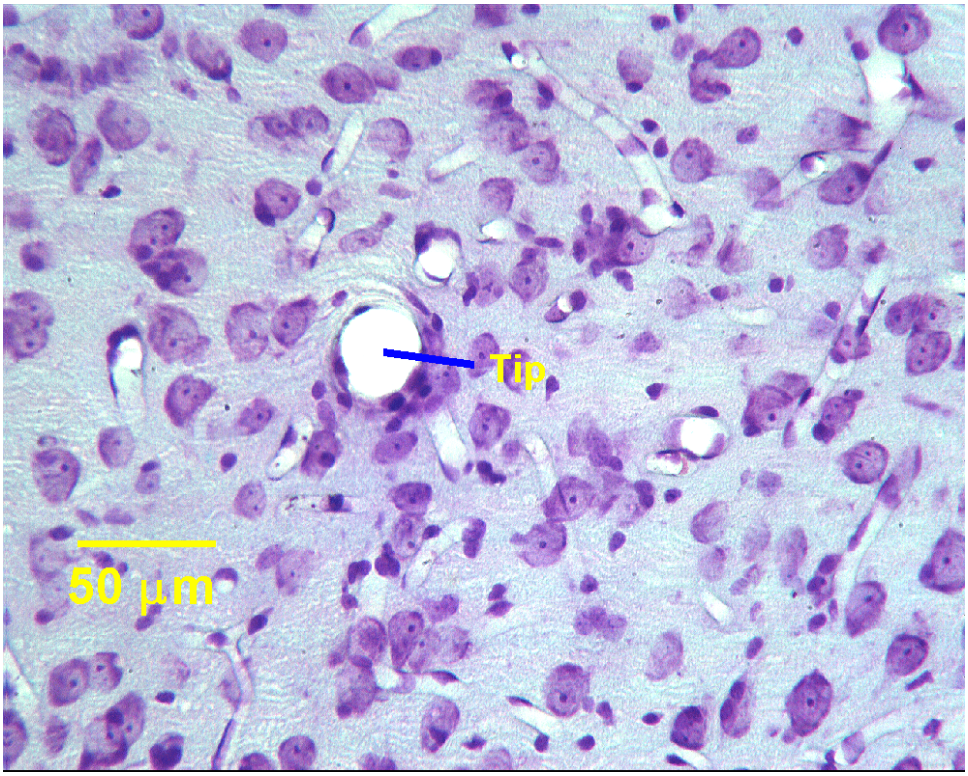


Figure 6A. Tip of uninsulated electrode p8

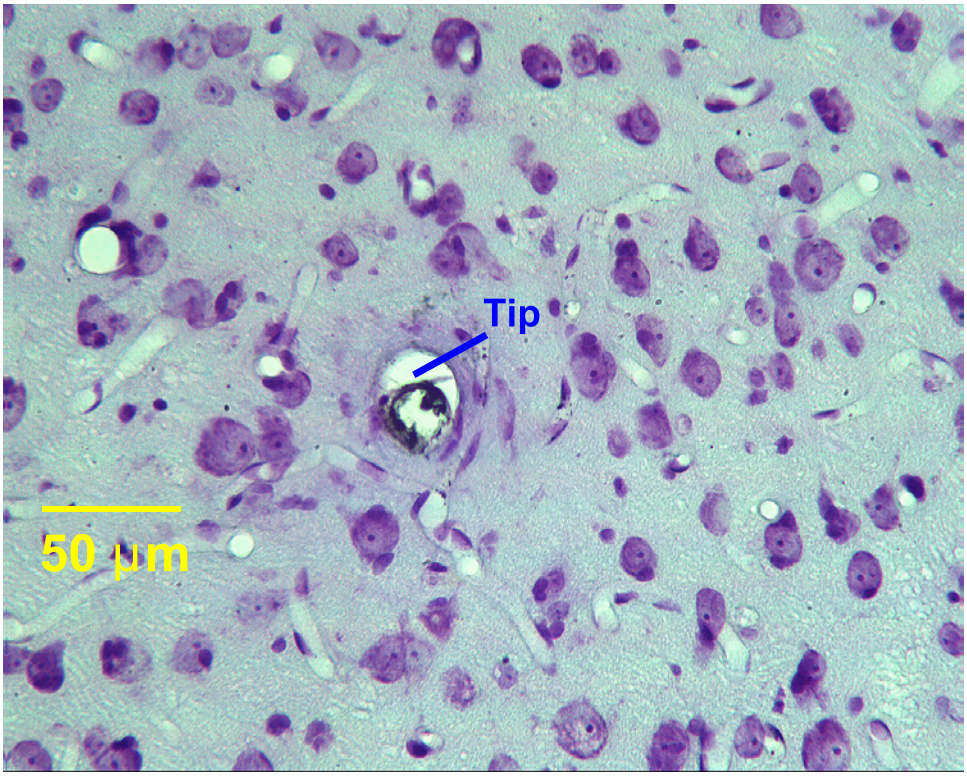


Figure 6B. Tip site of uninsulated electrode a8

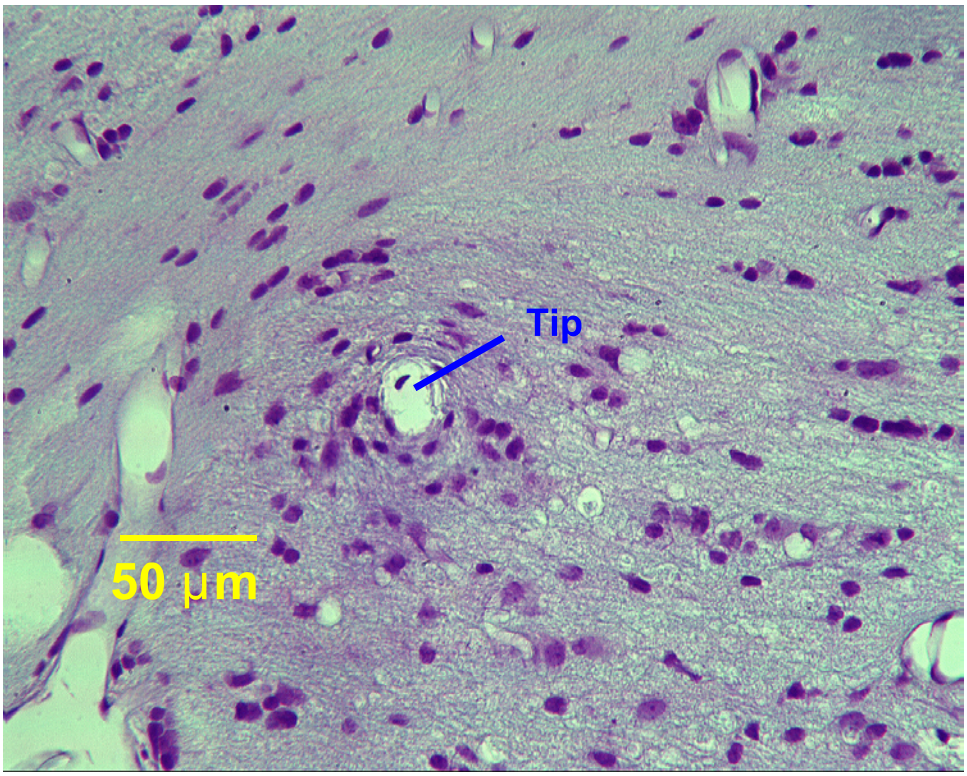


Figure 6C. tip of uninsulated electrode p3

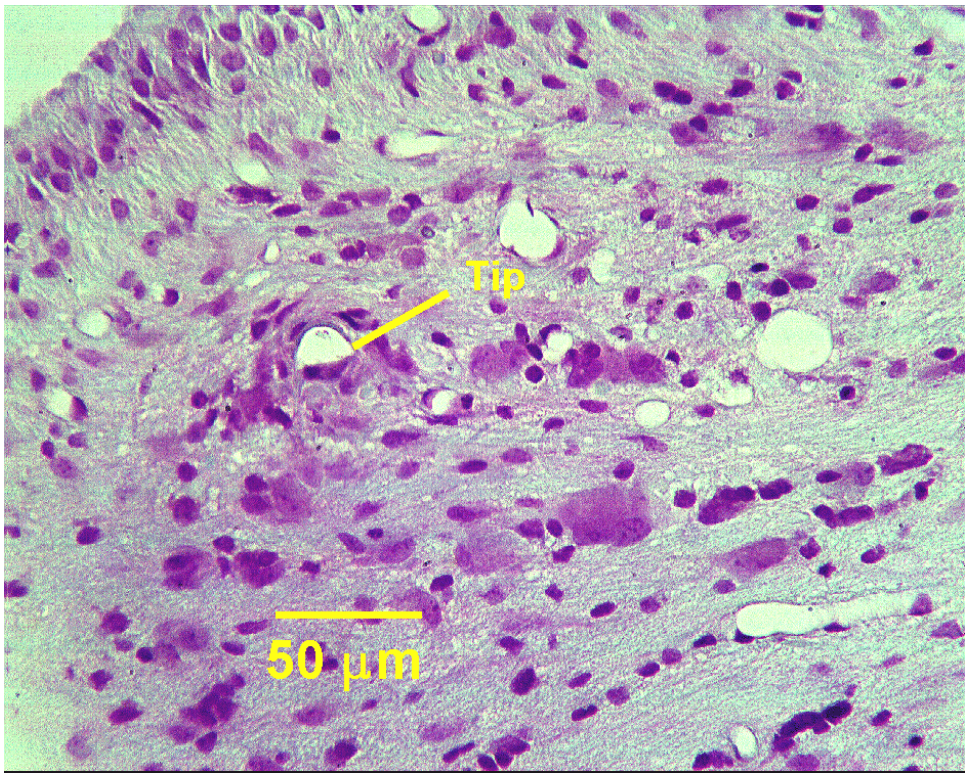


Figure 6D. Tip of uninsulated electrode a7

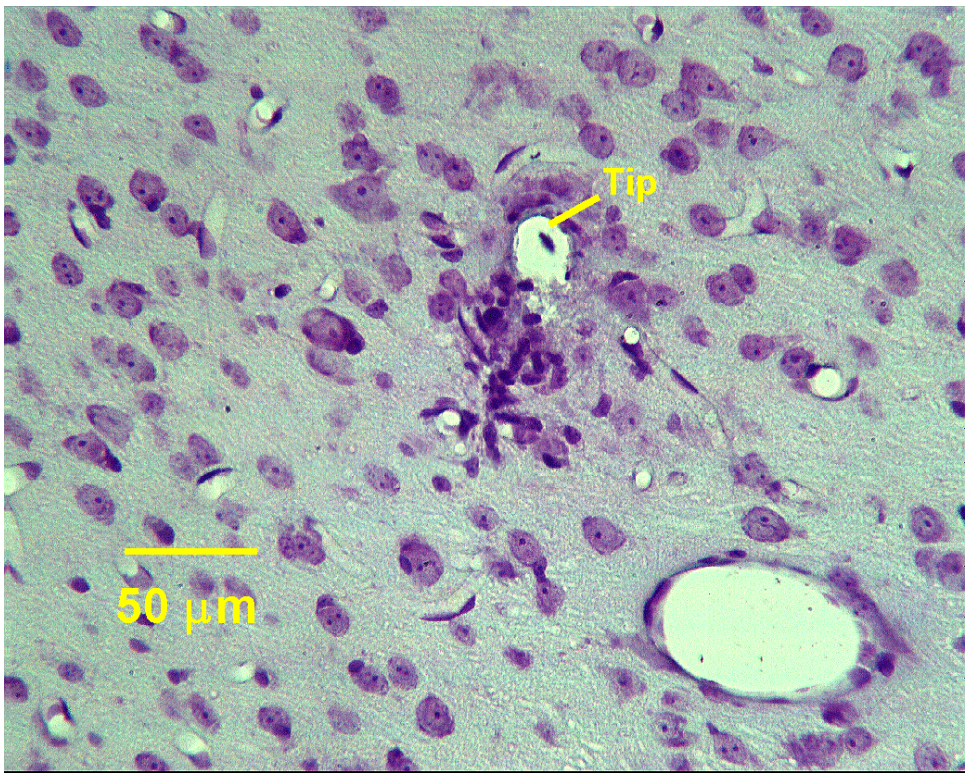


Figure 7A. Tip of insulated electrode a1

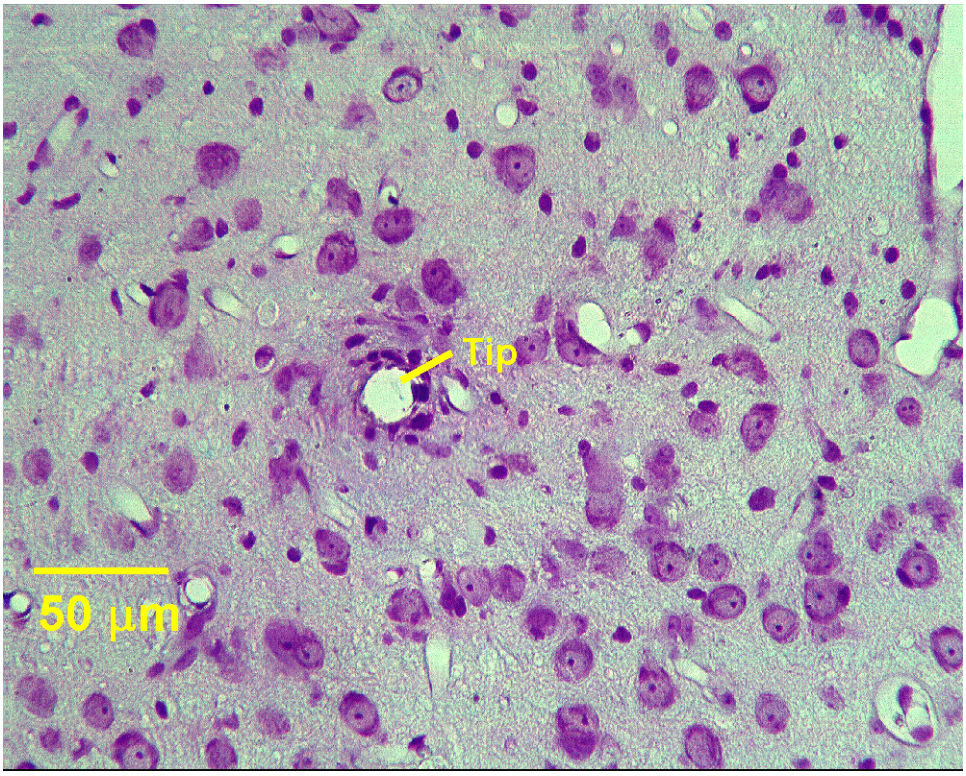


Figure 7B. Tip of insulated electrode a4

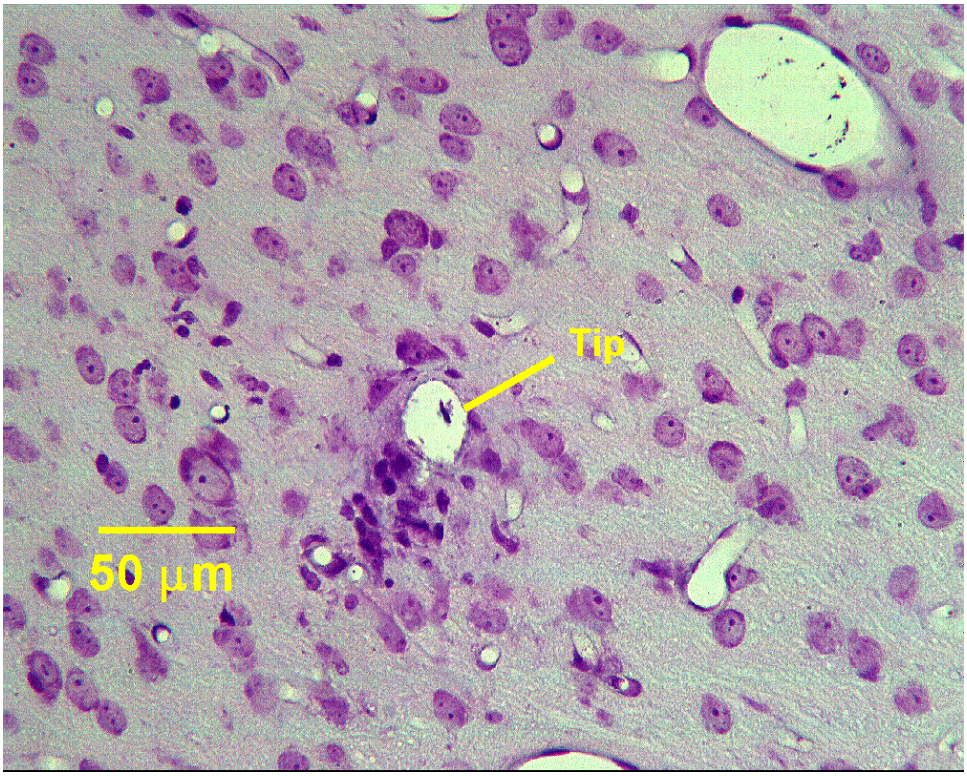


Figure 7C.Tip of insulated electrode p6

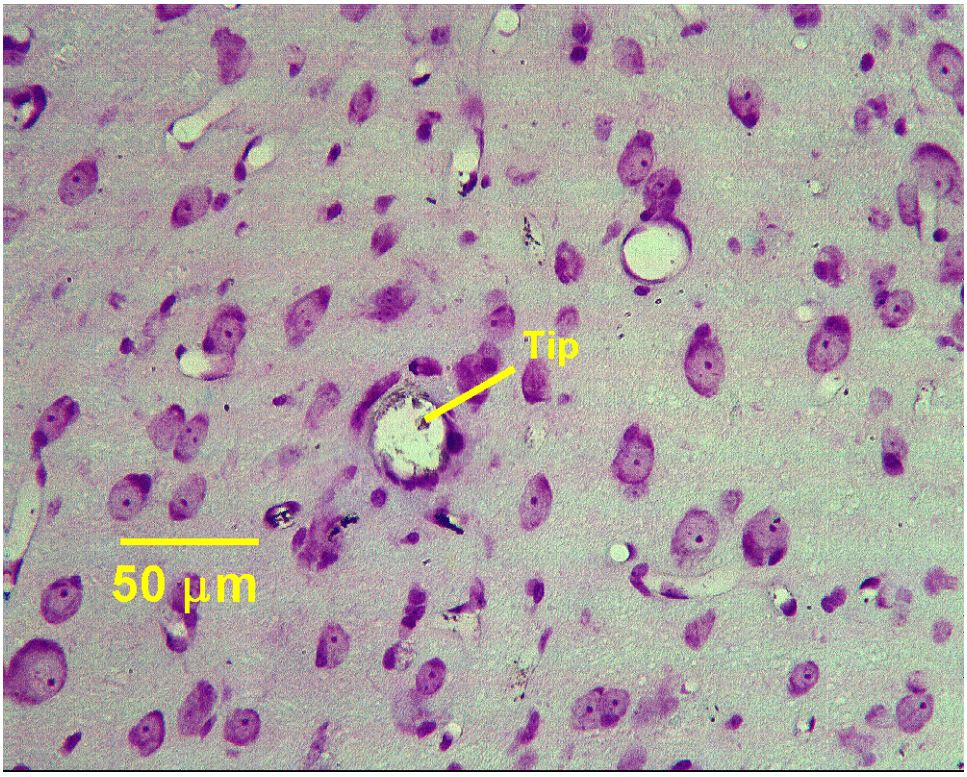
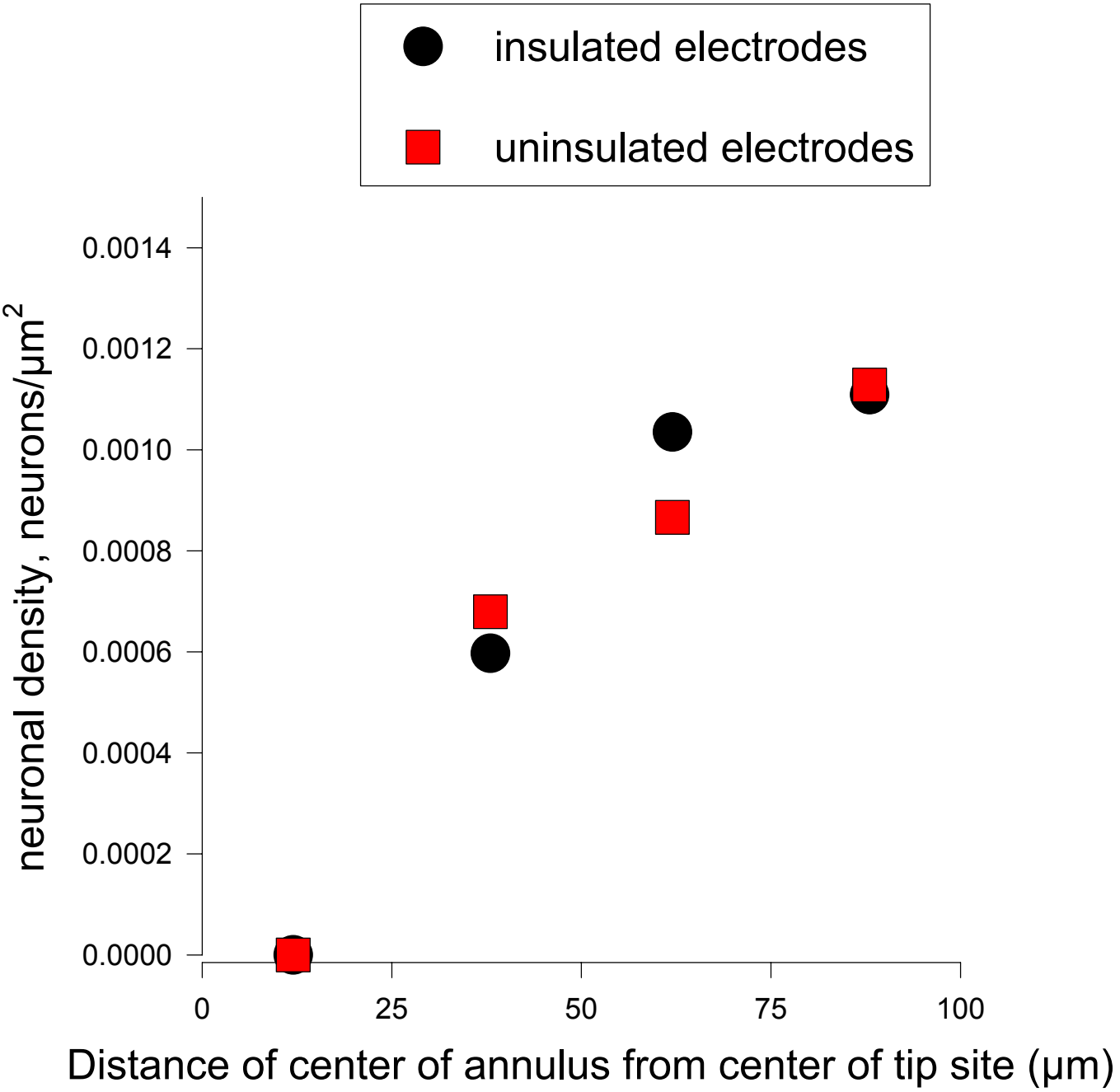


Figure7D. Tip of insulated electrode p2


insulated electrodes
uninsulated electrodes
neuronal density, neurons/μm2
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
0
25
50
75
100
Distance of center of annulus from center of tip site (μm)