"A Cochlear Nucleus Auditory prosthesis based on microstimulation"

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SUMMARY

We sacrificed cat cn74, seven years and one month after implanting 3 iridium stimulating microelectrodes into the posteroventral cochlear nucleus. The threshold of the electrical response evoked from one of the microelectrodes had remained very stable throughout the first 5 years of implantation, whereupon the electrical connection between the electrode and the percutaneous connector was lost. Another stimulating microelectrode continued to function well, and the slope of its response growth function remained very constant throughout 7 years. During the final few months *in vivo*, the threshold of the evoked response increased slightly, to approximately 8-10 μA. This longevity of the intranuclear electrodes is encouraging. Histologic evaluation revealed that the electrode shafts and tips were surrounded by gliotic sheaths that were not more than 10 μm in thickness, and this is consistent with the low threshold of the electrically-evoked response. Neurons and neuropil outside of the sheath appeared to be healthy, and this is consistent with the stable slope of the response growth functions through the 7 years *in vivo*.

In a second project, we used the hand-held inserter instrument to implant, into the feline cerebral cortex, "floating" microelectrode arrays that were scaled for the human ventral cochlear nucleus. This is part of our evaluation of the safety of the hand-held inserter tool when it is used to implant the electrodes into a very vascular part of the brain. The electrodes were not pulsed. The histologic evaluation conducted 31 days after implantation did not reveal any evidence of microhemorrhages. There was minimum gliosis surrounding the shafts and tips of the microelectrodes that were 1 mm in length. There was more gliosis surrounding the tips (but not the shafts) of microelectrodes up to 2 mm in length, although neurons and neuropil 25 to 100 μm from the tip appeared to be normal. Scarring was most extensive near the tips of the long (3.2 mm) electrically-inactive stabilizer pins which penetrated far down into the white matter underlying the cerebral cortex. Had these electrodes been implanted into the human auditory brainstem, these scars would have been ventral to the cochlear nucleus and within the middle cerebellar peduncle. The white matter surrounding the scars appeared to be normal, and there was no evidence that many axons had been damaged; they may have simply been pushed aside as the scar developed.

I: Long-term implantation of stimulating microelectrodes into the ventral cochlear nucleus of cat cn74.

INTRODUCTION AND METHODS

On May 14, 1993, three iridium microelectrodes insulated with Epoxylite varnish were implanted by stereotaxis into the left posteroventral cochlear nucleus of cat cn74. These microelectrodes had beveled ellipsoidal tips, rather than the blunt conical tips that we have used in recent years. A pair of recording electrodes was also implanted into the right inferior colliculus. One of the stimulating electrodes never functioned properly; it was subsequently shown to be at the extreme lateral margin of the nucleus. Another functioned for 5 years, but approximately 5 ½ years after implantation, the connector was damaged and it became an electrical open circuit. The third electrode continued to function until the cat was sacrificed on June 20, 2000, 2588 days (7 years and 1 month) after implantation of the electrodes.

At intervals throughout the 7 years, the responses evoked from the stimulating microelectrodes in the PVCN were recorded in the inferior colliculus, and these responses were summated (averaged) to obtain averaged evoked responses (AERs). The first component of the AER has a latency less than 1 ms, and on this basis it is assumed to represent neuronal activity evoked directly in the neurons projecting from the PVCN to the inferior colliculus. Response growth functions (RGFs), which represent the recruitment of neural elements surrounding the microelectrodes, were generated for each microelectrode, by plotting the amplitude of the first component of the AER against the amplitude of the stimulus current that evoked the AER. The "probe" stimulus applied to the intranuclear microelectrodes and used to evoked the neural responses was a train of cathodic-first, biphasic current pulses, 150 μs/ph in duration and at a rate of 50 Hz.

RESULTS

Figure 1A shows RGFs recorded in the IC from electrode #3, at various times after implantation. The threshold and slope of the RGFs remained quite constant throughout the first 1811 days (5 years). Shortly thereafter, the electrical connections between the electrodes and the percutaneous connector were damaged. The connection to electrode #3 was lost, but we managed to repair the connection to electrode #2. Figure 1B shows the RGFs of the responses evoked from this microelectrode and recorded in the IC. At 14 days after implantation, the threshold of the response was approximately 6 μA. By day 1247, it had decreased to approximately 4 μA. The threshold and slope of the RGF remain quite constant, until approximately day 2170. By day 2588, the threshold of the RGF had increased slightly, to approximately 8 μA, and the electrode's access resistance had decreased from approximately 60,000 to about 45,000 ohms. We were concerned that the electrode's Epoxylite insulation might be deteriorating or retracting from the electrode's tip after more than 7 years *in vivo*, and therefore, we decided to sacrifice the cat. The animal was deeply anesthetized and perfused through the aorta with ½-strength Karnovsky's fixative. The electrodes were removed from the cochlear nucleus, the nucleus was embedded into paraffin, sectioned at 8 μm and stained with Toluidine blue or with hematoxylin and eosin. Figure 2 shows sections cut obliquely through the track of electrodes #2 and #3 near the point at which electrode #2 had penetrated through the pia overlying the nucleus. The medial track is surrounded by a thin gliotic sheath approximately 3 μm in thickness. Figure 3 shows the site of the tip of microelectrode #3, whose RGFs are shown in Figure 1A. There is a small, nearly acellular gliotic scar ventral and lateral to the tip site, but neurons and neuropil ventromedial to the tip appear to be quite normal. Figure 4 shows the site of the tip of microelectrode #2 whose RGFs are shown in Figure 1B. The tip site is surrounded by a gliotic sheath approximately 10 μm in thickness. Outside of this capsule, the neuropil ventral to the tip site is somewhat compressed and distorted ventrally but otherwise appears to be normal and healthy.

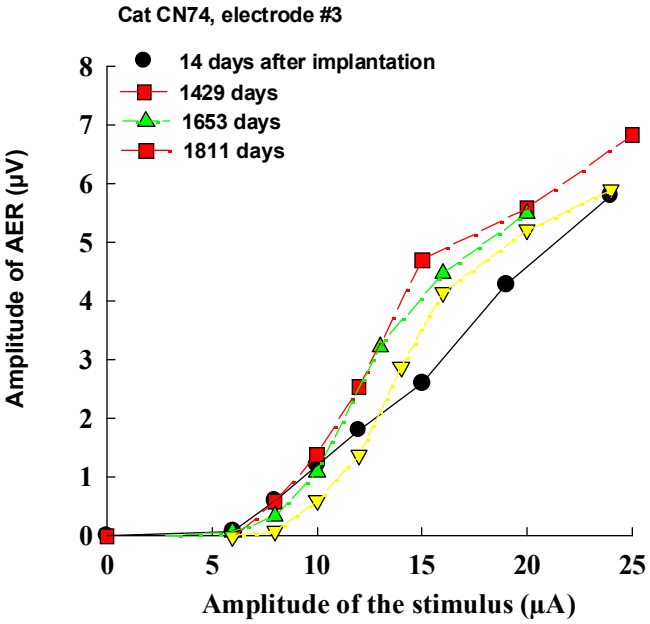

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Figure 1A

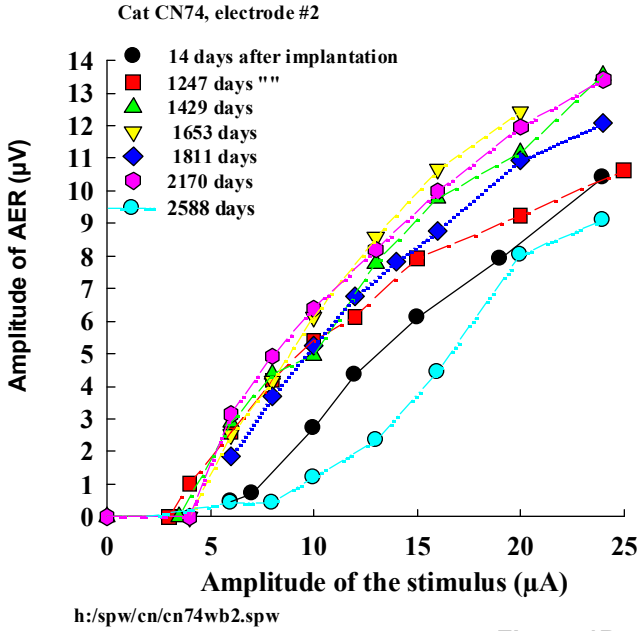

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Figure 1B

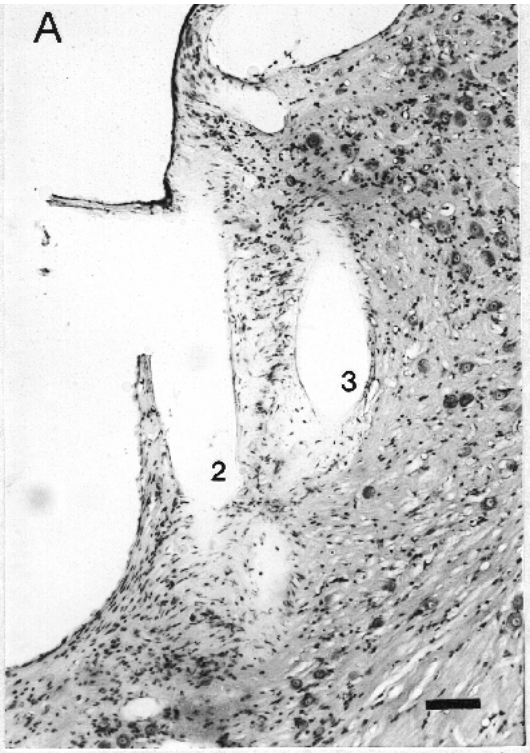

Figure 2. Histologic section cut slightly obliquely through the tracks of microelectrodes #2 and #3 in the posteroventral cochlear nucleus of cat cn74. Nissl stain. Bar = 100 µm .

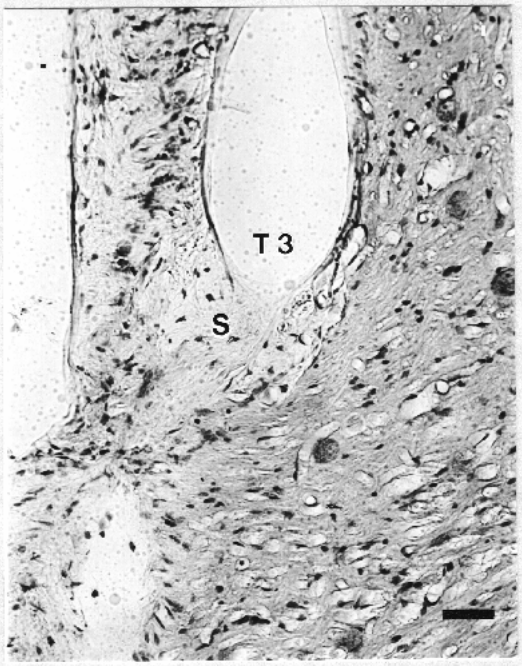

Figure 3. The site of the tip of microelectrode #3 in the PVCN of cat CN74. There is a small, nearly acellular gliotic scar (S) ventral and lateral to the tip site, but neurons and neuropil µm medial to the tip appear to be quite normal. Bar = 50 µm

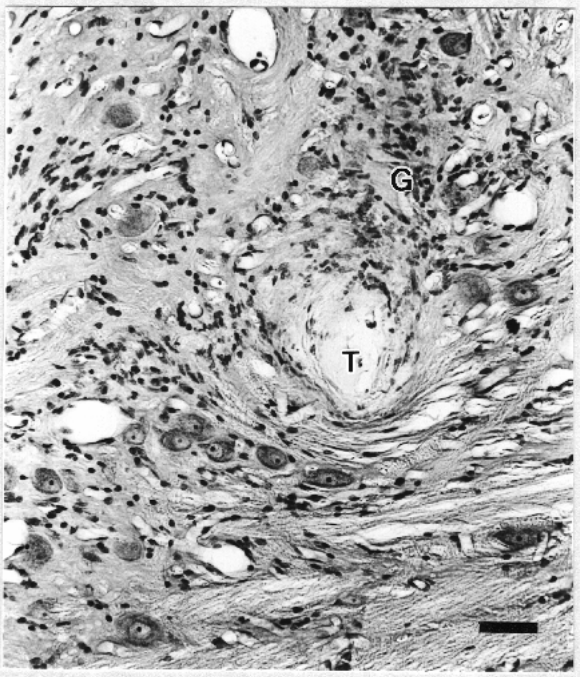

Figure 4. The site of the tip of microelectrode #2 in the PVCN of cat CN74. The tip site is surrounded by a gliotic sheath approximately 5 µm in thickness. Outside of this capsule, the neuropil ventral to the tip site is somewhat compressed and distorted ventrally but otherwise appears to be normal and healthy.
Bar = 50 µm

II: Implantation of human- type microelectrode arrays into the feline cerebral cortex.

INTRODUCTION

The feline ventral cochlear nucleus contains cell types that are very similar to those in the human VCN, but it is not a good site in which to evaulate the human array and the inserter tool, due to its shape and location as a pendulous structure on the dorsolateral surface of the brainstem. We have therefore implanted the human-type cochlear nucleus arrays into the feline lumbar spinal cord and also into the cerebral cortex (QPR#6). For this type of evaluation, the spinal cord is probably the the best feline model for the human auditory brainstem, but one difference is that it is covered by dura rather than by the lateral cerebellum, as is the human auditory brainstem (cats have only a small subarachnoid space over their lumbar enlargement). The second site, the feline cerebral cortex, allows us to evaluate the arrays when they are implanted into very vascular brain tissue. Also, the cortical gray matter spans nearly the same range of depths beneath the pia as does the human ventral cn.

In QPR #6, we described the results of implanting human-type arrays in which the individual microelectrodes were 70 µm in diameter. The electrodes were implanted into the cerebral cortex and also into the spinal cord. We noted that gliotic scars always surrounded the tips of the longest microelectrodes. The microelectrodes comprising the two arrays implanted into the cat described in this report (Animal CNH12) were 50 µm in diameter, in order to determine if the more slender electrodes would induce less tissue injury.

METHODS

Arrays of stimulating microelectrodes were fabricated from sections of iridium wire 50 µm in diameter. The tips of the microelectrodes were shaped electrolytically to a rather blunt point, with a radius of curvature of 6 ± 1µm. The electrodes were coated with Epoxylite electrode varnish which was removed from the tip with the aid of an erbium laser. The individual microelectrodes were assembled into arrays of 6 which ranged in length from 1 to 2.7 mm. These lengths were selected in order to span the

full dorsoventral extent of the human ventral cochlear nucleus, when the electrodes are inserted through the taenia choroidea. This range of electrode lengths allows for some variation between patients and also for the growth of up to 0.5 mm of connective tissue beneath the electrode superstructure. In addition to the 6 electrically active electrodes, the arrays contain 2 slightly longer (3.2 mm) stabilizing pins, whose tips are identical to the 6 working electrodes, but are not insulated. All 8 pins extend downwards from an epoxy button superstructure which is 2.5 mm in diameter. This array is designed to be implanted into the ventral cochlear nucleus with the aid of a hand-held instrument, which has been described previously.

The cats were anesthetized with nitrous oxide and Halothane. Using aseptic technique, a 25 × 25 mm craniectomy was made over the parietal cortex. Just prior to loading the array into the inserter tool, the array and the barrel of the tool were sonicated for 3 minutes in 100% ethanol and then for 1 minute in sterile water. After being inserted into the parietal cortex, the arrays were covered with a patch of fascia resected from the temporalis muscle, and the muscle layers and scalp were closed in layers. The bone flap was not replaced.

In the present series, none of the electrodes were pulsed. Thirty-one days after implantation, the cat was deeply anesthetized with Pentobarbital and perfused through the aorta with ½-strength Karnvosky's fixative. The electrode arrays were removed from the brain, and blocks of cortex containing the array tracks were embedded into paraffin and sectioned at 8 µA, approximately parallel to the electrode tracks. The sections were stained with Nissl stain.

RESULTS

Overall, the histologic findings from the cat CNH12 were very similar to those reported previously in QPR #6. At 31 days after electrode implantation, there was very little tissue injury or gliosis adjacent to the shafts and tips of any of the 1 mm electrodes. Figure 5A shows a tissue section that is nearly parallel to the track (TR) of one of the 1 mm electrodes from the posterior array. The tip site of the electrode was approximately 775 µm beneath the pia, and it apparently had been shimmed up by the

growth of a capsule of connective tissue, approximately 200-250 µm in thickness, on the underside of the array's superstructure. A remnant of this capsule (C) can be seen in the upper part of the photograph. The human ventral cochlear nucleus extends from approximately 0.3 - 0.5 mm to 2 mm beneath the surface of the brainstem, so if this array had been implanted through the taenia choroidea, the tip of this electrode would have been within the nucleus. The electrode track is lined with a thin sheath of glial cells approximately 2 to 3 µm in thickness. This sheath is slightly thicker near the tip (T) of the microelectrode (Figure 5B). The fissure in the tissue ventral to the tip site is a postmortem artifact that was observed beneath most of the tip sites from this array. It probably occurred when the array was accidentally jostled just prior to being withdrawn from the fixed tissue. Neurons and neuropil very close to the track and those within 25 µm of the tip site appear to be normal.

Figure 6 shows part of the track (TR) and site of the tip (T) of a slightly longer microelectrode from the same array. The tip site was 1115 µm beneath the pia. There is slightly more gliotic scarring near the tip, and there is greater proliferation of capillaries ("vascular hyperplasia"), an indication of a resolving injury. A very slender gliotic scar (S) extends ventrally beneath the tip site. It probably was formed as the electrode was gradually shimmed upwards during the formation of the connective tissue capsule on the underside of the array button. However, neurons and neuropil within 25 µm of the tip site appear quite normal. Figure 7 shows another tip site, 1250 µm beneath the pia. A fissure in the tissue also is present in this section, but neurons and neuropil within 50-100 µm of the tip appear to be normal.

The gliotic scarring was more severe near the tips of the longer electrodes. Figure 8 shows the tip site of a microelectrode from the same array, 1900 µm beneath the pia. The gliotic response extends at least 100 µm lateral to the tip site, although the actual scar is less extensive. However, neurons and neuropil within 150 µm of the tip appear to be normal. Scarring was most severe around the tips of the long (3.2 mm) stabilizing pins which penetrated deep into the subcortical white matter. Figure 9A shows a grazing cut through the track of one of the longest pins which penetrated 3 mm into the brain. A spheroidal scar (S), nearly 500 µm in diameter, and composed of

glial cells, fibroblasts, and connective tissue surrounds the tip site. However, the injury is confined to the vicinity of the tip site and there is little gliosis along most of the shaft. Figure 9B shows a high magnification of a section just lateral to the lower end of the track and tip site, which are encapsulated by a layer of elongated fibroblasts (F). Despite the large capsule of connective tissue, there is no indication of recent or old hemorrhage (no erythrocytes or hemosiderin).

The findings from the second (anterior) array were very similar. For this array, the histologic sections were slightly oblique to the electrode tracks. Figure 10A and 10B show sections through the tip sites of two of the 1 mm electrodes. Both sites are approximately 750 µm beneath the pia. Neurons and neuropil within 25 µm of the tips appear to be normal. Figure 11 shows a section through the tip site of a long electrode, approximately 1775 µm ventral to the pia. There is more scarring and some vascular hyperplasia surrounding the tip site, but neurons and neuropil within 75 µm of the tip appear to be quite normal.

During the past year, we have modified the process of cleaning the microelectrode arrays in order to reduce the incidence of inflammatory reaction and reactive gliosis that has occurred sporadically adjacent to the electrode shafts. The protocol changes include sonicating the array for 3 minutes in ethanol just before implantation. These modifications, and other changes in the procedure for cleaning the arrays prior to packaging and sterilization, have reduced, but have not entirely eliminated, the incidence of the inflammatory reactions. Thus, Figure 12 shows the only focus of inflammatory reaction (R) observed in cat CNH12. The reaction occurred approximately 200 µm ventral to the pia, surrounding one of the electrode tracks. There is an aggregation of cells which appear to be glia (probably astrocytes) and an infiltration of lymphocytes. The infiltrate extends approximately 50 µm outwards from the track. Unlike the gliotic scars at the tips of the microelectrodes, it contains little or no connective (scar) tissue and many lymphocytes. Lateral to the cellular aggregate, the neurons and neuropil appear to be quite normal.

DISCUSSION

Because of the difficulties inherent in using the feline cochlear nucleus as a site for testing the human array and its insertion tool, we have been implanting the arrays into the feline cerebral cortex or into the lumbar spinal cord. The histologic findings from the two arrays implanted into the cerebral cortex of cat CNH12 were very similar to those from the arrays implanted into the cerebral cortex of cat CNH6 and CNH7, as reported in QPR #6. In cat CNH12, the short (1 mm) electrodes inflicted only minimal damage in the neuropil surrounding the tip sites. The tip sites of (unpulsed) electrodes that were between 1 and 1.5 mm in length were surrounded by localized gliotic scars but neurons and neuropil 25-50 μm lateral to the tip sites appeared to be normal. The tip sites that were 2-3 mm beneath the pia were surrounded by more extensive gliotic scars, and at the tips of the long stabilizing pins, the scars were up to 500 μm in diameter. However, we believe that these long pins will be necessary to ensure that the array does not become dislodged from the human brainstem by manipulations of the array cable during the surgical procedure or during the immediate postoperative interval, before the array is stabilized by the formation of the connective tissue.

In all cases, the scars were confined to the immediate vicinities of the electrode tips. This raises the question of whether the active stimulation site should be placed at the tips of long microelectrodes. We have always placed the active (exposed) site at the tip because, when the electrodes are pulsed in order to determine parameters for safe and effective stimulation, it is quite easy to locate the site of the stimulation in the histologic material. This is more difficult when the active site is situated part way up the shaft. However, with modern photo-ablation laser techniques, Parylene or Epoxylite insulation can be removed from any part of the shaft, and if the objective is to stimulate at a site that is 2,000 μm beneath the pia, it may be advantageous to implant electrodes that are 2,200 μm in length, with the active site about 200 μm above the tip.

We are uncertain as to the mechanisms that are responsible for the tissue injury surrounding the tips of the longest electrodes. The phenomenon cannot be attributed simply to the length of the electrodes, because the electrodes that are stereotaxically-implanted into the cat cochlear nucleus are 3-4 mm in length but do not induce gliotic

scarring at the tips. The two electrode systems differ in at least four ways. The stereotaxically implanted arrays are inserted slowly (at approximately 1 mm/sec), while the human-type array is injected rapidly, at about 1 m/sec. It seems unlikely that the injury could be due to the higher insertion velocity *per se* since all of the microelectrodes are inserted at the same velocity but only the longer ones inflict injury. A second difference is that the human-type cochlear nucleus arrays were inserted by means of a hand held instrument, and the stereotaxically-implanted electrodes are inserted into the ventral cochlear nucleus using an introducer that is mounted on the stereotaxic head frame. The latter arrangement allows better control of dimpling and of the micromanipulation of the tissue before and during implantation of the array. Since the tips of the longest electrodes are farther from the array matrix, there may be more relative motion between the brain and the tips of the longer electrodes, if there is lateral or rotational movement of the surgeon's hands during electrode insertion. We should note that anatomical considerations dictate that the array must be implanted into the human cochlear nucleus using a hand-held tool and this also dictates that the array must be inserted at a fairly high velocity, lest the surgeon's hand move during the insertion process.

A third difference is that we have been evaluating the human-type cochlear nucleus arrays at sites that are normally covered by dura (the feline lumbar spinal cord or cerebral cortex). In order to reduce manipulation of the array after implantation, we have not replaced the dura, but instead have covered the array with a patch of muscle fascia. This has proved to be an effective dural substitute, sealing the dura completely and not binding to the pia. However, fascia does provide a matrix for the growth of connective tissue, and this tends to entrap the array cable and sometimes part of the array matrix itself. This entrapment may contribute to relative movement between the electrodes and the brain, when the brain moves with respect to the layer of dura/fascia and the partially immobilized array superstructure. Furthermore, the relative motion is likely to be greatest near the tip of the longest electrodes. Recently, we have modified our procedure for implanting the floating array so that the array superstructure and the

distal part of the cable are covered by dura rather than by fascia. Sutures are placed in the dura prior to inserting the array, so that there is minimal manipulation after the array is implanted; the sutures must only be drawn up and tied off. During the next quarter, we will implant 2 of the human-style cochlear nucleus arrays into the feline lumbar spinal cord and 2 into the cerebral cortex using the hand held tool. The dura will be closed in the manner described above, and we will determine if this modification reduces the injury near the tips of the longest electrodes.

The shafts of the electrodes comprising the arrays implanted into cat CNH12 were 50 µm in diameter while the shaft of those implanted into cat CNH7 (reported previously) were 70 µm in diameter. The gliotic scarring near the tips of the longest electrodes was essentially identical in the two animals. Short (1 mm) electrodes of either diameter induced very little injury. The 70 µm electrodes are sturdier and probably less vulnerable to damage or to becoming misaligned during handling, and therefore we will specify that the diameter of the electrode shafts in the human array should be 70 µm.

Our primary reason for implanting the human-type cochlear nucleus arrays into the feline cerebral cortex is to evaluate the potential for interstitial hemorrhage when the inserter tools is used to implant the electrodes into a very vascular region of the brain. To date, 24 electrode tracks from 4 such arrays have been examined, and we have found little evidence of new or resolved hemorrhages (erythrocytes or hemosiderin). In cat CNH7, a few very small aggregates of hemosiderin were observed near 3 or 4 of the cortical capillaries, 30 days after implanting the arrays, but no evidence of space-occupying microhemorrhages. No hemosiderin or erythrocytes was observed in any of the histologic sections from cat CNH12. This speaks well for the safety of implanting the arrays using the hand held tool.

If these electrode had been implanted into the human brainstem, the glial scars near the tip of the longest electrodes would not be within the ventral cochlear nucleus, which spans a range of depths between approximately 0.3 - 0.5 mm and 2 mm beneath the surface. The longest electrodes and the stabilizing pins would have passed through the ventral cochlear nucleus into the underlying middle cerebellar

peduncle and our findings indicate that they may produce small (0.5 mm) scars within this structure. The white matter surrounding the glial scars in the the cat cerebrum appears normal. There were no indications of degenerating axons, no macrophage activity, or other evidence that many of the axons have been damaged; they may have simply been pushed aside as the scar developed.

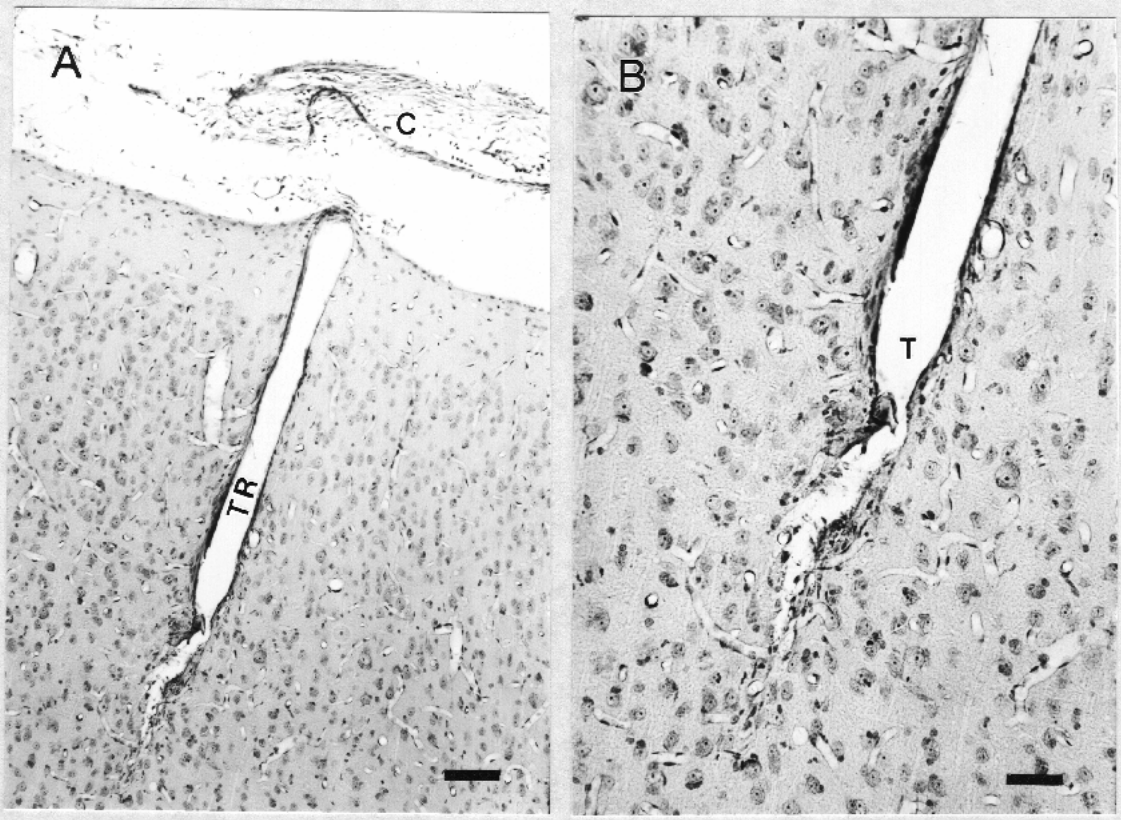

Figure 5. **A/** A section nearly parallel to the track (TR) of one of the short (1mm) electrodes in the posterior array of cat CNH12.The tip site of the electrode was approximately 775 µm beneath the pia, and it apparently had been shimmed up by the growth of a capsule of connective tissue, approximately 200-250 µm in thickness, on the underside of the array's superstructure. A remnant of this capsule (C) can be seen in the upper part of the photograph. The electrode track is lined with a thin sheath of glial cells approximately 2 to 3 µm in thickness. Bar = 100 µm

B/ A view of the electrode's tip site (T), at higher magnification. The fissure in the tissue ventral to the tip site is a postmortem artifact that was observed beneath most of the tip sites from this array. It probably occurred when the array was accidentally jostled just prior to being withdrawn from the fixed tissue. Neurons and neuropil near the track and within 25 µm of the tip site appear to be normal. Bar = 50 µm

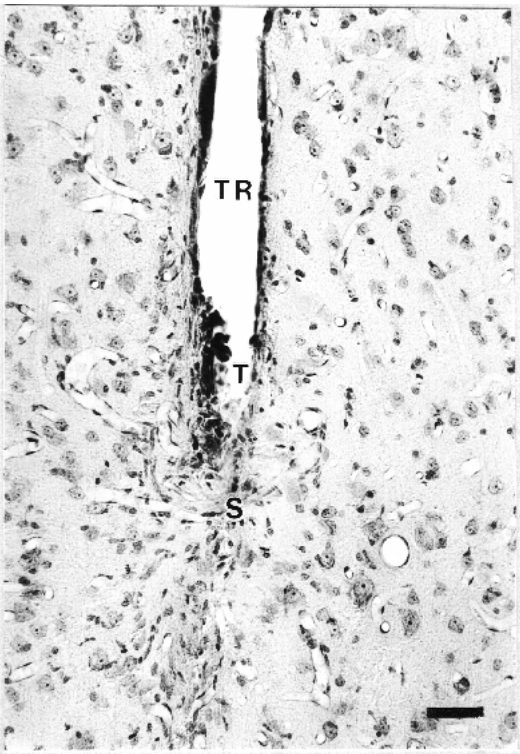

Figure 6. The track(TR) and tip site (T) of a slightly longer electrode, also from the posterior array. There is slightly more gliotic scarring and proliferation of small blood vessels near the tips site, which is 1115 µm beneath the pia. However, neurons and neuropil 50 µm from the tip appear to be normal. Bar = 50 µm.

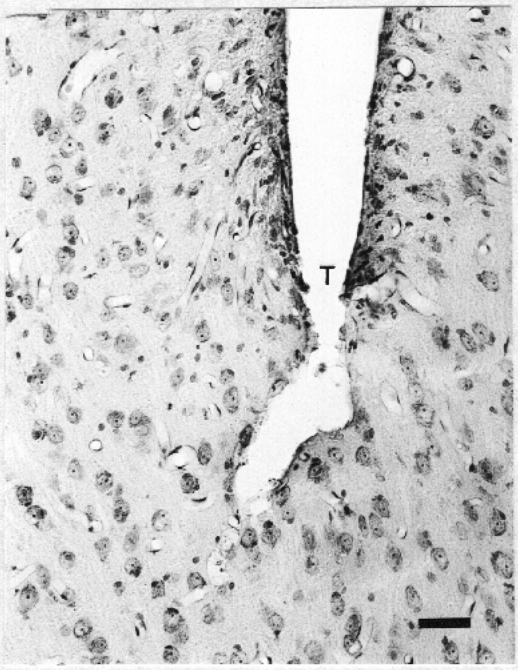

Figure 7. Another tip site from the posterior array, 1250 µm beneath the pia. Neurons and neuropil within 50-100 µm of the tip appear normal. Bar =50 µm

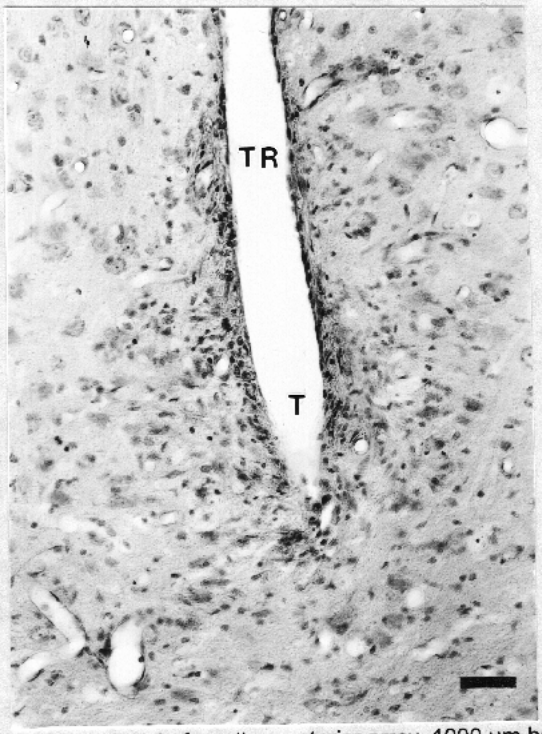

Figure 8. The tips site of a longer electrode from the posterior array, 1990 µm beneath the pia. The gliotic reaction extends at least 150 µm from the tip site, but the actual gliotic scar is less extensive. Neurons and neuropil within 150 µm of the tip appear to be normal. Bar = 50 µm

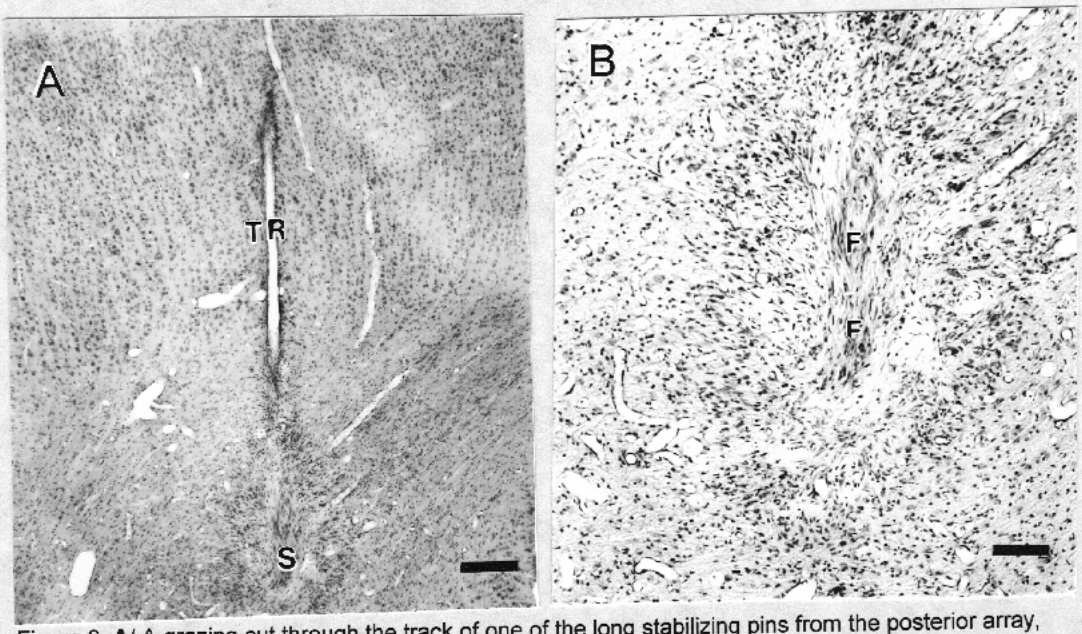

Figure 9. **A**/ A grazing cut through the track of one of the long stabilizing pins from the posterior array, which penetrated 3 mm into the brain. A symmetrical spheroid of glial cells, fibroblasts, and connective tissue, nearly 500 µm in diameter, surrounds the tip site. However, the injury is confined to the vicinity of the tip site and there is little gliosis along most of the shaft. Bar = 250 µm
B:/ A higher magnification view of a section just lateral to the lower end of the track and tip site, which are encapsulated by a layer of elongated fibroblasts (F). However, there is no indication of recent or old hemorrhage (no erythrocytes or hemosiderin). Bar =100 µm

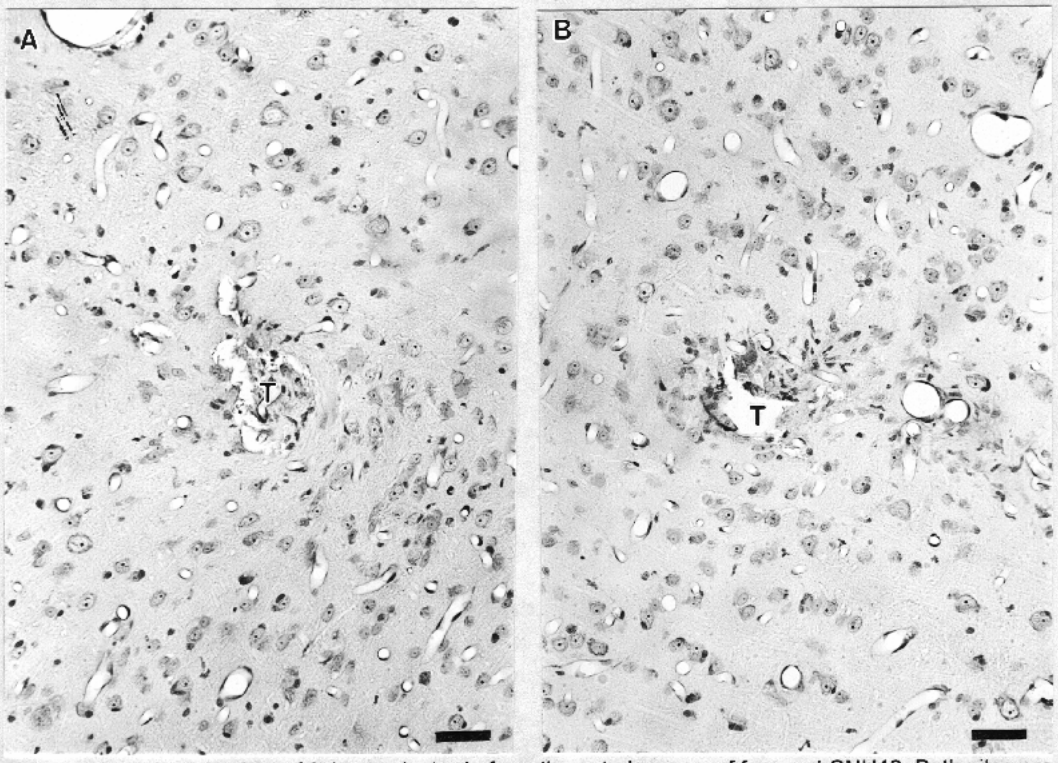

Figure 10A, B. The tip sites of 2 1 mm electrode from the anterior array of from cat CNH12. Both sites are approximately 750 µm beneath the pia. Neurons and neuropil within 25 µm of the tip appear to be normal. Bar = 50 µm

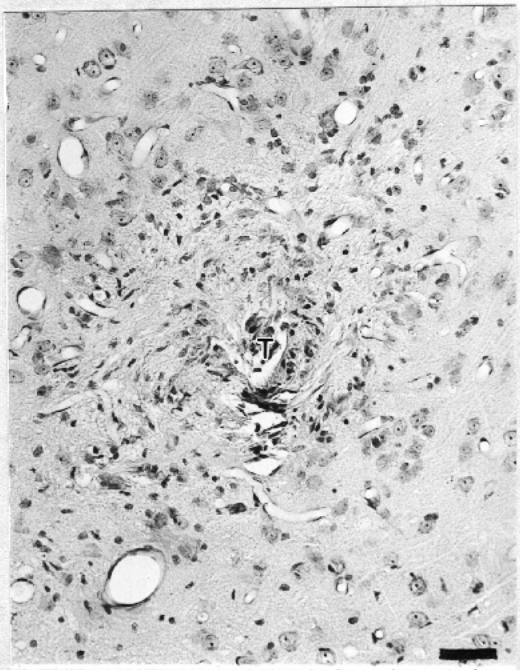

Figure 11. A section through the tip site of a long electrode from the anterior array, approximately 1775 µm ventral to the pia. There is more scarring and vascular hyperplasia surrounding the tip of this longer electrodes, but neurons and neuropil within 75 µm of the tip appear to be quite normal. Bar = 50 µm.

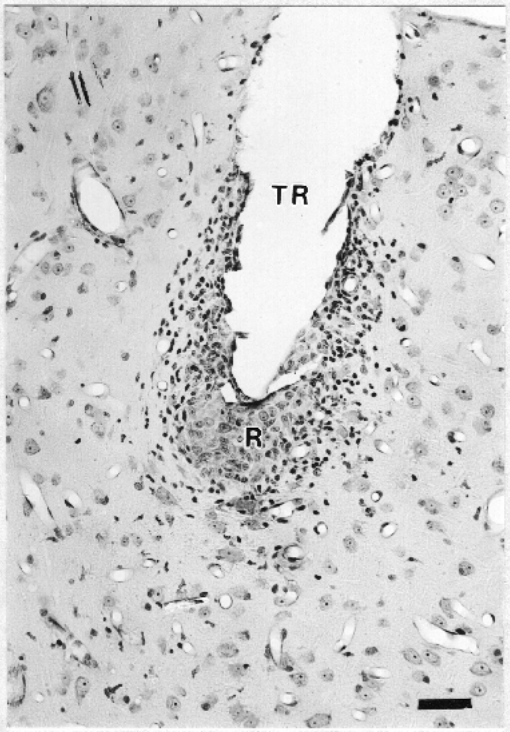

Figure 12. A section through the track of a microelectrodes from the posterior array of cat CNH12. There is a focal inflammatory reaction composed on an aggregate cells which appear to be glia (probably astrocytes) and lymphocytes. The infiltrate extends approximately 50 µm outwards from the track. Lateral to the cellular aggregate, the neurons and neuropil appear to be normal. Bar =50 µm