Chronic tissue response to untethered microelectrode implants in the rat brain and spinal cord

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Abstract

Objective. Microelectrodes implanted in the central nervous system (CNS) often fail in long term implants due to the immunological tissue response caused by tethering forces of the connecting wires. In addition to the tethering effect, there is a mechanical stress that occurs at the device–tissue interface simply because the microelectrode is a rigid body floating in soft tissue and it cannot reshape itself to comply with changes in the surrounding tissue. In the current study we evaluated the scar tissue formation to tetherless devices with two significantly different geometries in the rat brain and spinal cord in order to investigate the effects of device geometry.

Approach. One of the implant geometries resembled the wireless, floating microstimulators that we are currently developing in our laboratory and the other was a (shank only) Michigan probe for comparison. Both electrodes were implanted into either the cervical spinal cord or the motor cortices, one on each side. Main results. The most pronounced astroglial and microglial reactions occurred within 20 μm from the device and decreased sharply at larger distances. Both cell types displayed the morphology of non-activated cells past the 100 μm perimeter. Even though the aspect ratios of the implants were different, the astroglial and microglial responses to both microelectrode types were very mild in the brain, stronger and yet limited in the spinal cord.

Significance. These observations confirm previous reports and further suggest that tethering may be responsible for most of the tissue response in chronic implants and that the electrode size has a smaller contribution with floating electrodes. The electrode size may be playing primarily an amplifying role to the tethering forces in the brain whereas the size itself may induce chronic response in the spinal cord where the movement of surrounding tissues is more significant.

Keywords: floating microstimulators, optical stimulation, astroglial and microglial response, wire interconnects

(Some figures may appear in colour only in the online journal)

1. Introduction

Neuroprosthetics research has the potential to increase the quality of life for individuals with neurological disorders [1]. Chronically implantable microfabricated devices for neural stimulation [2] and recording [3] are important technologies with several emerging applications in substitution of motor
sensory and cognitive modalities that may be damaged as a result of an injury or a disease. While these neural electrodes are being developed for neuroprosthetics, there are some critical issues that need to be addressed, such as the long term stability and foreign body response of the electrodes.

An acute phase is observed after the implant where the immune system tries to repair damage to the micro vessels induced by insertion of the electrode and remove the cellular debris. The long term immune reaction is an ongoing response primarily to the mechanical perturbations generated by the implant and the chemical factors on the device surface. The encapsulated tissue eventually surrounds implanted electrodes in the neural tissue which contains a variety of cell types including microglia, macrophages, meningeal fibroblast, reactive astrocytes and the effectors released by these cells over time. Histological evaluation would show a zone of activated astrocytes surrounding a core of activated microglia adjacent to the implant. Incremental reduction of neuronal density has also been reported around the implant due to glial scar formation. Many reports have characterized the glial cells forming around chronic electrodes and the long term stability of electrophysiological recordings and stimulations with them. A potential explanation for the degradation observed in the recording and stimulation characteristics in these chronic studies is that the encapsulation of the electrode by reactive astrocytes may physically push the nearby neurons away from the electrode.

The fine wires that connect the microelectrodes to the external world present a two-fold problem. Microelectrode implants often fail either due to the chronic tissue response caused by the tethering forces of the connecting wires or their breakage. Thus, our laboratory is developing a floating light activated micro electric stimulator (FLAMES) which is a wireless implantable micro device for neural stimulation that uses near-infrared (NIR) light for energy transfer through neural tissue. The FLAMES was acutely tested in the rat spinal cord for feasibility of the main concept. The Monte Carlo method was used to simulate light–tissue interactions and predict the amount of light that could be harvested by the implant needed to generate sufficient electric currents for neural stimulation. To determine the maximum allowable optical power, temperature elevation profile was measured experimentally using a micro thermoprobe inside the rat brain induced by an NIR laser beam. These studies have produced supportive evidence for a wireless, optically activated microstimulator that is implanted into the neural tissue. This study attempts to quantify the long term biological response to such a floating device in the central nervous system (CNS). It was claimed that untethered devices elicit less of a foreign body response. In the current study, we designed an experimental paradigm to evaluate the glial reaction when untethered devices were implanted in the spinal cord and brain.

The foreign body response around each chronic implant was evaluated by studying the astroglial and microglial reaction using immunocytochemistry to identify each cell type. Glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba-1) were used as specific markers for astrocytes and microglia/macrophages, respectively. The results are compared with reports of tethered devices in the literature.

2. Methods

2.1. Implant specifications

Silicon based dummy floating electrodes (FL) that are similar to floating light activated micro-stimulators in shape and size were fabricated at Photonics Center, Boston University, USA with dimensions shown in figure 1(A). The fabrication started with a 4 inch wafer that was patterned with a MEMs
photoresist (SPR-220, Microchem Corp., Malborough, MA) with a target photoresist thickness of approximately 20 μm. A 4 inch p-type silicon wafer was descummed using an oxygen plasma asher (400 sccm O1, 500 W, 5 min). Hexamethyldisilazane was spun on at 2000 rpm for 15 s to promote adhesion. Thick photoresist (SPR-220-7) was spun on for 60 s at 2000 rpm. The wafer was then soft baked on a hot plate at 90 °C for 100 s. The wafer was placed in a humid chamber for 3 h before patterning the wafer. Using an appropriate dose (Channel 1, 28 s on MA-6 from Karl Seuss, Germany) the mask pattern was exposed onto the photoresist. The exposed wafer was placed in a humid chamber for one to two hours. Another soft bake was performed on a hotplate at 90 °C for 100 s. The photoresist was developed using MF319 (Shipley Company, Malborough, MA) with two separate baths, each 90 s. Finally, one more ‘hard’ bake on a hot plate at 90 °C for 30–60 min was performed.

After inspecting the photolithography process, the devices were released using a deep reactive ion etcher (DRIE, ASE HRM, Surface Technology Systems). To release the devices from the wafer, a top-side etch was performed using the standard silicon etching recipe. This recipe will result in an 8 μm min⁻¹ etch rate; the target depth is 100 μm. A second blank wafer was then attached to the top-side of the patterned wafer using Crystalbond 555-HMP adhesive. The blank wafer and adhesives are used to hold the micro devices in place once they have been released from the wafer. The back-side of the wafer was slowly etched until the devices separated from the wafer. They were carefully cleaned with acetone, isopropanol, and deionized water. Finally, the devices were descummed with oxygen plasma (300 sccm O1, 500 W, 10 min).

To create a biocompatible layer, 5 μm of Parylene-C was deposited as a conformal film. This was done using the PDS 2010 (Specialty Coating Systems, Indianapolis, IN). The system used had a deposition rate of 0.5 μm g⁻¹ of Parylene-C dimer. The devices were placed on cleanroom wipes while coating, which provided a conformal coating that, was removable from the cotton substrate by gently grabbing the devices with tweezers.

All implanted micro devices were cleaned by immersion in 70% ethanol, followed by rinsing in distilled water and sonicating 5 min, and sterilizing by ethylene oxide gas prior to surgery.

2.2. Animal preparation and surgical procedure

Rats were anaesthetized by intraperitoneal administration of ketamine (80 mg kg⁻¹) and xylazine (12 mg kg⁻¹) mixture diluted with saline and further doses of ketamine were given as needed. The head and back hair of the rats were shaved and swabbed with three alternating washes of iodine with sterile gauze. To avoid corneal drying, ophthalmic ointment was applied to the eyes. Rats were placed on a temperature regulated heating pad and the rectal temperature was maintained at 36 °C while they were mounted onto a stereotaxic frame. Marcaine (0.2 mL) was injected at the site of incision as a local analgesic. The end-tidal CO₂ was observed to maintain normal range throughout the surgery, which usually lasted about two hours, using a pulse oximeter attached to a foot.

Six Sprague-Dawley rats were used for implantation of microelectrodes. One FL and one MI electrode were implanted in opposite hemispheres and in opposite sides of the cervical spinal cord; thus a total of four micro devices were implanted in each rat, which allowed parallel comparison of the glial reaction to the FL and MI electrodes simultaneously in both CNS regions.

For the brain implants, an incision was made along the midline of the cranium using a scalpel. The underlying muscle and connective tissue were removed to expose the skull. Two craniotomies, roughly 1 mm in diameter, were made with their centers 2 mm anterior and 2 mm lateral to the bregma bilaterally using a #15 scalpel and a round-tip bone cutter. The broken skull pieces and debris were removed with fine forceps and cotton swaps. The connective tissue over the dura was removed while leaving the dura intact. Small punctures were made on the dura using a 32 g needle to insert the implants. One FL was implanted into the skull opening on the right side, and one Michigan probe (A1 × 16, 3 mm100, silicon substrate with silicon dioxide insulation, CNCT, U. of Michigan) was implanted on the left (figure 1(B)). The Michigan probe (MI) was a single shank 2.5 mm × 123 μm × 15 μm electrode (connection pad was broken off), tapering for the last 1.5 mm from the tip. Both implants were held with a pair of fine forceps and inserted slowly perpendicular into the cortex by hand until the body of the implant was fully immersed into tissue, avoiding any major blood vessels. A piece of connective tissue was used for sealing the dural openings over untethered electrodes without using any adhesives.

For the spinal cord implants, about a 4 cm incision was made along the midline behind the neck. The muscle and connective tissue were dissected with surgical scissors and partially removed with rongeurs to access the cervical vertebra. A laminectomy was performed at the C4 level to expose the spinal cord. The connective tissue over the dura was removed while leaving the dura intact. Two small punctures were made bilaterally into the dura matter over the dorsal column using a 32 g needle to insert the electrodes. One FL was inserted through the dural opening 0.5 mm right to the midline, and one untethered MI was cut at 1.5 mm length (since dorsoventral dimension of cervical cord is only 2.5 mm) and inserted symmetrically on the left side (figure 1(C)) slowly by hand using a pair of fine forceps until the body of the implant was fully immersed into the tissue (figure 1(D)). A piece of connective tissue was placed for sealing over the dural openings without any adhesives. The surgical sites were closed using absorbable sutures. All experimental procedures were approved by the Animal Care Committee at Rutgers University, NJ.

2.3. Tissue processing

Six age-matched Sprague-Dawley male rats all weighing between 300 and 400 g at implant time were used in this study.
(three rats for 4 weeks and three rats for 8 weeks). All subjects survived until termination. There were no overt motor deficits or infections at the surgery site at the time of termination. The rats were in intraperitoneally administered with ketamine (80 mg kg⁻¹) and xylazine (12 mg kg⁻¹) mixture diluted with saline and perfused transcardially with 150 mL phosphate buffer saline (PBS) at a pressure of 80 mmHg followed by 150 mL of 10% formalin at a pressure of 120 mmHg. The brain and spinal cord tissue were carefully dissected, post-fixed overnight in 10% formalin at 4 °C, washed with PBS, and cryoprotected in 27% (w/v) sucrose. Tissue samples were frozen in optimal cutting temperature compound on dry ice and 30 μm thick, horizontal cryostat sections were mounted slides and stored at −80 °C for immunohistochemical labeling.

2.4. Immunohistochemistry

Frozen sections were allowed to thaw for 30 min at 40 °C. The slides were rinsed with PBS at room temperature (RT). The tissue sections were blocked with 10% normal goat serum (NGS) or normal horse serum (NHS) in 0.1% T-PBS (0.1% Triton-X 100 in PBS) for an hour at RT. The sections were then incubated overnight at 4 °C in anti-GFAP (Dako, Glostrup, Denmark; 1:1000) or anti-Iba-1 (Wako Chemicals USA, Inc. VA, USA; 1:1000) antibodies diluted in 1.5% NGS + 0.1% T-PBS. The sections were then rinsed three times in PBS and incubated in Alexa Fluor® 488 (Life Technologies, OR, USA) or Alexa Fluor® 594 (Life Technologies, OR, USA)—labeled anti rabbit IgG (H + L) diluted 1:500 in 1.5% NGS (or NHS) + 0.1% T-PBS. The sections were then incubated one hour at RT in the dark to prevent photobleaching. The tissue sections were washed three times with PBS at RT for 10 min each wash. The slides were coverslipped with ProLong® Gold (Life Technologies, OR, USA) with diamidino-2-phenylindole (DAPI) mounting medium for fluorescence. Then slides were stored in the dark at 4 °C until images were collected.

2.5. Imaging and analysis

Five horizontal sections were selected for each group at similar depths; two slides at 200 μm, two slides at 500 μm, and one slide at 800 μm from the dorsal pial surface (see figure 1(A)). Fluorescent images were captured using a Leica fluorescent microscope under the same illumination level with 10X/20X/60X lens so that the overall background light intensities were comparable, and then converted into grayscale images using ImageJ® software. A 100 × 400 μm rectangular shape window starting from the long edge of the electrode footprint was marked as shown in figure 2. In the spinal cord samples, the rectangular window was placed on the side of the electrode cavity that would exclude the white matter.

The staining intensities within the window were quantified by averaging the pixels across the width of the rectangle in ImageJ® and plotting them as a function of distance from the electrode (longitudinal axis of the rectangle) in Matlab®.

The averages of intensities from 200 to 280 μm were taken as the baseline since the intensity profile plateaued well before this point. Each intensity curve was normalized to its own baseline.

Following this procedure, five normalized intensity curves were obtained from each implant corresponding to the slides taken at five different depths. The areas under the intensity curves from 0–100 μm were calculated and taken as representative values of tissue response in the vicinity of the electrodes, which produced a total of 15 data points (3 implants × 5 slides) per electrode type, per implant location, and per implant duration. These last three parameters were compared statistically using IBM SPSS (Statistical Package for Social Sciences) software at a significance level of $p<0.05$.

3. Results

3.1. Microglia/macrophage response to FL electrodes

To assess the microglial reaction elicited by the FL and MI electrodes, we labeled the brain and spinal cord sections containing the implantation sites with an antibody against Iba-1, a specific marker for microglia. Sections were prepared and analyzed at 4 and 8 weeks post implantation of the electrodes (figure 3).

In the brain sections, the FL electrode cavities had well defined shapes that resembled the device cross-section (figures 3(A) and (D)). Iba-1 positive cells, exhibiting the characteristic amoeboid morphology of reactive, macrophage-like microglia, and showing strong immunostaining, were localized to the immediate perimeter of the electrode cavity.
Figure 3. Representative Iba-1 + confocal images for characterization of horizontal sections perpendicular to implants in brain (A) and spinal cord (D) of rats receiving FL at 8 weeks after implantation. Tissue sections shown are taken at ∼200 μm from the pial surface both in the brain and spinal cord. High magnified images to illustrate amoeboid microglial cells (▲) proximal to implant (B) and (E), activated microglia cells (●) and ramified microglial cells (■) distal to implant (C) and (F). BR: brain, SC: spinal cord. Scale bar = 100 μm (A) and (D), 25 μm (B), (C), (E) and (F).

Figure 4. Representative Iba-1 + images in the brain (A)–(D) and spinal cord (E)–(H) of rats receiving the floating microelectrode (A), (C), (E) and (G) and Michigan probe (B), (D), (F) and (H) at 4 weeks (A), (B), (E) and (F) and 8 weeks (C), (D), (G) and (H) after implantation. (A), (B), (E) and (F) are from the same rat and (C), (D), (G) and (H) are from another rat. FL: floating microelectrode, MI: Michigan probe, GM = gray matter, WM = white matter. Scale bar = 100 μm.
These cells were contained within ~20 μm thick rim. Most Iba-1 positive cells outside this rim exhibited the characteristic, ramified morphology of resting microglia even though a few cells in the vicinity of the rim showed the activated morphology with thick and short processes (figure 3(B) ( ). Regions beyond 100 μm distance from the cavity were populated only with ramified microglia (figure 3(C) ( )). Overall, the microglial reaction to FL electrodes did not appear stronger than that to the MI electrode at both time points post-implantation (figures 4(A)–(D)) despite the difference in the size of the electrodes and their aspect ratios.

In contrast to the brain, in the spinal cord sections, the FL electrode cavities exhibited rounder and larger boundaries suggesting electrode motion within the cavity, most likely due to the perturbations that occur during the locomotion of the rat (figures 3(A) and (D)). In addition to the amoeboid microglia that formed a ~35 μm rim around the cavities (figures 3(D) and (E) ( )), many activated microglia were found in the penumbra even at 8 weeks post-implantation (figures 3(D) and (E) ( )). Outside the penumbra, microglia exhibited primarily ramified morphology (figure 3(F) ( )). Overall, the reaction to FL or MI electrode implantation appeared stronger in the spinal cord than the reaction in the brain, regardless of the electrode type (figure 4).

To quantify the overall microglial reaction at 8 weeks to the FL electrode implantation, we measured the Iba-1 intensity as a function of distance from the cavity. In both brain and spinal cord sections, the intensity analysis included only the gray matter (figure 5). These studies indicated that maximum intensities were measured within 10 μm and then declined with a distance of 50 μm (inset figure 5) regardless of the microglia activation state (amoeboid, activated or ramified).

To determine whether the microglial reaction is altered over time, we compared the intensity of staining at 4 and 8 week after electrode implantation. In the brain sections, the Iba-1 staining appeared similar at 4 and 8 weeks post-implantation regardless of the electrode type or implant site (one-way ANOVA $F_{(1119)} = 0.34, p = 0.56$).

Intensity data within 100 μm from the implants satisfied assumptions; normally distributed ($n > 5$ for each group and $p > 0.05$ for Shapiro–Wilk) and equal variance (same group size and $p < 0.05$ for Levene’s test). Multi-factorial ANOVA indicated that at least one group was statistically different ($F_{(7119)} = 3.72, p = .001$). Tukey post-hoc test was applied to compare implant type in nervous tissue between 4 and 8 weeks post implantation. The data did not support the hypothesis that the microglial activation, elicited by any type of implant, increased over time. $p$ values; BR/FL = 0.91, BR/MI = 0.89, SC/FL = 0.67 SC/MI = 0.73 ($n = 15$ for each group).

### 3.2. Astroglial reaction to the FL electrodes

The brain and spinal cord sections, adjacent to those labeled with Iba-1, were used for immunolabeling with an antibody against GFAP, an astroglial marker. In the brain sections, electrode cavities were surrounded by a dense rim of thick GFAP positive processes within ~20 μm (figures 6(A) and (B) ( )). This was seen both at 4 and 8 week post-implantation. Outside this rim, a few cells showed reactive astrocytes with thicker processes or the morphological characteristics of activated astrocytes even at 8 weeks post-implantation (figure 6(B) ( )). At distances greater than 100 μm from the cavity, astrocytes exhibited their non-reactive morphology (figure 6(C) ( )). However, the astroglial reaction to the FL and MI electrodes appeared similar (figures 7(A)–(D)). It is worth noting that the astroglial reaction in the brain showed inter-subject variability with some rats showing stronger reactions than others.

As in the case of microglia, in the spinal cord, the strongly immunopositive rim which contained the thick GFAP positive processes (figures 6(D) and (E) ( )) was confined to a 35 μm rim. A 100 μm thick penumbra contained reactive astrocytes (figure 6(E) ( )) at 4 (not shown) and 8 weeks post-implantation. Beyond the penumbra, astroglia showed their non-reactive morphology (figure 6(F) ( )). In general, the reaction of astrocytes to electrode implantation in the spinal cord appeared stronger (figures 7(E)–(H)).

The GFAP intensities (figure 8) declined sharply by distance, as in the Iba-1 stains both in the brain and the spinal cord, and descended to the baseline levels (observed at distant locations) by ~200 μm. The brain implants had sharper peaks in the normalized curves at the device surface for both electrode types (inlet in figure 8). However, the spinal cord implants induced the astroglial activation at longer distances. A majority of cells were found within a narrow band around the electrode perimeter and thus the statistical comparisons were limited to 0–100 μm intensity measurements.
Figure 6. Representative GFAP + images of adjacent sections to Iba-1 + images for characterization of astrocytes. Thick GFAP positive processes (□) proximal to implants (B) and (E), reactive astrocytes (●) proximal to implants (B) and (E) and non-reactive morphology (▲) as characteristic astrocytes (C) and (F). BR: brain, SC: spinal cord. Scale bar = 100 μm (A) and (D), 25 μm (B), (C), (E) and (F).

Figure 7. Representative GFAP + images obtained from sections adjacent to those labeled with Iba-1 + illustrating the astroglial reaction in the brain (A)–(D) and spinal cord (E)–(H) of rats receiving the floating microelectrode (A), (C), (E) and (G) and Michigan probe (B), (D), (F) and (H) at 4 weeks (A), (B), (E) and (F) and 8 weeks (C), (D), (G) and (H) after implantation. FL: floating microelectrode, MI: Michigan probe, GM = gray matter, WM = white matter. Scale bar = 100 μm.
Figure 8. Normalized fluorescent GFAP + intensities at 8 weeks after implantation in the brain and spinal cord. The area marked in gray (left) is shown at a larger scale in the inset. Each trace is the average of n = 15 curves from three rats. BR: brain, SC: spinal cord, FL: floating microelectrode, MI: Michigan probe, SE: standard error.

Similar to Iba-1 results, GFAP data met the assumptions; normally distributed (n = 15 for each group and p > 0.05 for Shapiro–Wilk) and equal variance (same group size and p > 0.05 for Levene’s test). The difference between 4 week and 8 week GFAP results, all groups combined, have not reached a level of significance (one-way ANOVA, $F_{(1119)} = 2.05$, p = 0.16). Also fANOVA results indicated at least one group with a significant difference ($F_{(7110)} = 7.47$, p < 0.001). Tukey post-hoc test did not show significant differences between groups to indicate increasing astrogial activation over time. p-values BR/FL = 0.81, BR/MI = 0.85, SC/FL = 0.47 SC/MI = 0.62 (n = 15 for each group).

3.3. DAPI on retrieved electrodes

Following perfusion of the rats, all implants were successfully retrieved with fine forceps without breaking and devices were saved for further analysis. In a few cases, retrieved implants were labeled with DAPI at 4 weeks to reveal any adherent material of cellular origin and observed under fluorescent microscopy. DAPI staining revealed a few cell nuclei on the MI probes, and almost no cells in the case of FLs (images not shown). This indicated that the activated tissue adjacent to the electrode surfaces was left behind in the slices stained and not on the device surface during removal of the device.

4. Discussion

In this study, we investigated glial reactions in the CNS in response to chronically implanted untethered FLs of our own design and MIs, as a comparison since a large amount of publications exist on the latter [29]. Neither of the device types had tethering wires attached, although the device profiles and aspect ratios were quite different. The rat brain gray matter as a site of neural implant testing has previously been reported [11, 13, 20, 30, 31]. However, immunohistochemical data from spinal cord [32] electrode implants is very rare. In most studies, epidural [33], or subdural [34, 35] stimulation approaches are taken in human subjects due to the high risk of neural trauma that may result from the mechanical stress induced by any rigid body implanted into the spinal cord.

The glial reaction in the neural tissue starts within hours after electrode implantation and continues for weeks. After six weeks no further increase in glial response around the implant is expected in the brain [31]. The glial cell morphologies observed in this study indicated stronger and longer-lasting reaction in the spinal cord compared to the brain. The responses of neurons, astrocytes and microglia in the brain and spinal cord to electrode implantation can be different since neurons and glia in distinct CNS regions have been reported to have distinct properties [36–39]. On the other hand, it is possible that the glial response in the spinal cord might have been exacerbated by the larger displacements inflicted on the implant [40–42] in a behaving animal. It should be noted that FL devices were relatively large for the rat spinal cord, presenting the worst case scenario. We anticipate that the actual FLAMES to be implanted in the rats will be smaller, thus potentially inducing less trauma than what has been observed here. Although the rat did not exhibit overt behavioral deficits following electrode implantation, specific behavioral tests could reveal more subtle motor anomalies and need to be studied further in future investigations.

The size, shape, and the chemical composition of the implant, the tethering forces, as well as the implantation technique are critical factors that play a role in foreign body response to chronically implanted neural electrodes [11, 12, 29, 43, 44]. Previous reports also demonstrated that the tethering forces generated by the wires that were used to mount the device to the skull triggered the immunological reaction and increased the encapsulation radius up to a few hundred microns around the implant [11, 12]. Although the two electrode geometries of this study had substantially different cross sectional areas and aspect ratios, the most severe responses in the brain were similar and were contained within 20 μm from the device surface according to the intensity profiles. The comparative analysis of glial intensity and morphology at 4 weeks versus 8 weeks post-implantation did not indicate worsening of the astroglial and microglial reactivity over time. Note that the spinal cord responses were different from that of the brain for both implant types. These results suggest that the future studies should put emphasis on understanding the tethering effect, perhaps more so than the device size or geometry.

A strong relation between neuronal loss and glial scar formation was reported by Biran et al [11] where 40% of the neurons were lost in a radius of 100 μm around tethered devices, where the most severe glial responses were observed. Seymour et al [29] investigated various microelectrodes with different cross-sections and shapes and found 12–17%
neuronal loss within a radius of 75 μm, which also correlated with the spatial extent of the glial response. The neuronal loss was not quantified in the present study. However, it can be extrapolated from the results cited above that viable neurons should be present at closer distances around the floating electrodes compared to the tethered ones. Reducing the thickness of the ‘dead zone’ around the electrode, for instance, from 60 to 20 μm would increase the voltage field experienced by the nearest neurons from a monopolar electrode (circular contact on a 30 μm wide shank) by more than three times [45] (their figure 3(C)). The voltage field of a bipolar, floating micro-stimulator would increase by similar amounts from 40 to 20 μm [45] (their figure 4(A)). This suggests that the stimulus current can be cut down significantly by reducing the glial response. A decrease in the threshold current would in turn improve the spatial selectivity of stimulation.

Finally, it is worth mentioning that device migration may also be a concern with floating microstimulators since this would alter the neuronal population activated by the device.

5. Conclusions

Current literature strongly suggests that neural electrode sizes should be in the same order as the neuronal cells to be considered ‘stealthy’ with minimal chronic neuroimmunological response. Our results indicate that chronic tissue response to much larger electrodes implanted both in the brain and spinal cord may be kept at a minimum so long as they are made to float by eliminating the wire connections. Further research is warranted to investigate these issues with active devices and longer implantation times.

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