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Chronic recordings from the rat spinal cord descending tracts with microwires

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Abstract

This study investigated the feasibility of chronically recording descending signals from the rat spinal cord using microwire electrodes. Eight 25μm diameter Pt-Ir microwires were implanted in the dorsolateral rubrospinal tract (RST) bilaterally at the c5 level in each of the four adult Long Evans rats trained for food reach-to-grasp task. Signal stability was assessed by calculating the signal-to-noise ratio (SNR) and mean signal amplitude during the four week recording period. The results of ANOVA did not suggest significant difference between sessions for any of the electrodes, indicating stability. Immunohistology suggested minimal tissue response to these microwires during the four week implant period. The results of this study show that microwire electrodes can be used for short-term chronic recordings of signals from the descending motor tracts in experimental animals.

I. Introduction

Spinal cord injury (SCI) results in a loss of function and sensation below the injury level. The mean life expectancy of those surviving the initial injury is nearly 40 years [1], as a result of which there are considerable costs associated with primary care and loss of income. These individuals can greatly benefit from a neuroprosthetic technology that can either replace missing limbs with prosthetics or restore some level of motor function using volitional command signals. The brain-computer interface (BCI) technique is one way of generating these command signals and it has achieved tremendous success in the past [2]–[8]. The spinal cord lateral descending pathways involved in producing skilled forelimb movements [9]–[12] can provide an alternate site for tapping neural signals as a means of generating the lost command signals [13]. Evidence in literature has shown survival of fibers proximal to the injury even several years after SCI [14]–[19], also suggesting availability of neural control information in the spinal cord long after injury. A recent study [20] showed that spinal cord tissue well tolerates microwire implants and chronic application of microstimulation for one month post-implant. We have also been able to record spinal cord signals using Utah microelectrode arrays for up to three months in rats [13].

In this study, we recorded descending signals from the rat cervical spinal cord for a period of four weeks. The rationale for choosing the implant site and the animal model has been discussed elsewhere [13]. The main focus of this study was to show that stable signals could be recorded using microwires from the spinal cord. Signal-to-noise ratios and mean signal amplitudes during the behavioral task were used to assess signal stability. Astroglial and

microglial response caused by the implants was investigated by immunostaining the explanted spinal cord sections.

II. Experimental methods

A. Microwire Electrode Fabrication

Twenty-five μm diameter, 90/10 Pt-Ir wires (A-M Systems, Inc.) were used as the electrode material. Fabrication of one such electrode is shown in Figure 1. The Pt-Ir wires were de-insulated a few millimeters at the ends and soldered to the microconnector terminals (Omnetics, Inc). A thin layer of epoxy was applied to provide insulation at the back of the microconnector. The wires were then bundled in groups by applying a thin layer of medical grade silicone (Med 4211, Nusil, Inc) to keep each group separate from the other. Electrode impedances were measured (IMP-2, BAK Electronics, Inc) at 1kHz and 100nA current *ex vivo* and *in vivo*. The microwire electrodes were gas (ethylene oxide) sterilized prior to surgery. Individual wires were implanted in groups of 3–4 in each rubrospinal tract in the dorsolateral funiculus (Fig. 1 bottom).

B. Behavioral Training

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC), Rutgers University, Newark, NJ. Four adult male Long Evans rats weighing between 300–350 grams were used in the study. The animals were trained to reach and grasp food pellets through an aperture. Animals were considered trained when they attained approximately 90% success level in food reaching and grasping.

C. Surgical Procedure

Anesthesia was induced using sodium pentobarbital (30 mg/kg, IP). Bupivacaine (0.2 ml, SC) at the incision site for local anesthesia, dexamethasone (0.2 mg/kg, IM) to prevent CNS edema, and atropine (6mg/kg, IM) to improve respiratory function were injected prior to surgery. Six holes were drilled into the skull and metal screws (Plastics One Inc, VA) were screwed after fixing the animal's head in a standard stereotaxic frame and exposing the skull by scraping the periosteum using a scalpel. Stainless steel wires (50μm) wound around the screws served as the ground connection. The head-stage micro-connector (Omnetics, Inc) was then fixed to the skull using dental acrylic. A second incision (4–5cms) was made from the end of the micro-connector on the animal's back down at the midline. Muscle layers were separated to access the vertebra and laminectomy was performed at c5 level to expose the spinal cord. The dura was punctured using a 30-gauge needle and then slit using microscissors. The wire bundles were then lightly tied at two separate locations to the muscles close to where they were to be implanted using size 6.0 non-absorbable sutures. Wire tips were cut at 45° angle with a pair of microscissors that served as the recording part of the wires. Wires were bent 1mm from the tip at 90° angle and then individually pushed into the spinal cord under 40x microscopic vision and a small drop of cyanoacrylate glue was applied around the wires to fix it in place. Figure 1 (bottom) shows the location of the microwires in the dorsolateral funiculus of the rat on each side of the spinal cord. After all the wires were inserted, a few more drops of glue were used to cover the implant area and seal the dura.

D. Recording Procedure

Recording sessions began about a week after the animals completely recovered from surgery and continued for four weeks. Each recording session usually consisted of 80–90 trials. Animals were numbered as W1 through W4. A 32-channel, 100-gain headstage amplifier (Triangle Biosystems, Inc) interfaced the microwire electrode to the data acquisition card (National Instruments PCI 6255) and the computer. A custom-written Matlab code was used

to simultaneously acquire five seconds of neural data (30kHz sampling rate) and video images (30frames/s deinterlaced to form 60fields/s) for each reach-to-grasp trial. Neural data and video images were time stamped using Matlab toolboxes.

III. Data analysis and results

Signals were recorded in non-referenced single-ended (NRSE) mode with respect to a stainless steel wire tied to the skull screw. Data analysis was performed using MATLAB. Power spectral density (PSD) of the signals was calculated using the Welch averaged periodogram method. Figure 2 (top) shows the PSD estimate of signals recorded from the right and left RST when the animal was performing the reaching behavior with the right hand. The ipsilateral RST spectrum has more power below 1kHz. The trace in black shows the spectrum when the animal was quiet and resting, which has significantly less power than the other two traces. Signals can be seen extending well up to 5kHz similar to our recordings with the Utah microelectrode arrays [13].

Two channels of neural signals (bandpass filtered between 300Hz-3kHz) recorded with wire electrodes during the reaching task in W1 are also plotted in Figure 2 (bottom). The arrow shows the duration of the behavior. An increase in activity in the ipsilateral white matter during the behavior suggests the involvement of RST fibers during the skilled reached-to-grasp task. The recordings from the contralateral dorsolateral funiculus registering very little increase in activity suggest that electrodes record local activity and not common-mode contamination from distant sources.

Raw signals were bandpass filtered between 300Hz-3kHz to obtain multi-unit activity recorded by the microwires. Signal-to-noise ratio was calculated as divided by the standard deviation of the channel noise in the resting animal. The mean signal-to-noise ratio (SNR) was calculated and plotted in Figure 3 for W4 (2 recording sessions). The SNR usually varied between 1.5 to 2.5 across the electrodes, but remained fairly stable during the 4-week recording period in all electrodes of W4 and in all the other animals (not shown) as suggested by the small standard deviations.

The mean amplitude of neural signals during the behavior was calculated from the rectified-averaged (50ms moving averager) signal for each trial to determine if the mean signal amplitudes remained stable across sessions. One-way ANOVA was performed between the mean amplitudes from all trials between sessions. The mean signal amplitude for all electrodes (2 recording sessions) is plotted in Figure 4 for W3. The small standard deviations suggest that the signal amplitude recorded from these electrodes remained fairly stable during the recording period. The results of ANOVA did not suggest significant difference between sessions for any of the electrodes (p-value = 0.05).

IV. Immunohistology

The animals, four weeks post-implant, were anesthetized and perfused with heparinized saline followed by 4% neutral buffered formaldehyde (NBF). The spinal cord segment containing the microwires was explanted and fixed for 24 hours in the NBF and then rinsed in phosphate buffered saline, pH 7.4. The cord segment, embedded in paraffin was oriented such that coronal sections could be taken perpendicular to the microwires tips. Serial 6μm sections were stained with Luxol fast blue-cresyl violet (LFB) for evaluation of myelin status and tissue reaction. Antigen retrieval was performed after deparrafinizing the sections using a computer controlled pressure cooker (2100 Retriever, Pick Cell Laboratory, Netherlands). Sections were stained for astrocytes using rabbit-anti-GFAP (Dako, CA) as the primary antibody and goat-anti-rabbit IgG (Alexa Fluor 488, Invitrogen, CA) as the secondary antibody. Microglia was stained with goat-anti-Iba1 (Abcam, MA) as the primary

antibody and rabbit-anti-goat IgG (Invitrogen, CA) as the secondary antibody. Non-specific nuclei were stained with 7-aminoactinomycin (7AAD, Invitrogen).

Figure 5 shows LFB stained images taken at 20x for two different wires (holes) at the same depth of 540μm from the dorsal surface of the spinal cord in W3. The wires were located, upon histological observation, in the white matter of the spinal cord. Minimal disruption of myelin was observed in the images around the implant site. Figure 6 shows an immuno-stained image for two wires in separate animals (W1 and W3) where the astrocytes are shown in green, the microglia in red, and the non-specific cells in blue (presumably oligodendrocytes). Minimal tissue response from the implanted wires was observed similar to other animals with wire implants in this study.

V. Discussion

We have reported stable recordings from the rat spinal cord descending tracts using Utah microelectrode arrays (MEAs) (Blackrock Systems, UT) for up to 3-months post implant [13]. Switching from Utah MEAs to microwires was a major change in methodology mainly influenced by the difference in the tissue response generated by these two types of electrodes. The Utah MEAs were designed for cortical implants and due to their rigidity and size they invoked greater tissue response in the rat spinal cord. Microwire implants, as shown by histological images in a small group of animals, caused significantly reduced astrocytic and gliotic responses. No motor deficits were observed in the animals investigated in this study. Post-operative recovery time was faster in animals implanted with microwires.

Microwires were able to record signals with slightly better SNR (Fig. 3) compared to Utah MEAs. The mean signal amplitudes during the reaching behavior were larger for all microwires (25–40μV) (Fig. 4) compared to Utah MEAs (6–12μV). Larger amplitude signals and better signal-to-noise ratios recorded from microwires can be attributed to their lower impedance.

Therefore, microwire electrodes may be a viable choice for a spinal cord neural interface in experimental animals. The animals were tested for only 4 weeks post-implant in this preliminary study. A longer implant period may be required to establish the viability of these electrodes. Microwires did not elicit significant tissue response in general, agreeing with the results in [20]. Staining for astrocytes and microglia also did not suggest a prolific tissue response around the microwire tip or along the length of the wires (Fig. 6). The astrocytes were found to be in their non-reactive state and very few microglia were present at the implant site. Neural tissue displaced by these microwires was also significantly less than the Utah MEAs because of the small diameter of the wires. Neuron viability was not tested around the microwires in this study. However, LFB staining (Fig. 5) did not show any damage in the normal tissue structure in the grey matter region, usually several hundred microns away from the wire tips. Demyelination was not observed in the LFB stained images in any of the animals with the microwire implants.

VI. Conclusion

Neural recording from the white matter of the spinal cord presents challenges in behaving animals, especially in rats. This study has demonstrated that stable recordings over a period of few weeks is feasible with microwire implants.

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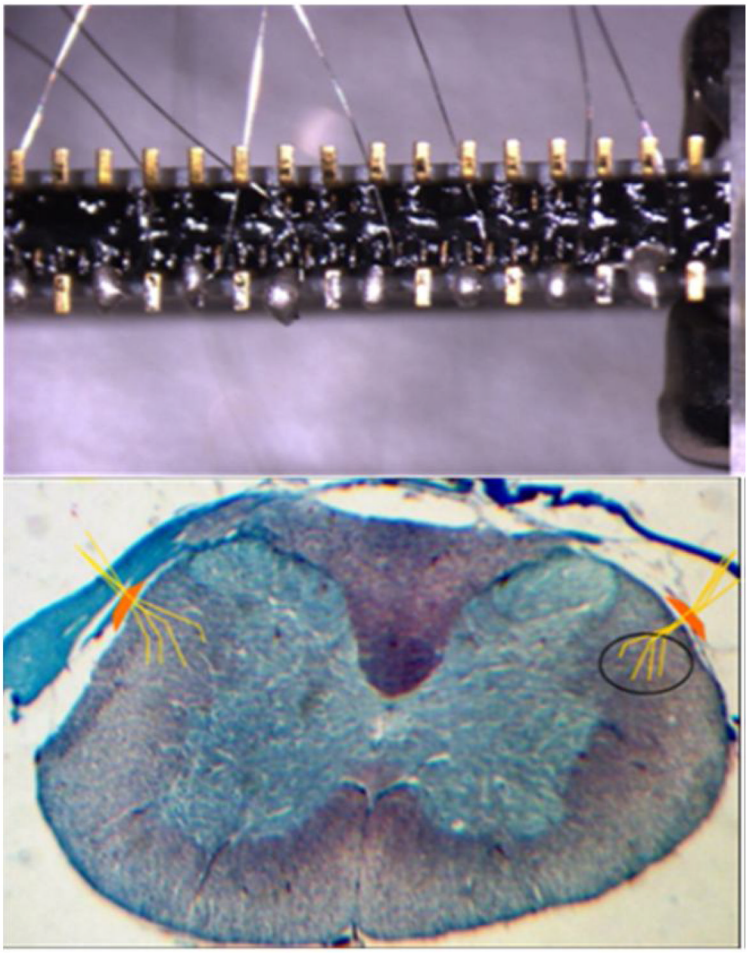

Fig. 1.
Microwire attachments shown on the top. Bottom image shows the implant locations of the microwires in the RST superimposed over a rat spinal cord histology.

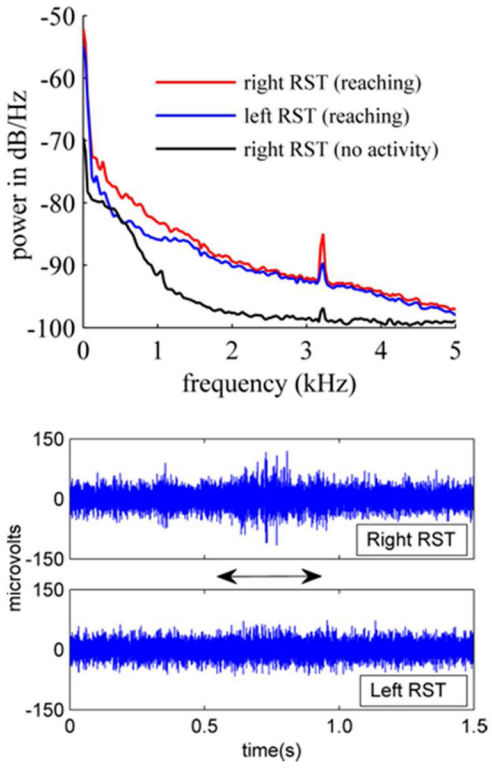

Fig. 2.

Top: PSD of neural activity from right and left RST during the reaching task. Bottom: Two channels of neural signals in W1 from the ipsilateral and contralateral dorsolateral funiculus to the forelimb used during the reaching task. The recordings in the bottom plot from the contralateral RST shows very little increase in activity. Arrow marks the duration of the behavior.

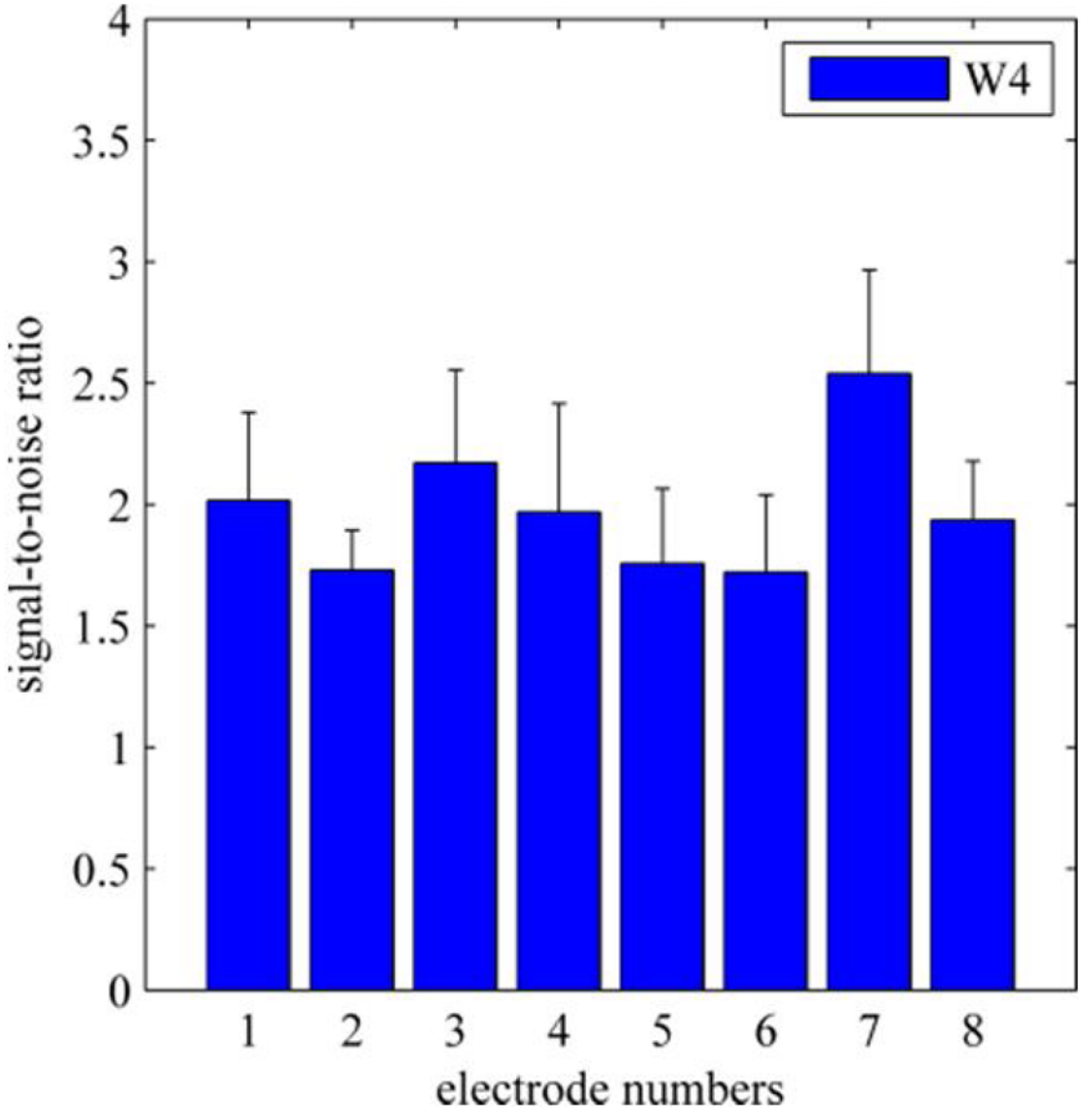

Fig. 3.
Signal-to-noise ratio calculated for animal W4.

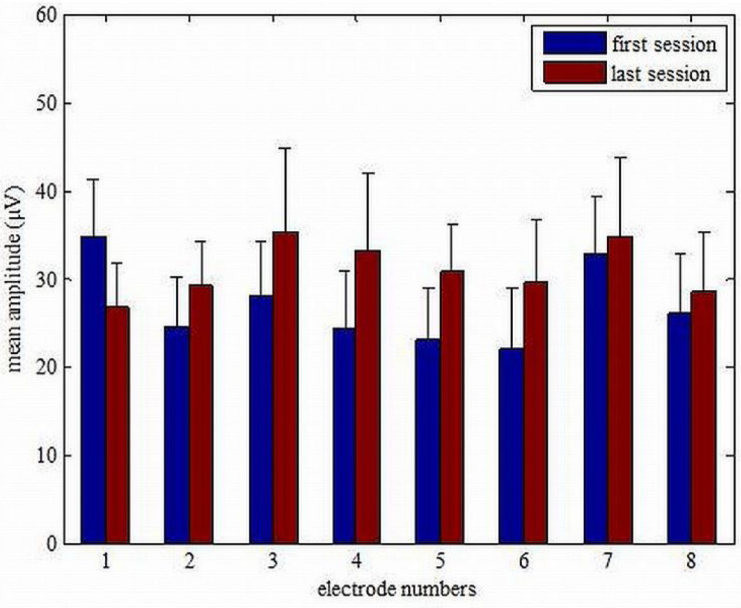

Fig. 4.
Mean signal amplitudes from 20 trials each in two recording sessions in W3.

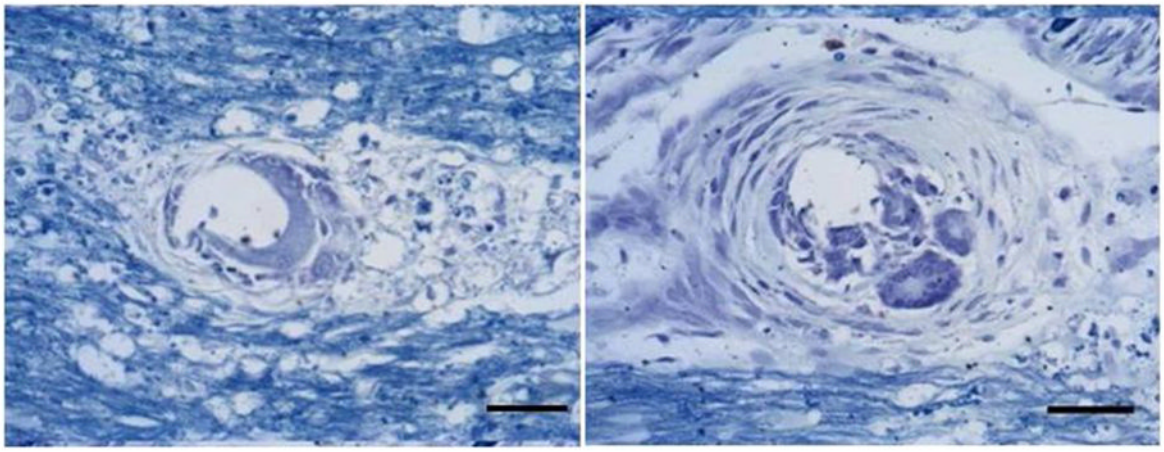

Fig. 5.
Two separate microwires (holes) are shown at 20x magnification from W3. Scale bars are 25μm. Minimal disruption of myelin and minimal tissue response can be observed in these images with Luxol fast blue staining.

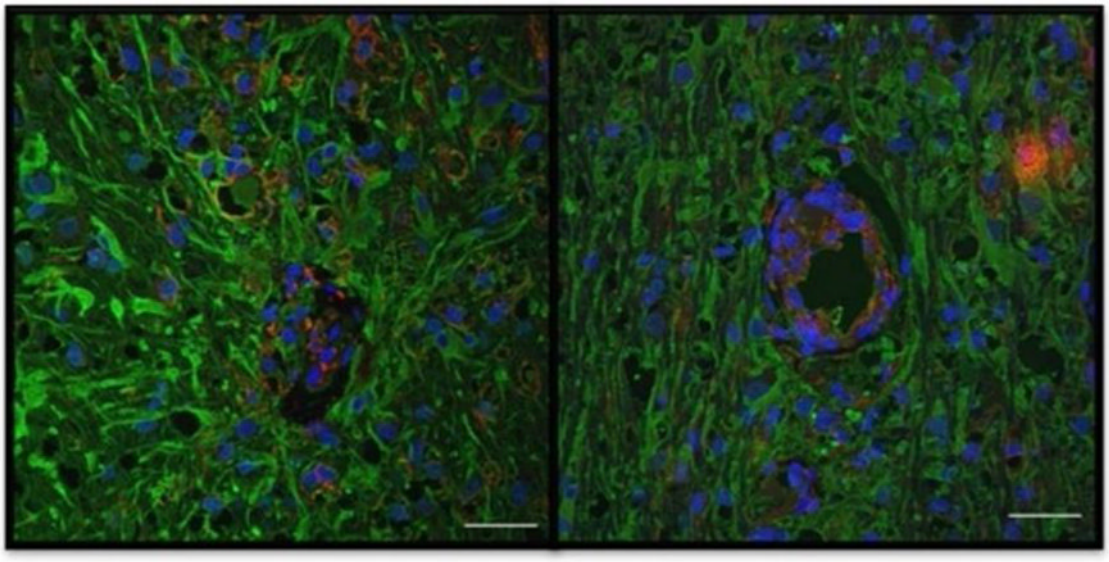

Fig. 6.

Fluorescent image taken at 40x magnification at the microwire tip (hole) implanted in W1 (left) and W3 (right). Astrocytes are shown in green (GFAP), microglia in red (Iba1), and the cell bodies in blue. Minimal microglial activation can be observed in the image (very few red colored cell bodies). Scale bar is 25μm.