Published in final edited form as:
J Neurosci Methods. 2007 July 30; 163(2): 321–325. doi:10.1016/j.jneumeth.2007.03.017.

A chronically implantable, hybrid cannula-electrode device for assessing the effects of molecules on electrophysiological signals in freely behaving animals

Bradley Greger[1,2,*], **Babak Kateb**[3,4,*], **Peter Gruen**[3], and **Paul H. Patterson**[2]

1*University of Utah, Department of Bioengineering, Salt Lake City, UT 84112*

2*California Institute of Technology, Division of Biology, Pasadena, CA 91125*

3*USC-Keck school of Medicine, Department of Neurological Surgery, Los Angeles, CA 90033*

4*City of Hope National Comprehensive Cancer Center, Brain Tumor Program, Duarte, CA 90101*

Abstract

We describe a device for assessing the effects of diffusible molecules on electrophysiological recordings from multiple neurons. This device allows for the infusion of reagents through a cannula located among an array of micro-electrodes. The device can easily be customized to target specific neural structures. It is designed to be chronically implanted so that isolated neural units and local field potentials are recorded over the course of several weeks or months. Multivariate statistical and spectral analysis of electrophysiological signals acquired using this system could quantitatively identify electrical “signatures” of therapeutically useful drugs.

Keywords

Drug delivery; Single-unit recording; Local field potential recording; Drug screening; Neural prosthetics

1. Introduction

For many decades, animal models have been used for the identification of drugs that ameliorate psychiatric, neuropathological and neuro-degenerative disorders. The principle means of assessing efficacy has been the measurement of behavioral responses. The development of anti-depressant drugs is an excellent example of the successful application of this methodology (Cryan et al. 2002). Similarly, the development of drugs for the treatment of epilepsy uses behavioral assays of seizure activity (White 2002, 2003). However, behavioral assessment is an indirect measurement of drug effects on neural circuitry. Recent data have shown that

Bradley Greger, Bioengineering, University of Utah , Salt Lake City, UT 84112, bradley.greger@utah.edu, phone: (801) 585-5795, fax: (801) 581 8966
Babak Kateb, City of Hope National Comprehensive Cancer Center, Brain Tumor Program, Duarte, CA 90101
Peter Gruen, USC-Keck school of Medicine, Department of Neurological Surgery, Los Angeles, CA 90033
Paul H. Patterson, Division of Biology, California Institute of Technology, Pasadena, CA 91125
*These authors contributed equally to this work

electrophysiological signals are modulated by anti-depressant drugs (Szabo and Blier 2001; Szabo et al. 1999) and serve as a predictor of drug efficacy (Gallinat et al. 2000; Hegerl et al. 2001; Hegerl and Juckel 2000). In addition, the effects of infusing substances into the striatum have been quantified using electrophysiology to understand their relationship to disorders such as Parkinson's disease and schizophrenia (Pierce and Rebec 1995; Stanford et al. 2005, In Press). These results suggest that systematic and quantitative electrophysiological screening of pharmaceuticals may prove to be a useful tool in drug development for a variety of neurological and psychological pathologies.

More recently, due to the rapidly developing field of neural prosthetics and brain stimulation a need has arisen to maintain chronic, i.e. several years, electrophysiological contact with neurons in the brain. Currently available, chronically implanted micro-electrode arrays for recording single neural units in neural prosthetic applications lose signals over time. In most cases these micro-electrodes fail completely after being implanted in the brain for several months to a few years. This loss of signal is thought to be primarily due to the inflammatory response engendered by insertion of the electrodes into the brain and subsequent relative motion of the electrodes and the brain (Polikov et al. 2005; Szarowski et al. 2003; Turner et al. 1999). Even arrays that float with the brain suffer from inflammatory responses that could be ameliorated by a pharmacological intervention (House et al. 2006; Kim et al. 2006; Vetter et al. 2004; Warren et al. 2004).

The device described here offers a simple and effective way to approach both drug development and electrode contact longevity issues. Although several cannula-eletrode devices have been designed for use in both behaving rats (Laird et al. 1979; Rebec et al. 1993) and monkeys (Kliem and Wichmann 2004), the device presented here possesses several significant advantages. It its extremely light weight, simple to use, highly configurable, bio-compatible, and can acquire both isolated neural units and local field potentials (LFPs), while delivering drugs through a cannula.

2. Materials and Methods

2.1 Assembly of the cannula-multielectrode array

An apparatus for simultaneously measuring electrophysiological signals and for infusing reagents in close proximity to the electrodes is described. The device is comprised of a body, a cannula, and electrodes mounted on the body so that reagents supplied by the cannula are delivered in proximity of the electrodes. The cannula and electrode can be arbitrarily configured with respect to each other in order to allow the device to be customized for optimal implantation in specific brain regions.

The device (Fig. 1) is based upon a commercially available cannula system (Brain Infusion kit II, Alzet). The electrodes are made up first, as single long "hat pins". Holes are drilled at the desired location into one of the electrode mounting disks supplied with the Alzet kit. The rigid hat pin electrode is placed through the pre-drilled hole with the desired length extending below the electrode mounting disk and tacked in place using a small amount of biomedical grade cyanoacrylate glue. The length of electrode above the electrode mounting disk is trimmed to a shaft of approximately 1mm and stripped of insulation. A flexible 33 gauge insulated copper wire lead is soldered to the electrode shaft so that it is at a right angle to the shaft and parallel to the electrode mounting disk. The other end of the copper lead wire can then be attached to any convenient electrical connector. The cannula is then slid into the central hole of the electrode mounting disk, until the desired length of the cannula is protruding below the disk, and tacked in place using the cyanoacrylate glue. The gap between the electrode mounting disk and the base of the cannula assembly is filled with Loctite M-31CL Medical Device Epoxy to protect wire leads and strengthen the device.

The electrodes are manufactured from the biocompatible materials, platinum/iridium alloy and Paralene-C insulation. The units tested utilize 75 micron diameter electrodes sharpened to 1 - 2 microns with impedance of ~0.3 megaohms. The electrodes and cannula extended 2.5mm and 2.0mm below the electrode mounting disk. Electrode materials and construction can also be customized according to the needs for insertion into different brain structures, e.g. longer electrodes for recording from deep brain structures. The electrode manufacturing and device assembly is carried out by Micro Probe Inc. (Gaithersburg, MD, USA).

Using the current version of the device, saline is infused using an osmotic mini-pump (Alzet). This pump uses the force generated by an osmotic gradient to slowly infuse liquid over the course of several days-to-weeks with no intervention.

2.2 Surgical implantation

The surgical implantation of the device is performed using a minimally invasive procedure. An extended borehole procedure is performed. The device is then stereotaxically implanted through the craniotomy. The duramater is pierced by the cannula and electrodes, but is otherwise left intact. The device is anchored to the skull using titanium bone screws (Osteomed, Addison, TX) and an island of methyl methacrylate forming a small head cap. A pocket is formed by blunt dissection of a subcutaneous space between the scapulae and the osmotic pump is placed into this pocket and connected to the cannula-electrode device with plastic tubing. The scalp is sutured around the head-cap, leaving the electrical connector exposed (Fig. 2). A skilled operator can implant the device in approximately 20 minutes from the onset of anesthesia.

It was reported that cyanoacrylate gel (loctite 454) is a more effective and easier means of cannula-electrode fixation since it does not require the use of skull screws for anchoring (Criado et al. 2003). This would greatly reduce the time required for implantation.

2.3 Data acquisition and analysis

Since astrocytes often form a barrier around chronically implanted electrodes, we use immunohistochemical staining for the astrocyte marker, glial fibrillary acidic protein (GFAP) (Jankowsky et al. 2000). Briefly, animals are given an anesthetic overdose and transcardially perfused with 10% formalin. Brains are then removed and immediately frozen in pre-chilled isopentane. After embedding in Cryo-M-Bed (Bright, Huntingdon, UK), 20 μm frozen sagittal sections through the region where the cannula-electrode device was implanted are collected. The sections are incubated overnight at 4°C with anti-GFAP antibody diluted 1:500 in blocking solution, and then incubated with a secondary antibody conjugated to a fluorescence marker for visualization.

Electrophysiological data can be acquired using standard amplification, filtering, and analog to digital converting systems. We recorded isolated neural-units and LFP using two signal paths and with different filters applied to each path. We used a Dam-80 isolation amplifier and filter (World Precision Instruments) and a National Instruments DAQ card. Electrical signals are amplified with a gain of 10k and filtered at either 100 – 10,000 Hertz for recording neural units, or 0.1 – 10,000 Hertz to acquire LFPs. Alternatively, a single broadband neural signal could be recorded and differentially digitally filtered offline.

3. Results

We successfully implanted this device into the frontal or parietal cortices of five rats, and obtained both electrophysiological and histological data. Activated astrocytes are a key part of the inflammatory response to neural injury, and increased GFAP staining is a reliable maker

of this response (Eng et al. 2000; Polikov et al. 2005; Szarowski et al. 2003; Turner et al. 1999). Several weeks post-implantation, we sacrificed the rats and performed GFAP immunohistochemistry. As expected, compared to the non-implanted hemisphere, the tissue around the electrode exhibits increased GFAP immunostaining (Fig. 3). We also collected electrophysiological data at two - five time points over many weeks post-implantation (Figs. 4 and 5). Even though an increase in the inflammatory response was detected by imunohistochemistry, we are able to collect high quality electrophysiological data. As calculated by spike peak-to-peak divided by the RMS of the whole recording, the signal to noise ratio of the recordings displayed in Fig. 4 is 19:1 for rat 2 and 25:1 for rat 3. Both the high frequency spike data and the spectral analysis of the LFP demonstrate electrophysiological activity two weeks post-implantation.

4. Discussion

The cannula-electrode device described here allows recording of the electrical signal from single neural units, and the more global LFP signal, at multiple sites. The recordings of electrical activity are made while a reagent is infused in close proximity to the recording electrodes. Similar devices used by others are capable of recording at only a single location (Kliem and Wichmann 2004; Rebec et al. 1993), or only EEG signals (Laird et al. 1979). The present device is highly configurable so that electrical recordings and reagent infusion can be targeted to specific neural structures.

We recorded electrical activity from, and infused saline into, the cerebral cortex, which served as a proof of concept for the functionality of the device. Further work is, however, needed to determine the effects of specific reagents on neural activity using this device, as well as the ability of this device to record from multiple structures, e.g. the basal ganglia and cerebral cortex, simultaneously. In addition, since cytokines such as interleukin (IL)-1, -4, -8, -10 and tumor necrosis factor–α (TNF–α) can enhance repair of injured tissue (Spera et al. 1998; Tanuma et al. 1997; Wang et al. 2002; Wang and Shuaib 2002), it would be interesting to use the described cannula-electrode device to determine if such anti-inflammatory agents can prolong the useful lifespan of the electrode arrays.

Recent studies have shown that electrophysiological signals from isolated neurons are affected by neuroactive drugs such as anti-depressants (Szabo and Blier 2001; Szabo et al. 1999), and that evoked potential responses can serve as a marker of anti-depressant efficacy (Gallinat et al. 2000; Hegerl et al. 2001; Hegerl and Juckel 2000; Linka et al. 2004; Linka et al. 2005). Such results suggest that there are likely to be electrophysiological signatures for neuro-active drugs effective against a variety of neuro-pathologies. Recordings of neural units and LFP may allow for the detection of such signatures in localized neural structures. The effects of intracerebral infusion of pharmaceutical agents could then be examined for their effects upon electrophysiological signatures.

This device could also serve as a tool for determining pharmaceutical methods of improving the longevity of chronically implanted electrodes used in neural prosthetic applications. When coupled with telemetry for wireless transmission of the neural signals (Harrison et al. 2007; Neihart and Harrison 2005), there is no need for a transcutaneous electrical connector, so the skin can be sutured completely closed over the acrylic head-cap. In such a configuration the device could provide continuous infusion of reagents and monitoring of signals in the freely behaving animal without requiring a wired connection and a commutator.

Acknowledgements

We thank Richard Andersen for his support of this work. This work was funded by a McGrath Foundation grant to Paul H. Patterson, NIH grants R01 EY013337 and R01 EY0155445 to Richard Andersen, and NIH training grant

NS007251-18 support for Bradley Greger, and the brain injury fund of Dr. Peter Gruen, which supported Babak Kateb. This work would not have been possible without the precise work of Martin Bak (Micro Probe, Inc.), who implemented the design of the cannula-electrode-pump hybrid system.

References

Criado A, Barford J, Parker F, Bate S, Whelan G. Use of Cyanoacrylate Gel as a Substitute for Dental Cement in Intracerebroventricular Cannulations in Rats. Contemporary Topics in Laboratory Animal Science 2003;42:13–16. [PubMed: 12906396]

Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 2002;23:238–245. [PubMed: 12008002]

Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). Neurochem Res 2000;25:1439–1451. [PubMed: 11059815]

Gallinat J, Bottlender R, Juckel G, Munke-Puchner A, Stotz G, Kuss HJ, Mavrogiorgou P, Hegerl U. The loudness dependency of the auditory evoked N1/P2-component as a predictor of the acute SSRI response in depression. Psychopharmacology (Berl) 2000;148:404–411. [PubMed: 10928314]

Harrison RR, Watkins PT, Kier RJ, Lovejoy RO, Black DJ, Greger B, Solzbacher F. A Low-Power Integrated Circuit for a Wireless 100-Electrode Neural Recording System. Solid-State Circuits, IEEE Journal of 2007;42:123–133.

Hegerl U, Gallinat J, Juckel G. Event-related potentials. Do they reflect central serotonergic neurotransmission and do they predict clinical response to serotonin agonists? J Affect Disord 2001;62:93–100. [PubMed: 11172876]

Hegerl U, Juckel G. Identifying psychiatric patients with serotonergic dysfunctions by event-related potentials. World J Biol Psychiatry 2000;1:112–118. [PubMed: 12607207]

House PA, MacDonald JD, Tresco PA, Normann RA. Acute microelectrode array implantation into human neocortex: preliminary technique and histological considerations. Neurosurg Focus 2006;20:E4. [PubMed: 16711661]

Jankowsky JL, Derrick BE, Patterson PH. Cytokine responses to LTP induction in the rat hippocampus: a comparison of in vitro and in vivo techniques. Learn Mem 2000;7:400–412. [PubMed: 11112799]

Kim SJ, Manyam SC, Warren DJ, Normann RA. Electrophysiological mapping of cat primary auditory cortex with multielectrode arrays. Ann Biomed Eng 2006;34:300–309. [PubMed: 16496084]

Kliem MA, Wichmann T. A method to record changes in local neuronal discharge in response to infusion of small drug quantities in awake monkeys. Journal of Neuroscience Methods 2004;138:45. [PubMed: 15325110]

Laird HE 2nd, Hermansen JE, Huxtable RJ. An electrode-cannula unit for intracerebral electrical stimulation, EEG recording and drug administration in small animals. Pharmacol Biochem Behav 1979;10:429–431. [PubMed: 450953]

Linka T, Muller BW, Bender S, Sartory G. The intensity dependence of the auditory evoked N1 component as a predictor of response to Citalopram treatment in patients with major depression. Neurosci Lett 2004;367:375–378. [PubMed: 15337269]

Linka T, Muller BW, Bender S, Sartory G, Gastpar M. The intensity dependence of auditory evoked ERP components predicts responsiveness to reboxetine treatment in major depression. Pharmacopsychiatry 2005;38:139–143. [PubMed: 15902586]

Neihart NM, Harrison RR. Micropower circuits for bidirectional wireless telemetry in neural recording applications. IEEE Trans Biomed Eng 2005;52:1950–1959. [PubMed: 16285399]

Pierce RC, Rebec GV. Iontophoresis in the neostriatum of awake, unrestrained rats: differential effects of dopamine, glutamate and ascorbate on motor- and nonmotor-related neurons. Neuroscience 1995;67:313–324. [PubMed: 7675172]

Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. J Neurosci Methods 2005;148:1–18. [PubMed: 16198003]

Rebec GV, Langley PE, Christopher Pierce R, Wang Z, Heidenreich BA. A simple micromanipulator for multiple uses in freely moving rats: electrophysiology, voltammetry, and simultaneous intracerebral infusions. Journal of Neuroscience Methods 1993;47:53. [PubMed: 8321014]

Spera PA, Ellison JA, Feuerstein GZ, Barone FC. IL-10 reduces rat brain injury following focal stroke. Neurosci Lett 1998;251:189–192. [PubMed: 9726375]

Stanford JA, Salvatore MF, Joyce BM, Zhang H, Gash DM, Gerhardt GA. Bilateral effects of unilateral intrastriatal GDNF on locomotor-excited and nonlocomotor-related striatal neurons in aged F344 rats. Neurobiology of Aging. 2005In Press

Szabo ST, Blier P. Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons. Brain Res 2001;922:9–20. [PubMed: 11730697]

Szabo ST, de Montigny C, Blier P. Modulation of noradrenergic neuronal firing by selective serotonin reuptake blockers. Br J Pharmacol 1999;126:568–571. [PubMed: 10188964]

Szarowski DH, Andersen MD, Retterer S, Spence AJ, Isaacson M, Craighead HG, Turner JN, Shain W. Brain responses to micro-machined silicon devices. Brain Res 2003;983:23–35. [PubMed: 12914963]

Tanuma N, Kojima T, Shin T, Aikawa Y, Kohji T, Ishihara Y, Matsumoto Y. Competitive PCR quantification of pro- and anti-inflammatory cytokine mRNA in the central nervous system during autoimmune encephalomyelitis. J Neuroimmunol 1997;73:197–206. [PubMed: 9058777]

Turner JN, Shain W, Szarowski DH, Andersen M, Martins S, Isaacson M, Craighead H. Cerebral astrocyte response to micromachined silicon implants. Exp Neurol 1999;156:33–49. [PubMed: 10192775]

Vetter RJ, Williams JC, Hetke JF, Nunamaker EA, Kipke DR. Chronic neural recording using silicon-substrate microelectrode arrays implanted in cerebral cortex. IEEE Trans Biomed Eng 2004;51:896–904. [PubMed: 15188856]

Wang CX, Reece C, Wrathall JR, Shuaib A, Olschowka JA, Hao C. Expression of tumor necrosis factor alpha and its mRNA in the spinal cord following a weight-drop injury. Neuroreport 2002;13:1391–1393. [PubMed: 12167759]

Wang CX, Shuaib A. Involvement of inflammatory cytokines in central nervous system injury. Prog Neurobiol 2002;67:161–172. [PubMed: 12126659]

Warren DJ, Koulakov A, Normann RA. Spatiotemporal encoding of a bar's direction of motion by neural ensembles in cat primary visual cortex. Ann Biomed Eng 2004;32:1265–1275. [PubMed: 15493513]

White HS. Animal models of epileptogenesis. Neurology 2002;59:S7–S14. [PubMed: 12428026]

White HS. Preclinical development of antiepileptic drugs: past, present, and future directions. Epilepsia 2003;44(Suppl 7):2–8. [PubMed: 12919332]

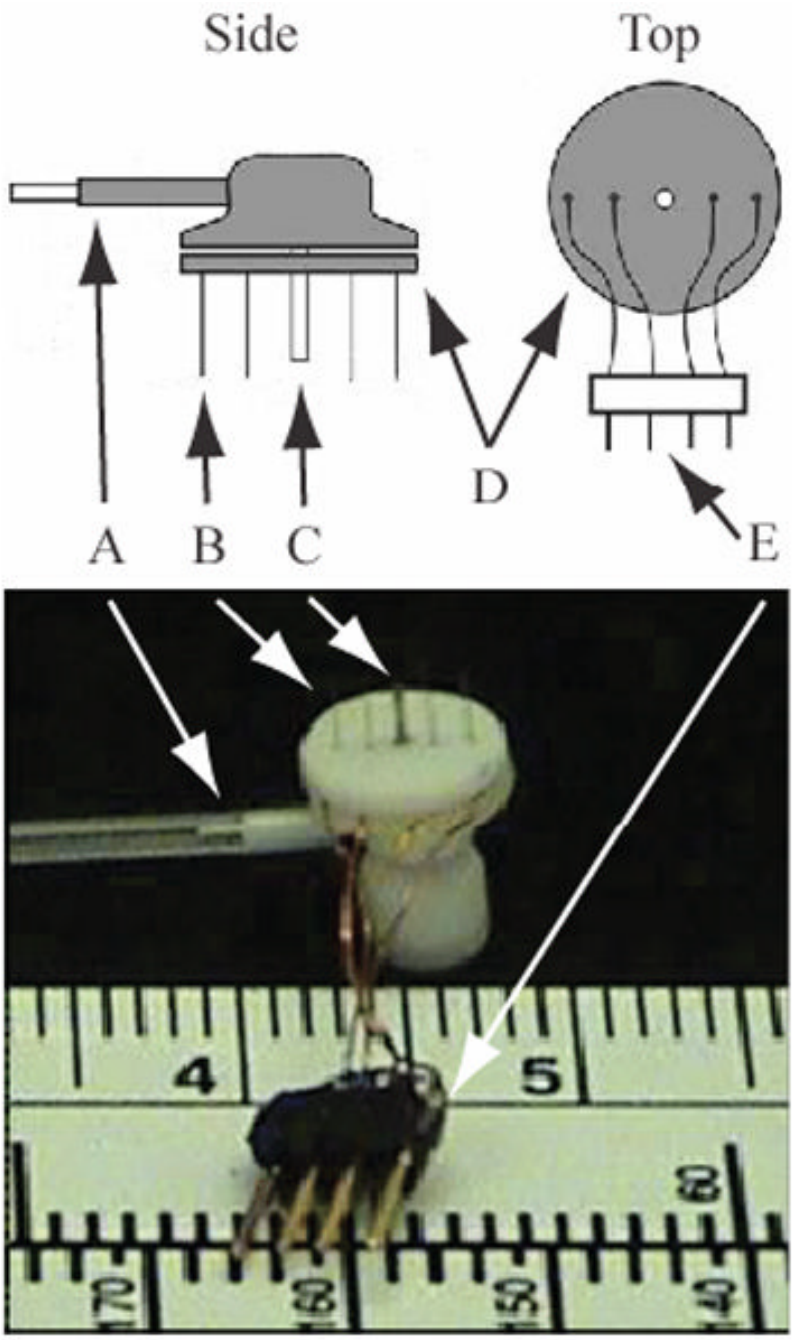

Figure 1.

A schematic and picture of the cannula-electrode device are shown. The schematic shows a side view of the entire device and a top view of the disk upon which the electrodes are mounted, and the electrical connector. The picture displays the complete device next to a scale in centimeters. A – coupling to osmotic pump, B – electrode, C – cannula, D – electrode mounting disk, E – electrical connector.

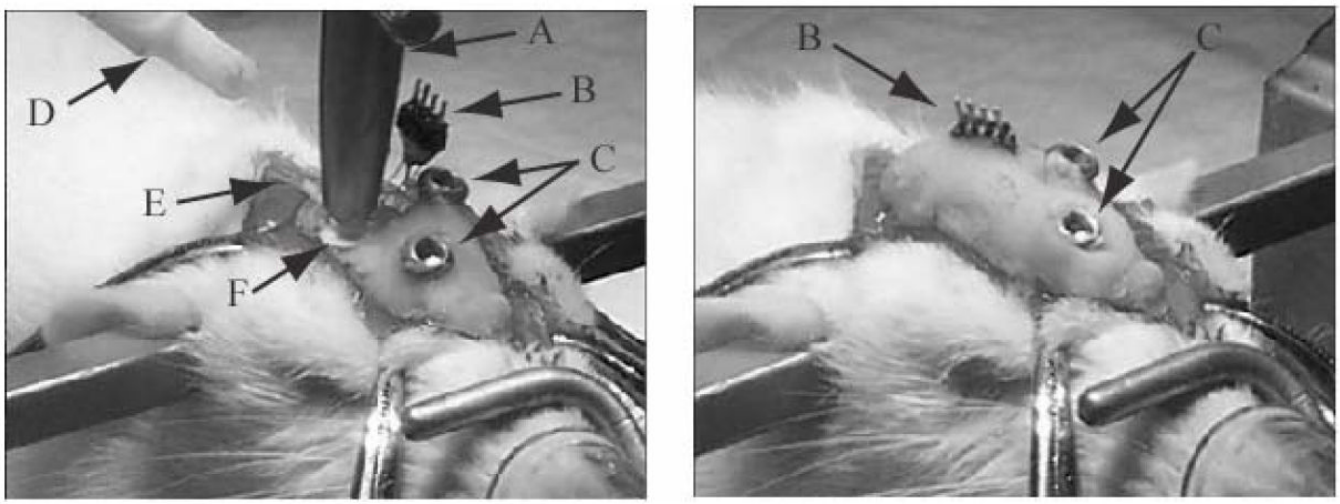

Figure 2.

The left panel shows the insertion of the cannula-multielectrode device using a stereotaxic arm after placement of the osmotic pump sub-cutaneously between the scapulae. The right panel shows the completed procedure with the electrical connector embedded in the acrylic head-cap. A – stereotaxic arm, B – Electrical Connector, C – Titanium Screws, D – Syringe Applying Acrylic, E – Tube Connecting to Osmotic Pump, F – Cannula-multielectrode device.

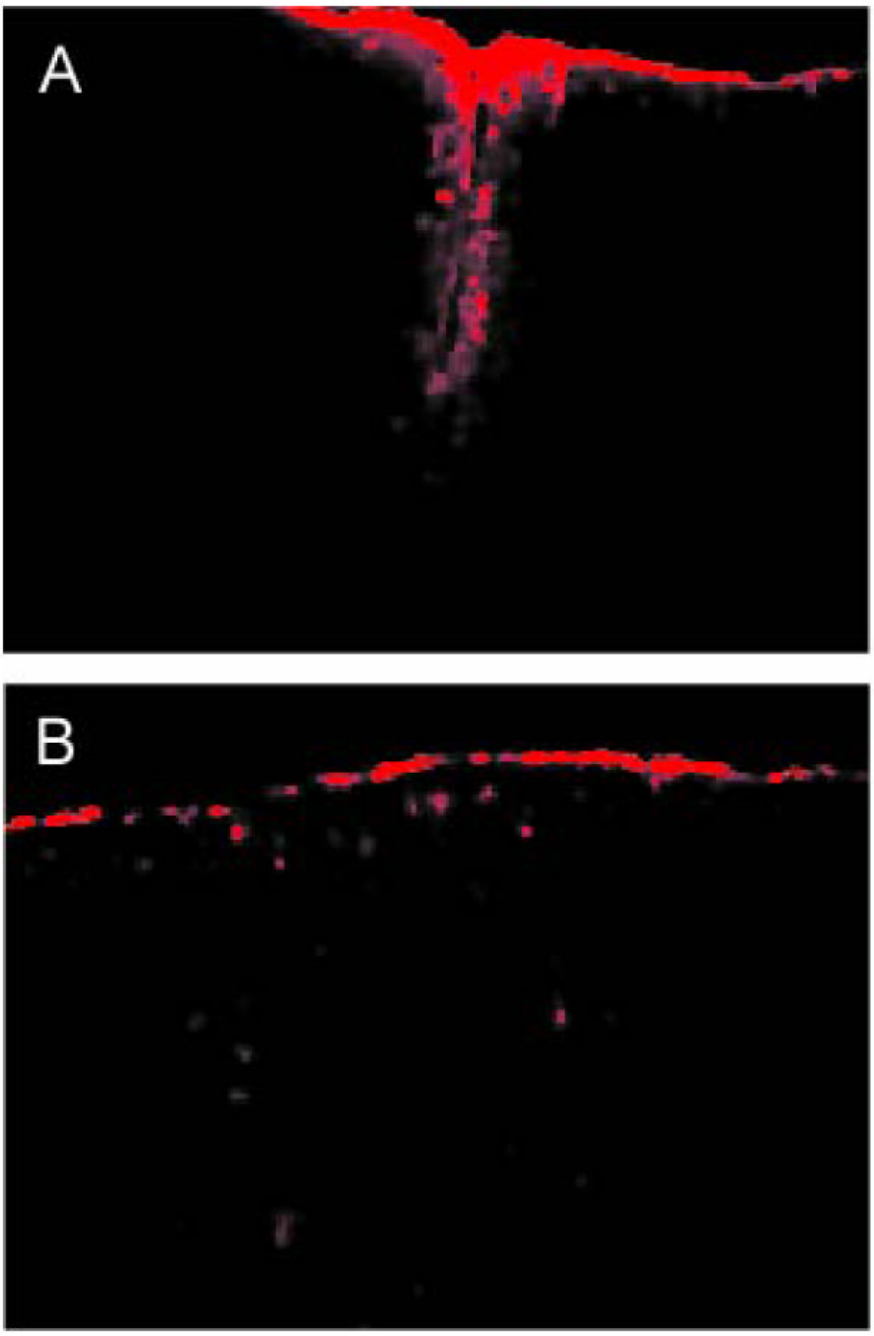

Figure 3.
Immunohistological staining for GFAP shows (A) an increased inflammatory response at the site of one of the electrodes in comparison with (B) the contralateral hemisphere were no electrodes were placed. Animal was sacrificed at 30 days post device implantation.

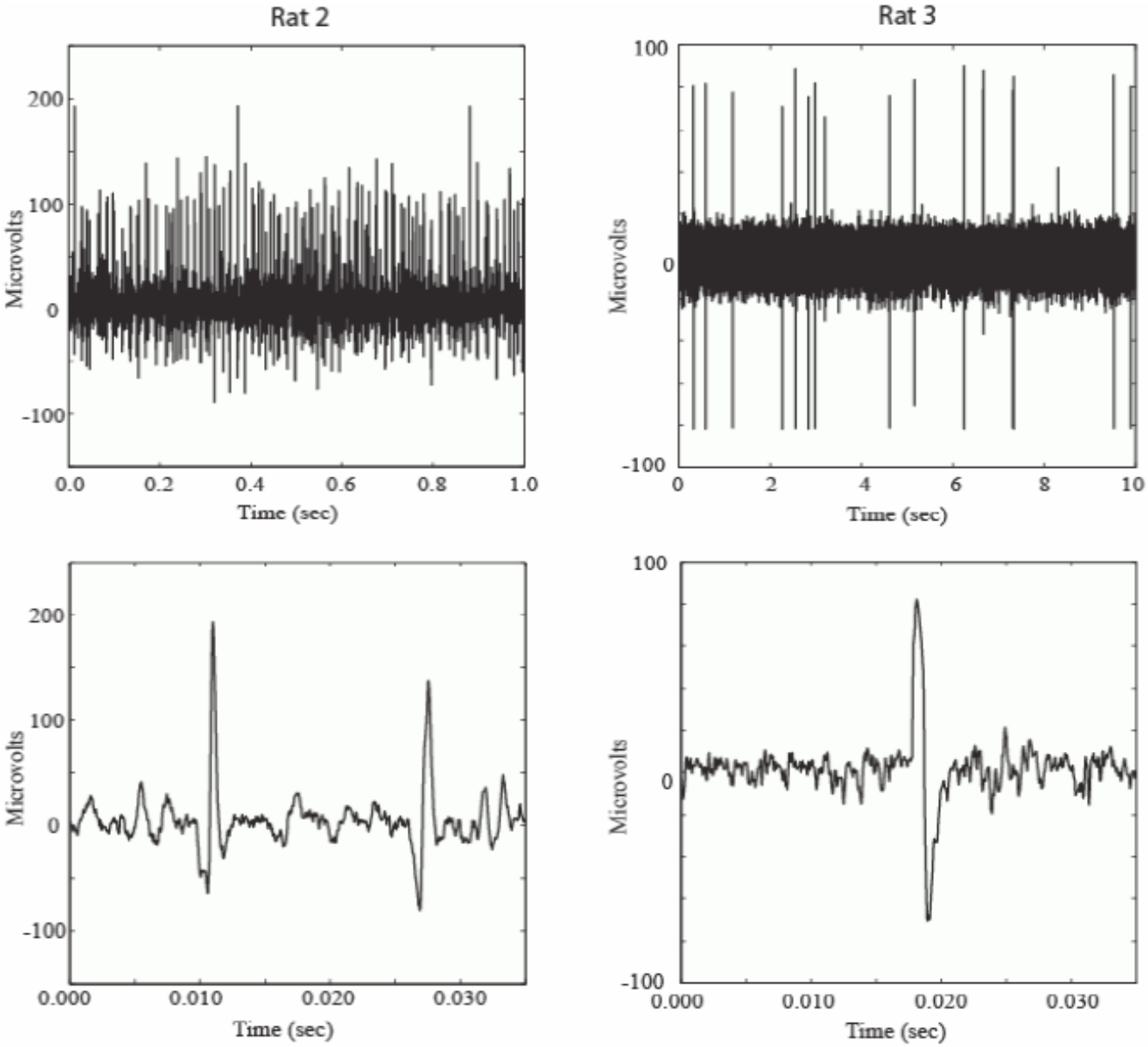

Figure 4.

Electrophysiological data collected from the cannula-electrode device from two rats (band pass filtered 300 – 10000 Hertz). The top panels show multiple spikes over the course of one second for Rat 2 and ten seconds for Rat 3. The middle panels zoom in on the temporal scale to show two single spike discharges. This data was collected at 12 days (Rat 3) and 7 months (Rat 2) post array implantation.

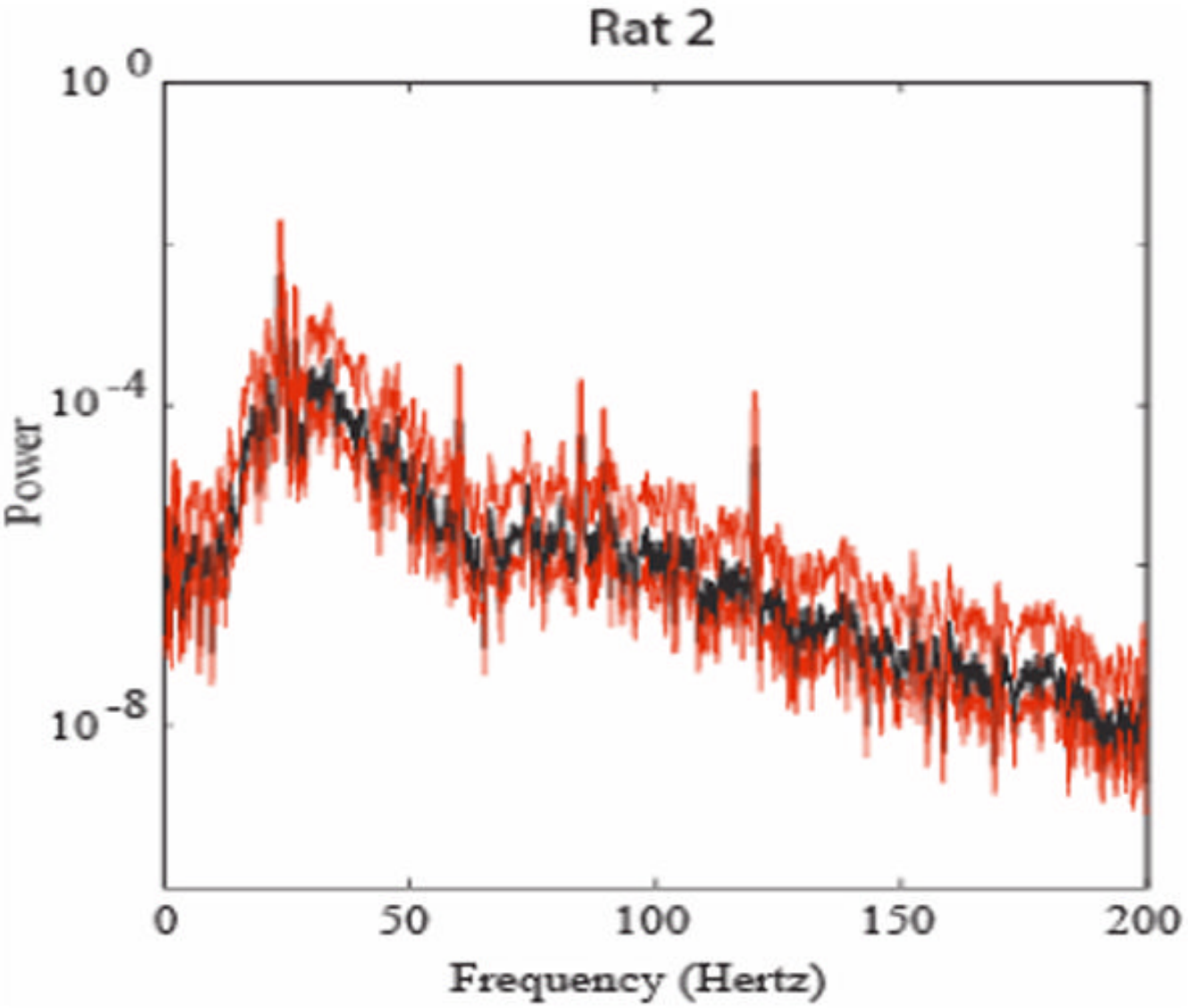

Figure 5.
Spectral analysis of electrophysiological data collected from the cannula-electrode device from one rat (wideband filtered 0.1 – 10000 Hertz). The LFP exhibits a peak in the power spectrum in the beta and low gamma frequencies (10 – 50 Hertz) typical of recordings from the cerebral cortex. The data was acquired 15 days post array implantation. Red lines are equal to one standard error.