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

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Abstract

We describe a device for accessing the effects of diffusible molecules on electrophysiological recordings from multiple neurons. This device allows for the infusion of reagents through a cannula approximate to 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. When coupled with wireless telemetry, the device can be implanted completely sub-cutaneously and provides continuous infusion of reagents and monitoring of signals in the freely behaving animal. 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). However, behavioral assessment is an indirect measurement of drug effects on neural circuitry. Recent data have shown that 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). These results suggest that a systematic and quantitative electro-physiological 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 provide chronic, i.e. several years, access to electro-physiological signals in the brain. All currently available chronically implanted electrode arrays for neural prosthetic applications lose signals over time and in some cases 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). However, low levels of certain 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 is therefore important to identify anti-inflammatory agents that can block this response and prolong the useful lifespan of the electrode arrays used in neural prosthetic applications.

The device describe here offers a simple and effective way to approach both drug development and electrode 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, can acquire both isolated neural units and local field potentials (LFPs), and is implantable sub-cutaneously when coupled with telemetry.

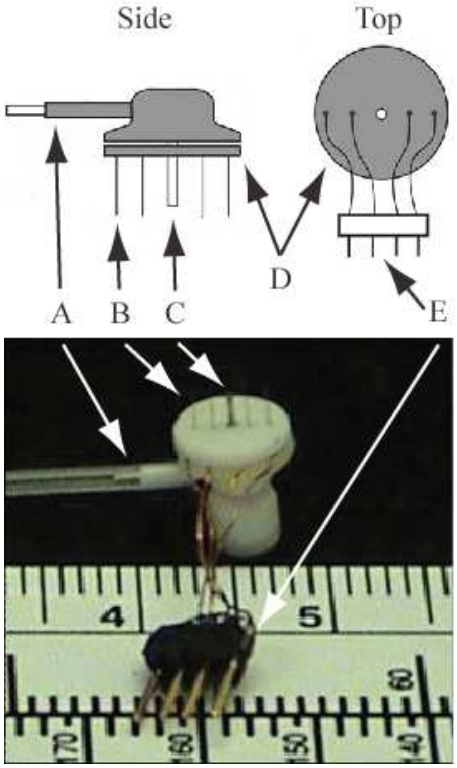

Figure 1

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 mount 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.

2. Materials and Methods

2.1 Assembly of the cannula-multielectrode

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 mounted on the body and electrodes place in proximity to the cannula and mounted on the body so that a reagent supplied by the cannula is 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.

As illustrated (Fig. 1), the device was 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 gel cyanoacrylate glue. The length of electrode above the electrode mounting disk is trimmed to approximately 1mm shaft 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 the slide into the central hole of the electrode mounting disk, until the desired length of the cannula is protruding bellow the disk, and tacked in place using the cyanoacrylate gel. 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 highly biocompatible materials platinum/iridium alloy and Paralene-C insulation. The units tested utilized 75 micron diameter electrodes sharpened to 1 - 2 microns with impedances of ~0.3 megaohms. The electrodes and cannula extended 2.5mm and 2.0mm below the electrode mounting disk. Electrode materials and construction could also be customized according to the needs for insertion into different brain structures. The electrode manufacturing and device assembly was carried out by Micro Probe Inc. (Gaithersburg, MD, USA).

Using the current version of the device, saline was infused using an osmotic minipump (Alzet). This pump uses the force generated by an osmotic gradient to slowly infuse substances over the course of several days to weeks with no intervention required. Other sources of fluids could be connected to this device in a similar fashion.

2.1 Surgical implantation

The surgical implantation of the device is performed using a minimally invasive procedure. A craniotomy 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 and island of methyl methacrylate forming a small head-cap. An osmotic pump is also implanted sub-cutaneously by blunt dissection of a sub-cutaneous space between the scapulae. The scalp is sutured around the head-cap, leaving the electrical connector exposed (Fig. 2). A skilled technician can implant the device in approximately 20 minutes from the onset of anesthesia. Alternatively, it is possible using telemetry to implant the entire device subcutaneously.

A recent study reported that cyanoacrylate gel (loctite 454) may be a more effective and easier means for cannula-electrode fixation since it does not

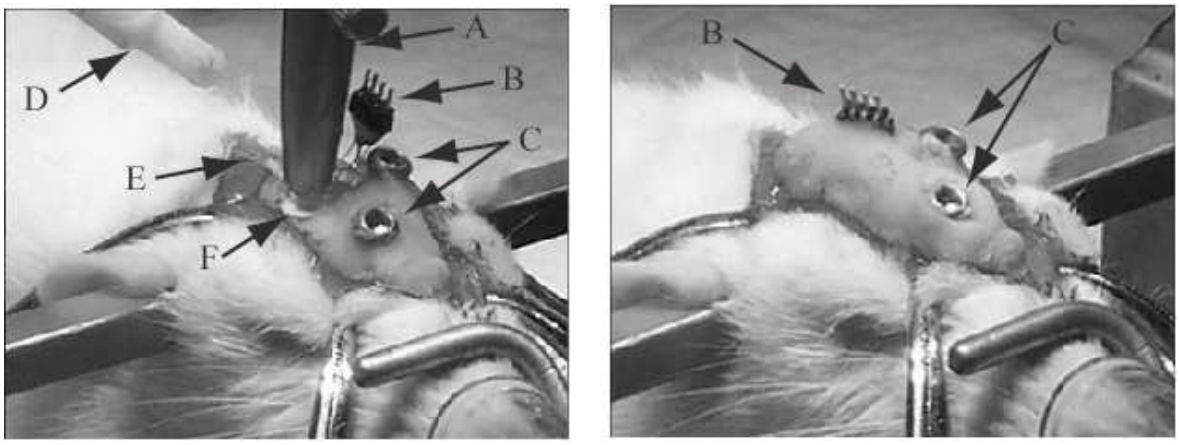

Figure 2

The left panel shows the insertions 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.

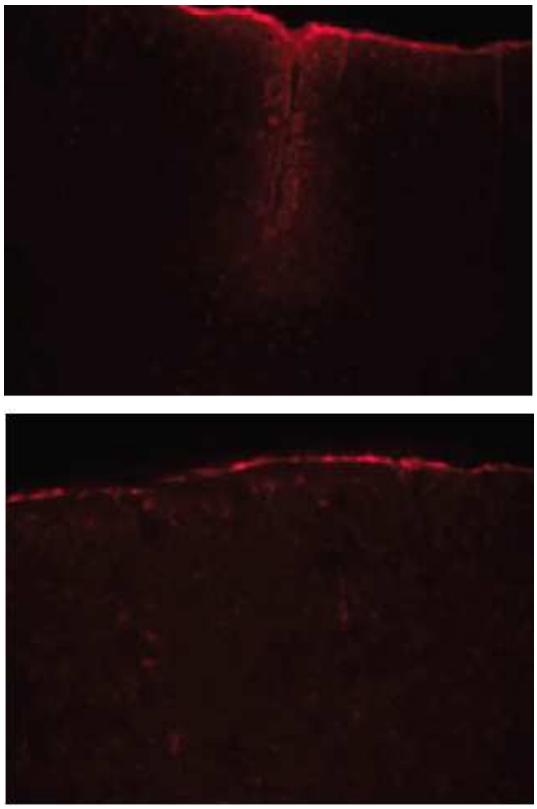

Figure 3

Immunohistological staining for GFAP shows an increased inflammatory response at the site of one of the electrodes in comparison with the contralateral hemisphere were no electrodes were placed. Animal was sacrificed at 30 day post device implantation.

require the use of skull screw for anchoring (Criado et al. 2003). Using cyanoacrylate gel to anchor the device would greatly reduce the time require for implantation.

2.1 Data acquisition and analysis

Since astrocytes are often found to form a barrier around chronically implanted electrodes, we performed immuno-histochemical staining for the astrocyte marker glial fibrillary acidic protein (GFAP) using standard methodology (Jankowsky et al. 2000). Briefly, animals were given an anesthetic overdose and transcardially perfused with 10% formalin. Brains were 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 were collected. The sections were incubated overnight at 4°C with anti-GFAP antibody diluted 1:500 in blocking solution. Sections were then incubated with a secondary antibody conjugated to a fluorescence marker for visualization.

Electrophysiological data can be acquired using any standard amplification, filtering, and analog to digital converting system. However, to acquire both isolated neural-units and LFP requires the ability to split the signal into to paths and apply different filters to each path. Alternatively, the device could be coupled with telemetry for wireless transmission of neural signals (Neihart and Harrison 2005) to a data logging system. We used a Dam-80 isolation amplifier and filter (World Precision Instruments) and a National Instruments DAQ card. Electrical signals were 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.

3. Results

We have successfully implanted this device into the frontal and parietal cortexes of several rats, and have obtained both electrophysiological and histological data from these rats. 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 the tissue around the electrode exhibits increased GFAP immunostaining when compared to the non-implanted hemisphere (Fig. 3). We also collected electrophysiological data at several time points prior to sacrificing the animals (Fig. 4). Even

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Figure 4

The top two panels display electrophysiological data collected from the cannula-electrode device (high pass filtered 300 – 10000 Hertz). The top panel shows multiple spike firings over the course of one second. The middle panel zooms in on the temporal scale to show two different single spike discharges. The bottom panel displays the power spectrum of a recording from the cannula-electrode device (wideband filtered 0.1 – 10000 Hertz). The spectrum exhibits an increase in beta and gamma band power typical of recordings from the cerebral cortex. Dashed lines are equal to one standard error.

though an increase in the inflammatory response was detected by imuno-histochemisty, we were able to collect high quality electrophysiological data. Both high frequency spike data and spectral analysis of the LFP showed robust electrophysiological activity several 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 have been proposed, but these devices 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). This device is highly configurable so that electrical recordings and reagent infusion can be targeted to specific neural structures. Here 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. However, further work is needed to determine the effects of specific reagents on neural activity using this device, and the ability of this device to record from multiple structures, e.g. the basal ganglia and cerebral cortex, simultaneously.

Recent studies have shown that electrophysiological signals from isolated neurons are affected by neuro-active drugs such as anti-depressant (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). These 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 intra-cerebral 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.

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