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Relationship between intracortical electrode design and chronic recording function

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ABSTRACT

Intracortical electrodes record neural signals directly from local populations of neurons in the brain, and conduct them to external electronics that control prosthetics. However, the relationship between electrode design, defined by shape, size and tethering; and long-term (chronic) stability of the neuron–electrode interface is poorly understood. Here, we studied the effects of various commercially available intracortical electrode designs that vary in shape (cylindrical, planar), size (15 μm, 50 μm and 75 μm), and tethering [electrode connections to connector with (tethered) and without tethering cable (untethered)] using histological, transcriptomic, and electrophysiological analyses over acute (3 day) and chronic (12 week) timepoints. Quantitative analysis of histological sections indicated that Michigan 50 μm (M50) and Michigan tethered (MT) electrodes induced significantly ($p < 0.01$) higher glial scarring, and lesser survival of neurons in regions of blood–brain barrier (BBB) breach when compared to microwire (MW) and Michigan 15 μm (M15) electrodes acutely and chronically. Gene expression analysis of the neurotoxic cytokines interleukin (*Il*)*1* (*Il1α*, *Il1β*), *Il6*, *Il17* (*Il17a, Il17b, Il17f*), and tumor necrosis factor alpha (*Tnf*) indicated that MW electrodes induced significantly ($p < 0.05$) reduced expression of these transcripts when compared to M15, M50 and FMAA electrodes chronically. Finally, electrophysiological assessment of electrode function indicated that MW electrodes performed significantly ($p < 0.05$) better than all other electrodes over a period of 12 weeks. These studies reveal that intracortical electrodes with smaller size, cylindrical shape, and without tethering cables produce significantly diminished inflammatory responses when compared to large, planar and tethered electrodes. These studies provide a platform for the rational design and assessment of chronically functional intracortical electrode implants in the future.

1. Introduction

Recent advances in the field of neural prosthetics have led to a renewed interest in the use of brain-computer interfaces (BCIs) to provide paralyzed patients with the capacity to communicate with and control external neuroprosthetics [1]. A range of non-invasive electroencephalogram (EEG) and electrocorticographic (ECoG) electrode arrays have been developed for various BCI applications [2–4]. However, the ability of invasive intracortical electrodes to communicate directly with local neuronal populations and to provide more precise control to BCI systems capable of controlling a robotic limb makes them more attractive and preferred over non-invasive electrodes.

Outside the realm of BCI related applications, chronically implanted electrode arrays have been widely used over the past several decades to measure the activity of individual neurons and populations of neurons in circuits relevant for perception and behavior, and form the crux of a wealth of basic neuroscience studies. While magnetic resonance imaging [5], optical imaging [6], EEG [7], and ECoG [8] electrode arrays are being employed for various basic neuroscience applications, chronically implanted electrodes are the gold standard for studying systems level aspects of brain function [9–13]. The chronic presence of intracortical electrodes in the brain tissue is nevertheless hypothesized to

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trigger a strong and sustained foreign body response which compromises function, ultimately leading to electrode recording failure. The widespread acceptance and development of this technology is therefore entirely dependent on the ability of implanted neural interfaces to function reliably in a chronic setting.

While signal acquisition, signal processing, and device control continue to evolve rapidly, the design of invasive intracortical electrodes has evolved little since early implantation studies in cat and primate brains by Clark and Ward [14], and Delgado [15]. Modern intracortical electrodes of different designs are largely based on three major design formats: a) cylindrical insulated metal electrodes with exposed recording site tips [16]; b) planar silicon multi-recording site microelectrodes [17]; and c) needle shaped conductive silicon based multi-electrode arrays [18], that are connected to a headstage via a tethering cable and pneumatically inserted into the cerebral cortex [19].

A number of studies have reported the benefits of small implant size [20,21], implant flexibility [22–29] and the use of neuro-integrative and anti-inflammatory bioactive coatings [30–33]. While these strategies lend themselves to increasing neuronal viability and ameliorating reactive gliosis around intracortical electrode implants with varying degrees of success, the ultimate goal of facilitating chronic electrode function nevertheless still remains elusive, as most studies have used non-functional electrodes. Therefore, a comprehensive study addressing the effects of electrode design on tissue response, and the temporal expression of neurotoxic and proinflammatory cytokines is germane to informing electrode design, and facilitating chronic recordings from indwelling electrodes.

In this study, we investigated the role of intracortical electrode design on the triggering of brain tissue foreign body responses, and expression of pro-inflammatory and neurotoxic cytokines over acute (3 day) and chronic (12 week) timepoints. Using multiple unique analysis measures, we interrogated the relationship between the chronic expression of neurotoxic pro-inflammatory cytokines and chronic electrode recording failure.

2. Materials and methods

2.1. Intracortical electrodes

A range of commercially available intracortical electrodes covering the current design space of size and shape were acquired from different vendors and classified into tethered and untethered groups (Fig.1 & Table 1). Further classifications of size and shape were made within each major group. In the untethered group, Michigan electrodes [M15 (15 μm thickness), Fig. 1A; and M50 (50 μm thickness), Fig. 1B; silicon non-functional and functional CM32 probes] were obtained from NeuroNexus (Ann Arbor, MI); and Microwire Arrays (MW; tungsten w/30° tip angle, 50 μm diameter functional probes; Fig. 1C) were obtained from Tucker-Davis Technologies (Alachua, FL). In the tethered group, Michigan tethered [(MT; non-functional silicon H-series probe, 15 μm thickness with tethering cable), Fig. 1D] were obtained from NeuroNexus (Ann Arbor, MI) and Floating Microwire Arrays [FMAA (Pt/Ir fast tapered, 75 μm diameter with tethering cable) and FMAB (Pt/Ir slow tapered, 75 μm diameter with tethering cable) non-functional and functional probes; Fig. 1E] were obtained from MicroProbes for Life Sciences (Gaithersburg, MD); and Utah Arrays (UA; Silicon, 6 × 6 functional arrays with tethering cable; Fig. 1F) used as the clinical gold standard for electrophysiological comparison were obtained from Blackrock Microsystems (Salt Lake City, UT).

2.2. Animal surgeries

All animal procedures were approved by the Institutional Animal Care and Use Committee at the Georgia Institute of Technology. Different cohorts of animals were implanted with intracortical electrodes of various designs for acute and chronic histological, cytokine and electrophysiological analyses (Fig. 2), amounting to a total of 124 animals across all groups. Each of these procedures was conducted using methods previously described [34]. Briefly, each adult male rat was anesthetized using 2% isofluorane and its head depilated. The animal was subsequently transferred to a stereotaxic frame (David Kopf Instruments, CA) fitted with temperature regulation, heart-rate and respiration monitoring systems, and isofluorane delivery systems. Lidocaine was injected sub-dermally as a local anesthetic, following which a single midline incision was made. A craniotomy was made ~1.5 mm posterior from anterior bregma and ~4 mm lateral from the midline, after retracting the skin flaps and cleaning the skull surface with 3% hydrogen peroxide. Uniform insertion techniques were used for implantation of untethered (M15, M50, and MW) and tethered (MT, FMAA, and FMAB) electrodes into the rat barrel cortex using a hydraulic micropositioner (David Kopf Instruments, CA) and vacuum inserter tool (for FMA electrodes). Untethered electrodes M15, M50 and MW were implanted stereotaxically to depths of ~1200 μm (M15 & M50) and ~800 μm (MW); and tethered electrodes MT, FMAA and FMAB were implanted to depths of ~1200 μm (MT) and ~800 μm (FMAA & FMAB) in the rat barrel cortex respectively following the removal of the dura-mater and placement of bone screws. The tethered Utah arrays were stereotaxically implanted using a pneumatically-actuated array inserter without retracting the dura-mater as recommended by the manufacturer (Blackrock Microsystems, UT). In the case of all tethered electrodes, the tethering cable was immediately secured to a bone screw with dental cement following electrode implantation to avoid electrode displacement. The craniotomy was covered with sterile 2% SeaKem agarose (Lonza, NJ) and sealed with UV curing dental cement which was also used to build the head cap (Fig. 1G). The hemostats were subsequently removed and the skin flap sutured back. The animal was injected with buprenorphine HCl and allowed to recover before returning it to the cage. Animals assigned for acute and chronic histological analysis were additionally subjected to a stereotaxically inflicted stab wound on the hemisphere contralateral to the electrode implant, and had non-functional electrodes implanted.

2.3. Tissue preparation and immunohistochemistry

Animals were anesthetized using ketamine (50 mg/kg)/xylazine (10 mg/kg)/acepromazine (1.67 mg/kg) 3 days and 12 weeks post electrode implantation, and transcardially perfused with phosphate buffered saline (PBS), followed by 30% sucrose in PBS, followed by 4% paraformaldehyde. The brains with implanted electrodes were post-fixed in 4% paraformaldehyde, following which electrodes were carefully extracted and brain tissue ($n = 6$/electrode type) prepared for immunohistochemical analysis as described previously [34]. For laser capture microdissection (LCM) studies, animals implanted with intracortical electrodes for 3 days and 12 weeks were anesthetized as above and transcardially perfused with PBS followed by 30% sucrose in PBS. The electrodes were carefully extracted and the brains were snap frozen and stored at −80 °C. Brain tissue was sectioned at a thickness of 15 μm using a cryostat, and serial sections were collected on polyethylene naphthalate (PEN) membrane slides (Life Technologies, NY) for LCM, and on charged glass slides (VWR, PA) for immunohistochemical analysis. Serial sections were subsequently blocked with blocking buffer (PBS containing 4% goat serum and 0.5% Triton-X100) for 1 h and incubated overnight in blocking buffer containing groups of antibodies (Table 2). Following primary antibody incubation, the sections were washed thrice with PBS, blocked for 1 h in blocking buffer containing 1:220 dilutions of appropriate secondary antibodies (Table 2). After 1 h, sections were washed several times with PBS and stained for 15 min with 4′,6-diamidino-2-phenylindole (DAPI) nuclear stain (Life Technologies, NY). Tissue sections were subsequently washed thrice with PBS and coverslipped with Fluormount-G (Southern Biotech, AL). Sections were stored at −20 °C until imaged. Tissue sections were imaged on a Zeiss Axiovert 200 M (Carl Zeiss, NY) using equal exposure times for all fluorescent markers across all timepoints.

2.4. Laser-capture microdissection and qRT-PCR

LCM of brain tissue of interest was conducted as described previously [34]. Briefly, brain tissue sections were placed on a cold block and fixed for 2 min in ice-cold 75% ethanol, followed by three rinses with ice cold nuclease free PBS (Life Technologies, NY). Sections were then stained for 1 h with 1:220 dilution of goat anti-rat IgG + 500 U RNase inhibitor (Life Technologies, NY). The sections were rinsed thrice with nuclease free PBS and subsequently serially dehydrated with 75%, 95% and 100% ethanol for 15 s each. Sections were allowed to air dry for 5 min on the ice block before LCM using an Arcturus XT Laser Capture Microdissection System (Life Technologies, NY).

Tissue sections from multiple 3 day and 12 week and naïve ($n = 4$/group) animals were laser capture microdissected, and total RNA extracted for qRT-PCR analysis of cytokine gene expression using PCR arrays (Qiagen, CA) as described previously [34]. Gene expression data was analyzed using the ΔΔCt method, and a fold difference of >3 or −3 fold in quantities of cytokine mRNA expression in electrode implanted animals when compared to naive uninjured animals was considered biologically significant. Gene expression data was also normalized against the average expression of five endogenous controls.

2.5. Electrophysiology

Sensory evoked extracellular (single and multi-unit) recordings from the rat barrel cortex obtained during vibrissal deflections were amplified and collected using a 32–channel data–acquisition system (Plexon Inc., TX). Neuronal signals were amplified, band–pass filtered (500–5 kHz), and digitized at 40 kHz/channel. Recordings were analyzed using the OfflineSorter software suite (Plexon Inc., TX) to assign the recorded spike waveforms to single–units on the basis of standard template matching techniques and physiologically plausible refractory periods. All