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MRI compatible optrodes for simultaneous LFP and optogenetic fMRI investigation of seizure-like afterdischarges

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ABSTRACT

In preclinical studies, implanted electrodes can cause severe degradation of MRI images and hence are seldom used for chronic studies employing functional magnetic resonance imaging. In this study, we developed carbon fiber optrodes (optical fiber and electrode hybrid devices), which can be utilised in chronic longitudinal studies aiming to take advantage of emerging optogenetic technologies, and compared them with the more widely used tungsten optrodes. We find that optrodes constructed using small diameter (~130 μm) carbon fiber electrodes cause significantly reduced artifact on functional MRI images compared to those made with 50 μm diameter tungsten wire and at the same time the carbon electrodes have lower impedance, which leads to higher quality LFP recordings. In order to validate this approach, we use these devices to study optogenetically-induced seizure-like afterdischarges in rats sedated with dexmedetomidine and compare these to sub (seizure) threshold stimulations in the same animals. The results indicate that seizure-like afterdischarges involve several extrahippocampal brain regions that are not recruited by subthreshold optogenetic stimulation of the hippocampus at 20 Hz. Subthreshold stimulation led to activation of the entire ipsilateral hippocampus and septum, whereas afterdischarges additionally produced activations in the contralateral hippocampal formation, neocortex, cerebellum, nucleus accumbens, and thalamus. Although we demonstrate just one application, given the ease of fabrication, we anticipate that carbon fiber optrodes could be utilised in a variety of studies that could benefit from longitudinal optogenetic functional magnetic resonance imaging.

Introduction

Optogenetic functional magnetic resonance imaging (ofMRI) is a powerful new technique based on combining optogenetics with functional magnetic resonance imaging (fMRI) (Desai et al., 2011; Lee, 2011, 2012; Lee et al., 2010; Weitz and Lee, 2013). Optogenetics allows temporally precise and cell-type specific modulation of neural activity, while fMRI allows us to visualize this at the whole-brain level. ofMRI is likely to play an important role in dissecting functional networks. However, it relies on measuring hemodynamic changes, in particular the blood oxygenation level-dependent (BOLD) signal, which is a surrogate measure of changes in neural activity. In order to fully take advantage of this technique, simultaneous electrophysiological recordings will be highly beneficial. Most studies using fMRI in animal models do not employ other measures of neural activity, e.g. electroencephalography (EEG), local field potentials (LFPs), multi-unit recordings or single-unit recordings. This is primarily because implanted electrodes and connectors can cause severe degradation of magnetic resonance images due to differences in magnetic susceptibility, which in turn leads to static field inhomogeneity and susceptibility artifacts.

Many studies have attempted to reduce the artifacts associated with electrodes for electrophysiological recordings in small animals. Successful attempts at MRI compatible recordings include the use of carbon fiber (CF) electrodes placed on the skull or surface of the brain (Austin et al., 2003; David et al., 2008; Mirsattari et al., 2005; Nersesyan et al., 2004; Opdam et al., 2002), calomel electrodes anchored to the skull (Brinker et al., 1999), platinum wire electrodes covering the scalp (Sumiyoshi et al., 2011), saline-filled (Canals et al., 2009; Moreno et al., 2015) or carbon fiber-threaded (Moreno et al., 2015; Shyu et al., 2004) glass micropipettes inserted into the brain. Many of these designs are only suited to recording in head-fixed animals and are therefore not suitable for chronic optogenetics studies. There have been far fewer reports on the use of high-field MRI compatible depth electrodes for long-term LFP recording and/or stimulation. Recently, Dunn et al. demonstrated that this is achievable without causing significant artifacts by coating carbon fiber bundles in polyvinylidene fluoride (PVDF) for insulation and rigidity (Dunn et al., 2009). Further studies have shown

Abbreviations: ofMRI, optogenetic functional magnetic resonance imaging; CF, carbon fiber; ChR2, channelrhodpsin-2.

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that susceptibility artifacts caused by chronically implanted ultra-fine (36–50 μm) tungsten electrodes can be tolerable, even in highly T2* weighted images that are particularly sensitive to magnetic field inhomogeneity (Chao et al., 2014; Huttunen et al., 2008; Lai et al., 2015). Alternatively, to minimize susceptibility effects from implanted electrodes, some researchers insert electrodes at a less than 90° angle from the rostral-caudal plane, although this requires a more skillful surgical procedure (Englot et al., 2008). Despite the multitude of studies using implanted electrodes, there has yet to be a systematic comparison between different implantable electrodes for long-term recording or combined stimulation including optogenetics.

In this study, we investigate different optical fiber and electrode hybrid devices (commonly known as optrodes) (Gradinaru et al., 2007) for performing both optical stimulation and LFP recording within the MRI environment. These devices find application in both optogenetic functional magnetic resonance imaging (ofMRI) and in experimental studies employing optogenetics and long-term electrophysiological recordings where MRI compatibility is desired. Here, we compare different optrode designs and show that those constructed using small-diameter (~130 μm) carbon fiber electrodes are well suited for the task as they have low impedance and also cause minimal susceptibility artifacts in functional MRI images. In order to validate this approach, we use these devices in combination with simultaneous LFP-ofMRI to study optogenetically-induced seizure-like afterdischarges. Our previous work based on ofMRI demonstrated that optogenetic stimulation of the hippocampus revealed the subregion and frequency dependent nature of hippocampal networks (Weitz et al., 2014) and Osawa et al. have used optogenetics and LFP recordings to study afterdischarge dynamics *in vivo* (Osawa et al., 2013). In this work, we aim to extend these approaches to encompass simultaneous fMRI and extracellular field recordings in for the purpose of studying optogenetically-induced seizure-like afterdischarges at the whole brain level. Lastly, we believe that the simplicity of the design and fabrication of these devices will allow them to be exploited in a variety of different neuroscience studies.

Materials and methods

The aim of this study was first, to fabricate and compare different devices for optogenetic stimulation and electrical recording in the MRI environment and second to employ these optrodes to study optogenetically-induced seizure-like afterdischarges using fMRI. In this section, we first consider the design and fabrication of these optrodes and subsequently go on to outline the *in vivo* testing and optogenetics experiments.

Implantable optical fiber

For optogenetic stimulation, light may be delivered through an optical fiber that is surgically implanted into the desired brain region (Sparta et al., 2012). We constructed optical fibers for implantation using a 105 μm core diameter multimode optical fiber (FG105LCA, Thor Labs) inserted and secured into 1.25 mm diameter ceramic stick ferrules (Thor Labs, Newton, NJ). The ferrules have a convex end, which connects to the light source, and a concave end, which is ultimately directed towards the brain. First, the optical fibers were stripped of their plastic coating and cleaved to the desired length (11 mm) using a high-precision fiber cleaver (Fujikura, CT-05, Tokyo, Japan) in order to maximize the reproducibility of light delivery to the brain. The cleaved optical fiber was examined to ensure that the ends were flat and cleanly cut (Fig. 1a). Next, the section of fiber was then inserted into the ferrule through its concave end until the fiber was level with the convex face of the ferrule. Epoxy adhesive was applied on the concave side of the ferrule to secure the optical fiber in place (Fig. 1b). The completed optical fiber implant was inspected using a light microscope to ensure that the fiber was not damaged and was free of debris. Examining the fiber along the optical axis, it was ensured that the fiber core remained intact and that light could pass unobstructed through the optical fiber (Fig. 1c). Finally, light transmission was tested using an optical power meter (Newport Corp, CA) to ensure that it was greater than 80%.

Carbon fiber optrode design and fabrication

Optrodes used in optogenetics studies are typically comprised of materials that can create artifacts during MR imaging. The carbon fiber design discussed here is an attempt to reduce these artifacts by replacing components with large magnetic susceptibilities. The electrode design discussed here has been adapted from the carbon fiber-based MR-compatible electrodes described by Dunn et al. (Dunn et al., 2009). There are several key differences in our design. First, the previous carbon electrode design employed a brass screw as an electrical contact to the carbon fiber bundle. We found that this metallic screw caused artifacts in spiral readout fMRI images, which distorted the image in the cortex above the electrode and we therefore replaced it by a single wire to alleviate this problem. Second, we found that the silver print used by the aforementioned study to affix the carbon fiber bundle, was too fragile and difficult to handle. Therefore, to improve the strength and ease of fabrication of the electrode we used silver epoxy as a conducting adhesive. Lastly, the 400 μm diameter electrodes designed by Dunn et al. caused too much brain injury and were not suitable for our chronic studies. For this reason, we decided to explore a range of electrode diameters.

Individual carbon fiber electrodes were constructed out of 20–30 mm sections of 1K carbon tow (CST Composites, Tehachapi, California, USA). 1K tow consists of approximately 1000 carbon filaments per tow (bundle). In order to produce electrodes with different diameters, the 1K tow was split in half once to produce 0.5K bundles and twice to produce 0.25K bundles of carbon fiber. (Fig. 1d). Individual carbon fiber bundles were cold soldered to a 10 mm section of stripped 30 AWG wrapping wire using conductive silver epoxy (MG Chemicals, Ontario, Canada). The epoxy was allowed to cure for at least 24 hours and following this the carbon fiber bundles were coated in a solution of the thermoplastic PVDF diluted with methyl isobutyl ketone at a 2:1 ratio. The bundles were dipped into PVDF, baked at 200 °C for 20 minutes and cooled at room temperature for 20 minutes. This process was repeated 3 times to ensure ample coating and insulation of the carbon fiber bundle (Fig. 1e).

Carbon fiber electrodes were cut using surgical scissors to expose the contact point and were then fastened to the implantable fiber optic ferrules using epoxy adhesive, ensuring that they remained parallel and that the ends of the fiber optic and carbon fiber electrode met at the same point (Fig. 1f). This ensured that the LFP recording takes place at the site of optical stimulation. The wire attached to the carbon fiber electrodes was then soldered to a 3–4 cm section of wire attached to a press fit connector (part number: H3909-ND, Digi-Key, MN). A brass screw was soldered to the connector via a 30 AWG wire to serve as a cerebellar reference electrode (Fig. 1g). Finally, the optrode was implanted into male adult rats for *in vivo* testing, as described below (Fig. 1h).

Tungsten optrode fabrication

Tungsten optrodes were constructed using a method similar to that described by Armstrong et al. (Armstrong et al., 2013). Briefly, 50 μm perfluoroalkoxy alkane (PFA) insulated tungsten wire (A-M systems, WA) was attached to the implantable fiber optic (described above) using fine thread and epoxy adhesive. The tungsten microwire was cut so that the end of the electrode was in line with the end of the fiber. The other end of the microwire was soldered to the same connector used for the carbon fiber optrodes (Fig. 1g) and similarly a brass screw was used as a reference electrode.