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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 those made with 50 μm diameter tungsten wire and at the same time the carbon electrodes have lower impedance, which leads to higher quality intracranial 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, whereas afterdischarges additionally produced activations in the contralateral hippocampal formation, septum, 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.

Keywords

fMRI; electrode; optogenetics; optrode; carbon fiber; epilepsy; hippocampus; ofMRI

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1 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 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., 2014). 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.

2 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.

2.1 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%.

2.2 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 different 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).

2.3 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.

2.4 Impedance measurements

The contact impedance of the electrodes was tested in 0.9% NaCl in distilled water. Briefly, a function generator (33210a, Agilent Technologies, Palo Alto, CA) was used to produce a constant voltage (1V peak-to-peak amplitude, 100 Hz) sine wave. The output terminal of the function generator was connected to a true root mean squared (RMS) digital multimeter (Fluke 87V, Fluke Corporation, WA) to measure the current flowing through the circuit. The other terminal of the ammeter was connected to a AgCl reference electrode (immersed in saline solution). The (carbon fiber or tungsten) working electrode was immersed in the saline solution to complete the circuit. The contact impedance magnitude of the electrode was calculated as the ratio of the RMS voltage across the test electrode (measured using an oscilloscope) and the RMS current flowing through the circuit.

2.5 MRI phantom construction, imaging and analysis

To compare the imaging artifacts induced by each electrode, a phantom was constructed in which tungsten (50 μm) and carbon fiber electrodes (approximate number of fibers: 1 K, 0.5K and 0.25K) were embedded in 2% agarose (G-Biosciences, St. Louis, MO) within a 50 ml Falcon tube. All magnetic resonance imaging was carried out at Stanford University using a 7T MR901 horizontal bore scanner (Agilent Technologies, Palo Alto, CA) and a shielded gradient system (600 mT/m, 6000 T/m/s). A two-channel 30 mm diameter Millipede transmit/receive volume coil (Agilent Technologies) was used for data acquisition. The phantom was oriented such that the electrodes lay perpendicular to the main field. A fast spin echo (FSE) sequence was used to assess the artifacts caused by each electrode. The sequence parameters were as follows: TR = 5 s, Echo train length (ETL) = 8, effective TE (TE_{eff}) = 42 ms, matrix = 384×384 , field-of-view (FOV) = 35×35 mm, slice thickness = 0.5 mm, number of slices = 23, number of averages = 2. The acquired data was first smoothed using a Gaussian kernel with a full-width at half-maximum of 200 μm to reduce the effects of high-frequency noise. Following this, 1-dimensional signal intensity profiles were generated by taking the row of voxels at the center of the hypointense region and averaging across 7 consecutive slices, as these slices each contained all 4 of the electrodes. The center voxel for each electrode was taken to be the voxel located within the hypointense region that had the lowest signal intensity.

2.6 Virus injection and optrode implantation

Adult male Sprague-Dawley rats ($n = 15$ in total; 300–620 g; Charles River Laboratories, Wilmington, MA) were used for *in vivo* experiments. Animals were housed under a 12-hour light-dark cycle and provided with food and water *ad libitum*. Animal husbandry and experimental manipulation were in strict accordance with the National Institutes of Health (NIH) and Stanford University's Institutional Animal Care and Use Committee (IACUC) guidelines.

Optogenetics (Boyden et al., 2005; Zemelman et al., 2002) is a technique that utilizes opsins (light-sensitive proteins) to achieve millisecond-precise cell-type specific manipulation of neural activity *in vivo*. Here, we used channelrhodopsin2 (ChR2)-EYFP (enhanced yellow fluorescent protein) fusion protein viral expression system in order to express ChR2, a light-sensitive cation-selective channel (Nagel et al., 2003) under control of the Ca^{2+} /calmodulin-

dependent protein kinase II α (CaMKII) promoter (expressed primarily in excitatory neurons). The viral plasmid was constructed by cloning CaMKII α -Chr2(H134R)-EYFP into an adeno-associated virus (AAV) backbone using MluI and EcoRI restriction sites (map available online at www.optogenetics.org). The recombinant AAV vector was serotyped with AAV5 coat proteins and packaged by the University of North Carolina viral vector core (titer of $\sim 4 \times 10^{12}$ vg/ml). This viral construct was administered to the right-side of the hippocampus as described below.

During surgery, rats were anesthetized with isoflurane (induction 5%, maintenance 2–3%; Sigma-Aldrich, St. Louis, Missouri, USA) and secured in a stereotactic frame. Following a midline incision, a small craniotomy and viral injection/optrode implantation were performed at the intermediate hippocampus (5.8 mm posterior to Bregma, 5.2 mm from the midline, and 3.1 mm from the dura). 2 μ l of virus (n=11) or saline in controls (n=4) was delivered through a 34-gauge needle (World Precision Instruments Inc., FL) attached to a 10 μ l Hamilton syringe at a rate of 150 nl/min. The syringe needle was left in place for 5 minutes before being slowly withdrawn.

The optrode was slowly inserted through an opening in the dura mater at the aforementioned coordinates, leaving 1–2 mm of uncoated fiber protruding from the brain. After cleaning the surface of the skull with hydrogen peroxide the optrode was secured to the skull using light-curable dental cement (Clearfil Liner Bond 2V, Kuraray America, Inc. NY). Just enough dental cement was added to cover the uncoated fiber as well as approximately half of the ferrule. As a result, the ferrule was held securely while at the same time there was enough space to connect the ferrule to the fiber optic patch cable. In earlier experiments, we found that in the case of inadequate dental cement securing the ferrule, there was a risk of the ferrule becoming detached from the dental cement. A brass screw was used as a support for the dental cement and also used as the reference electrode. This screw was fixed to the skull above the cerebellum at approximately 10 mm posterior to Bregma and 3 mm from the midline, and finally the connector, which was used to connect the electrodes to the lead wires, was mounted on the skull and secured with dental cement. Buprenorphine was injected subcutaneously pre and postoperatively to minimize discomfort induced by the surgical procedure. In order to allow time for viral-mediated Chr2 expression, all optogenetics experiments were performed at least 6 weeks following virus injections.

2.7 In vivo assessment of MRI artifacts and data analysis

In order to investigate the artifacts caused by 3 of the electrode designs (tungsten (n=5), 1K CF (n=4) and 0.25K CF (n=4)), implanted rats were anesthetized with 4 % isoflurane and maintained at 2 % throughout the duration of the experiment. Structural imaging was carried out using a FSE sequence with the following parameters: TR = 5 s, TE_{eff} = 42 ms, ETL = 8, FOV = 30 $\times$ 30 mm, slice thickness = 0.75 mm, number of slices = 30, matrix = 256 $\times$ 256, in-plane resolution = 117 $\times$ 117 μ m². Spoiled gradient echo (SPGR) with rectilinear sampling was also used to compare tungsten to 0.25K CF electrodes using the following sequence parameters: TR = 0.6 s, TE = 10 ms, FOV = 30 $\times$ 30 mm², matrix = 128 $\times$ 128, in-plane resolution = 234 $\times$ 234 μ m². Assessment of artifacts on fMRI scans was carried out using the functional imaging sequence described below. Following data acquisition, all of

the reconstructed images were smoothed for noise reduction with a low bandwidth Gaussian kernel of 0.2 mm at FWHM to ensure the artifacts were not oversmoothed. In order to assess the artifact over a number of subjects, 1D signal intensity profiles were generated from one fMRI image frame in each subject. This was achieved by selecting the row of voxels in the middle of the hypointense region and normalizing this row of voxels to the average local signal intensity, where the local signal intensity was taken to be those voxels located between 4–8 voxels from the center voxel.

2.8 optogenetic fMRI experiments

Out of 13 rats used in the previous experiment, in 3 rats with 1K carbon fiber electrodes implanted, the ferrule holding the fiber optic was not held tightly enough by the dental cement and consequently became loose upon connecting the fiber optic patch cable. These rats were therefore excluded from this part of the study along with 2 rats implanted with tungsten optrodes and 4 control rats without virus injection. 4 of the rats previously injected with virus and implanted with CF optrodes, which were used for the experiments assessing MRI artifacts, were used for the ofMRI experiments. An additional 2 rats were also implanted with 0.25K CF electrodes for this part of the study (in total: 0.25K, n=5 and 1K, n=1). Previously, we showed that seizure-like activity can be elicited in rats anesthetized using a mixture of nitrous oxide and isoflurane (Weitz et al., 2014). However, isoflurane can have significant anti-convulsant properties (Kofke et al., 1989). Therefore, in this experiment, we explored sedating the rats using a different anesthetic, dexmedetomidine hydrochloride, as it is known that rats have a higher propensity for seizures under dexmedetomidine sedation (Airaksinen et al., 2010; Choy et al., 2010; Fukuda et al., 2013; Mirski et al., 1994). Briefly, this regimen entailed initially anaesthetizing the animal using 4% isoflurane, followed by a 0.05 mg/kg bolus subcutaneous (s.c.) injection of dexmedetomidine (Dexdomitor, Pfizer, NY), followed by a continuous intravenous (i.v.) infusion of dexmedetomidine at a rate of 0.1 mg/kg/h via a 24G catheter inserted into the lateral tail vein. After the initial induction, isoflurane concentration was gradually reduced to zero over a 10 min period. Rats were allowed to breathe room air spontaneously throughout the imaging sessions.

A custom-designed transmit/receive single-loop surface coil (inner diameter = 22 mm, outer diameter = 40 mm) was used for data acquisition. The coil was placed around the connector and ferrule, as close as possible to the rat's head. Functional imaging was implemented using a multi-slice Gradient Recalled Echo (GRE) sequence with a four-interleave spiral readout (Glover and Lai, 1998) using the following parameters: TR = 750 ms, TE = 12 ms, FOV = 30 × 30 mm, number of slices = 23, slice thickness = 0.75 mm, inplane resolution = 0.43 × 0.43 mm, number of frames = 130. Images were reconstructed using 2-dimensional gridding (Fang and Lee, 2013; Jackson et al., 1991) and a sliding window reconstruction (Nayak et al., 2004; Riederer et al., 1988). Following image reconstruction, image realignment was achieved using our previously reported method (Fang and Lee, 2013).

2.9 ofMRI stimulation paradigms

In this study we employed a 473 nm diode-pumped solid-state laser (Laserglow technologies, Toronto, Canada) for optogenetic stimulation and two different stimulation

paradigms. The first paradigm was a subthreshold paradigm designed not to induce seizure-like activity. For this, we used a 6-cycle block design with a period of 60 s (20 s on, 40 s rest) preceded by a 30 s baseline. The stimulation parameters consisted of a 20 Hz pulse train with a 15 ms pulse duration. The light intensity per laser pulse entering the brain for each rat was set at a level, which was below the threshold for inducing afterdischarges (74–185 mW/mm²). Light intensity entering the brain was estimated by assuming 80% of the light exiting the fiber patch cable was transmitted to the brain. 80% was used as a conservative estimate because upon testing before implantation, all ferrules transmitted at least 80% of the input light from the fiber optic cable. A second paradigm was used to investigate seizure-like afterdischarges. For this, the afterdischarge threshold was found by increasing the light intensity in steps of 92 mW/mm², until an afterdischarge resulted from a 20 s stimulation. At the light-intensity required for inducing afterdischarges, the stimulation paradigm consisted of a 30 s baseline followed by a single 20 s stimulation (20 Hz, 15 ms pulse width, light intensity = 92–555 mW/mm² at the fiber tip). A single-stimulus response measurement enables monitoring of seizure progression at different stages throughout afterdischarges and also minimizes the interaction between consecutive responses.

In order to control for potential heating-related artifacts (Christie et al., 2013; Desai et al., 2011), 3 of the 4 control animals, which were used earlier for assessment of MRI artifacts and which had been injected with sterile saline in place of AAV5, were imaged using the block design described above. One of the rats was not imaged because there was no evidence of any significant heating-induced signal changes in the first 3 rats that were imaged. A range of light intensities was tested, which included: 1293, 1663, 2310, 2587 mW/mm² per laser pulse at the fiber tip. At the 30% duty cycle used here, this is equivalent to time-averaged power densities at the fiber tip of 388, 499, 693 and 776 mW/mm² respectively. In 2 of the 3 animals, a single experiment using a 99% duty cycle and a time-averaged power density of 2561 mW/mm² at 10 Hz was carried out to determine the effect of very high light intensity on the fMRI response.

2.10 ofMRI data analysis

fMRI data analysis was performed using SPM 12 (<http://www.fil.ion.ucl.ac.uk/spm>) using a general linear model (GLM) in MATLAB 2014a (Mathworks, MA). Images were initially smoothed using a Gaussian kernel with a FWHM of 0.4 mm to improve the signal-to-noise ratio (SNR). For the block-design paradigm, the design matrix was created by convolving the stimulation period with the canonical haemodynamic response function (HRF). For the single-stimulation paradigm, two different analysis methods were used. First, to study activation dynamics, a time-resolved GLM analysis was used for the single-subject data. A sequence of three boxcar functions were used. These included the 20 s during the stimulation, 20 s post-stimulation and the rest of the afterdischarge as defined on the LFP recording. These were convolved with the canonical HRF to take into account the haemodynamic delay and used as a design matrix for the GLM. Activation maps were generated by comparing these active periods vs. baseline. Our goal was to compare the activation map during the 20 s suprathreshold stimulation against the activation map generated during the first 20 s of the afterdischarge. Second, for the group analysis, because the number of subjects was low, a multi-subject 1st level design (fixed-effects model) was

used. In this analysis, we were interested in the afterdischarge itself and therefore regressors included the stimulation period and the entire period during the afterdischarge as defined on the simultaneously recorded LFP. At the subject level, in order to correct for multiple comparisons, voxels were deemed to have a significant response if their voxel-wise false discovery rate (FDR) corrected p-value was less than 0.01. At the group-level analysis, a stricter threshold of $p < 0.001$ (FDR corrected) was used.

2.11 LFP recordings and Analysis

Monopolar single channel intracranial LFP was recorded at a sampling rate of 5 kHz from the hippocampal depth electrodes using the Biopac MP150 data acquisition system and EEG100C-MRI amplifier (Biopac Systems, CA). The cerebellar screw electrode was used as the reference electrode. For recordings taking place in the MRI scanner, a ground electrode was not needed due to the electrically quiet environment. For the awake recordings, a subcutaneous electrode was placed under the skin and used as a ground electrode. LFP quality was tested by using awake recordings in 6 rats ($n=3$ 0.25K CF and $n=3$ tungsten). Average power across the 4 different frequency bands was calculated by using the bandpower function in MATLAB from 60 s of recording. Subthreshold and suprathreshold stimulations were carried out in 2 awake rats using the same paradigm as the fMRI experiment. To calculate the LFP spectral power over time, band power was calculated over 3 s windows corresponding to 4 TRs and this was normalized to the baseline period. The 0.1 Hz high-pass, 35 Hz low-pass filters on the amplifier were used in conjunction with the Biopac radiofrequency filtered cable system. In order to minimize gradient-induced artifacts, the electrode leads were used in a twisted pair configuration. Using this setup, in combination with the low-pass filter and the signal processors on the EEG100C-MRI amplifier reduced the gradient artifact to amplitudes comparable to or below the amplitude of the LFP signal. Where necessary, artifacts were further reduced using the FMRIB plug-in for EEGLAB (Allen et al., 2000; Niazy et al., 2005) using timing triggers from the radiofrequency amplifiers. In general, the use of a hardware filter at such a low cutoff frequency can remove valuable information from the gamma frequency range and therefore may not be desirable depending on the study requirements.

2.12 Histology

In order to confirm ChR2 expression in the targeted region, 2 rats were perfused with 0.1M phosphate-buffered saline (PBS) and ice-cold 4% paraformaldehyde in PBS. 50 μ m coronal sections were prepared on a freezing microtome (HM 430, ThermoScientific) and imaged using a widefield fluorescence microscope (Leica EL6000).

3 Results

3.1 Impedance testing

The aim of this study was to investigate different strategies for MRI compatible, chronic extracellular field recordings for ofMRI. First, in order to determine their suitability for field recordings, we tested the contact impedance magnitude of the 4 different electrodes on the bench by immersing the electrodes in an electrolyte consisting of 0.9% saline and passing a 100 Hz alternating current through a circuit formed with a reference electrode. The results

from this experiment (displayed in Table 1) indicated that the 50 μm diameter tungsten wire electrodes had the highest impedance ($591 \pm 110 \text{ k}\Omega$) and as was expected, the impedance of the carbon fiber electrodes increased with decreasing diameter. Even the smallest diameter (128 μm) 0.25K carbon fiber electrodes tested here had a lower impedance ($79 \pm 4 \text{ k}\Omega$) than the 50 μm diameter tungsten wire electrodes commonly used for LFP recordings, indicating that these CF electrodes should lead to higher SNR LFP recordings (Castagnola et al., 2015; Ferguson et al., 2009; Keefer et al., 2008).

3.2 MRI imaging of electrodes embedded in an agarose phantom

The FSE image of the MRI phantom is shown in Fig. 2a. The size of the artifacts caused by the 1K CF electrode and the 50 μm diameter tungsten electrode are of a similar magnitude. The 0.5K and 0.25K CF electrodes caused significantly less artifact than the tungsten or 1K CF electrode. The 1-dimensional profiles, averaged across several slices are displayed in Fig. 2b. From this figure it can be seen that the distortion caused by the tungsten and 1K CF electrodes is extensive and spreads to approximately 5 voxels from the center of electrode. The distortion caused by the 0.5K and 0.25K CF electrodes is much more modest and only spreads to approximately 2 voxels from the center. In order to further investigate this, optrodes constructed using the tungsten, 1K CF and 0.25K CF electrodes were implanted into live animals for *in vivo* validation. As other studies (Dunn et al., 2009; Jupp et al., 2006) have used thicker diameter carbon electrodes ($>0.4 \text{ mm}$), 1K CF and 0.25K CF optrodes were both tested in order to determine the most suitable diameter for *in vivo* studies.

3.3 In vivo assessment of optrode artifacts on MRI

In order to assess the imaging artifacts caused by each of the different optrode designs, structural (FSE) and functional spiral readout GRE imaging was carried out on a high-field 7T MRI scanner. *In vivo* images of the implanted optrodes are shown in Fig. 2c,d,e for the tungsten, 1K CF and 0.25K CF electrodes respectively. Artifacts from the optrodes are much more evident on the GRE images compared to the FSE images for all 3 designs (Fig. 2c,d,e,f), which indicates the artifacts are primarily caused by magnetic field inhomogeneity. As we hypothesized from the phantom data, the tungsten optrodes caused the most significant degradation of GRE images, followed by the 1K CF and 0.25K CF electrodes. The 50- μm diameter tungsten electrodes eliminated signal from approximately half of the ipsilateral hippocampus from the imaging slice in which it was located (Fig. 2c). Similar to the phantom data, quantification across animals was achieved by averaging the 1D profiles along the axis perpendicular to the electrode (Fig. 2g). The plot of averaged 1D profiles shows that 0.25K CF implanted optrodes caused consistently less image distortion surrounding the electrode than either the optrodes constructed using the 1K CF or tungsten electrodes.

3.4 Awake LFP recordings

Between 2–3 months following optrode implantation, awake intracranial LFP recordings were carried out in 6 rats implanted with either tungsten ($n=3$) or 0.25K CF electrodes ($n=3$). Examples of these awake LFP recordings are shown in Fig. 2h. The average power within 4 frequency bands: delta, theta, alpha and beta was used as a measure of data quality. 1 rat in the tungsten group was excluded as no signal could be obtained. As to be expected

from the impedance measurements in saline, the average power was higher across all frequency bands in the 0.25K CF group compared to the tungsten electrode group (Fig. 2h). In one rat implanted with a 0.25K CF electrode, the total RMS noise was estimated to be 5 μ V by recording shortly after death, indicating that these increases in LFP amplitudes are unlikely to be due to increased noise.

As one of our aims was to investigate seizure-like afterdischarges using LFP and fMRI, two rats were tested awake and under dexmedetomidine to compare seizure thresholds in the awake vs. sedated state. Previous studies have suggested that dexmedetomidine lowers seizure thresholds (Mirski et al., 1994), however in both of the rats tested here, the light power density needed to elicit seizures was marginally higher (~ 92 – 185 mW/mm³) in the sedated compared to the awake state, indicating that dexmedetomidine did not significantly potentiate seizures using our stimulation protocol.

3.5 Control of fMRI experiments

As our group and other research groups have previously reported the potential for artifactual fMRI responses (Christie et al., 2013; Desai et al., 2011; Lee et al., 2010) when delivering light to the naïve (or opsin-negative) brain, we decided to characterise these responses under our current experimental setup to ensure that the results presented here are solely due to optogenetic manipulation. Upon preliminary investigation, we could not observe any heating artifacts at light intensities below 500 mW/mm². Therefore, to determine the full relationship between light intensity and fMRI response we investigated a range of (time-averaged) light intensities between 388 and 2561 mW/mm². We found that even time-averaged light power densities as high as 499 mW/mm² were unable to generate a measureable response and no voxels reached the significance level at the site of stimulation in any of the 3 rats tested (FDR corrected $p < 0.01$) (Fig. S1a,b). On the other hand power densities of 693 mW/mm² or greater resulted in a small clusters of negative fMRI signal change directly below the fiber optic tip (S1a,b). In 2 experiments ($n = 2$), a very high time-averaged power density of 2561 mW/mm² was investigated. In one of these acquisitions, there was an extensive pattern of negative fMRI signal changes surrounded by positive fMRI signal changes (S1a, b). In the other animal, this same laser power provoked only negative fMRI signal changes. The range of time-averaged light intensities required for the ofMRI experiments reported in this study is 56–167 mW/mm² which is far below the threshold required to generate artifactual fMRI signal changes (~ 693 mW/mm²). Hence, the data from these control experiments rule out the possibility of artifactual responses in the ofMRI experiments.

3.6 ofMRI investigation into seizure-like afterdischarges

Initially, two rats were perfused in order to confirm histologically that the surgical procedure led to ChR2-EYFP expression in the right-side of the intermediate hippocampus (Fig. 3a). Next, in order to validate the CF optrodes for use in chronic ofMRI studies, we performed optogenetic fMRI experiments using 2 different stimulation paradigms. We used simultaneous LFP-fMRI to investigate the difference between subthreshold stimulations and stimulations using light intensities capable of eliciting seizure-like afterdischarges in rats sedated with dexmedetomidine. Stimulation of one of the rats did not result in

afterdischarges even with high light intensity (1295 mW/mm^2) and this data was therefore excluded from the analysis. This could have occurred due to a possible mismatch between the virus injection and optrode locations. Subthreshold stimulation of the intermediate hippocampus resulted in activation confined primarily to the posterior ipsilateral hippocampal formation (HF)—primarily the dentate gyrus and CA3 subregions—as well as to the septum (Sep), most significantly within the lateral septum ipsilateral to the site of stimulation (Fig. 3b). Simultaneous LFP recording using the CF electrodes at the site of stimulation confirmed an increase in amplitude in the Beta band (13–30 Hz) during the stimulation and confirmed an absence of seizure-like afterdischarges (Fig. 3d,e). During a suprathreshold stimulation, in addition to the ipsilateral hippocampus and septum, positive BOLD signal changes presented throughout the ipsilateral and contralateral hippocampus (septal and temporal regions), and the retrosplenial (RS) cortex (Fig. 4b). During the optogenetically-induced afterdischarge, significant activation was much more widespread throughout the HF and septum in both hemispheres and also throughout the cortex and in addition included the cingulate (Cg) primary somatosensory (S1) and cerebellum (Cb) (Fig. 4c). Within the basal ganglia, significant activation was present in the accumbens nucleus (Acb) and there were limited regions of negative signal change within the caudate putamen (CPu). Finally, the subject-level activation map showed that there was limited activation in the midline nuclei of the thalamus (Thal). To investigate the time course of activation, 4 of the most significantly activated regions were segmented on the structural images: ipsilateral hippocampus, contralateral hippocampus and septum, and the site of stimulation (Fig. 4d) and the ROI at the site of stimulation shows a large BOLD response during the stimulation and throughout the afterdischarge (Fig. 4e) whilst the presence of epileptiform afterdischarges was confirmed using simultaneous LFP recording at the stimulation site (Fig. 4f,g). These manifested as mid-low frequency $< 20 \text{ Hz}$, high amplitude fluctuations lasting $79 \pm 12 \text{ s}$ (13 afterdischarges in 5 rats). The time course of BOLD signal change throughout the stimulation and afterdischarge shows time delays between the fMRI responses in different brain regions during an epileptic discharge e.g. activity in the contralateral hippocampus follows the septum, which in turn follows the ipsilateral HF (Fig. 4h).

In order to quantify these effects at the group level, the subjects were coregistered together in order to generate group-level activation maps. These group-level activation maps (Fig. 5a,b) also indicate that during subthreshold stimulations, activity is localized to the ipsilateral HF and septum. On the other hand, during afterdischarges, activation is located throughout the entire HF, septum, retrosplenial, cingulate and cerebellar cortices, as well as limited regions within the somatosensory and motor cortices. The fMRI time courses averaged across subjects for the subthreshold and suprathreshold stimulations are shown in Fig. 5c and e respectively. For the afterdischarge stimulation paradigm, the response in the septum appears to be slightly delayed relative to the ipsilateral hippocampus and similar to the single-subject data shown in Fig. 4h, there might exist a delay between the BOLD response in the septum and the response within the contralateral hippocampus. The averaged LFP band power shows increases in the Beta band during the subthreshold stimulation (Fig. 5d), whilst the suprathreshold stimulation results in increases in Theta, Alpha and Beta during the afterdischarge period (Fig. 5f). 13 different brain regions were segmented both contralaterally and ipsilaterally to the stimulation site (Fig. 5g) and percentage of positive

BOLD within a ROI was used to compare subthreshold stimulations to afterdischarges. The results from this analysis are shown in Fig. 5h. For the subthreshold stimulation, in all 5 rats activity was localized to the ipsilateral HF and in 2 of 5 rats it was also present in the septum (Fig. 5a,h). During the afterdischarge, activity was present throughout the contralateral HF in all rats (Fig. 5b,h). On average 52 % of the contralateral hippocampus ROI exhibited activation during the afterdischarge compared to 0.6 % during the subthreshold stimulation. The retrosplenial cortex (RS) and the ipsilateral cingulate, somatosensory and motor cortices were activated in all subjects during the afterdischarge. Within the thalamus, primarily the dorsal-lateral (DL), and medial-dorsal (MD) subregions displayed the most significant activation.

4 Discussion

The results from this study indicate that small diameter carbon fiber optrodes are well-suited for long-term recording in animal studies employing both optogenetics and MRI. In order to demonstrate their potential utility we use them to investigate seizure-like afterdischarges using LFP and fMRI. We also show that the artifacts these electrodes cause on MRI images are significantly reduced compared to larger diameter CF electrodes and also tungsten microwire electrodes. Importantly, these electrodes have consistently low impedance and have lower impedance than tungsten microwire electrodes, which are commonly used in neuroscience studies, demonstrating the suitability of CF electrodes for high-quality long-term intracranial LFP recordings. In the current study, we also show that carbon fiber electrodes can be fabricated at a much smaller diameter than previously thought (Dunn et al., 2009; Jupp et al., 2006), whilst maintaining excellent quality of recordings, thus reducing potential brain injury caused by large diameter electrodes. The increased LFP amplitudes that we measured for CF electrodes *in vivo* is consistent with the lower contact impedance measured in saline. This is in line with other experimental studies, which found that lower impedance electrodes result in greater signal amplitudes (Castagnola et al., 2015; Ferguson et al., 2009; Keefer et al., 2008). It is well accepted that lower impedance electrodes give rise to higher SNR recordings e.g. by reducing the amplitude of power line interference and thermal noise and reducing loss of signal due to the voltage divide caused by the electrode impedance and the amplifier input impedance (Ludwig et al., 2006). Despite this, there does not appear to be a simple relationship between the impedance and SNR (Baranauskas et al., 2011). Carbon fiber has a graphite-like structure and is therefore diamagnetic with a much lower magnetic susceptibility than tungsten, which exhibits paramagnetism (Endruweit et al., 2005; Haynes, 2013). Given this, our data are in line with theory that susceptibility artifacts should be significantly improved when using CF over tungsten wire.

One of the issues associated with using depth electrodes for long-term recording is that their impedance and thus the recording quality changes over time. This occurs both due to corrosion at the electrode tip and also because of glial scarring and tissue encapsulation (Sankar et al., 2014). Carbon fiber electrodes do not suffer from corrosion, and their lower impedance compared to tungsten microwires is likely to offer a significant advantage for long-term recordings. New polymer nanocomposites promise to alleviate the problem of brain injury due to chronic implantations, by softening over time to more closely match the mechanical properties of the brain (Capadona et al., 2008). Future studies could involve

novel surface coatings such as carbon nanotubes in order to maximize the surface area and further reduce the impedance at the electrode interface (Keefer et al., 2008). In order to improve the consistency of the contact impedance, it might be possible to use an alternative method of cleaving the electrodes such as fire-sharpening (Guitchounts et al., 2013), which consists of holding the electrode underwater with the tip exposed above the water and burning the tip down to the water level. To improve the consistency of the electrode diameter, electrodes could be fabricated from the same continuous bundle of carbon fiber.

In order to test the potential of CF electrodes to be used in chronic optogenetic studies, these devices were implanted into the hippocampus of rats transduced with ChR2 under the CaMKII promoter. Optogenetic fMRI experiments were carried out 8 weeks after surgery demonstrating the suitability of these optrodes to be used in chronic optogenetic studies. Stimulation of the hippocampus at 20 Hz resulted in either a 20 Hz pattern of evoked potentials on LFP recordings or, at higher laser powers, resulted in seizure-like afterdischarges, which continued upon cessation of the stimulus. Subthreshold stimulation of the hippocampus resulted in BOLD signal changes which were localized to the ipsilateral hippocampus and lateral septum, whilst optogenetically-induced afterdischarges resulted in widespread activation throughout the contralateral HF and also activation within the retrosplenial, somatosensory and cingulate cortices, thalamus, basal ganglia and cerebellum. Activation maps indicated that during the transition to epileptiform activity i.e. during the stimulation, the septum and contralateral hippocampus are both activated, indicating that these regions are recruited early in the epileptiform afterdischarge.

The results from the current study are in agreement with Takata et al. who recently showed that optogenetic stimulation of CA1 pyramidal neurons in the mouse results in activation of the ipsilateral hippocampus, lateral septum and retrosplenial cortex (Takata et al., 2015). Our results also agree with previously reported studies based on electrical stimulation of the rat perforant pathway (Angenstein et al., 2007; Angenstein et al., 2009; Canals et al., 2008), which indicated that subthreshold stimulation primarily leads to a significant BOLD response throughout the entire ipsilateral HF. These investigations found that the amplitude and spread of activation depends strongly on the pattern of the stimulus and in line with the current study, found that extrahippocampal polysynaptic spread of activity is limited and of low amplitude. Interestingly, following a high frequency stimulation protocol designed to induce long-term potentiation (LTP), BOLD activity in the contralateral HF and extrahippocampal regions could be dramatically increased (Alvarez-Salvado et al., 2014; Canals et al., 2009). Moreno et al. found that electrical stimulation of the CA3 subregion at 10 or 20 Hz results in widespread activity throughout the cortex and contralateral hippocampus (Moreno et al., 2015), whereas stimulation at frequencies below or above this did not. At 10 or 20 Hz, they observed increases in population spike amplitudes with consecutive stimulation pulses, indicative of short-term plasticity (STP), and therefore this frequency dependence was attributed to the effects of STP. In future studies, it might be interesting to utilize the proposed method to determine whether similar results can be achieved using optogenetics.

Most studies investigating seizure-like activity in rats indicate that during seizures induced by hippocampal stimulation, BOLD signal changes are bilateral and involve primarily the

HF and the cortex. Electrically-induced afterdischarges in ketamine/xylazine anaesthetized rats, causes widespread positive BOLD changes in both hippocampi, the anterior hypothalamus and septum (Motelow et al., 2015). Surprisingly, Motelow et al. found that this was accompanied by negative changes in the centrolateral thalamus, midbrain tegmentum and throughout the cortex. The functional response in systemic chemoconvulsant models appears to vary with the chemical used to induce seizures as well as the anesthetic used for imaging. For example, kainic acid-induced seizures under medetomidine anesthesia evokes activity throughout the hippocampus and neocortex (Airaksinen et al., 2012; Airaksinen et al., 2010), while pentylenetetrazol (PTZ) induced seizures under halothane (Keogh et al., 2005; Van Camp et al., 2003) affects primarily the neocortex. Contrary to this, systemic bicuculline principally causes positive fMRI signal changes in the cortex and thalamus (DeSalvo et al., 2010; Nersesyan et al., 2004; Reese et al., 2000). Finally, intracerebroventricular administration of PTZ in awake rats generates seizures involving the thalamus, basal ganglia, amygdala and cortex (Brevard et al., 2006).

The wide-ranging outcomes from these aforementioned studies indicates a possible need for more specific models of epilepsy, which are able to better mimic the wide-ranging subtypes of seizures and epilepsy in humans. For example, optogenetics enables generation of seizures from a specific location as well as from specific cell-types. Previously, we reported that optogenetic stimulation of excitatory neurons in the intermediate hippocampus under isoflurane results in bilateral activation of the hippocampus, hypothalamus, retrosplenial and cingulate cortices (Weitz et al., 2014). The results from the current study support the conclusion that these previously reported data represent seizure-like afterdischarges, albeit afterdischarges which are shorter in duration due to the seizure-suppressing nature of isoflurane.

While most researchers have found an absence of light-induced fMRI activation in opsin-negative animals (Desai et al., 2011; Iordanova et al., 2015; Liang et al., 2015), one study has indicated that heating-induced artifacts might sometimes confound the conclusions from ofMRI studies (Christie et al., 2013). However, the results presented here are in disagreement to Christie et al. in that light intensities typically used for ofMRI experiments did not result in significant modulation of the fMRI signal. However, discrepancies could be explained by differences in the optical fibers used for stimulation as well as differences in experimental setups.

The advantages of using optogenetics over electrical stimulation are numerous. First, at this time, only optogenetics offers the possibility of stimulating specific neuronal or glial cell types *in vivo*. This is will be hugely important in future studies, for example for understanding the contribution of different cell types to the BOLD response which at this time is not well understood (Attwell et al., 2010). However, for studies seeking to disentangle BOLD mechanisms in different brain regions, simultaneous ofMRI and LFP recordings, which are possible using the carbon fiber optrodes described here, will be highly beneficial. Second, optogenetics allows for simultaneous stimulation and recording, which is not possible using electrical stimulation due to stimulation artifacts. Third, the use of step-function opsins (Berndt et al., 2009) enables the enhancement of neuronal excitability without enforcing specific firing patterns and lastly, the use of light-driven hyperpolarizing

channels (Zhang et al., 2007) permits inhibition of neural activity. In future investigations, CF optrodes could be used as a stimulation electrode and therefore as a means to compare optogenetic stimulation to electrical stimulation.

5 Conclusion

In this study, we developed high-field MR compatible optrodes for simultaneous optogenetic stimulation and electrophysiological readout. We showed that the carbon fiber optrodes have significantly less MRI susceptibility artifacts than implanted tungsten electrodes. Furthermore, their suitability for high quality LFP recordings was demonstrated by their lower contact impedance than traditional tungsten electrodes. In order to validate these devices *in vivo*, optogenetic fMRI was used to compare seizure-like afterdischarges to subthreshold optogenetic stimulation of the hippocampus. To our knowledge, this is the first study to demonstrate the feasibility of using MRI compatible carbon fiber optrodes for chronic optogenetic studies. Furthermore, these results suggest the possibility of multi-site recordings with minimal brain injury and without significant degradation of fMRI image quality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ofMRI	optogenetic functional magnetic resonance imaging
CF	carbon fiber
ChR2	channelrhodopsin-2

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Fig. 1.

Assembly of carbon fiber optrodes. (a) 105 μm core diameter fiber optic was stripped of its plastic coating, and cleaved to a predetermined length. The end of the fiber (black triangle) appears to be flat and free of cracks when viewed under a light microscope. (b) The fiber was then inserted into the concave end of a 1.25 mm ceramic ferrule and secured with epoxy adhesive. A correctly inserted fiber optic appears flush with the convex end of the ferrule. (c) The end of the ferrule can be checked under a light microscope to ensure that light passes unobstructed through the fiber optic. (d) 1K carbon fiber tow was separated into two bundles, and each bundle was separated again to make four 0.25K bundles from one 1K bundle. (e) Each 0.25K bundle was then attached to a section of wire using silver conductive epoxy, and coated with three layers of a PVDF solution. Finished carbon fiber electrodes appear straight and evenly coated. (f) A Carbon fiber electrode and implantable fiber optic were secured together using epoxy adhesive. When viewing the optrode under a light microscope (right panel), the electrode and fiber optic ran parallel to each other. (g) Unused contacts were removed from the press fit connector, and to complete the assembly, the implant was soldered opposite a brass screw, which was used as a reference electrode. (h) Completed implants were surgically implanted into Sprague-Dawley rats.

Fig. 2.

Comparison of MRI artifacts and LFP quality for tungsten and carbon fiber optrodes. (a) FSE MRI image of different electrodes embedded in an agarose phantom. (b) 1D profiles of the signal intensity through the center of each electrode in the phantom averaged across 7 slices showing signal void (as a percentage of local signal intensity) vs. distance from center of electrode. (c)–(e) *In vivo* structural (FSE) and functional 4-interleave spiral readout GRE (average of 520 frames) MRI images showing rats implanted with optrodes constructed out of (c) tungsten microwire, (d) 1K CF and (e) 0.25K CF electrodes. (f) Standard SPGR with rectilinear sampling comparing tungsten and 0.25K CF electrodes. (g) Mean 1D profiles for the spiral readout functional MRI images of the signal intensity through the center of each optrode for each of the different designs. Error bars represent the standard error of the mean. Tungsten (n=5), 1K CF (n=4), 0.25K CF (n=4). (h) Example LFP recordings and average power within different LFP frequency bands for tungsten (n=2) and 0.25K CF electrodes (n=3) measured in awake rats 2–3 months after implantation.

Fig. 3.

Single subject simultaneous LFP and optogenetic fMRI during subthreshold stimulation of the hippocampus. (a) Left panel - schematic indicating location of stimulation (blue triangle) and recording electrode line (black line). Middle panel - 50 μm thick coronal section showing EYFP expression in the right hippocampus. Right panel - location of imaging slices 1–20. (b) T-statistic map from block-design (20s-on, 40s-off) subthreshold stimulation of the hippocampus (average over 3 trials). (c) fMRI time course (average of 3 trials and single trial) shown for the block-design stimulation paradigm. (d) Single trial simultaneously recorded EEG shown for the Beta band 13–30 Hz. (e) Spectrogram of the EEG recording during fMRI acquisition. Abbreviations: HF - Hippocampal Formation, Sep - Septum.

Fig. 4.

Single subject simultaneous LFP and optogenetic fMRI during seizure-inducing (suprathreshold) stimulation of the hippocampus. (a) GLM design matrix for the fMRI analysis. (b) T-statistic map showing regions of significant BOLD signal change during a seizure-inducing stimulation (average of 2 trials). (c) T-statistic map showing regions of significant BOLD signal change during the first 20 s of an epileptiform afterdischarge. Site of optical stimulation is marked by the white triangle. (d) Segmentation of 4 different ROIs. (e) fMRI time course shown for a single trial. (f) Single trial simultaneously recorded LFP shown for the Beta band 13–30 Hz. (g) Spectrogram of the LFP recording during fMRI acquisition. (h) fMRI time course for the single trial shown from the ipsilateral hippocampus, septum and contralateral hippocampus. Duration of optical stimulations are marked by blue bars. T-statistic maps are thresholded at a significance level of $p < 0.01$, voxel-wise FDR corrected. Abbreviations: Acb - Accumbens Nucleus, Cpu - Caudate Putamen, RS - Retrosplenial Cortex, Thal - Thalamus, Cg - Cingulate Cortex, HF - Hippocampal Formation, S1 - Primary Somatosensory Cortex, Sep - Septum.

Fig. 5.

Group-level analysis of fMRI data. (a) First-level (fixed-effects) t-statistic map showing voxels which are significantly activated during subthreshold optogenetic stimulation at 20 Hz. (b) First-level (fixed-effects) t-statistic map showing voxels which are significantly activated during seizure-like afterdischarges. Group-level T-statistic maps are thresholded at a significance level of $p < 0.001$, voxel-wise FDR corrected. (c) fMRI time courses for subthreshold block-design stimulation from the ipsilateral hippocampus averaged across subjects. (d) Average LFP band power change from baseline (calculated over each 3 second period) for subthreshold stimulation in the Beta and Theta and Alpha bands. (Error-bars are shown as $\pm$ S.E.M.) (e) fMRI time courses from the ipsi- and contralateral hippocampi and septum during optogenetically-induced afterdischarges (averaged across subjects). (f) Average LFP band power change from baseline for the supra threshold stimulation in the Beta and Theta and Alpha bands (Error-bars are shown as $\pm$ S.E.M.) (g) Segmentation of

MRI images into different brain regions. Segmented regions are overlaid as colored ROIs on a structural (FSE) MRI image. (h) Scatter/bar graph showing percentage of significantly activated voxels within a ROI *vs.* Region of interest for both subthreshold stimulations and seizure-like afterdischarges. Bars indicate the mean value across all 5 subjects and error bars represent $\pm$ S.E.M. Significantly activated voxels were considered to be those with a p-value of <0.01 , voxel-wise FDR corrected. All panels include $n=5$ rats. Abbreviations: Acb - Accumbens Nucleus, Amyg - Amygdala, Cpu - Caudate Putamen, M - Motor Cortex, RS - Retrosplenial Cortex, Thal DL - Thalamus Dorsal-Lateral, Thal VM - Thalamus Ventral-Medial, Cg - Cingulate Cortex, Ent - Entorhinal Cortex, HF - Hippocampal Formation, S1 - Primary Somatosensory Cortex, Sep - Septum.

Table 1

Impedance magnitude measurements at 100 Hz in saline for tungsten and carbon fiber electrodes constructed at different diameters. Diameters and impedances are reported as $\pm$ standard error of the mean. Tungsten electrodes (n = 5), carbon fiber 1K (n = 5), carbon fiber 0.5K (n = 6), carbon fiber 0.25K (n=6).

Electrode	Mean Diameter (μm)	Impedance magnitude ($\text{k}\Omega$)
Tungsten	50	591 ± 98
Carbon fiber 1K	283 ± 11.6	28.9 ± 1.6
Carbon fiber 0.5 K	171 ± 15.4	47.2 ± 5.6
Carbon fiber 0.25K	128 ± 9.9	79.1 ± 4.0