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Direct detection of neural activity *in vitro* using magnetic resonance electrical impedance tomography (MREIT)

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ABSTRACT

We describe a sequence of experiments performed *in vitro* to verify the existence of a new magnetic resonance imaging contrast — Magnetic Resonance Electrical Impedance Tomography (MREIT) —sensitive to changes in active membrane conductivity. We compared standard deviations in MREIT phase data from spontaneously active *Aplysia* abdominal ganglia in an artificial seawater background solution (ASW) with those found after treatment with an excitotoxic solution (KCl). We found significant increases in MREIT treatment cases, compared to control ganglia subject to extra ASW. This distinction was not found in phase images from the same ganglia using no imaging current. Further, significance and effect size depended on the amplitude of MREIT imaging current used. We conclude that our observations were linked to changes in cell conductivity caused by activity. Functional MREIT may have promise as a more direct method of functional neuroimaging than existing methods that image correlates of blood flow such as BOLD fMRI.

1. Introduction

Direct methods for functional neural imaging are critical to advancements in understanding neural behavior, plasticity, connectivity and pathology. Many methods have sought to directly image neural activity *in vivo* using magnetic resonance methods. These include the area of neural current magnetic resonance imaging (ncMRI), where disturbances in the main magnetic field of an MR system caused by intrinsic neural currents have been observed to produce artifacts in magnitude or phase images (Bandettini et al., 2005; Huang, 2014; Huang and Zhu, 2015; Jiang et al., 2014; Luo and Gao, 2009; Park et al., 2006; Petridou et al., 2006; Sundaram et al., 2016). More recently, attempts have been made to detect the effects of ion flow using Lorentz force imaging (Pourtaheri et al., 2013; Truong et al., 2008) and Mg enhanced MR imaging (Radecki et al., 2014). All these contrasts typically produce changes near or below noise floors of high field systems and require creative strategies for their recovery. The largest ncMRI signals are predicted to occur in coherent white matter (Huang and Zhu, 2015), however complex neural architecture may cause loss of signal due to self-cancellation of multiple overlapping neural current fields (Cassarà et al., 2008).

We examined Magnetic Resonance Electrical Impedance Tomography (MREIT) to determine if this approach has potential to detect neural activity. MREIT, which is sensitive to conductivity contrast (a scalar), involves administration of external currents to probe conductivity properties. In the case of neural activity, MREIT may be able to detect changes in membrane conductance associated with neural spiking (functional MREIT, fMREIT) in a similar manner to the related technique of fast neural electrical impedance tomography (Aristovich et al., 2016; Vongerichten et al., 2016). While the contrast mechanisms of fMREIT and fast neural EIT are the same, fMREIT has the advantage that signals from deep cortical structures can potentially be recovered, and implanted electrodes need not be used. MREIT signal size can be controlled by changing imaging current, so this strategy allows acquisitions to be tailored to different imaging environments. Moreover, because conductivity contrast is scalar, the method should be less sensitive to neural magnetic field architecture, potentially offering a direct functional imaging method that is robust to scaling.

In MREIT, small external currents are applied to an object as MR

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Abbreviations

MREIT	Magnetic Resonance Electrical Impedance Tomography
fMREIT	functional MREIT
ASW	Artificial Seawater
ncMRI	neural current MRI
SNR	Signal to noise ratio
ROI	Region of interest
EPI	Echo planar imaging
NC	Image gathered with no imaging current
MREIT	Image gathered using either 100 μA or 1 mA imaging current
PRE	Image acquired before addition of KCl solution
POST	Image acquired approximately 30 min after addition of KCl solution
DEAD	Image acquired approximately 19 h after experiment initiated
AP	Region of interest identified containing ganglion tissue
BK	Region of interest containing background media
RSD	Relative standard deviation

imaging is performed. The magnetic flux density changes caused by this current flow are encoded in MR phase data. Reconstructed phase data is then converted to conductivity or current density slice images (Seo and Woo, 2014; Seo et al., 2003; Woo and Seo, 2008). The large (ca. thirty-fold) changes in membrane conductance that occur during neural activity also cause changes in paths of externally applied currents. If activity occurs during MREIT imaging, increased neural activity rates should become visible as small increases in apparent conductivities of voxels coincident with active cell regions. MREIT voxels within active tissues are sensitive to these small conductivity shifts. Computer simulations have indicated (Sadleir et al., 2010) that imaging of small cell preparations may be feasible with high signal to noise ratio (SNR) levels and moderate resolutions. MREIT imaging necessarily involves application of current, which in general may change the underlying activity levels, thus making MREIT naturally suitable for studying the effects of electrical stimulation therapies or for the study of differential activity caused by application of current combined with a drug or other intervention.

In this study, we demonstrated the existence of MREIT neural activity contrasts *in vitro* using the abdominal ganglion of *Aplysia californica*, a commonly studied neural complex (Frazier et al., 1967; Grant et al., 2000; Novak and Wheeler, 1986; Radecki et al., 2014). MREIT phase data from a test chamber containing the isolated ganglion were gathered, first, when the ganglion was spontaneously spiking in seawater background solution (PRE), and secondly in a state where increased activity was provoked by injection of a presumed excitotoxic solution (KCl-doped seawater) into the test chamber (POST). The effect of this agent was confirmed by separate microelectrode array (MEA) recordings. In a final phase of each experiment, performed approximately 19 h after the sample was placed in the machine, the same image set was acquired from the ganglion remains (DEAD). Within each phase of each experiment, data were also gathered from ganglia with no injected current (NC), providing an opportunity to qualitatively compare findings with those in ncMRI studies (Huang, 2014; Huang and Zhu, 2015; Jiang et al., 2014; Luo et al., 2009).

We provide an overview of MREIT image parameters and factors affecting signals and contrast in Appendices A and B. Because the effect of passive tissue conductivity dominates current flow, it was only possible to confirm the contrast caused by activity by applying a treatment to modify spike rates. Experiments were performed at two different MREIT imaging current amplitudes, and matched controls were employed to further validate results. Data were analyzed in terms of absolute and relative standard deviations observed in phase data in regions of interest corresponding to the *Aplysia* tissue (AP) or background media (BK). Because MREIT sequences used were long relative to typical interspike intervals, and only spontaneous activity was studied, we did not expect any coherent association between activity location or timing. We therefore anticipated that phase changes accumulated over an entire imaging sequence would be more variable over voxels within active tissue when there was more spiking activity, than during a state with less average activity.

Since the mechanism of the MREIT image contrast is scalar, and therefore not diminished by superposition of multiple neural fields, it can be applied in large tissue samples and *in vivo*. This study thus serves as an *in vitro* proof of concept exercise to confirm the viability of this predicted contrast mechanism.

2. Materials and methods

2.1. Animal preparation, controls and treatments

Thirty small juvenile (<100 g) *Aplysia* were obtained from the National Institutes of Health/University of Miami National Resource for *Aplysia* Facility. Animals were anesthetized with $MgCl_2$ solution (77 g/L of $MgCl_2$ and 3.6 g/L of HEPES buffer) injected into the foot process, middle, and head as a paralytic, followed by a mid-dorsal, longitudinal incision to remove the abdominal ganglion, located on the posterior side of the animal near the gonopore. Following removal, the extensions of the abdominal ganglion were trimmed and the ganglion body was placed into a solution of artificial seawater (ASW).

2.2. Control and treatment solutions

Two solutions were used in this study. One was ASW, which was the native medium of the animals. The composition of ASW was as follows: NaCl (0.35 mol/L), $CaCl_2$ (0.011 mol/L), $MgCl_2$ (0.055 mol/L), KCl (0.010 mol/L) and HEPES (0.015 mol/L). The conductivity of this solution was calculated to be approximately 5.8 S/m at a temperature of 25 °C. ASW was used as the initial environment for ganglia in all experiments, and extra ASW was added as a control solution. A similar solution that had a larger concentration of potassium ions added was used as a treatment. This KCl-doped solution contained NaCl (0.35 mol/L), KCl (0.45 mol/L), $MgCl_2$ (0.055 mol/L), $CaCl_2$ (0.011 mol/L) and HEPES (0.015 mol/L). The approximate conductivity of this solution at 25 °C was calculated to be 6.6 S/m.

2.3. Microelectrode array reference experiment

Before MREIT experiments commenced, the effect of adding treatment or control media to abdominal ganglion cells on average spiking rates was tested by administering solutions to 6 ganglion samples placed into the center of a microelectrode array (MEA) dish (MEA60-200-30-3D, Qwane, Lausanne, Switzerland). The surface of the MEA was treated with polyethyleneamine to improve tissue adhesion. The ganglia were initially placed in the center of the dish in approximately 500 μl ASW, and activity was recorded using a standard MEA amplifier system (MEA-60, Multi Channel Systems, Reutlingen, Germany). Recordings were made continuously before and after treatment with 500 μl KCl or ASW solution (added via a syringe over a period of 50 s), and continued for approximately 60 min. No current was applied to ganglia used in these experiments. Spike detection was performed on each recording using MEABench software (http://www.danielwagenaar.net/res/software/meabench/) at a 5σ threshold for treatment animals, and a 4σ threshold for controls.

2.4. MREIT test chamber

The remaining 24 ganglia were used in MREIT experiments. A custom