Magnetic Resonance Imaging of Neuronal Currents in an in vitro Turtle Cerebellum

Padmavathi Sundaram[1], William Wells[2], Darren Orbach[1], Daniel Orringer[3], Mukund Balasubramanian[1], Robert Mulkern[1], and Yoshio Okada[4]

[1]Radiology, Children's Hospital Boston, Harvard Medical School, Boston, MA, United States, [2]Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States, [3]Neurosurgery, University of Michigan, Ann Arbor, MI, United States, [4]Neurology, Children's Hospital Boston, Harvard Medical School, Boston, MA, United States

Purpose: This work investigates the relationship between neuronal currents and MR signals in an in vitro preparation of a bloodless turtle cerebellum (Cb). Prior work has demonstrated sensitivity of the MR signal to currents in phantoms [1, 2] and to biological currents in cell cultures [3]. While these studies are encouraging, the spatiotemporal relationship between MR phase and neuronal currents remains to be characterized in intact brain tissue. The isolated turtle Cb is ideal for this study because: (1) there are no MR signal contributions from blood flow, respiration or motion, (2) unlike a slice preparation, the entire cellular circuit is intact, (3) the preparation can withstand anoxia and is physiologically functional for several hours without perfusion of oxygen during imaging, and (4) the geometry of the Cb is ideal as it is a flat tissue with the principal magnetic field generators (the Purkinje neurons) oriented parallel to each other and perpendicular to the cerebellar surface.

Theory: *(1) MR phase shift from local magnetic fields:* Neuronal currents generate local magnetic fields. The component ΔB_z of these local magnetic fields that lies along B_o modulates MR phase Φ. For a gradient-echo sequence, the phase shift $\Delta\Phi$ is given by: $\Delta\Phi = \gamma\, \Delta B_z\, TE$, where TE is the echo time, and γ is the gyromagnetic ratio. *(2) Estimating local neuronal magnetic fields:* We assume the current dipole moment density in the tissue to be 1 nA•m/mm^2, based on prior measurements [4, 5]. When one of the cerebellar peduncles is stimulated, it creates an active area ≈ 2 mm X 5 mm in the ipsilateral hemisphere. Using this distributed dipole source [6] and the Biot-Savart law, we estimated a maximum neuronal magnetic field of 0.65 nT in the Cb which would result in a local MR phase shift of 0.26° at a TE of 26 ms.

Methods: *(1) Cb preparation:* The surgical procedure was approved by our institution's Subcommittee on Research Animal Care (SRAC). The brain of the red-eared slider turtle (Pseudemys scripta elegans) was removed after decapitation and placed in cold Ringer solution (in mM: 100 NaCl, 5 KCl, 40 $NaHCO_3$, 1.25 $MgCl_2$, 2.5 $CaCl_2$, 20 D-glucose). The Cb was surgically resected from the brain under a microscope. *(2) Imaging chamber/recording setup:* The Cb was placed in a Ringer-filled acrylic chamber. **Fig 1** shows stimulating (red), recording (blue) and ground (green) electrodes (Teflon-coated Ag wires insulated everywhere except at the end). The stimulating and recording electrodes are twisted pairs and half-way in the tissue. *(3) LFP recording during MRI:* We interfaced the chamber to an MR-compatible differential amplifier (BrainProducts) to record local field potentials (LFPs) in the Cb during MRI. LFPs recorded during scanning are contaminated with artifacts. These were removed using low pass filtering (10 Hz cutoff) since our signal of interest is slow. *(4) Evoking slow LFPs:* We evoked slow LFPs (excitatory postsynaptic potentials) in the Cb [7] using tetanic stimulation (six 100-µs biphasic current pulses at 100 Hz; total stimulus duration 55 ms; inter-stimulus time 20 sec). The slow LFPs have a time-to-peak of 2-3 seconds after the stimulus and enable imaging without interference from stimulus artifacts. To evoke these LFPs, we added picrotoxin (PTX, 1 mM) and tetraethylammonium (TEA, 10 mM) to the Ringer. *(5) Gradient-echo (GE) EPI:* We used a 4.7T Bruker MRI scanner for imaging. A 1.5 cm-diameter surface coil placed flat on top of the chamber was used for transmit/receive **(Fig 1)**. We acquired single slice GE-EPI with: TR 100 ms, TE 26 ms, flip angle 25 °, 96 x 96 matrix, 0.33 mm in-plane voxel size, 1 mm slice thickness. We acquired concurrent LFPs and GE-EPI under 3 conditions: (1) "PTX" (evoked slow LFP), (2) "CNQX" (pharmacological block), (3) "WASH" (removal of block, partial response recovery). In each case, we stimulated the tissue 150 times and obtained MR magnitude and phase data. *(6) Analysis:* Linear detrending was used to remove scanner drifts from the images. We computed an average phase change map during the slow LFP by averaging the phase images corresponding to the slow LFP (> 100 µV for PTX, > 80 µV for WASH; 5264 $\Delta\Phi$ images in each case). For the "CNQX" case, we took an identical number of $\Delta\Phi$ images to compute the average $\Delta\Phi$ map. We manually defined the region-of-interest (ROI) containing the Cb and computed the average $\Delta\Phi$ and %-change magnitude time course for the entire ROI.

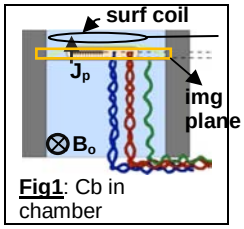

Fig1: Cb in chamber

Results: **Fig 2** shows the average $\Delta\Phi$ map during the response and the average concurrently recorded LFP for each of the conditions. We see phase changes in the tissue during the PTX and the WASH conditions. The response was only partially recovered in WASH resulting in lower signal. We do not see any activity related phase changes in the Cb in CNQX. In PTX, we see positive and negative phase changes in the tissue. The phase changes appear to show some symmetry about the B_o axis. In **Fig 3**, the average $\Delta\Phi$ and magnitude time courses (both in red) across the entire Cb are shown overlaid on the concurrently recorded LFP (solid black). The latency to peak (~2.5 sec) of the MR phase and magnitude time courses match that of the LFP. The peak $\Delta\Phi$ change is approximately 0.27°, in agreement with our predictions. We also see a small magnitude change of 0.3%. Our results suggest that the phase changes are related to the postsynaptic currents in the tissue during the evoked response; this represents MR detection of neuronal currents in vitro, a step towards in vivo detection.

References: **1.** Bodurka et al., JMR 1999 **2.** Witzel et al., Neuroimage 2008 **3.** Petridou et al., PNAS 2006 **4.** Okada et al., Brain Res 1987 **5.** Okada et al., Biophys J., 1988 **6.** Sundaram et al., ISMRM 2012 (2073) **7.** Larson-Prior et al., Neurosci Lett, 1989

Acknowledgements: This work was sponsored by an ISMRM-ASNR Seed grant.

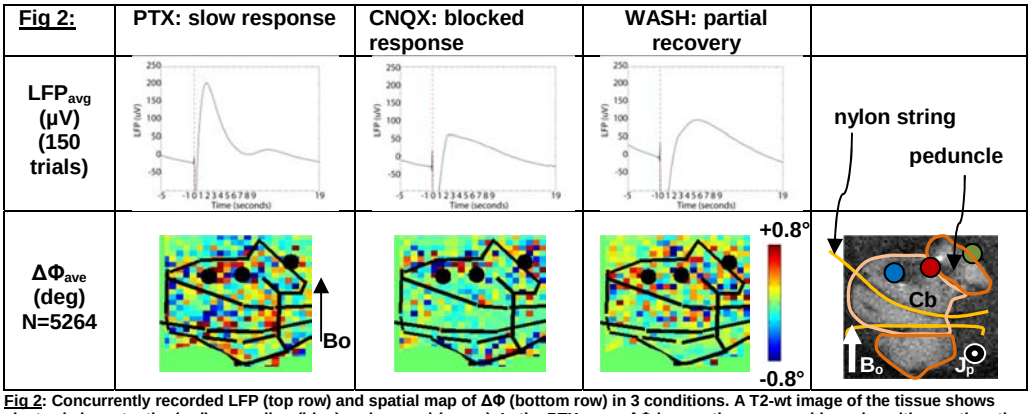

Fig 2: Concurrently recorded LFP (top row) and spatial map of $\Delta\Phi$ (bottom row) in 3 conditions. A T2-wt image of the tissue shows electrode layout: stim (red), recording (blue) and ground (green). In the PTX case, $\Delta\Phi$ is negative on one side and positive on the other side of the center of neuronal activity near the recording electrode in the hemisphere ipsilateral to the stim electrode.

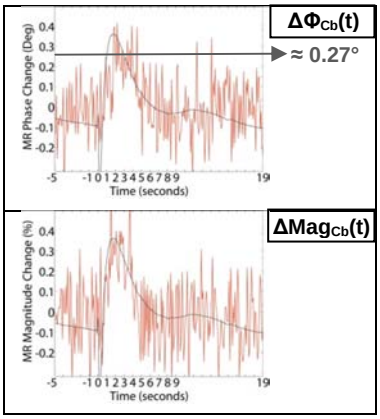

Fig 3: Average $\Delta\Phi(t)$ and Mag(t) across the Cb superimposed on concurrently recorded LFP in the "PTX" condition