Magnetic Resonance Microscopy of Rat Hippocampal Slice Using Manganese as Neuronal Activity Indicator and Magnetic Contrast Agent

S. Huang[1,2], L. Giocomo[3], C. T. Farrar[2], G. Dai[2], P. K. Liu[2], M. Hasselmo[3], B. Rosen[2,4]

[1]Health Science and Technology, MIT, Cambridge, MA, United States, [2]Martinos Center for Biomedical Imaging, Masscheusetts General Hospital, Charlestown, MA, United States, [3]Psychology, Boston University, Bosotn, MA, United States, [4]Health Science and Technology, MIT/Harvard University, Cambridge, MA, United States

Introduction:

Functional Magnetic Resonant Imaging (fMRI) has been widely used to study brain activity noninvasively. However, fMRI is limited by its inherent dependence on the hemodynamic change, which is only indirectly coupled to the underlying neuronal activity. As neurons are activated, voltage-gated/or ligand-gated calcium channels open which results in an instantaneous increase in intracellular calcium concentration. Therefore, calcium can act as a good indicator of neuronal activity in brain. Manganese is a calcium analog that can enter neurons through voltage-gated/or ligand-gated calcium channels. It is also a good MRI contrast agent with strong scalar coupling relaxation effect at high magnetic field. This dual role of manganese as a calcium analog as well as MRI contrast agent makes it possible to study neuronal pathways directly with Magnetic Resonant Microscopy (MRM).

We use rat hippocampal slice as in vitro model system of a living functional brain unit. This allows us to overcome issues such as toxicity and delivery of manganese into the brain and study brain activity without disrupting the integrity of the neuronal network of interest. The goal of our study is to investigate the feasibility of manganese as a functional MRM contrast agent at high field and study neuronal activity in our model system. Our results demonstrated the selective uptake of manganese by neurons and its transportation from neuron to its projecting target after local injection.

Materials and Methods:

Male Sprague Dawley rat (three to four week old) was anesthetized using halothane and decapitated after it failed to respond to hard tail pinch. The brain was quickly taken out and sliced using vibroslicer (World Precision Instrument, FL USA) at 4°C. Hippocampal slices were left for recovery in oxygenated artificial cerebral spinal fluid (ACSF) at room temperature for about one hour before the experiment.

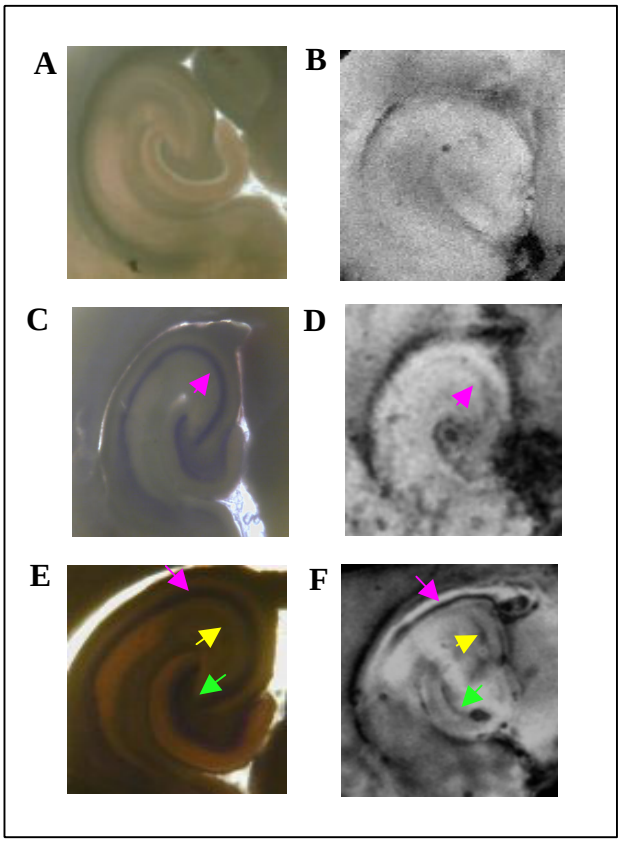

figure 1: images of rat hippocampal slices showing manganese selective uptake and neuron tracing. A) light microscope image of normal slice, B) MRM of normal slice, C) Nissl stained slice with two injection sites, D) MRM of the same slice as that in C, E) Nissl Stained slice with one injection site, F) MRM of the same slice as that in E

10 µl $MnCl_2$ (1mM in ACSF) was locally injected into selective regions of hippocampal slices using micro pipette with a tip of 5 µm in diameter under light microscope. Slices were then put back into oxygenated ACSF for recovery and active manganese uptake and transport at room temperature for another two hours before fixation with four percent paraformaldehye solution. MRM was done at 14T Bruker Biospin System. Because of the strong scalar coupling term of manganese at high magnetic field, our phantom data suggested that MR image will be more sensitive to T2*(T2) weighting. High resolution T2* weighted images were thus acquired using gradient echo pulse sequence (TR/TE = 300/12ms, FOV = 5.5×5.5 mm, slice thickness = 400 µm, resolution = 20 × 20 µm). After MR imaging, the same slices were stained with Thionin overnight. Pictures were taken under light microscope with magnification of forty.

Results and Discussion:

Figure 1 shows the MR images from three brain slices along with the histological comparison. Only subtle intrinsic tissue contrast prior to manganese injection is evident (1B) using these MRI pulse sequence parameters. Following manganese injection, however, distinct tracks of diminished T2* are clearly radiating from the injection sites (1D & 1F). The appearance of dentate gyrus granular cell layer (green arrow) and its projection target, CA pyramidal cell layer (pink arrow), after local injection into dentate gyrus region indicates that manganese was uptake by active neurons in dentate gyrus and transported to their targets (figure 1D and 1F). With two injections at two different sites, additional structure emerged, which can be seen from 1F (yellow arrow). Our results suggest that selective manganese regional uptake and local transport can be visualized in our in vitro slice preparation. Therefore, manganese can be used as neuronal activity indicator. This may allow us to study dynamic neuronal function and image detailed neural networks under a wide variety of physiological conditions, including electrical stimulation, influence of drugs or neural transmitter antagonists, etc.

1. Timothy M. Shepherd, Stephen J. Blackband, and Edward D. Wirth III, MRM 48:565 - 569 (2002)

2. Ichio Aoki, Yi-Jen Lin Wu, Afonso C. Silva, Ronald M. Lynch, and Alan P. Koretsky, NeuroImage 22 (2004) 1046 - 1059