Magnetic brain cell stimulation using an MRI contrast agent: superparamagnetic iron oxide nanoparticles (SPIONs)

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TARGET AUDIENCE

This work should be of interest to researchers who are studying, neuroscience, the MRI use of SPIONs, and who are interested in the stimulation of the brain cells.

PURPOSE

There is a growing interest in using an MRI system not only for imaging nanoparticles, but also for cellular actuation through them [1], owing to its ability to manipulate SPIONs *in vivo* using the magnetic field gradients [2]. Our goal is to develop a new technology that uses magnetic fields to remotely move iron oxide particles and alter cell activity; we are currently exploring external magnets and the MRI system as sources of the magnetic field. This will be a less invasive approach to selective control of cellular events compared to optogenetics, which necessitates optic fibre implantation for light delivery. Here we propose a novel method that uses magnetic 'action at a distance' to directly stimulate an increase in intracellular Ca^{2+} concentration in astrocytes, which are the most abundant type of glial cells in the brain and are known to give metabolic and structural support to neuronal networks. We successfully performed the first magnetic stimulation of astrocytes by producing a force on astrocytic cell membrane using an MRI contrast agent and an applied magnetic field. We also targeted SPIONs to astrocytes *in vivo* using specific antibodies and monitored their biodistribution over 9 days using a 1T benchtop MRI system.

METHODS

Astrocyte stimulation: Primary cortical cell cultures are made from post-natal day 3 rat pups. Iron (II,III) oxide powder (Fe_3O_4, particle size < 5μm, Sigma-Aldrich) is coated with collagen (Sigma-Aldrich) based on a previous study [3]. Before Ca^{2+} imaging, cells are loaded with 4μM Fura-2 AM (Life Technologies) and 0.04% Pluronic F-127 (Life Technologies) in HBSS for 0.5 hr, and then 0.2-1 mg/ml collagen-coated Fe_3O_4 powder is added for another half hour's incubation. An Olympus IX71 inverted microscope is used for excitation ratiometric imaging of Fura-2, and magnetic field is applied by lowering an N42 Neodymium permanent magnet (first4magnets) upon the cell culture.

SPION biodistribution study: After anti-GLAST (ACSA-1, Miltenyi Biotec) is conjugated to SiMAG particles (500nm, chemicell), 1μl of the particle solution (0.2mg/ml) is injected 1mm below the cortical surface of a 50g Sprague-Dawley male rat using a stereotaxic frame and fine glass pipette on the right hand side. On the left hand side in a corresponding location, 1μl of saline is injected as a control. The animal is imaged in a 1T ICON™ desktop MRI scanner (Bruker) at post-operative day (POD) 1, 3, 4 and 9 with a FLASH sequence (TE = 3.7ms, TR = 305.3ms, Number of Averages = 6, Number of Repetitions = 1, FA = 30°).

RESULTS

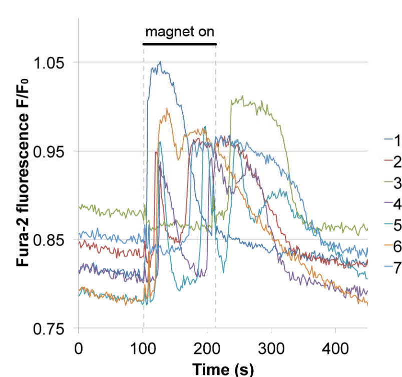

Figure 1. *Ca^{2+} concentration increases in cultured astrocytes in response to magnetic field application*

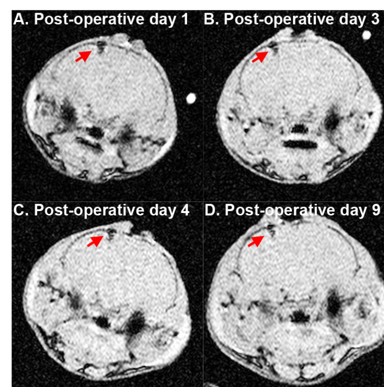

Figure 2.*SPIONs targeted to astrocytes remain in situ for at least 9 days after stereotaxic injection into the cortex.*

Astrocyte stimulation: We were able to robustly stimulate cultured astrocytes. When the magnetic field was applied, there was a sharp rise in the ratio between Fura-2 fluorescence intensity at excitation wavelength 340nm and 380nm, indicating an increase in the intracellular Ca^{2+} concentration (Figure 1).

SPION biodistribution study: The spatial distribution of hypointensity stays relatively unchanged for at least 9 days post injection, indicating targeting of the SPIONs and favourable biodistribution for actuation (Figure 2, indicated by red arrows).

DISCUSSION & CONCLUSION

We have demonstrated, for the first time, that astrocytes can be stimulated by an external magnetic field acting on iron oxide particles attached to their cell membrane. We have also shown that SiMAG particles conjugated to an antibody specific for a transmembrane protein expressed by astrocytes remain for at least 9 days after they are injected into the cortex. These results are very encouraging and indicate that it is feasible to image SPIONs for the remote magnetic stimulation of astrocytes *in vivo* using a 1T "benchtop" MRI system. Further work will quantify the force threshold needed to stimulate astrocytes. If this technology indeed proves to be successful, it will be a powerful, minimally invasive tool for astrocyte research and more, as it allows researchers to conduct imaging and actuation with a single system.

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