MRI Visualization of Anatomical Connections in vivo using a Gadolinium Chelated Neural Tracer

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Introduction: Defining the anatomical connections between different sets of neurons is crucial for understanding the brain, in both the normal and modified states. However, conventional mapping of anatomical connections require staining of fixed tissue from *ex vivo* brains, and therefore are unsuitable for any chronic experiment. To solve this problem, manganese enhanced MRI (MEMRI) has been used to reveal neural connections, at a spatial resolution adequate to distinguish specific layers **(1).** However, MEMRI requires careful timing of the imaging to disambiguate the order of connections, because manganese can be transported across synapses. Here, our goal was to develop novel MRI-visible anatomical tracers that would furnish easy and reliable tracing of connected brain regions in target-specific, mono-synaptically. Gadolinium chelates have been widely used as MRI contrast agents in human research. Here we tested a new compound that was designed to make the conventional neuro-anatomical tracer cholera toxin subunite B (CTB) **(2)** detectable by MRI by conjugation with GdDOTA **(**gadolinium- tetraazacyclododecanetetraacetic acid**)**. Specifically, we tested whether 1) the CTB component of the bioconjugate would be taken up and transported by neurons, and 2) the GdDOTA component of the conjugate would reveal location of the transported compound using MRI.

Experimental Design: Four experiments were conducted. In the first experiment, CTBGdDOTA was injected into primary somatosensory cortex (S1). Following a systematically-varied delay, the known thalamic targets of S1 (the ventroposterior thalamic nucleus VP, and the reticular thalamic nucleus RTN) were tested for T_1 weighted MRI contrast to test for specific transport of the CTBGdDOTA, and (if any), to measure the transport dynamics. This experiment confirmed specific transport of the CTBGdDOTA, with a time course similar to that of histologically-based CTB. In the second experiment, the clearance rate of CTBGdDOTA was compared with the clearance rate of GdDOTA from the brain, by injecting GdDOTA into the cerebral ventricles. In the third experiment, MRI results were validated by comparing the CTB results based on histology, to those based on the MRI, in the same animals. This experiment showed an excellent correlation between the MRI and histological results, consistent with monosynaptic transport in at least the anterograde direction. Finally, the generalizability of the CTBGdDOTA transport was tested in other regions of the brain, including the olfactory and auditory pathways of the rat.

Materials and Methods: CTBGdDOTA bioconjugate was made at the Imaging Probe Development Center (NHLBI, NIH). The ratio of GdDOTA to CTB was found to be 1:1 or 1:2. Sprague-Dawley rats received unilateral intra-cortical injections of CTBGdDOTA (0.5-1 µl, final concentration of 20%, w/v) into the forelimb representation of S1, using a microsyringe. In control measurements of extracellular diffusion, GdDOTA alone was injected into the 3rd ventricle (0.5 mol/L, 15 µl coordinate: -3.8; -5.2; +6.8). In independent control experiments, CTBGdDOTA was placed in the olfactory bulb (0.5 µl) or nostril cavity (15 µl), and into the auditory thalamic nucleus (0.1-0.2 µl). Animals were imaged at baseline prior to injection, immediately (~2 hr) post injection, and at days 2-7, at 1 month, and up to 2 months post-injection. The MRI imaging was performed on an 11.7T, 31-cm bore magnet with a Bruker Advance console. A 15-mm-diameter receive-only surface coil and a 90-mm-diameter birdcage transmit coil were used. Multislice-multiecho (MSME) pulse sequence was applied to acquire T1-weighted spin-echo 2D or 3D high-resolution images with the following parameters: for 2D, TE/TR=7.9/1000 ms, matrix size=256x256, FOV=2.56x2.56, or TE/TR=8.0/450 ms, matrix size=25.6x25.6 FOV=128x128, slice thickness=0.5mm. For 3D, TE/TR=9.1/300 ms, matrix size=256x256x180, FOV=25.6x25.6x18 mm, or TE/TR=8.0/300 ms, matrix size =192x128x112, FOV=38.4x25.6x22.4. In addition, a modified driven equilibrium Fourier transform (MDEFT) pulse sequence with 100 µm isotropic resolution was applied to enhance and differentiate the gray versus white matter contrast of the brain using the following parameters: TR=4000 ms, echo TR/TE=15/5 ms, TI=1000 ms, number of segments=4, averages=4, matrix size =256x256x128, FOV=25.6x25.6x12.8 mm. Image reconstruction and data analysis were performed using ParaVision (Bruker Medical GmbH), Amide (The free software Foundation, Inc, Boston, MA), and ImageJ (NIH). Following the final MRI, animals were sacrificed, and the brains were perfused and cut into frozen sections. MRI results were verified with immunocytochemical staining methods for CTB **(3).**

Results: Following S1 injection, target-specific neuronal transport of CTBGdDOTA was found in the ipsilateral thalamic target zones that are known to have a mono-synaptic connection with S1. In addition, we found that: 1) the label at the injection site was very sharply-defined, and it remained stable for up to one month following injection. This is consistent with the localization of CTB histologically, and inconsistent with passive diffusion. 2) It took 5-7 days to fully enhance thalamic nuclei, comparable transport of CTB alone in the rat. This suggests that CTBGdDOTA is transported by the same neural mechanism as CTB alone. 3) GdDOTA injected to the ventricles was cleared from the extracellular space 24 hrs post injection. In contrast, CTBGdDOTA enhancement remained stable for at least 4 weeks, and enhancement was visible for up to 2 months. 4) At corresponding locations, MRI of transported CTBGdDOTA was in good agreement with CTB staining. For example, CTB-labeled neurons were found in the ventroposterior thalamic nucleus VP, indicating that CTBGdDOTA can be used for both retrograde and anterograde tracing. 5) In addition to somatosensory circuitary, CTBGdDOTA also traced connections in the olfactory and auditory pathways. Seven days after injection into the olfactory bulb, strong enhancement was detected in other parts of the olfactory bulb, the anterior olfactory nucleus, and the pyriform cortex. Unilateral nostril cavity injection of CTBGdDOTA, strongly enhanced olfactory epithelium ipsilateral to the injection side as early as 12 hrs post injection, and in the outer layer of the olfactory bulb 7 days post injection. When injection was placed in the auditory thalamus, clear enhancement was detected in the auditory cortex and brain stem 7 days post injection.

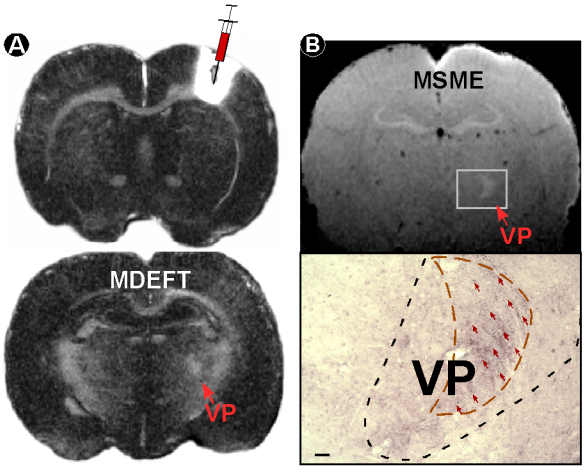

Fig A). Example of MDEFT images showing S1 injection site and thalamic enhancement 7 days post injection. At the location of VP, signal intensity is much higher in the injection side, as compared to the opposite, no-injection side. **Fig B).** CTB-histology staining verifies MRI results. Top: MSME image reveals a triangular shape of enhancement in VP nucleus. Bottom: CTB-stained terminal fields (red arrowheads) was in excellent agreement with MRI. The corresponding location of MRI enhancement in is outlined with red dashed lines, and the approximate border of nucleus VP is outlined with black dashed lines. Scale bar=0.1 mm. **Fig C).**Time course of MRI signal changes in thalamic nucleus VP after S1 injection. It takes 5-7 days to fully enhance the thalamic targets, and the enhancement remains in the thalamus for up to 1 month and clears up 2 months post injection.

Conclusions: Our study shows that CTBGdDOTA is a MRI neural tracer that allows reliable visualization of mono-synaptically connected circuits of the brain *in vivo*. The transport of CTBGdDOTA is target-specific, bi-directional, very reproducible, and stable over a relatively long period of time. This agent opens the possibility for repetitive, chronic, and longitudinal studies of monosynaptic connections in animal brains. For instance, this should be useful for using MRI to guide electrode placement for physiological recordings of synaptically connected sites, as well as to investigate how the brain is rewired following injury and during development.

References: (1) Tucciarone et al. (2009) *Neuroimage* 44:923-932. (2) Ericson and Blomqvist (1988). *J. Neurosci. Methods* 24:225-235. (3) Angelucci et al. (1996) *J. Neurosci. Methods* 65:101-112.