In vivo Tracing Anatomical Circuitry of the Brains using Gadolinium

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Introduction: The brain is composed of billions of neurons and thousands of synapses for each of those neurons that allow communication between neurons. Since the pioneering times of Golgi and Cajal, tracking neuronal connections has been a challenge in neuroanatomy, with profound implication for the study of neural function and the development and adult plasticity of the nervous system. Modern approaches to study neuronal circuitry require directly injecting tracers into a specific region of the brain in the living animals and examine the anterograde labeled nerve terminals and retrograde labeled neurons in the histologically processed tissue after animals are sacrificed. Recent advances using manganese-enhanced-MRI has opened the possibility to visualize anatomical connections *in vivo* (1-3). However, the shortcoming of MEMRI is that it requires precise timing to image the brain because manganese can be transported across synapses. Gadolinium complex meglumine, Gd-chelates, has been widely applied to a number of clinical problems. Gd-DOTA, has recently been used as an MRI contrast agent in humans (4). The goal of this work is to expand the MRI contrast media available for neuronal track tracing. Here we demonstrate that, under certain conditions, Gd-DOTA can be used as a MRI visible anatomical track tracing agent that reliably trace well-understood anatomical circuitry.

Materials and Methods: Twenty adult Sprague Dawley rats were used for this study. Surgery for Gd-DOTA injection was carried out under aseptic conditions while the animals were anesthetized with isofluorane (5% induction followed by 2% maintaining, in 60 air/40 oxygen mixture) with a nose cone. After animals' heads were fixed in the stereotaxic apparatus, a craniotomy was performed, and Gd-DOTA (0.3-0.5µl, 200 mM, dissolved in PBS) was slowly injected into the different representations (forelimb cortex coordinates: 0.3, 3.8, 3.4; orofacital cortex coordinates,1.0, 5.5, 5.2) of the primary somatosensory cortex, S1, through a Hamilton microsyringe. After intra-cortical Gd-DOTA injection, the opening in the skull was sealed, and animals were imaged immediately after surgery and imaged for between 2-14 days under isofluorane anesthesia. Animals were carefully monitored during recovery from anesthesia and throughout the survival time. The MRI experiment was performed on an 11.7T, 31-cm bore magnet interfaced to a Bruker Advance console. A 15-mm-diameter receive only surface coil and a 90-mm-diameter birdcage transmit coil were used. T1-weighted spin echo 2D or 3D images were obtained with the following parameters: 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. 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. This yielded voxel size with 100^2 or 200^2µm resolution. Image reconstruction was performed using ParaVision (Bruker Medical GmbH) and Amide (The free software Foundation, Inc, Boston, MA). In the control experiment, anatomical tracer biocytin, alone, or mixture of biocytin and Gd-DOTA was injected into the brains in order to verify the location and direction of transport. These animals were perfused transcardially with PBS (pH 7.4), followed by 4 % paraformaldehyde in PBS, and then followed by the fixative solution with 10% sucrose. Brains were stored in 20% sucrose in PBS overnight before sectioning and cut frozen on a cryostat at 40 µm thickness. Brain sections were incubated with ABC kit (vector) and finally visualized with DAB-GOD-glucose products. Histologically reacted sections were viewed under high magnification and captured using a digital scanning camera (Hamamatsu, C4742-95) mounted on a Leica microscope (MZ FL III) and scanned with Q-capture software (v2.68).

Results and Discussion: (1) Prominent T1-weighted enhancement is found in the expected brain regions connected with S1, as early as 24 hours following injection. These regions include major somatosensory thalamic ventroposterior nuclei (VP), associative thalamic nucleus such as posterior medial nuclei (Pom), reticular nuclei (RT) and zona incerta (ZI). Major subcortical transport is also found in caudate putamen (CPu). (2) Fiber tract bundles were prominently enhanced. These include the corpus callosum (cc) that connects to the other cortical hemisphere, and the internal capsule (ic) that connects cortex with the brainstem and spinal cord. (3) The transport was only observed when Gd-DOTA was applied to deeper cortical layers and the injection site included white matter underneath. However when applied to the superficial cortical layers, no evidence of transport was detected. (4) Transported Gd-DOTA clears up within a week. (5) Based on evidence of transport found in RT, Gd-DOTA can trace anatomical connections in the anterograde direction. (5) There was no evidence of transport across synapses. (6) Preliminary results suggest that Gd-DOTA can trace connections in a somatotopic manner. (7) The location of transported Gd-DOTA is comparable to conventional anatomical tracer as verified by biocytin histology stains. It is generally accepted that Gd-chelates remains extracellular, therefore the specific tracking was unexpected because specific tracing normally requires intracellular uptake and transport. Therefore, it is likely that, similar to some anatomical tracers, successful transport of Gd-DOTA requires physical damage of the fiber that is necessary for uptake and transport. We have started to investigate the mechanisms mediating intracellular transport of Gd-DOTA.

Conclusions: We demonstrate, for the first time, that Gd-DOTA can be used as an *in vivo* anatomical track tracing MRI contrast agent. This finding expands the usefulness of Gd-DOTA for noninvasive imaging of the brain to visualize anatomically connected circuitry, and therefore will be useful to longitudinally study brain wiring during different developmental stages, and to study how the brain rewires following injuries.

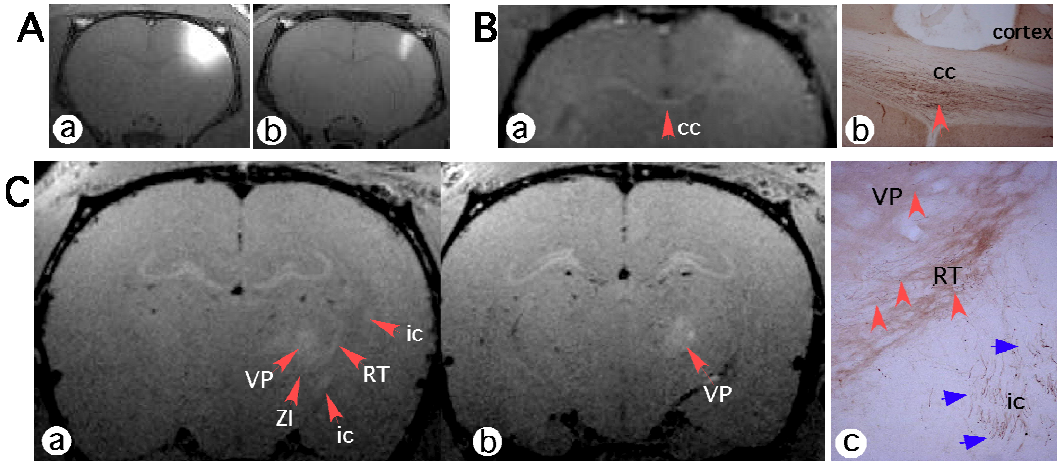

Fig A, Injection sites immediately (a) and 48 hr (b) following Gd-DOTA administration. **Fig B**, Corpus callosum is evident enhanced in Gd-DOTA image (a) and is clearly labeled in the histology verified biocytin section (b). **Fig C**, Major transport in the thalamus can be easily detected. Patches of labeled were found in groups of thalamic nucleus in 2D coronal image plan (a-b, a is a more rostral than b). Detailed nerve terminals (orange arrowheads) and fiber bundles (blue arrows) are observed in the biocytin stained section (c), confirming that Gd-DOTA is transported in the anterograde direction.

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