Mapping MRI Contrast Enhancement with Histology

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Identification and quantification of anatomical, functional and molecular changes are important for understanding the development and aging, detecting disease, evaluating pathology, and assessing treatment efficacy. Conventionally, these rely on invasive or destructive methods like histological staining or biochemical assays. Although MRI provides excellent soft tissue contrast, fine organization within a tissue, such as laminar layers or cortical columns in the gray matter of the brain, are difficult to identify using conventional anatomical MRI. With the advances in high-field MRI, new contrast mechanisms, and the use of contrast agents, it is potential to visualize structure or even functional structure similar to histology using *in vivo* or *ex vivo* MRI.

The first challenge is the high spatial resolution (eg, ≤ 50 μm) and SNR required for delineating microscopic structures. Strategies such as optimizing pulse sequence, minimizing motion/vibration, improving gradient and RF coils, using stronger magnetic field or extracting the tissue for ex vivo imaging are need to be considered. Previous attempts to identify fine cortical structures in the brain utilized T_1 contrast between gray matter and myelinated axons in gray matter to differentiate myelin-rich regions in the cortex, such as the stripe of Gennari in the primary visual cortex in human (1). At 3T, it

took 45 min to acquire image of 350 x 350 x 600 μm^3 resolution *in vivo* using a surface coil. The scan time for acquiring 500-μm isotropic resolution can be reduced to 10 min using a 7T scanner with 24-channel array coils (2). Since T_1 contrast is reduced at higher field, T_2 contrast can be optimized to better visualize such myelin-rich and/or cell density dependent contrast in the cortex (3). However, it shall be noted that the layers detected is not the same as the cortical layers defined based on cellular organization in histology.

The other challenge for resolving cytoarchitecture is the lack of significant difference in proton density, spin-lattice relaxation time (T_1), spin-spin relaxation time (T_2) and diffusion coefficients with surrounding tissues. With the development of ultra-high magnetic field system, minute difference in tissue susceptibility starts to show interesting contrasts in T_2*-weighted (4), phase sensitive (5, 6), and susceptibility quantified (7) imaging at 7 Tesla and above. These studies show promise for enabling *in vivo* visualization of laminar architecture in the gray matter (8), axonal structure (9) and myelin dependency (10, 11) in white matter and iron content (12) in tissue due to the endogenous differences in magnetic susceptibility. Accumulated iron in certain amyloid-β plaque has also been suggested as a contrast source for detecting the plaques in Alzheimer's disease models (13).

In addition to intrinsic contrasts, cell/tissue-dependent uptake and accumulation of contrast agents can also provide a means to visualize structures with MRI. Typical contrast agents, such as Gd-chelates or iron oxide particles, change T_1 and T_2 relaxation times. Initially, Gd-chelates are used to shorten the T_1 to achieve optimal SNR in short scan time (14, 15). Recently, Gd-chelates, especially with high dose, have been demonstrated to discriminate cortical layers, subregions in subcortical areas like hippocampus, and white matter structures in perfusion fixed *ex vivo* brain and body indicating tissue dependent binding (16-18). Especially, T_2 and T_2* are reduced significantly, making stronger T_2

contrast between myelinated axonal fibers and myelin-rich gray matter layers (19). However, due to low tissue permeability and short blood half-life *in vivo*, the 'staining' by Gd-chelate can only be done by perfusion/fixation and immersion in fixative that doped with Gd-chelate for a long period and imaged *ex vivo*. Besides, not entire brain can be penetrated well by Gd-chelate even with very long (eg, 9 weeks) immersion. Such unequal distribution would contribute to the contrast seen in certain subregions.

Mn^{2+} has been demonstrated to be very useful for mapping neuronal function, connections and brain anatomy *in vivo*. As a Ca^{2+} analogue and a required trace metal in the body, Mn^{2+} can enter cells via Ca^{2+} channel and other transport mechanisms. In the neuron, Mn^{2+} can be transported through microtubule in axons and cross synapses to be taken up by the postsynaptic neurons (for review, see 20). Due to its activity, cell type and density dependent uptake, a number of cytoarchitectural features in the brain, such as layers in the hippocampus, cerebral cortex, olfactory bulb, and cerebellum have been differentiated *in vivo* after simple systemic administration of Mn^{2+} (21-23). Even smaller neural units within a functional layer, such as the individual olfactory glomeruli in the rodent olfactory bulb, could be detected (24). With intraocular injection, cellular layers in the retina can be differentiated (25). Furthermore, since the Mn^{2+} transport across a synapse relies on presynaptic release and postsynaptic uptake, and depends on the strength of connections and plasticity in a neural system, it should thus be possible to produce quantitative indices of Mn^{2+} movement through a neural system after an activity-based representation is initiated, and hence map the strongest functional connections through that system (26). This is demonstrated in mapping odorant induced enhancement in the specific glomeruli and mitral cell layers in the mouse olfactory bulb (27). An advantage of the manganese-enhanced MRI (MEMRI) method is that the biological tissue half-life of Mn^{2+} is about a week in the brain (28), it allow injection in normal behaving animal and acquisition with

high-resolution imaging afterward. However, toxicity has always been a concern for Mn^{2+}, especially when high dose is needed to optimize the contrast. Since tissue half-life of Mn^{2+} is quite long, fractionated injection across several days (29) or slow infusion by osmotic pump (30) could be considered to maximize the dose while minimize toxicity. Besides *in vivo* application, *ex vivo* staining using Mn^{2+} is also demonstrated and shown to enhance structures different from Gd-chelates that may be due to their penetration, compartmentalization and binding properties (16, 17).

With these endogenous and exogenous contrasts, new information could be derived to characterize diseases at exquisite resolution. However, certain contrast mechanisms are still unclear or non-specific, and hence will need to be investigated further or combined with other methods to understand more specific biological information they may reflect. Further development of contrast agents for 'active' or 'targeted' staining will be needed.

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