New Application of Nanoparticle based 21 Tesla MRI of Mouse Brain

Structural Segmentation and Volumetrics

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ABSTRACT

The intact mouse brain high resolution MRI is becoming state of art. Two major approaches are used: 1. ultrahigh 21 Tesla magnetic field MRI in visualizing anatomical structural details; 2. the use of nanoparticle based datasets revolve around the creation of a probabilistic brain atlas as a normalization anatomical templet useful in variety of applications. One of the major applications is localization of anatomical landmarks and visualization of brain structures to identify them with validation process. In brain, cortex, hippocampus structures such as dentate gyrus made of granule cells are widely accepted as possible MRI visible structures. These major anatomical sites predict the drug or neuropharmaceutical compounds induced morphological changes. Coregistration of MRI visible neuroanatomical regions with histological identification provides finer details and different MRI contrast mechanisms.

Key words: Mice, brain,MRI,nanoparticle

1.2. Nanoparticle imaging contrast agent Preparation

In general, polymeric nanoencapsulation methods, which combine sonication and nonsolvent temperature induced crystallization steps include (a) providing active agent nanoparticles having an average diameter between about 5 and about 100 nm; (b) treating said active agent nanoparticles (e.g., a superparamagnetic material) with an anionic surfactant to form modified active agent nanoparticles; (c) mixing the modified active agent nanoparticles with a solution of a polymer in a solvent at a first temperature, which is greater than the melting temperature of the polymer and less than the boiling point of the solvent to form a first mixture, said mixing comprising the use of sonication; (d) mixing a non-solvent with the first mixture to form a second mixture, the non-solvent being a non-solvent for the solvent and for the polymer and having a boiling point greater than the melting temperature of the polymer; (e) sonicating the second mixture to form an emulsion; and (f) cooling the emulsion to a second temperature and at a rate effective to precipitate polymeric nanoparticles comprising the polymer with the modified active agent nanoparticles dispersed therein.

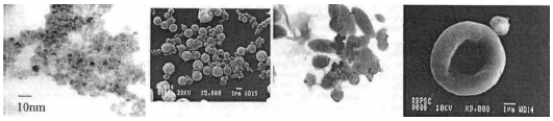

Figure 1: (on left) 35 nm sized paramagnetic iron oxide myoglobin particles.(on right) A comparison of nanoparticle with RBC is shown.

1.3 Advantages of ultrahigh resolution MRI microimaging:

Recently, several advantages of ultrahigh magnetic field have been reported to achieve high resolution microimaging [1]. Major advantages are high ultra high contrast, high SNR, multicontrast, high resoluiton DTI etc. However, inhomogeneity also linearly grows at high magnet field strength. Disadvantages of ultrahigh magnet field strength are not reported as safe technique or remains to establish the disadvantages of high magnetic fields.

1.4 Application of ultra high resolution MRI of mice brain

Notable examples are following: 1. Mice brain atlas in localization of site-specific damage: Alcoholic encephalopathy; 2. Transgenic knock-out mice brain and effect of alcohol, colchicine etc.

2. MATERIAL AND METHODS

2.1 Microimaging of brain:

Bruker 21 Tesla magnet and Micro-2.5 microimaging system is available in imaging studies. USPIO iron-oxide nanoparticle injected mice excised brains immersed in a perflourinated solution can generate T2*-weighted datasets with a three dimensional MSME and gradient-echo pulse sequence (TE/TR = 7.5/150 ms) at a 15 µm isotropic resolution in 5 hours and T2 and diffusion tensor weighted MR data sets with a 3 dimensional spin-echo pulse sequence

2.2 Validation of brain structures:

The mouse brains (n = 3) were perfusion-fixed for MRI microscopy. The dentate gyrus of dorsal hippocampus in both left and right sides showed collateral control. The susceptibility matching eliminates both extraneous proton signal and coil loading. (see the Figure 1).

The axial, coronal and sagittal planes in 2D slices reconstruct the 3D brain atlas by volume rendering. The main features at ultrahigh resolution are: distinct gray and white matter tissues and neuron proton density in different structures (shown in Fig 2).

2.3 Mice brain atlas:

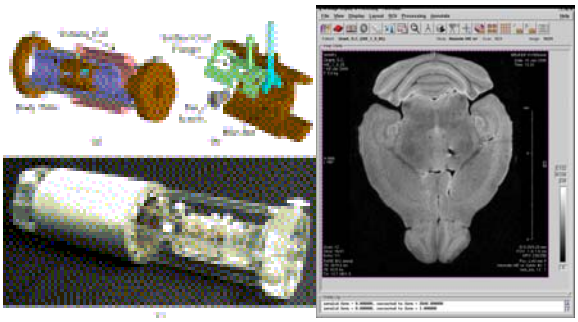

Figure 2: (Top on left) A Rf coil with microimaging; (Bottom on left) The attachment of animal holder for live Animal experiments; (on right)T2* weighted high resolution mice brain image is shown with clear layers of cortex, central semi ovale, hippo-campus, frontal, parital and occipital lobes with cerebellum

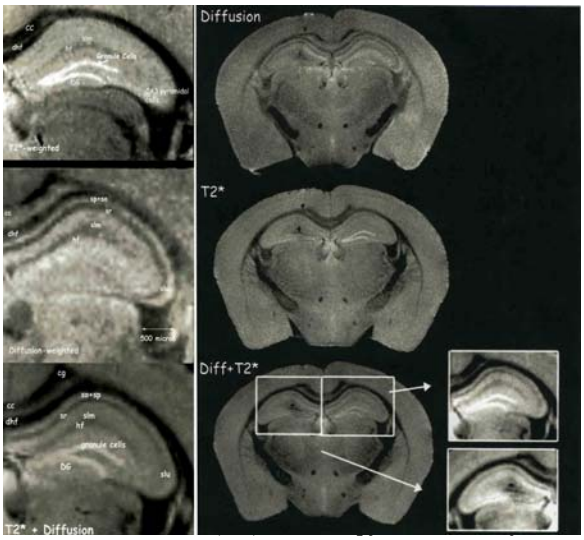

Figure 3: (on left panels) Enlarged section of dentate gyrus by Diffusion (top), T2*(middle) and Diff+T2*(bottom) (on right panels) Whole hippocampus is shown by diffusion-weighted MRI microimages (top) display the stratum oriens/stratum pyramidale (so+sp), stratum radiatum (sr), stratum lacunosum moleculare (slm) and lucidum (slu); T2* weighted images (middle); and Co-registered T2* - and diffusion-weighted MRI microimages (at bottom) provided the better information of hippocampus fissure (hf) and corpus callosum (cc).

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Figure 4: (on left panels) A 3D Gradient echo image set axial, coronal and sagittal planes. (on right panels) 3D-FLASH image set, are shown in 3 plane with clear structures within 15 seconds. Notice the less r and clear contrast as benefit over Gradient Echo images

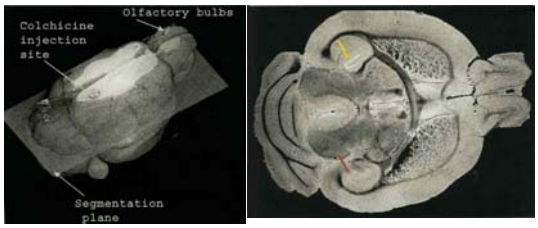

***Figure 5: (a) 3D reconstruction showing the CI site (3D GE);(b) axial slice through the 3D dataset at the segmentation plane of the top image. The elimination of the granule cells in CI hemisphere(red arrow) is evident in contrast to the contralateral control(yellow)**

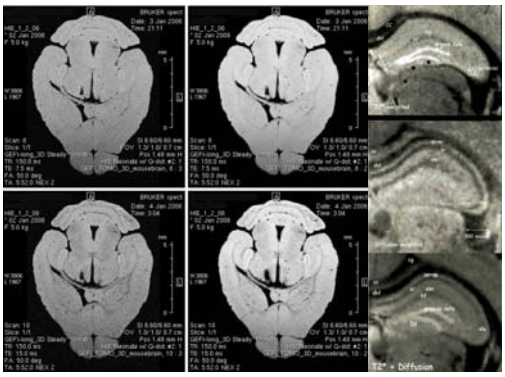

***Figure 6: T2* weighted, diffusion weighted and combined MRI images of a coronal view. The hyperintense line corresponds to granule cells of the dentate gyrus. In the hemisphere shows disruption of hippocampus layers is slightly apparent. Bottom: Comparison of normal hippocampus shows atrophy.**

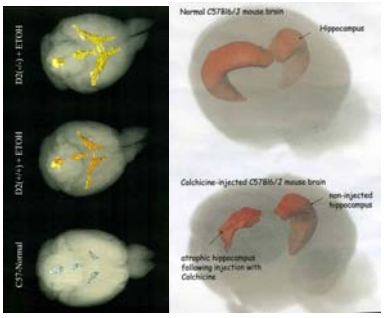

Figure 7: Cerebrospinal fluid (CSF) filled ventricular spaces have been highlighted in color. D2 knockout mouse brain shows large ventricles compared to the D2 (+/+) mouse brain(middle) following alcohol exposure. The control brain displays smaller ventricles than either of treatment groups. It suggests that D2 receptor modulate EtOH induced toxicity.

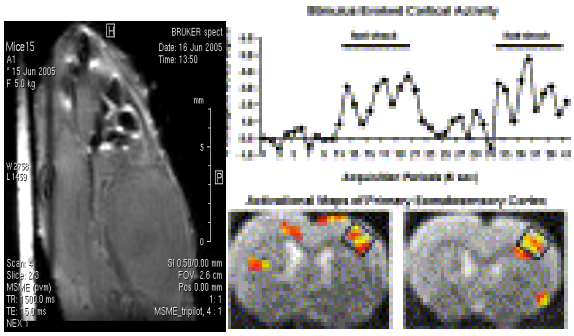

Figure 8: (on left) A nanoparticle filled tube with nanoparticle injected mice brain is shown. Notice the signal loss in air cavities.
(on right) A possibility of functional MRI is shown using live mice using light blinking event and neuroactivation in brain.

4. APPLICATION OF MRI MICROSCOPY:

4.1. D2(-/-) and D2 (+/+) Mutants and Influence of Ethanol

Dopamine (DA) transgenic mice have been genetically engineered and used to study the relevance of specific DA receptors for the acute response to drug abuse as well as predisposition, toxicity and relapse and neurodegenerative diseases. The dopamine appears to play a critical role through all phases of central nervous system ontogeny such as cell proliferation, neural migration growth maturation and synaptogenesis. However, it is not known how DA influences brain maturation and how dysfunction of certain DA receptors might influence overall brain morphology in DA transgenic mice using high resolution MR microscopy as part of longitudinal study.

4.2 D2 knockout mice (D2 (-/-) and overexpressed (D2 +/+) C57BL6/J mice

The animals were exposed to ethanol for 10 months. After exposure (age about 20 months) the mice were euthanized and formalin-perfusion fixed. MR microscopy was performed on the excised brains for gross volume measurement and identification of MRI visible regions.

4.3 Future attempts for Functional MRI Imaging

At low magnetic field, mice fMRI experiments showed promise to evaluate the brain functions and neuroactivation events. As of today, the possibility of 21 Tesla fMRI experiments is unknown.

5. MOLECULAR IMAGING WITH MRI

There is rapidly growing interest in making radiological imaging techniques sensitive to specific molecules or biological processes. The marriage of the wealth of information being generated from molecular genetics, the large number of mouse models of human diseases, and imaging is the engine driving the development of molecular imaging. Nuclear imaging techniques, optical imaging techniques, and MRI have all had aspects of molecular imaging as part of their makeup for a number of years; however, there is renewed vitality as more and more is learned about the pathophysiology of disease and more and more targets for therapeutic intervention become available.

Molecular imaging of the brain offers significant challenges, primarily due to the problem of delivering agents through the blood-brain barrier. Nonetheless, several recent studies demonstrate the potential. For example, amyloid plaque can be imaged in mice with targeted MRI contrast after disrupting the blood-brain barrier.

In transgenic mice engineered to produce a large number of amyloid plaques specific accumulation of the MRI agent enabled detection of plaques as an example. This strategy of coupling MRI contrast agents to peptides or antibodies that recognize specific targets is rapidly growing area in molecular imaging. Creative ways to get large molecules through the blood-brain barrier will be crucial to the general success of this strategy.

Another very promising area for molecular imaging is to monitor cell migration *in vivo*. Here the idea is to label a specific cell population, either *in vitro* or *in vivo*, with MRI contrast agents and then to follow the movement of these cells in the animal. Typically nanometer-sized iron-oxide-based contrast agents are used and some form of endocytosis is used to get these particles into cells. The advantage of these iron oxide particles is that it has been shown that single cells can cause sufficient contrast change to be detected by MRI. There have been recent examples of MRI-based cell tracking to study diseases of the brain.

5. CONCLUSION

MRI microscopy is extremely valuable in in vivo localization and identification of brain anatomical structures in the excised mouse brain. The present study demonstrates the hippocampus structures using T2* and diffusion weighted contrasts at 21 Tesla with details of neuroanatomical features throughout the brain. Within the hippocampus, site-specific, dose-specific, dose-dependent injections of colchicine selectively destroyed the granule cell layer of the dentate gyrus. The lesion volume measurement technique, however, suffers from the artifact of hyperintense cellular layer of hippocampus. The anisotropy of water diffusion in these ex vivo samples may visualize the mossy fiber pathways of the hippocampus. The possibility of fMRI at 21 Tesla is controversial due to mainly uncertain effects of ultrahigh magnetic fields on live or dead brain or any other tissues.

6. ACKOWLEDGEMENTS

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