High Throughput Microimaging of the Mouse Brain

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INTRODUCTION

With the growing interest in mouse models of human disease[1], especially neurological models, new methods to rapidly assess neuroanatomy need to be developed. Conventional histology, used to study tissue microstructure, is generally accepted as the gold standard for determining changes in anatomy**.** However, histology suffers from several drawbacks, including: distortions with cutting, complicated preparation, poor 3D structural visualization, and destruction of the original sample.

Magnetic resonance imaging (MRI) can circumvent these drawbacks. Unaffected by motion artifacts, fixed MR imaging achieves much higher resolution than live imaging and computer visualization of an isotropic 3D data set provides maximal flexibility. We present a robust experimental design to fix a mouse brain in the skull and methods for the parallel acquisition of three high-resolution MR datasets of such brains in overnight scanning sessions.

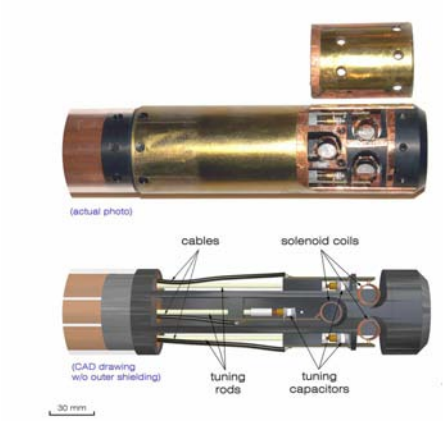

Figure 1. Design and implementation of three solenoid parallel coil probe for imaging of fixed brains in the skull.

METHODS

All animal protocols were approved by the Hospital for Sick Children Animal Care Committee. Mice were anesthetized by intraperitoneal injection of Rompun and Ketamine (20 and 100 mg/kg). The animals were then perfused with an left-ventricular catheterization with 30 ml of room temperature phosphate buffered solution (PBS), followed by a fixation pass using 30 ml of iced 4% paraformaldehyde (PFA). Once fixed, the mice were decapitated and the skin, lower jaw, ears, nose tip and zygomatic bones were removed. The remaining skull structure was placed in a 4% PFA bath for 12 hours at 4° C, and then transferred to a solution containing 1X PBS and 0.01% sodium azide for 5 days at 15° C on a nutator to remove excess PFA. The brains were then transferred to the same solution including a 2 mM concentration of Prohance© for at least 7 days to diffuse through the skull. Prior to imaging, each skull was blotted and immersed in Fluorinert© within a plastic tube diameter = 13 mm, length = 40 mm which was then vertically mounted in the probe head. The mean values of T1 and T2 in brain parenchyma were measured to be 175 ± 16 and 28 ± 2 ms.

A custom-built array of solenoid coils was used to image three samples concurrently. Fig. 1 shows the instrument with three transmit/receive coils, equally spaced and individually shielded. The 8-turn solenoidal coils are over wound at the ends to provide uniform sensitivity to within 10% over a length of 26 mm. The entire assembly was designed to fit within a 60 mm insert gradient set (rise time = 150 µs, maximum gradient strength = 1,000 mT/m). Imaging was completed on a 7 T Varian INOVA scanner using a fast spin-echo (FSE) sequence (TR/TE = 325/8 ms) with 6 echoes (the center of k-space acquired on the fourth echo, TE_{eff} = 32 ms), FOV = 12 mm x 12 mm x 25 mm, acquisition matrix of 432 x 432 x 780, and NEX = 4. A variable gain acquisition was used to accommodate the large dynamic range of the 3D acquisition[2]. The total imaging time was 14 hours, and yielded an image with 32 µm isotropic voxels.

RESULTS

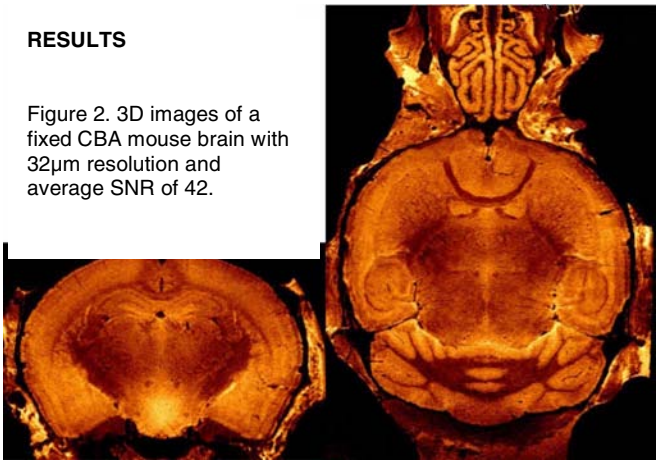

Figure 2. 3D images of a fixed CBA mouse brain with 32µm resolution and average SNR of 42.

APPLICATIONS

Over the past six months, we have used this method to image 225 brains. Although much of this data is still being analyzed, we have already shown that:

(1) the average RMS displacement of genetically identical brains registered to a common unbiased atlas is approximately 121 µm,

(2) there is a statistically significant difference in the volume of the striatum in a mutant model of Huntington's disease compared to wild-type littermates,

(3) a DISC1 mutant mouse model of Schizophrenia shows neuroanatomical anomalies.

CONCLUSION

The protocol outlined above yields high-resolution 3D neuroanatomical images in efficient scan time (overnight) and with good SNR. The brains are maintained in their natural conformation in the skull conserving original anatomy. With the three-coil array, our effective throughput is one brain every 4 hours with 32 µm isotropic resolution. This compares favorably in the literature where scan times per brain are 10 hours with 50 µm isotropic[3] or 5.5 hours with 47 µm isotropic resolution at 17.6 Tesla[4]. The design presented here provides an ideal method for fixed neuroanatomical imaging for phenotyping studies.

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