Diffusion Tensor Imaging and Tractography of the Isolated Rat Hippocampus

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INTRODUCTION

The hippocampus is a critical structure for semantic memory formation that is particularly vulnerable to insult. Characterizing the normal hippocampal cytoarchitecture with diffusion tensor imaging (DTI) is an important step to understanding how diverse disease processes, such as Alzheimer's or traumatic injury, structurally alter the hippocampus and damage its function. The hippocampus also provides an interesting tissue substrate for DTI because it is atypical compared to gray or white matter since it contains distinct layers with unique cytoarchitectural features; densely packed neuron cell body layers are surrounded by layers of neuropil, such as stratum radiatum, where relatively few neuron cell bodies are interspersed with glia and a complex interdigitation of dendrites and axons projecting from local or distant regions [1]. The hippocampus also contains well-characterized fiber tracks such as Schaeffer collaterals, perforant and mossy fibers that are significantly smaller than fiber bundles found in typical white matter structures such as the corpus callosum [1]. To better understand this unique, challenging structure, this study characterized the isolated rat hippocampus using diffusion tensor imaging with 50-µm in-plane resolution.

METHODS

Rats were perfusion-fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) (pH 7.4, 300 mOsm/kg). The hippocampi were dissected medially from each hemisphere and immersed in fresh fixative for 8-10 days, then washed overnight in PBS prior to imaging. Samples were imaged with a 5-mm birdcage coil inside a 600-MHz narrow-bore spectrometer. The imaging protocol used a pulsed gradient spin-echo pulse sequence (TR/TE = 1000/28.3 ms, 10-14 averages) to acquire continuous axial slices along the septo-temporal axis of the hippocampus with 50 x 50 x 200 µm resolution (total time = 12 hrs). After the first image was collected without diffusion-weighting, 21 diffusion-weighted images (diffusion time = 17 ms) were acquired with 305 mT/m diffusion gradients (b = 1250 s/mm^2) applied in directions determined by the tessellations of an icosahedron on the hemisphere. The resulting data were used to determine the apparent diffusion tensor at each voxel [2] from which mean bulk diffusivity (<D>), fractional anisotropy (FA) and fiber direction were calculated [3]. The <D> and FA indices were compared statistically using an ANOVA. Data from this study were also used to create colormaps and fiber-tracts were calculated in ortho- and retrograde directions from selected seed points using a streamline-based algorithm that employs 4th-order Runde-Kutta technique.

RESULTS

Six rat hippocampi were imaged for the diffusion tensor protocol with mean signal-to-noise ratios of 50.6 ± 4.4 in the diffusion-weighted images (b = 1250 s/mm^2). Fig. 1 illustrates typical data obtained from the DTI protocol used in this study (this slice was from the midpoint of the septo-temporal axis). The bulk mean diffusivity, fiber color map and fractional anisotropy appeared heterogeneous and will be discussed below. Table 1 shows <D> and FA values for selected regions of the rat hippocampus. Several of these differences appear statistically significant ($P < 0.05$). Fig. 2 illustrates tract-tracing from seeding a small region of CA3 apical dendrites.

DISCUSSION

Previous diffusion tensor studies at lower resolution suggested the presence of water diffusion anisotropy in the fixed mouse hippocampus [4], but did not describe individual layers within the hippocampus and may have been confounded by volume averaging with adjacent white matter structures. High resolution DTI, like the present study, is essential for investigating water diffusion in the hippocampus and its components since some hippocampal layers are only 50 – 70 µm thick. In Fig. 1B there appear to be at least 3 distinct mean bulk diffusivities (pyramidal neurons > molecular layer > fimbria). This data (table 1) contradicts previous reports that suggested mean bulk diffusivity in nervous tissue is homogeneous [5]. Fractional anisotropy also varies significantly for different regions of the rat hippocampus. Myelinated white matter fibers projecting to other brain regions, like the fimbria, were bright in Fig. 1D and have FA values (Table 1) similar to the corpus callosum, but other regions of the hippocampus, such as stratum radiatum, have intermediate FA values atypical of gray or white matter. Such regions are composed of many different fiber projections that interdigitate in complex ways on a very small scale [1]. The trilaminate appearance to the dentate gyrus blades in Fig. 1D suggests the polymorphic layer can be detected with this contrast method. The color fiber map also reveals several features not typically observed in MRI of rat hippocampus. For example, the mossy fibers projecting from granule cells to CA3 and the perforant pathway penetrating into the crest of the dentate gyrus can be observed. In the stratum lacunosum-moleculare and stratum oriens, the principal "fiber" orientation appears predominated by axonal projections running perpendicular to the primary dendritic axis of CA1 pyramidal neurons, whereas principal "fiber" orientation in stratum radiatum appears dominated by apical dendrite orientations perpendicular to the tissue plane. Fig. 2 illustrates a preliminary attempt at fiber tracking in the rat hippocampus and may reflect a divergent axonal connection from CA3 neurons to apical dendrites of several CA1 neurons in the stratum radiatum and axonal projections from those CA1 neurons to the alveus. These projections match anatomical descriptions [1] and provide preliminary evidence that high resolution DTI may permit tractography of the intrahippocampal fibers, but will require further confirmation.

REFERENCES

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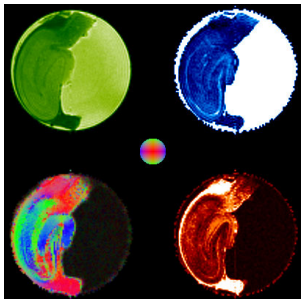

Figure 1 – Images of signal without diffusion weighting (A), bulk mean diffusivity (B), color fiber map (C) and fractional anisotropy (D).

Region	<D> (µm^2/ms)	FA (no units)
Fimbria	0.178 ± 0.065	0.91 ± 0.06
CA3	0.305 ± 0.044	0.39 ± 0.09
Molecular layer	0.206 ± 0.014	0.58 ± 0.06
Subiculum	0.227 ± 0.053	0.33 ± 0.03
PBS	1.743 ± 0.028	0.06 ± 0.00

Table 1 – bulk mean diffusivity and fractional anisotropy for selected regions of the hippocampus [mean ± SD].

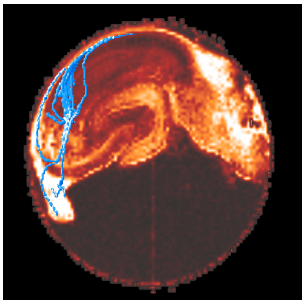

Figure 2 – Example of potential intrahippocampal fiber projections (blue surfaces) in the rat hippocampus (2D projection on FA image).