In vivo study of the development of hippocampal subregions by high-resolution diffusion weighted imaging

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Introduction

Hippocampus is a prominent component of the nervous system, whose neurons are arranged in distinctive layers and divided into subregions. The lesion in each hippocampal subregion is related to impaired memory storage. The diseases such as amnesia and Alzheimer's disease have been found to be accompanied by gradual loss of pyramidal neurons in hippocampal subregions (1). However, most studies of the hippocampal tissue architecture relied on histological techniques, which need to sacrifice animals thus renders longitudinally study the morphological changes in the brain unfeasible. Therefore, an *in vivo* non-invasive methodology is needed. Magnetic resonance (MR) imaging is able to provide high-resolution structural and functional information in the brain of a living animal. However, the fine structures of hippocampal subregions were failed to be distinguished on conventional MR contrast. Recently, diffusion tensor imaging (DTI) has been applied on the fixed mouse brain (2). Our study focused on the *in vivo* delineation of hippocampal subregions using high-resolution DWI with less scan time. Moreover, for the further understanding of the morphogenesis of hippocampal subregions, a longitudinal study using high-resolution DWI with three diffusion directions were performed to provide the temporal changes of ADC values in each subregions during development. The efficiencies of the ADC values of using three diffusion directions in the delineation of hippocampal structure were discussed.

Material and Method

Animal Preparation: Total four male SD rats were scanned at age periods of new born (P14), juvenile (P28), young adult (8 weeks) and adult (12 weeks). The anesthetization of animals were induced with 4 % isoflurane in O_2 (4 L/min) and maintained at 1.5 %. The body temperature was kept with warm water circulation at 37±1°C.

MRI Protocols: All experiments performed on a 9.4T Bruker Avance 400(Bruker Biospin, Germany). Axial images of T2WI and DWI were employed with matrix of 256*256, FOV of 2.56 cm, slice thickness of 1 mm and in plane resolution 100*100 μm^2. For DWI, TR of 1.5s, TE of 32 msec and 12 averages were used, four DWI images were acquired with different diffusion gradients (b0, x, y and z). For T2 map, TR of 8s, TE was from 10 msec to 80 msec (spacing was 10 msec) and one average was used. The ADC and T2 maps were performed by MRVision (MRVision Co.,MA,USA). The color map was calculated by AMIRA (TGS Inc., San Diego, CA) base on the signal intensity of DWIx.

Histology: Rat brains were perfused, removed, cryoprotected, sectioned at 20 μm, and stained with the antibody against voltage-gated calcium channel 1.2.

Result and Discussion

Fig. 1 displays the color map modified from DWIx, ADCx map, Trace map and brain section on a juvenile rat (P28). DWI results clearly showed superior contrast for the delineation of laminar structures of hippocampus than T2 and Trace map. This suggested the high anisotropy nature in hippocampus. Comparing with histology, layers in DWIx image were assigned: 1) stratum oriens (SO) of CA3; 2) pyramidal cell layer (PCL) of CA3; 3) stratum radiatum (SR) of CA3; 4) hippocampal fissure; 5) SR of CA1; 6) PCL of CA1. In our results, the contrasts of PCL and SR were invert on DWIx, which may be due to that these two adjacent structures was perpendicular (2). The layers in hippocampus can be more clearly observed upon the color map that based on the signal intensity of DWIx. The high efficiency of x direction DWI at P28 rat brain could be resulted from the x direction properly perpendicular to pyramidal cell layers and dendrites. Furthermore, we applied high-resolution DWI to study the morphogenesis of hippocampal pyramidal cells during the early postnatal period. Fig. 2 shows the ADC values of PCL and SR in CA3 at different postnatal stages, as the representatives of the postnatal development of pyramidal cell body and apical dendrites, respectively. Our results show that, after the quantitative measurement of the ADC values with three diffusion directions from P14 to 12 weeks old rat brains, the ADC values of PCL and SR in CA3 both decreased significantly after P14. It is known that PCL, SR and SO continuously to grow up at different rates until P21. The pyramidal cell shape changed from round to triangular, while the apical dendrites arborized and increased (3). The observed changes of ADC values probably reflected the spatial changes of pyramidal cell shape and the branching of apical dendrites, respectively. Interestingly, our results suggest that ADCx and ADCy may be more sensitive than ADCz, since the significant decreases of ADCx and ADCy was recognized from P28 in comparison with P14. After P28, the reduced changing of ADCxy in PCL may be due to the completed cell body formation. In contrast, the significantly decreased ADCy was still observed in apical dendrites of CA3 at 12W in comparison with P28, suggesting the proceeding maturation of dendrites. Our results indicate DWI with three different directions is efficient enough to delineate the morphological changes of hippocampal cell body and dendrites during early postnatal stages.

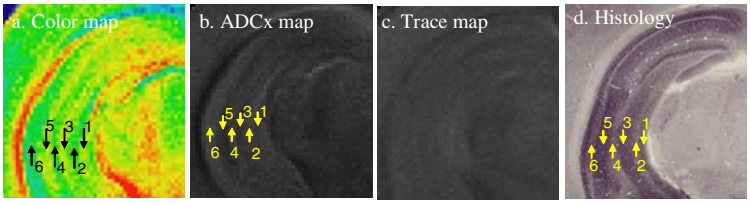

Fig.1 Representative images of the rat brain at juvenile (P28). (a) Color map modified from DWIx, (b) ADCx map, (c) Trace map, and (d) histology. Hippocampal subregions are : 1) stratum oriens (SO) of CA3; 2) pyramidal cell layer of CA3; 3) stratum radiatum (SR) of CA3; 4) hippocampal fissure; 5) stratum radiatum of CA1; 6) pyramidal cell layer of CA1

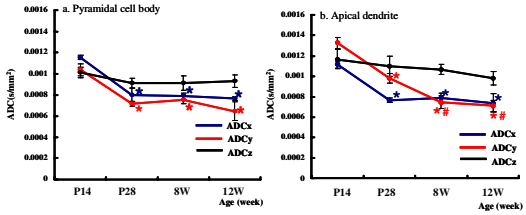

Fig.2 Changes of ADC values of hippocampal subregions at different age. (a) ADC values of the pyramidal cell layer of CA3, (b) ADC values of apical dendrites of CA3. Blue was ADCx, red was ADCy and black was ADCz. ANOVA was used to estimate ADC values between these four groups. (* p<0.001 vs. P14, # p<0.001 vs. P28)

Conclusions

The present study showed the ability of high-resolution DWI to delineate the development of hippocampal subregions. We demonstrated the individual layer structures of hippocampus *in vivo* using high-resolution DWI. We further showed that the temporal changes of ADC values reflect the maturation of pyramidal cell body and dendrites. Such a time-saving MR approach has potential for studying the neural plasticity such as axonal sprouting and neuronal degeneration in hippocampus *in vivo*.

References

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