Characterizing Brain Development in the Ferret *in vivo* Using Diffusion Tensor Imaging

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Introduction Ferrets are of special importance in studies of brain development because of the brain immaturity at birth. In addition to rapid growth in size, a ferret brain undergoes surface folding during the first month of life, leading to morphological maturity around 4 weeks of age. In previous studies formalin-fixed brains of young ferrets were used to characterize brain development with T_2-weighted MRI and diffusion tensor imaging (DTI) (1); furthermore, regional patterns of brain surface development were shown to be strongly correlated to the diffusion anisotropy (DA) in the cortex during folding (2). However, fixed brains have limited values in longitudinal studies, especially in monitoring disease progression. In this work we present the results of our first longitudinal study of the brain development in a young ferret using DTI. We investigate the relationships between surface folding, T_2, average ADC, and DA in the white matter (WM) and gray matter (GM) of the brain. Such investigations will help improve understanding of the causes for the folding-related brain diseases such as schizophrenia.

Methods A female young ferret (Marshal BioResources) was imaged in an 11.7 T magnet (Magnex) controlled by a Varian INOVA console at the ages of P9, P14, P21, and P30. The gradient insert is 15 cm in diameter, capable of 300 mT/m gradient with a 300-μm rise time. The ferret was kept under anesthesia for less than 3 hours using 1-1.5% isofluorine during each imaging session with the physiological conditions (pulse rate and oxygen saturation level) closely monitored by a pulse oximeter (Kent Scientific). Head motion was prevented by a home-built head holder which was secured to the experiment tray. A commercial (Helmut Stark) volume coil and a home-built surface coil on top of the brain were used to respectively transmit and receive RF signals. At P21 and P30 (and for the adult ferret) TTL-equipped cardiac gating (SA Instruments, Stony Brook, NY) was used to prevent artifacts caused by the pulsatile motion of the blood. All experimental procedures were approved by our institutional review board. A 2D spin-echo (SE) sequence with the diffusion-sensitizing gradients added on both sides of the π pulse was used for data acquisition. Gradients were applied in 7 different directions (including b=0). In-plane resolution was 0.25–0.27 mm isotropically; slice thickness was 0.25–0.4 mm. At P9 and P14 more than 25 slices were acquired to cover a large portion of the brain; at P21 and P30 (and for the adult), due to the delay caused by acquisitions synchronized with the cardiac motion, only 6 to 8 slices were imaged to keep the acquisition time short. Other imaging parameters include: TR/TE = 2000/31 ms, imaging bandwidth = 40 kHz, gradient duration (δ) = 8.4 ms, amplitude = 9 G/cm, separation (Δ) = 13.8 ms, b = 900 s/mm^2.

Results The spin-echo images (b=0, top), the average ADC maps (middle), and the RGB plots of the diffusion tensors (bottom) at similar positions of the same ferret at P9, P14, P21, and P30, along with the images of a different adult ferret (> 3 months of age) are shown in the Fig. 1 (the adult brain is shown at slightly smaller scale). Either SE or ADC maps show that the ferret brain with a fairly smooth surface at P9 became highly folded and morphologically mature at P30. The SE images also show similar contrast between the WM and GM for the young ferret brain at all 4 time points. The reversed contrast in the adult brain indicates that although the brain is fully folded by P30, the axons in the WM have not been fully myelinated. This is confirmed by the ADC maps: by P30 the average ADC in WM (~2.5X10^{-3} mm^2/s) is much higher than that in GM (<1.0X10^{-3} mm^2/s), while in adult ferret the average ADC in WM decreased significantly and WM is indistinguishable from GM in the ADC map. From the RGB maps it is clear that water diffusion is strongly anisotropic in the GM at P9 (indicated by arrow heads). This DA decreased with time and disappeared completely at P30. For the WM, no significant difference was observed among the 4 time points of the young ferret. However, DA in WM becomes much more prominent in the adult ferret brain, which is a result of myelination.

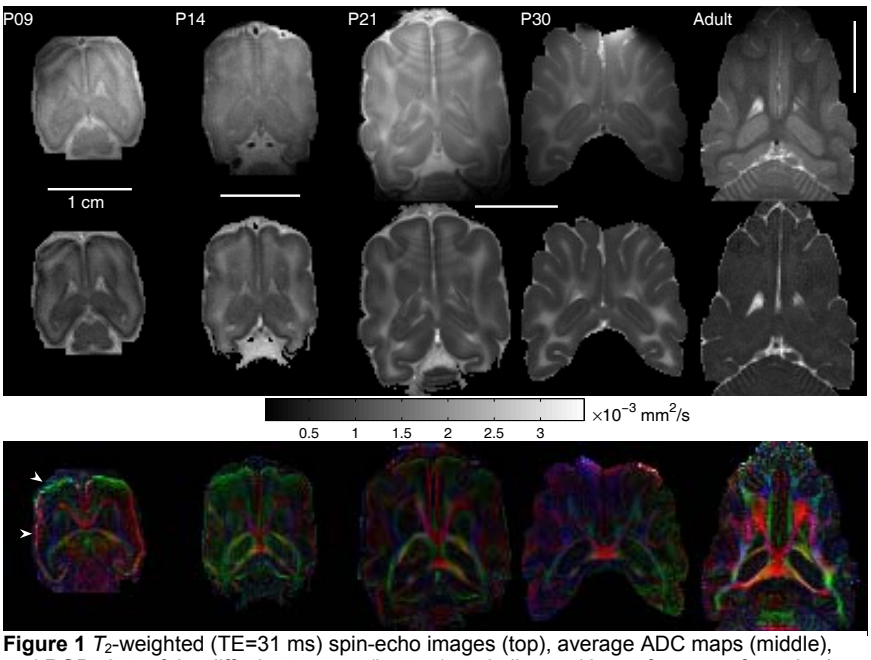

Figure 1 T_2-weighted (TE=31 ms) spin-echo images (top), average ADC maps (middle), and RGB plots of the diffusion tensors (bottom) at similar positions of a young ferret brain at P9, P14, P21, P30, and an adult ferret brain. The colors in the RGB plots represent: red: left–right; green: superior–inferior; blue: ventral–dorsal. All scale bars are 1 cm long. The arrow heads indicate areas in the cortex where diffusion anisotropy vanishes during brain development.

Discussion Our results of the young ferret at 4 time points demonstrate that during the first month after birth the ferret brain becomes fully folded. Folding is simultaneously accompanied by the vanishing of the DA in GM. This indicates that folding is predominantly associated with increase in cortical area due to arborization of cortical neurons. During this period, no obvious changes in WM were observed. Our results are consistent with the previously reported data from fixed brains (1,2). These findings lead us to suggest that the WM myelination is independent of the folding processes since it only happens after the brain is fully folded. This observation is relevant to the proposed tension-based theory of cortical folding (3) since tension must be borne by unmyelinated axons (4). Further studies with more quantitative analysis of the diffusion tensor will be conducted to consolidate our findings. The main problem of our current imaging method is the long acquisition time caused by both acquisition scheme (SE) and cardiac gating, which prevents us from imaging the entire brain. By contrast, echo-planar imaging (EPI) DTI is faster and navigator echoes (5) instead of cardiac gating can be used for motion correction. These acquisition methods will be tested in future studies.

Conclusions We have demonstrated the feasibility of characterizing brain development in young ferrets using DTI *in vivo*. We investigated the changes in T_2, average ADC, and DA during cortical folding. Our results are consistent with previously reported findings on fixed brains. The results suggest that cortical folding and WM myelination happen sequentially and are independent.

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References (1) Barnette AR et al. Pediatric Research 2009;66:80 (2) Kroenke CD et al. Cerebral Cortex 2009;19:2916 (3) Van Essen DC et al. Nature 1997;385:313 (4) Xu G et al. J Biomech Eng 2010;132:071013 (5) Holdsworth SJ et al. MRM 2009;62:1629