T2-Weighted MRI Visualizes Cortical Layers in Living Mice

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Introduction

Based on its cyto- and myeloarchitecture the mammalian neocortex is organized into 6 layers. The molecular and cellular mechanisms leading to this organization have only recently begun to be understood (1). For example, genetically modified mice were used to analyze the influence of distinct transcription factors on the fate of cortical neurons. Complementing histological analyses, MRI allows for the detection and individual follow-up of more global aspects of the cortical architecture in living animals without suffering from shrinking or other preparation artifacts. Of course, the visualization of cortical layers by MRI is challenging and requires both high spatial resolution and an adequate contrast-to-noise ratio. So far, most MRI studies were therefore performed in vitro or relied on the administration of Mn^{2+} to enhance the layer contrast. Recently, the delineation of up to 5 cortical layers in adult C57/bl6 mice could be shown by strongly T2-weighted MRI (2). The aim of this study was to explore this capability to monitor age-related differences in the cortex of normal C57/bl6 mice and layer alterations in cortex-specific Pax6 conditional knockout (*Pax6cKO*) mice (3).

Methods

C57/bl6 mice (at the age of 15 weeks and 1 year) as well as adult *Pax6cKO* mice (1.5 years) and their control litter mates were anesthetized by 1-1.5% isoflurane in a mixture of oxygen and ambient air and positive pressure ventilated via endotracheal tube. T2-weighted MRI was performed at 9.4 T (Bruker Biospin) and 40 × 40 × 300 μm^3 spatial resolution with the use of a fast spin-echo sequence (TR = 4200 ms, effective TE = 65 ms, 8 differentially phase-encoded echoes, 14 sections, matrix 512 × 512, measurement time 1 hour). Signal reception employed a 4-element phased-array surface coil (Bruker Biospin).

Results and Discussion

Horizontal T2-weighted images of adult C57/bl6 mice revealed a 5-layer structure of the cerebral cortex (Figure A and B, yellow bars). This layering partly correlates with the local cellular density as shown by a corresponding cresyl violet staining of a 10 months old C57/bl6 mouse. In contrast, young adult mice (Figure D) present with a small layer of higher signal intensity inside the two darker layers (Figure D, arrows), which presumably reflects a not yet fully completed myelination. *Pax6cKO* mice (Figure E) are characterized by an almost complete loss of cortical layers II, III, and IV and an ectopic superficial location of an enlarged set of pyramidal neurons. These histologically proven alterations nicely correspond to the observed 3-layer structure by MRI (Figure E, yellow bars).

Concluding remarks

High-resolution MRI with pronounced T2 contrast allows for the detection of cortical layer-like structures in mouse brain in vivo, which resemble the histologically defined 6-layer structure of the mammalian cortex. For the first time developmental changes in the cortical architecture of healthy mice as well as layer alterations in *Pax6cKO* mice could be visualized by in vivo MRI. Most likely the observed contrast depicts both cell density and myelin content. Although it cannot be expected that MRI contrasts completely match to the histological mapping of the cortex, the present findings further enhance the scientific potential of in vivo MRI techniques for studying experimental animals.

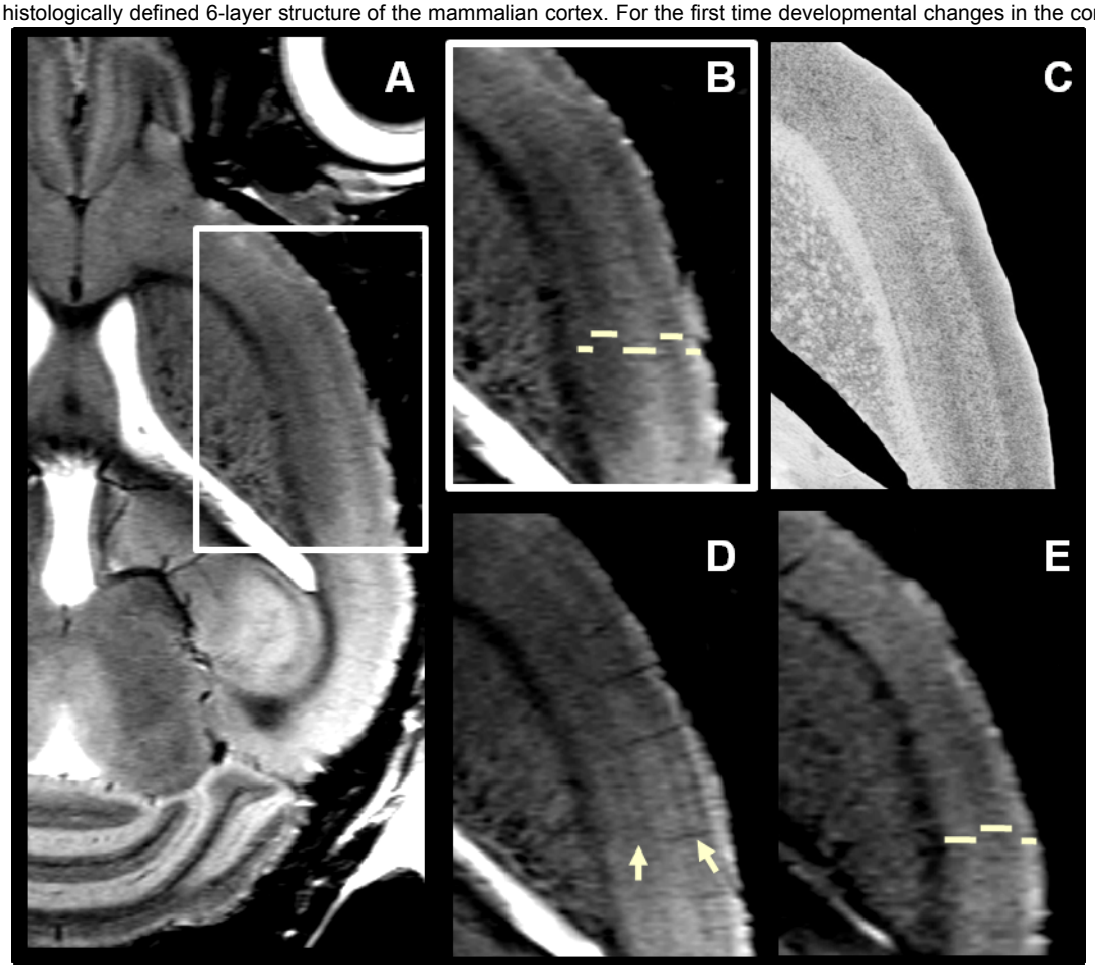

Figure: T2-weighted MRI (TE = 65 ms) of the mouse cortex in vivo at 40 × 40 × 300 μm^3 resolution. **A:** Adult C57/bl6 mouse, **B:** zoomed area. **C:** Cresyl violet staining of similar section (http://www.mbl.org.). **D:** Young C57bl6 mouse. **E:** Adult *Pax6cKO* mouse.

References

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2. Boretius et al, NeuroImage 47:1252-1260, 2009
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