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Morphological maturation of the mouse brain: An in vivo MRI and histology investigation

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ABSTRACT

With the wide access to studies of selected gene expressions in transgenic animals, mice have become the dominant species as cerebral disease models. Many of these studies are performed on animals of not more than eight weeks, declared as adult animals. Based on the earlier reports that full brain maturation requires at least three months in rats, there is a clear need to discern the corresponding minimal animal age to provide an "adult brain" in mice in order to avoid modulation of disease progression/therapy studies by ongoing developmental changes. For this purpose, we have studied anatomical brain alterations of mice during their first six months of age. Using T2-weighted and diffusion-weighted MRI, structural and volume changes of the brain were identified and compared with histological analysis of myelination. Mouse brain volume was found to be almost stable already at three weeks, but cortex thickness kept decreasing continuously with maximal changes during the first three months. Myelination is still increasing between three and six months, although most dramatic changes are over by three months. While our results emphasize that mice should be at least three months old when adult animals are needed for brain studies, preferred choice of one particular metric for future investigation goals will result in somewhat varying age windows of stabilization.

Introduction

The development of the brain continues well beyond birth and goes through various phases of adolescent changes (Andersen, 2003; Meaney and Stewart, 1981; Spear and Brake, 1983) before it reaches a state in adulthood which is considered a steady state condition. Such steady state is usually assumed when adult animals are studied, be it in the course of neurodevelopmental studies (Tissir and Goffinet, 2003) or for the investigation into cerebral disease evolutions (Adamczak et al., 2014; van Dorsten et al., 1999) and development of corresponding therapeutic strategies (Bockhorst et al., 2008). This requires the definition of a time window in early age when this cerebral maturation has been reached as well as the definition of a time window in progressed age when aging processes start to disturb this steady state. The definition of the onset of adulthood and the end of maturation is the goal of the present study. Often, adulthood of experimental animals was used equivalent to full sexual maturity or to body weight indications only. However, in an earlier study on rat brain maturation we have been able to point out a wide temporal discrepancy of sexual maturity to full brain maturation (Mengler et al., 2014).

With the wide access to studies of selected gene expressions in transgenic animals, mice have become the dominant species for experimental investigations on cerebral diseases, including stroke (Adamczak et al., 2014), neurodegenerative diseases such as Alzheimer's disease (Zempel and Mandelkow, 2014), trauma (Webster et al., 2015) and many more. Many of these studies are performed on animals of not more than eight weeks, declared as adult animals. Based on the earlier reported situation on rats that full brain maturation requires animals with an age of at least three months (Mengler et al., 2014), there is a clear need to discern

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the corresponding minimal animal age to provide an "adult brain" in mice.

Magnetic resonance imaging (MRI) has become an indispensible tool in neuroanatomy. MRI provides information on macrostructural as well as microstructural changes in the intact tissue, without suffering from shrinkage or other preparation artifacts, like shearing or cutting artifacts. The non-invasive character of MRI allows a longitudinal investigation of subjects and can complement histology when studying development, aging or disease models. Especially in postnatal development, MRI has proven useful for the detection and measurement of region specific growth and myelination (Wozniak and Lim, 2006). The relative water content of a tissue contributes greatly to contrast based on the spin–spin relaxation time contrast T2. Additionally, the different water diffusion characteristics in tissues are captured in diffusion tensor imaging (DTI); the structural complexity of a tissue not only changes the degree of diffusion, but also the directionality of the water movement. In recent years, postnatal development in rodent brains was frequently studied using DTI of ex vivo specimen (Mori et al., 2001; Zhang et al., 2003, 2005). These studies have built a fair basis for DTI-based morphometry of white matter. However, they lack detailed gray matter characterization and only few studies were performed with true in vivo measurements.

Neurodevelopmental studies of cerebral and in particular of cortical morphology can help identify structural, anatomic changes and reorganizations. Here, we have employed T2 relaxivity and DTI MRI in order to visualize microscopic as well as morphometric changes of the mouse brain and to unravel the time profile of the development and maturation of cortical structures.

Methods

Animals

All experiments were performed on C57Bl/6J mice (Janvier, Le Genest Saint Isle, Cedex, France) of four different postnatal ages: three, eight, twelve and twenty-four weeks.

Animals were allowed two weeks of habituation upon arrival. Weaning mice (P21) were born in the facility. They were housed in groups of four and were given access to food and water ad libitum, in an environment with controlled temperature (21 +/− 1 °C), humidity (55 +/− 10%), and light (12/12 h dark/light cycle). All animal experiments were conducted in accordance with the German Animal Welfare Act and approved by the local authorities (Landesamt für Naturschutz, Umwelt und Verbraucherschutz NRW).

MRI acquisition and data processing

MRI experiments were conducted on a 9.4 T Bruker BioSpec horizontal bore, dedicated animal scanner (Bruker Biospin, Ettlingen, Germany), equipped with a gradient system of 660 mT/m at 110 μs ramp time. For RF excitation a quadrature volume resonator (inner diameter 72 mm; Bruker Biospin) was used, for signal reception a quadrature mouse brain surface coil (Bruker Biospin) was applied. MRI data was acquired using Paravision 5.1 software. After induction of anesthesia, mice were placed in an MRI compatible cradle (Bruker Biospin), and the head was fixed with ear bars and a support ring for the upper incisors in order to reduce movement artifacts. Animals were anesthetized with 2% isoflurane (Forane, Baxter, Deerfield, IL, USA) in a 70/30 mixture of N_20 and O_2; vital functions were monitored during the whole anesthesia period using DASYLab (version 9.0, Measurement Computing Cooperation, Norton, MA, USA). The breathing rate was assessed via a breathing pillow, placed under the thorax, and kept at 100–120 breaths/min by adjusting the isoflurane concentration. Body temperature was recorded with a rectal temperature probe, and regulated by adjusting the temperature of a warm water circulation system (Medres, Cologne, Germany), feeding a heating blanket and the MR cradle.

T2WI and DTI protocols were set to cover the volume between the rhinal fissure and the anterior part of the cerebellum, and imaging data was acquired with identical geometry for both scans (field of view: 28 mm × 28 mm, matrix 192 × 192, 0.5 mm slice thickness, no inter-slice gaps). The number of slices was adjusted individually in every session according to age dependent brain size.

T2W images were acquired with a multi slice multi echo sequence (MSME TR/TE = 5000 ms/10 ms; 10 echoes, with 10 ms inter-echo spacing). For every voxel a monoexponential decay curve was determined from the ten echoes of the MSME (IDL version 6.4, Boulder, CO, USA) and the resulting spin–spin relaxation time was calculated pixelwise to obtain quantitative T2 maps.

Diffusion tensor imaging was recorded with an 8-shot spin echo EPI sequence (30 directions; b-value = 670 s/mm^2, and five supplementary A0 images), with a gradient scheme according to Jones 30 (Jones et al., 1999; Skare et al., 2000). DTI data is prone to distortions due to eddy currents, induced when strong gradient pulses are switched on and off, and to motion artifacts originating from the subject. Prior to tensor calculation, we therefore applied an eddy current correction (FSL version 4.1.7, FMRIB Centre, Oxford, UK) and visually inspected the single frames, discarding the corrupted ones. The occurrence of other distortions and artifacts was limited during image acquisition by applying an automatic ghost correction, the use of a fat suppression module (1.9 ms Gaussian pulse, 1,400 Hz bandwidth, 2 ms spoiler) and navigator echoes. The total acquisition time added up to 50 min, including adjustment scans and a full brain RARE sequence. The RARE data set was used for co-registration of measurements at separate time points.

From the diffusion tensor, three eigenvectors and the corresponding eigenvalues (λ_1, λ_2, λ_3) can be determined, representing in each voxel the main diffusion directions and the magnitude of diffusivity in all three directions. The largest eigenvalue (λ_1) represents the axial or parallel diffusivity ($\lambda_\parallel$), while the perpendicular or radial diffusivity ($\lambda_\perp$) is the average of the two minor values, the second (λ_2) and third eigenvalue (λ_3). The average of all three eigenvalues is a measure for the mean diffusivity (MD) (Basser et al., 1994). Based on the three eigenvalues, the fractional anisotropy (FA) is calculated, giving a measure for the anisotropy of diffusion within the voxel ($0 < FA < 1$, where 0 = isotropic condition).

Registration procedure and deformation-based morphometry (DBM)

The first echo of the MSME yields the best anatomical contrast and a high SNR, thus best qualifying for co-registration of individual image data sets. The images were stripped of external tissue (Smith, 2002) and normalized to a template brain compiled of n = 24 nine week old C57/Bl6 mice. The registration followed a hierarchical scheme (Mengler et al., 2014) and was implemented using Elastix (Klein et al., 2010). Detailed information on registration parameters can be found here (http://elastix.bigr.nl/wiki/index.php/Par0025). Two independent raters controlled the quality and success of the registration process using a custom-made graphic user-interface (Khmelinskii et al., 2013). Subsequently, the displacement field, containing the local deformations relative to the template, was applied to re-orient the calculated maps derived from the T2WI and DTI datasets.

The template was manually labeled with anatomical brain regions of interest (see below), and the corresponding volumes of interests (VOIs) were mapped in the native space of the individual datasets.

The displacement field encodes the anatomical differences between two ages of a brain, such that each voxel describes the transformation vector to the homologous position. A measure derived from the deformation matrix is the determinant of the Jacobian (detJac), representing the local volumetric changes. The detJac ranges from 0 (100% shrinkage) over 1 (no volume change) without upper boundary (volume increase). A logarithmic transform makes the Jacobian distribution symmetric, setting aside every a priori assumption on volume growth (Leow et al., 2006; Yanovsky et al., 2008). Volume changes were evaluated based