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A quantitative magnetic resonance histology atlas of postnatal rat brain development with regional estimates of growth and variability

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ABSTRACT

There has been growing interest in the role of postnatal brain development in the etiology of several neurologic diseases. The rat has long been recognized as a powerful model system for studying neuropathology and the safety of pharmacologic treatments. However, the complex spatiotemporal changes that occur during rat neurodevelopment remain to be elucidated. This work establishes the first magnetic resonance histology (MRH) atlas of the developing rat brain, with an emphasis on quantitation. The atlas comprises five specimens at each of nine time points, imaged with eight distinct MR contrasts and segmented into 26 developmentally defined brain regions. The atlas was used to establish a timeline of morphometric changes and variability throughout neurodevelopment and represents a quantitative database of rat neurodevelopment for characterizing rat models of human neurologic disease.

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Introduction and background

In contrast to virtually all other mammalian organ systems, the brain continues to develop and gain new functionality well into the postnatal period. Postnatal neurodevelopment allows the brain to adapt to the organism's surroundings, but the immature brain is vulnerable to developmental insults outside of the protective environment of the uterus. A wide range of toxic, vascular, traumatic, and even psychological insults are known to alter this crucial period of brain development (Friess et al., 2007; Herrmann et al., 2008; Jaaro-Peled et al., 2009; Maciag et al., 2006; O'Leary-Moore et al., 2011; Panzer, 2008). There has been growing interest in the role of early postnatal insults in neurodevelopmental diseases, such as autism spectrum disorders and attention deficit hyperactivity disorder (Courchesne et al., 2001; Giedd and Rapoport, 2010; Hazlett et al., 2005). The study of neurodevelopmental disorders requires an understanding of the relationship between the three spatial dimensions of the brain and the fourth temporal dimension. Rodent models, and in particular the rat, represent an invaluable tool for studying human brain disorders, but relatively little is known about the spatiotemporal development of the rat brain. Developmental brain atlases can provide unique insight into the normal course of neurodevelopment, and allow researchers to distinguish normative developmental change from pathologic changes.

The importance of a developmental atlas of the rat brain has been recognized for some time, and several notable examples have been published. Two well known histology atlases are the Sherwood and Timiras (1970) "A Stereotaxic Atlas of the Developing Rat Brain" and the Ashwell and Paxinos (2008) "Atlas of the Developing Rat Nervous System". These atlases are primarily focused on prenatal development. The Sherwood–Timiras atlas includes three postnatal time points (p10, p21, and p39), and the Ashwell–Paxinos atlas only one (p0). More recently, Ramachandra and Subramanian (2011) published their 2011 "Atlas of the Neonatal Rat Brain", which features three postnatal time points (p1, p7, and p14). There is also a large body of data on the normal postnatal growth and development of the rat brain starting with the work of H. H. Donaldson (Donaldson, 1915; Donaldson and Hatai, 1911; Jackson, 1913). These data are largely focused on changes in whole-brain mass rather than regional volumetric changes. A number of conventional histology studies have reported regional postnatal volume changes (Bayer, 1980; Eayrs and Goodhead, 1959; Smith, 1934); however these studies are generally limited to larger brain regions (i.e. neocortex, hippocampus) and are subject to the effects of fixation, sectioning and embedding, which can substantially affect the reported volumes (Hillman and Deutsch, 1978).

Magnetic resonance histology (MRH) is emerging as a powerful new technique for small animal brain atlasing. Although the term *histology* usually pertains to light microscopy studies, MRH refers to the use of high-resolution magnetic resonance imaging to study tissue microstructure. In contrast to the relative wealth of conventional histology atlases, to our knowledge there is currently no comparable MRH-based atlas of the developing rat brain. There are, however, at least

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two examples in the mouse (Chuang et al., 2011; Zhang et al., 2005). These atlases further highlight the need for a complementary MRH atlas of the developing rat brain with an emphasis on developing a quantitative database of normal neurodevelopment.

With the advent of MRH, rodent brain atlases have steadily been evolving from qualitative, histology-based, printed volumes to quantitative, searchable, digital entities. Notable examples of MR-based rat brain atlases include both in-vivo (Nie et al., in press; Schwarz et al., 2006; Valdés-Hernández et al., 2011) and higher-resolution ex-vivo templates (Veraart et al., 2011; Johnson et al., 2012). Although MRH has certainly not replaced conventional histology, it does allow a new type of brain atlasing with enticing possibilities. Modern MRH can provide quantitative, regional measurements of brain volume and tissue microstructure at a series of time points throughout postnatal neurodevelopment (Chuang et al., 2011; Zhang et al., 2006). MRH allows elaboration of several distinct image contrasts or "proton stains", each of which highlights different anatomical features. In addition, non-linear image registration (Avants et al., 2008) allows statistical measurements of variability in a population of animals, which is essential for distinguishing normal from pathologic variation (Badea et al., 2007; Johnson et al., 2007; Kovacevic et al., 2005). Despite this exciting potential, there is to date no comprehensive MRH atlas of postnatal rat neurodevelopment.

Virtually all MRI-based developmental brain atlases focus on neurodevelopmental volume changes. Although volume is not a direct measure of brain function, it can be used as a surrogate for neuropathology. For example, abnormal morphologic neurodevelopment has been identified in both autism spectrum disorders and schizophrenia (Courchesne et al., 2001; Mehler and Warnke, 2002). Unfortunately, manual segmentation of MRI volumes is tedious and time-consuming, and a majority of previous atlases track volume changes in only a small number of regions. Of the previously mentioned atlases of mouse brain development, Chuang et al. (2011) tracks volume change in three compartments (cortex, cerebellum and hippocampus) and Zhang et al. (2005) in four compartments (cortex, cerebellum, hippocampus and caudate/putamen). Nonetheless, these atlases provide essential, quantitative information about the normal course of rodent brain development. A comparable atlas of rat brain neurodevelopment would provide a framework for detecting developmental abnormalities in a variety of diseases that are more easily modeled in the rat.

Here, we present the first MRH-based atlas of the developing rat brain. Our atlas incorporates the best features of preceding MRH-based atlases, and introduces several new features designed to increase its utility as a quantitative database for neurodevelopmental research. We believe that our atlas presents the highest-resolution MRH images of the whole rat brain (25 μm isotropic spatial resolution). The atlas comprehensively covers postnatal neurodevelopment with nine postnatal time points chosen to accurately sample the full scope of postnatal volume changes in the rat brain. Each time point is represented by eight distinct image contrasts, five of which are quantitative in nature. Each time point has been manually segmented into 26 developmentally relevant brain regions to allow detailed region-of-interest (ROI)-based analyses, and population averages have been created for every image contrast at each time point to allow enhanced visualization and voxelwise analyses of variation.

Materials and methods

Experimental animals

All experiments and procedures were done with the approval of the Duke University Institutional Animal Care and Use Committee. To ensure accurate sampling of postnatal brain growth, we selected nine time points temporally spaced to allow a fixed percentage increase in brain volume between samples based on previously published rat brain growth curves. The nine time points selected for the atlas were p0, p2, p4, p8, p12, p18, p24, p40, and p80 (where "p" indicates postnatal day). Five male, Wistar rats were selected for imaging at each time point. All animals were from litters of 10–12 pups (average = 11) and had a body weight within one standard deviation of mean weight for age based on the growth curve provided by the supplier (Charles River Wistar Rat Page, 2012).

Specimen preparation

Animals were perfusion-fixed using the active staining technique (Johnson et al., 2002). Perfusion fixation was achieved using a 10% solution of Neutral Buffered Formalin (NBF) containing 10% (50 mM) Gadoteridol. After perfusion fixation, rat heads were removed and immersed in 10% NBF for 24 h. Finally, fixed rat heads were transferred to a 0.1 M solution of Phosphate Buffered Saline containing 1% (5 mM) Gadoteridol at 4 °C for 5–7 days. Prior to imaging, specimens were placed in custom-made, MRI-compatible tubes and immersed in fomblin liquid fluorocarbon for susceptibility matching and to prevent specimen dehydration. All imaging experiments were performed with the brain in the neurocranium to preserve tissue integrity and native spatial relationships.

Image acquisition

Imaging was performed on a 7 T small animal MRI system (Magnex Scientific, Yarnton, Oxford, UK) equipped with 650 mT/m Resonance Research gradient coils (Resonance Research, Inc., Billerica, MA, USA), and controlled with a General Electric Signa console (GE Medical Systems, Milwaukee, WI, USA). RF transmission and reception was achieved using a series of custom solenoid coils ranging from 15 mm diameter × 30 mm long to 30 mm diameter × 50 mm long. T2*-weighted gradient recalled echo (GRE) images were acquired using a 3D sequence (TR = 50 ms, TE = 8.3 ms, NEX = 2, $\alpha = 60°$). The acquisition matrix ranged from 1024 × 512 × 512 to 1600 × 800 × 800 and the field of view (FOV) ranged from 25.6 × 12.8 × 12.8 mm to 40 × 20 × 20 mm. Matrix size was chosen so that the Nyquist isotropic spatial resolution was 25 μm in all cases.

Diffusion-weighted images were acquired using a spin-echo pulse sequence (TR = 100 ms, TE = 16.2 ms, NEX = 1). Diffusion preparation was accomplished using a modified Tanner-Stejskal diffusion-encoding scheme (Stejskal and Tanner, 1965) with a pair of unipolar, half-sine diffusion gradient waveforms (width $\delta = 3$ ms, separation $\Delta = 8.5$ ms, gradient amplitude = 600 mT/m). One b0 image and 6 high b-value images ($b = 1492$ s/mm^2) were acquired with diffusion sensitization along each of six non-colinear diffusion gradient vectors: [1, 1, 0], [1, 0, 1], [0, 1, 1], [−1, 1, 0], [1, 0, −1], and [0, −1, 1]. In this paper, the b0 image is referred to as "T2-weighted" because the long TR and TE of the acquisition relative to the T1 and T2 of the specimen, respectively, result in strong T2-weighting. The acquisition matrix ranged from 512 × 256 × 256 to 800 × 400 × 400 and FOV ranged from 25.6 × 12.8 × 12.8 mm to 40 × 20 × 20 mm. The Nyquist isotropic spatial resolution was 50 μm for all diffusion-weighted images. All images were derived from fully sampled k-space data with no zero-filling.

Diffusion tensor image processing

Diffusion data were processed with a custom image-processing pipeline comprised of freely available software packages including Perl (http://www.perl.org), ANTs (http://www.picsl.upenn.edu/ANTS/), and Diffusion Toolkit (http://www.trackvis.org). This automated pipeline was created to ensure that all data were processed in the same way, and to reduce the potential for user error. First, diffusion-weighted image volumes were spatially normalized to the b0 volume using the ANTs translation only registration to correct for the linear portion of eddy current distortions. Next, diffusion tensor estimation, and calculation of tensor-derived data sets were performed using Diffusion