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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.

Keywords

Neurodevelopment; Rat; Brain; Atlas; DTI; MRI

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

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Appendix A. Supplementary data Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.neuroimage.2013.01.017>.

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 atlas. 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 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 atlas 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, $\alpha=60^\circ$). 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 μ m 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/mm²) were acquired with diffusion sensitization along each of six non-collinear 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 μ m 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 Toolkit. Finally, data were organized into a consistent file architecture and archived in NIFTI format (<http://nifti.nimh.nih.gov>) in an on-site Oracle database (Johnson et al., 2007).

Image registration and averaging

Inter-specimen registration of MR data was accomplished with the ANTs software package (Avants et al., 2008, 2011). Skull-stripped GRE and DWI images from each set of specimens (5 per time point) were aligned using an affine registration, followed by an iterative, viscous fluid model, non-linear registration. We employed a Minimum Deformation Template (MDT) strategy, which uses pairwise, non-linear image registrations to construct an average template requiring the minimum amount of deformation from each of the starting points (Kochunov et al., 2001). The similarity metric used for registration was cross-correlation computed for a kernel radius of 4 voxels. We used a multi-resolution scheme, and did a maximum of 4000 iterations at a down-sampling factor of three, 4000 iterations at a down-sampling factor of two, and 250 iterations at full resolution. We used the greedy symmetric normalization (SyN) model with a gradient step of 0.5 and a Gaussian regularization with $\sigma=3$ for the similarity gradient, and $\sigma=1$ for the deformation field. The algorithm converged at each step of the registration, usually with fewer than 500 iterations. The resultant transformations were applied directly to scalar images to create population averages.

Tensor averaging and tractography

To create population average tensor volumes we used a log-Euclidean transformation strategy (Arsigny et al., 2006). In short, the transformation is applied to the matrix logarithm of the tensor, and then the matrix exponent of the result is taken to create the transformed tensor. FACT tractography (Mori et al., 1999) was performed on each individual and each average tensor volume using a fractional anisotropy threshold of 0.25 and an angle threshold of 45°.

Alignment of image data to histologic atlases

MR data were manually aligned to existing conventional histology atlases to facilitate anatomic delineations. We chose to align the MR data to two atlases—the p80 data were aligned to the Paxinos and Watson adult rat atlas (Paxinos and Watson, 2007) and the p0 data were aligned to the Ashwell and Paxinos p0 rat atlas (Ashwell and Paxinos, 2008). The isotropic MR data were manually reoriented to match the coronal sectioning plans outlined in the introduction section of the two atlases. We then manually compared at least six coronal MR planes (three rostral and three caudal) with spatially unique structures near both the dorsal and ventral surfaces of the brain. Based on the observed error, we then reoriented the original MR data to more closely match the histology atlas and, once again, compared six coronal MR planes. We proceeded in this fashion until we could no longer decrease error by reorienting the MR data. MR data for time points other than p0 and p80 were automatically oriented using a rigid affine registration. Early time point data (i.e. p2, p4, p8, and p12) were registered to the aligned p0 data, while late time point data (i.e. p18, p24, and p40) were registered to the aligned p80 data.

Anatomic segmentation and labeling

Manual, whole-brain segmentation was performed on population-averaged images at each of the atlas time points. We segmented 26 distinct brain regions that are identifiable in MRH volumes throughout postnatal neurodevelopment (Calabrese et al., 2012). The segmented regions and their nomenclature are based on a large body of previous work on developmental mammalian neuroontology and brain atlasing (Paxinos and Watson, 2007; Puelles et al., 2007). Both the average T2*-weighted and diffusion-weighted images were used for segmentation to facilitate delineations using two different contrast mechanisms. Manual segmentation was performed with Avizo 3D segmentation software (<http://www.vsg3d.com>). The resulting population-average label sets were propagated back to the individual specimens using the inverse of the affine and diffeomorphic transforms used to create the population-average image volumes.

Quantitative volumetric analysis and statistics

To demonstrate the quantitative value of our atlas, we analyzed regional volume changes in the rat brain throughout postnatal neurodevelopment. Volumes were calculated for each of the 26 manually segmented brain regions in each of the 45 atlas specimens (five specimens at each of nine time points). Volumes were calculated by multiplying the number of voxels in a segmented region by the voxel volume (15.6 pl for the T2*-weighted image volumes). Regional volumetric data were then imported into JMP Pro 9 statistics software (<http://www.jmp.com>) for further analysis. For each region, we performed non-linear regression with a Gompertz sigmoidal growth model

$$f(t) = a \times \exp\left(-e^{c(b-t)}\right)$$

where t is time after birth measured in days (Jolicoeur et al., 1992). Fitting data to this model provides estimates of three parameters: a = the expected adult volume in μl , b = the abscissa of the inflection point of the growth curve, and c = the relative growth rate at the inflexion point. We also tested for statistical differences in regional brain volume between all time points using ANOVA and Tukey's HSD test. Finally, to assess local morphologic variance, we created mean positional difference (MPD) maps at each time point by taking the mean of the five specimen-to-average warp vector magnitudes (Kovacevic et al., 2005).

Results

Atlas orientation

Fig. 1 illustrates the orientation scheme for the atlas. Late time point data (i.e. p18 and older) were oriented to be consistent with the Paxinos and Watson adult rat atlas (column A) (Paxinos and Watson, 2007), while early time point data (i.e. p12 and younger) were oriented to be consistent with the Ashwell and Paxinos neonatal rat atlas (column B) (Ashwell and Paxinos, 2008). The sectioning diagram from the respective histology atlas is included at the top of each column for reference.

Atlas dimensionality

The final atlas is truly multidimensional, being comprised of the three spatial dimensions, the fourth dimension of time (neurodevelopment) and a fifth dimension of contrast. Fig. 2 attempts to capture the high level of dimensionality of the atlas. On the left side of the figure, T2-weighted horizontal slices through the genu of the corpus callosum are shown for each of the nine atlas time points, increasing from postnatal day zero (p0) at the top to postnatal day 80 (p80) at the bottom. Fig. 2A shows a three-plane view of the p0 gradient recalled echo volume, illustrating that each time point features isotropic data comprising the three spatial dimensions of the atlas. Fig. 2B displays the average whole-brain tractography set from the p18 time point. Tractography segments are colored based on orientation of the primary eigenvector following standard coloring convention (red = left/right; green = rostral/caudal; blue = dorsal/ventral). Each atlas time point has five individual tractography volumes and a sixth, average tractography volume derived from the average tensor. Fig. 2C demonstrates the MR contrast dimension of the atlas by displaying the seven other image contrasts (excluding T2-weighted) that exist for each time point. The abbreviations used for the different contrasts are listed in Table 1.

Anatomic segmentation and labeling

At each time point image data were manually segmented into 26 brain structures covering the entire 3D brain volume. The same 26 structures were segmented at all time points to allow direct of brain sub-regions throughout development. Table 2 lists each of the 2 comparisons of the 6 structures, along with the color code that is used to identify it throughout the atlas. Segmentation results are shown in Fig. 3. For each of the nine atlas time points the corresponding label sets are shown as both a 2D color overlay on a coronal slice through the mid-hypothalamus (top) and a 3D surface rendering (bottom). The 3D surface renderings are shown in dorsolateral view with the olfactory bulbs pointed towards the bottom-right of the figure. The isocortex and corpus callosum/external capsule labels are displayed in transparency to reveal underlying structures. 2D image slices are shown to scale with respect to each other, and 3D renderings are shown to scale with respect to each other; however, 2D images are displayed on a larger absolute scale than 3D renderings to show detail. Label volumes are available for download at <http://www.civm.duhs.duke.edu/ratbraindevatlas/>.

ROI-based volumetric analysis

We measured the volume of each of the 26 brain regions at all nine time points included in the atlas. The complete data table is provided in the supplement. The estimated Gompertz parameters for all 26 structures are presented in Table 3, along with the R^2 values for the regression. Fig. 4 shows volume data versus postnatal day for 12 major brain regions plotted alongside the estimated Gompertz growth function. Also shown are the predicted and measured 95% confidence intervals and the R^2 values for the regression.

Fig. 5 is intended to be a different way of visualizing the same morphometric data shown in Fig. 4. In Fig. 5, atlas time points are treated as nominal rather than continuous variables, so the x-axis is not proportional to age. Box and whisker diagrams are included to show the data distribution including the median (box band) the 75th and 25th percentile (top and bottom of box respectively) and the max and min (whiskers). For each brain region, all pairs of mean volumes were compared using Tukey's HSD test to correct for multiple comparisons. The mean comparison circles on the right side of each plot have a diameter equal to the 95% confidence interval of the corresponding data. Two means are statistically different (i.e. $p < 0.05$) if the comparison circles do not overlap, or if the outside angle of their intersection, θ , is less than 90° (see Fig. 5 legend).

Voxelwise estimates of morphologic variability

Fig. 6 provides voxelwise measurements of morphologic variability at each of the nine atlas time points spanning postnatal rat neurodevelopment. For each time point the mean positional difference (MPD) map, generated from the respective warp vector fields, is displayed as a heat-map color overlay on a three-plane view of the average DWI. Color overlays are set to the same scale, which is displayed at the bottom of the figure. Mean MPD across all ages was $129 \mu\text{m}$ and the maximum observed MPD was $445 \mu\text{m}$ at p8. Mean MPD values for each time point are included as part of the supplemental material.

Discussion

The atlas presented here is the first comprehensive MR-based atlas of postnatal rat brain development. This atlas has several important advantages over existing histology atlases. First, the atlas provides better time resolution than any other rat brain atlas. The magnetic resonance histology (MRH) techniques used to create the atlas are faster and simpler to perform than conventional histology, allowing us to image more specimens at more time points. Second, the atlas is quantitative. High-resolution MRH allows accurate regional volume measurements, and diffusion tensor imaging (DTI) provides the tissue contrast necessary to identify major brain regions throughout postnatal neurodevelopment. Together these techniques enable quantitative modeling of regional brain growth throughout postnatal life. Finally, the atlas includes estimates of population average values and variability. The digital nature of MRH and the use of non-linear image registration allow voxelwise statistical measurements both within and across time points, which are not possible with conventional histology. Together these features provide a robust, quantitative database of normal rat neurodevelopment that can be used to assess aberrant neurodevelopment in disease models and pharmacologic safety testing.

Atlas orientation and incorporation of previous work

MRH and conventional histology atlases are complementary in that each provides something that the other cannot. Histology offers superior resolution and cellular detail, while MRH provides quantification and statistical comparisons of volume and tissue microstructure parameters. The MRH atlas is not meant to replace histology atlases, but rather add to and expand upon existing work. It is precisely for this reason that we have carefully aligned our

image data into the same orientation as the most widely used conventional histology atlases of the rat brain. By incorporating our work into the same space as established histology atlases, as illustrated in Fig. 1, we have attempted to facilitate the use of both technologies by researchers. For example, it is possible to infer the location of small nuclei that may not be readily visible on MRH by simply referring to the corresponding histology plate. Conversely, it is possible to rapidly extract quantitative morphological or microstructural data from the MRH atlas that would not be possible with the histology atlas alone.

Unfortunately, for a majority of the time points covered in our atlas no corresponding optical histology atlas exists. Because of this, we have chosen to orient those time points that more closely resemble the adult (p18–p80) to the Paxinos and Watson (2007) atlas, and those time points that more closely resemble the neonate (p0–p12) to the Ashwell and Paxinos (2008). One fortunate result of this orientation strategy is that the middle time points (i.e. p12 and p18) are actually quite similarly oriented, as evident in Fig. 1 at the bottom of each column. This result is not entirely unexpected, as the orientation of p0 histology atlas was chosen to approximate that of the adult atlas (Ashwell and Paxinos, 2008). Our hope and intention is that future rat brain atlases, regardless of modality, will continue to use a universal and rational orientation scheme that facilitates intermodal comparison and collaboration. To facilitate these types of collaborations, we have provided the atlas images and label sets to the research community at <http://www.civm.duhs.duke.edu/ratbraindevatlas/>.

Anatomic segmentation and labeling

One of the most important features of any atlas is the precision and accuracy of the anatomic segmentation and labeling. The segmentation included in our atlas is meant to serve as both an anatomic map and a platform for quantitative, regional, anatomic comparisons. We believe that our atlas provides some of the most accurate measurements of brain volume currently available due to the high spatial and contrast resolution, and the non-destructive nature of our MRH techniques. The atlas segmentation includes 26 structures carefully chosen to be both developmentally relevant and identifiable throughout postnatal life. Importantly, several of these structures are difficult to accurately identify without the unique image contrast provided by DTI, particularly in early postnatal brains where T2 contrast is limited.

The fact that the atlas labels remain constant throughout development allows researchers to visualize and quantify differences in position, shape, and volume of different brain regions throughout postnatal life. For example, the label surface renderings in Fig. 3 show the cerebellum (rendered in turquoise) as a thin, flat, sliver in the p0 brain, which rapidly develops into a quite large and intricately foliated structure in the adult brain. In contrast, the midbrain (orange) remains relatively constant in volume, but becomes completely covered by the rapidly expanding isocortex (transparent green) as the brain matures. In addition to characterizing normal growth patterns, these labels will also allow researchers to identify subtle deviations from normal neurodevelopment in rat models of neurologic disease.

Quantitative volumetric analysis and statistics

One of the most important advantages of MRH atlasing is the possibility for quantitative morphometry. As part of our quantitative morphometric analysis, we found that our regional volumetric data was well modeled by a Gompertz sigmoidal growth function. The results of this analysis are presented in Table 3 and Fig. 4. All 26 brain regions displayed high coefficients of determination (R^2 values) for the regression; the lowest R^2 value was 0.68 for the bed nucleus of the stria terminalis (BST) and the average value for all structures was 0.93. The estimated growth parameters presented in Table 3 reveal remarkable

spatiotemporal heterogeneity in the developing rat brain. The relative growth rate (RGR) (parameter c) varies by almost an order of magnitude from 0.018 for the pineal gland to 0.153 for the septum. For some regions, the day of maximum RGR (parameter b) occurs within the first few days of postnatal life (i.e. midbrain, septum, hypothalamus, and preoptic area), while other regions lag by several weeks (i.e. pituitary, pineal gland, and optic pathway). Interestingly, the structures that reach their maximum RGR earlier tend to be axial structures involved in basic life support and homeostasis, while those that peak the latest are involved in endocrine and sensory functions that are only necessary after birth (e.g. growth hormone secretion, vision) (Calvo and Boya, 1984; Jailer, 1950). It is important to note that negative values of parameter b are not necessarily erroneous, as the growth curve inflection point may have occurred during prenatal development.

The growth curves presented in Fig. 4 reveal additional spatiotemporal nuances of rat brain development. For example, the volume of cortical structures such as the olfactory cortex, amygdala, hippocampus, and isocortex appear to plateau around p40 while most other structures show at least some growth between p40 and p80. In contrast, white-matter rich structures such as the hindbrain, fimbria/fornix, and anterior commissure exhibit nearly linear growth throughout postnatal development. These findings are consistent with the theory that late brain development is dominated by increased axonal connections between existing brain regions (Nagy et al., 2004; Toga et al., 2006); however, more studies are needed to establish whether our volume measurements of white-matter structures correlate with increased connectivity.

Interestingly, of structures plotted in Fig. 4, only the pituitary shows a significant lag phase at the beginning of the growth curve. This relatively late growth could reflect the fact that placental hormones largely supplant pituitary function during prenatal life (Jailer, 1950). The lack of a lag phase on other growth curves is either because these structures simply do not exhibit significant lag phase growth, or more likely, it occurs during prenatal neurodevelopment. Similar morphometry studies on the prenatal rat brain will be needed to capture the full range of neurodevelopment.

Direct comparison of our regional volume measurements to previous work based on conventional histology is problematic due to differences in technique. Volumes calculated with conventional histology are prone to error caused by brain extraction, freezing or embedding, sectioning and tissue dehydration during staining. For these reasons, histology-based estimates of regional brain volume tend to underestimate in-vivo volume. For example, Smith (1934) reported the volume of the p86 male, Wistar rat cortex to be $\sim 400 \mu\text{l}$, which is substantially smaller than the p80 isocortex volume reported here (average volume = $661 \mu\text{l}$). Similarly, histology-based estimates of hippocampal formation volume collected by S. A. Bayer (1980) are almost 50% lower than the MRH-based measurements presented here. The majority of previous MRH atlases of the rat brain do not report regional brain volumes (e.g. Johnson et al., 2012; Nie et al., in press; Schwarz et al., 2006; Veraart et al., 2011). Those that do report volume are difficult to compare with the current work because they use different sexes, different image resolution, and different ages/weights for their templates (e.g. Valdés-Hernández et al., 2011).

Fig. 5 demonstrates the statistical power of the atlas segmentation. Several factors contribute to the ability to identify a statistically significant volume change between two time points including magnitude of growth, structure variability, and segmentation reproducibility. We have taken several steps to ensure data consistency, such as controlling for litter size and animal body weight. These steps have likely contributed to our ability to detect differences with a relatively modest sample size at each time point ($n=5$). Higher n 's would almost certainly allow more time points to be statistically differentiated, however this is somewhat

impractical given the high cost and long scan time required for each specimen. Nonetheless, these data show researchers what they can reasonably expect using similar methods. Some regions, such as the cerebellum and olfactory structures, have statistically significant volume differences at nearly every time point. Several late-developing regions including the anterior commissure and pituitary have indistinguishable volumes in the pre-weaning period (i.e. before postnatal day 20) and relatively large statistical differences in the post-weaning period. It is also important to note that the time-interval between adjacent time points varies from two days (between p0 and p2) to 40 days (between p40 and p80), and statistically significant volume changes must be interpreted with regard to the intervening growth period. We also caution researchers on comparing these results with experimental data from different rat sub-species, as small but significant anatomic differences are known to exist among commonly used laboratory strains (Boss et al., 1987). Our intention is to provide a guide for the volume differences that researchers should expect using similar methodology. We suggest that these results will help design future experiments assessing regional volume differences in disease models.

Voxelwise measurements of variability

Fig. 6 reveals relatively constant morphometric variability in the rat brain throughout postnatal development. The average mean positional difference (MPD) across all time points was 129 μm , which is consistent with previous measurements of variability in adult rodent brains (Badea et al., 2007; Kovacevic et al., 2005). A few regions exhibit somewhat higher variability, notably the midbrain and olfactory bulbs. The high variability of the midbrain, especially in the p2-p12 range, is also reflected in the measured volume distribution displayed in Figs. 4–5. There are at least two explanations for this increased variability. The first is that it reflects true biologic variability; the midbrain is located at a flexure in the neuraxis and experiences its most rapid growth in the early prenatal period, as evident in Fig. 4. The second explanation is related to specimen preparation. Although specimens were imaged in the skull to prevent external deformation, the posterior fontanelle, which sits just above the midbrain, does not close until after the second week of postnatal life (Moss, 1954). It is certainly possible that external deformation through the open fontanelle could be contributing to midbrain variability. We still believe that these results are meaningful as this type of error is likely to be reproduced in future studies.

Conclusions

The work presented here is the first MRH atlas of postnatal rat brain development. We have attempted to highlight the quantitative nature of our atlas because we believe this is one of the most important advantages of MRH-based atlasing. We have demonstrated the use of the atlas as a database for quantitative morphometry throughout postnatal neurodevelopment; however, there are several other quantitative aspects of the atlas that remain to be explored. For example, diffusion tensor-derived contrasts such as fraction anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD), may provide insight into tissue microstructural changes throughout postnatal development (Giorgio et al., 2010; Kochunov et al., 2012). These studies, and others, will be the subject of future work on the developmental atlas by us, and hopefully by others in the community.

Ultimately, an atlas is only useful if it is available to the research community. With that in mind, we have shared what we believe to be the most important portions of the atlas on our website <http://www.civm.duhs.duke.edu/ratbraindevatlas/>, including full resolution average GRE volumes, label volumes, and morphometric data tables for all atlas time points. At nearly one terabyte in size, hosting the entire atlas is impractical given current resources; however, all atlas datasets will be available to researchers on request. We hope that studies

will add to and improve upon our atlas, and perhaps most importantly, use it to characterize and quantify rat models of developmental neuropathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

The orientation scheme of the atlas. Late time point data (p18-p80) were oriented to the Paxinos and Watson adult rat brain atlas (Column A). Early time point data (p0-p12) were oriented to the Ashwell and Paxinos p0 rat brain atlas. The sectioning diagrams from the respective histology atlases are shown at the top of each column.

Fig. 2.

The multidimensionality of the atlas. Horizontal, T2-weighted images through the genu of the corpus callosum for each atlas time point are shown to scale on the left of the figure. A highlights the three spatial dimensions of the atlas with a three-plane view of the p0 average GRE. B shows whole-brain tractography from the p18 average tensor. Each time point has associated tensor and tractography volumes. C demonstrates the eight image contrasts included in the atlas. A single horizontal slice from the p80 average dataset is shown for each contrast. See Table 1 for contrast abbreviations and descriptions.

Fig. 3.

2D and 3D views of the atlas label volumes for each time point. At each time point label volumes are presented as 2D color overlays on a coronal slice through the hypothalamus (top) and 3D surface renderings. Labels are color-coded according to Table 2. In the 3D surface renderings, the isocortex and corpus callosum are shown in transparency to reveal underlying structures.

Fig. 4.

Gompertz growth models for 12 selected brain regions. For each region we have plotted the raw volume data (open circles) over the estimated model (solid line). Also shown are the 95% confidence intervals for the model (small dashed line) and for the measured data (large dashed line), and the R^2 values for the regression. Estimated Gompertz parameters for all structures are shown in Table 3.

Fig. 5.

Comparison of means for 12 selected brain regions. Raw structure volume data are plotted along with min and max box and whisker plots to show data distribution. The comparison circles to the right of each plot were generated with Tukey's HSD comparison of means. Two groups are statistically different if their comparison circles do not overlap, or if the outside angle of their overlap is acute (see legend). Age is used as a nominal variable for these comparisons so the x-axis is not age proportional.

Fig. 6. Mean positional difference (MPD) maps for each time point. Calculated MPD maps are displayed as color overlays on three-plane views of the average DWI for each atlas time point. The MPD scale is consistent across all time points and is shown at the bottom of the figure. The maximum observed MPD was 450 μm .

Table 1

Abbreviations and descriptions of the eight different atlas contrasts.

Abbreviation	Contrast	Description
ADC [‡]	Apparent diffusion coefficient	Magnitude of water diffusion in mm ² /s
AD [‡]	Axial diffusivity	Magnitude of water diffusion along primary eigenvector of the tensor in mm ² /s
DWI	Mean diffusion weighted image	Average of the 6 diffusion weighted images
FA [‡]	Fractional anisotropy	A normalized ratio of eigenvalue differences to the apparent diffusion coefficient
FAC [‡]	Fractional anisotropy color map	The above colored based on fiber orientation
GRE	Gradient recalled echo	T2*-weighted gradient echo
RD [‡]	Radial diffusivity	The mean of the secondary and tertiary eigenvalues of the tensor in mm ² /s
T2	T2-weighted contrast	T2-weighted spin echo

[‡] indicates a quantitative contrast (i.e. voxel values represent a meaningful physical quantity).

Table 2

Color code for brain structures used throughout this document.

Structure	Color
Accumbens nucleus	Red
Amygdala	Olive
Anterior commissure	Light green
Axial hindbrain	Light green
Bed nucleus of the stria terminalis	Purple
Cerebellum	Brown
Cingulum	Red
Corpus callosum/deep cerebral white matter	Teal
Diagonal domain	Cyan
Diencephalon	Blue
Fimbria/fornix	Brown
Hippocampal formation	Magenta
Hypothalamus	Light blue
Internal capsule/cerebral peduncle/pyramids	Light purple
Isocortex	Olive
Mesencephalon	Orange
Olfactory structures	Blue-gray
Optic pathways	Light purple
Pallidum	Pink
Pineal gland	Red
Pituitary	Blue
Preoptic area	Light green
Septum	Light green
Striatum	Blue
Substantia nigra	Yellow
Ventricles	Purple

Table 3

Estimates of Gompertz growth parameters for all 26 atlas structures and R^2 values for the regression.

Region	<i>a</i>	<i>b</i>	<i>c</i>	R^2
Cingulum	6.27 μ l	9.05 days	0.063	0.94
Midbrain	96.63 μ l	1.19 days	0.067	0.89
Substantia nigra	7.52 μ l	10.00 days	0.079	0.97
Anterior commissure	4.57 μ l	13.91 days	0.046	0.97
Hindbrain	275.34 μ l	13.56 days	0.045	0.99
Septum	13.38 μ l	0.85 days	0.153	0.84
Diagonal domain	5.70 μ l	3.51 days	0.057	0.89
Hypothalamus	38.67 μ l	0.40 days	0.100	0.87
Striatum	93.47 μ l	6.71 days	0.076	0.96
Diencephalon	113.54 μ l	4.71 days	0.085	0.98
Internal capsule	38.37 μ l	14.29 days	0.056	0.99
Hippocampal formation	108.44 μ l	7.95 days	0.088	0.96
Pallidum	19.86 μ l	8.67 days	0.060	0.96
Accumbens	15.00 μ l	8.81 days	0.059	0.93
Fimbria fornix	20.13 μ l	13.80 days	0.050	0.97
Corpus Callosum	86.52 μ l	6.46 days	0.048	0.91
Amygdala	46.23 μ l	2.90 days	0.138	0.95
Preoptic area	9.54 μ l	−1.18 days	0.119	0.88
Isocortex	624.32 μ l	6.41 days	0.128	0.97
Cerebellum	340.51 μ l	13.45 days	0.084	0.97
Olfactory structures	228.34 μ l	9.14 days	0.081	0.98
BNST	3.46 μ l	−9.03 days	0.039	0.68
Pituitary	14.35 μ l	30.77 days	0.052	0.98
Ventricles	10.54 μ l	1.50 days	0.107	0.81
Optic pathway	6.84 μ l	26.39 days	0.036	0.97
Pineal gland	3.42 μ l	44.44 days	0.018	0.88