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An ontology-based segmentation scheme for tracking postnatal changes in the developing rodent brain with MRI

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Abstract

The postnatal period of neurodevelopment has been implicated in a number of brain disorders including autism and schizophrenia. Rodent models have proven to be invaluable in advancing our understanding of the human brain, and will almost certainly play a pivotal role in future studies on postnatal neurodevelopment. The growing field of magnetic resonance microscopy has the potential to revolutionize our understanding of neurodevelopment, if it can be successfully and appropriately assimilated into the vast body of existing neuroscience research. In this study, we demonstrate the utility of a developmental neuro-ontology designed specifically for tracking regional changes in MR biomarkers throughout postnatal neurodevelopment. Using this ontological classification as a segmentation guide, we track regional changes in brain volume in rats between postnatal day zero and postnatal day 80 and demonstrate differential growth rates in axial versus paraxial brain regions. Both the ontology and the associated label volumes are provided as a foundation for future MR-based studies of postnatal neurodevelopment in normal and disease states.

Keywords

MRI; Neurodevelopment; Postnatal; Rat brain; Diffusion tensor imaging

1. Introduction

In recent years, the United States has seen a dramatic rise in the prevalence of neurodevelopmental disorders including Autism Spectrum Disorders (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2006 Principal Investigators Centers for Disease Control and Prevention (CDC), 2009; Centers for Disease Control and Prevention (CDC),

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2010). These and other neurodevelopmental disorders are difficult to study because mammalian neurodevelopment is a complex process that spans a long time period, from early organogenesis to late adolescence. Virtually all mammals undergo significant postnatal brain growth and differentiation (Clancy et al., 2007), yet surprisingly little is known about this period of neurodevelopment. Importantly, both mice and rats undergo a relatively large portion of neurodevelopment in the postnatal period, including the majority of cerebellar growth and differentiation. In humans, similar developmental processes typically occur in the third trimester of gestation (Tiemeier et al., 2010; White and Sillitoe, 2012). For this reason, rodent postnatal neurodevelopment can serve as a model for human neurodevelopment from late gestation through adolescence. This critical period of brain development is highly susceptible to a variety of insults including toxic, traumatic, and vascular events (Clancy et al., 2007; Herrmann et al., 2008; Jaaro-Peled et al., 2009; O'Leary-Moore et al., 2011). In order to effectively characterize aberrant postnatal neurodevelopment resulting from such insults, one needs both a well-defined "normal" model and a set of quantitative tools for assessing differences between exposed subjects and controls.

Magnetic resonance histology (MRH) has emerged as a powerful, quantitative tool for studying brain structure in small mammals; however, the application of this technique to postnatal brain development presents a unique set of problems (Johnson et al., 2002a; Badea et al., 2007; Johnson et al., 2007; Petiet et al., 2008; Chuang et al., 2011). Typically, comparisons between quantitative image sets are done using whole-brain, voxel-wise, or region of interest (ROI)-based approaches (Giuliani et al., 2005). Large postnatal changes in brain shape and volume make whole-brain and voxel-wise approaches problematic, leaving the ROI-based approach the most straightforward type of comparison. Unfortunately, manual segmentation is difficult and time-consuming, and because of this, previous attempts to track postnatal imaging biomarkers have focused on only a handful of major brain regions (Zhang et al., 2005; Chuang et al., 2011). Further, the chosen segments are often based on what is convenient and not necessarily on biology.

To appropriately characterize postnatal brain changes, one must consider the developmental origins of different parts of the brain. For example, it does not make sense to track developmental changes in the collection of deep motor nuclei commonly known as the "basal ganglia," when in fact this functionally defined group contains descendents of at least three distinct major embryonic subdivisions—the striatum (arising from the subpallial telencephalon), the subthalamic nucleus (arising from the basal hypothalamus), and the substantia nigra which has components arising from the hindbrain, mesencephalon, and diencephalon (Watson, 2012; Puelles et al., 2012a; 2012b; 2012c). Clearly it would be difficult to sort out whether quantitative developmental changes in the "basal ganglia" were arising from the telencephalon, diencephalon, mesencephalon, hindbrain, or some combination thereof. For this reason, when studying neurodevelopment, researchers must move away from dated classifications of brain regions, and instead classify regions based on a developmentally defined brain ontology.

A developmental brain ontology is a hierarchically organized set of brain regions defined based on their embryonic origins, an excellent example of which is the chick brain ontology (Puelles et al., 2007). A number of attempts have been made to create a mammalian brain ontology in the recent past, the most notable of which include the NeuroNames ontology (Bowden and Martin, 1995), the Brain Architecture Knowledge Management System (BAMS) (Bota and Swanson, 2008), Biomedical Informatics Research Network (BIRN) (Bug et al., 2008), and the Allen Brain Atlas ontology (Dong, 2008). These four ontologies are limited in that they are based on a traditional topological classification of brain components. Another issue is that all of these ontologies, including the chick brain ontology,

are based in large part on conventional histology, which allows identification of subtle borders and small nuclei. Because MRH has limited resolution (10-25 μm) and uses a variety of contrast mechanisms, none of which exactly recapitulates conventional histology stains, the range of structures that can be readily identified in MRH volumes of the mammalian brain is limited. Fortunately, an exhaustive collection of structures is not necessarily desirable for quantifying postnatal neurodevelopment because of the time involved in manual segmentation at several different time points. Large-scale screening and quantification studies require a comprehensive, yet tractable, segmentation that can be preformed rapidly and reliably throughout postnatal life.

An ideal segmentation scheme for tracking imaging biomarkers throughout postnatal neurodevelopment would be comprehensive, ontologically based, technically realizable, and flexible enough to accommodate further advances in MRH. Here we introduce a developmental ontology and segmentation guide that attempts to satisfy each of these requirements. The ontology presented here is comprehensive in that it covers the entirety of the mammalian brain. It is ontologically based in that the brain divisions are based on a wide range of developmental neuroscience research including histology, gene marker studies, fate mapping, and in-situ hybridization, which have been compiled in the avian brain ontology of (Puelles et al., 2007). We demonstrate the technical feasibility of this ontologically based segmentation scheme throughout postnatal neurodevelopment by successfully segmenting MRH volumes of the rat brain immediately after birth (postnatal day 0-p0) and at adulthood (postnatal day 80-p80). Finally, we discuss the flexibility of this ontology in accommodating new imaging technologies and brain images of other mammalian species.

2. Material and methods

2.1 Specimen preparation

All experiments and procedures were approved by the Duke University Institutional Animal Care and Use Committee. Five p0 and five p80 male, Wistar rats were selected for imaging studies. 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. Animals were perfusion-fixed using the active staining technique (Johnson et al., 2002b). 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 hours. 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 native spatial relationships.

2.2 Image acquisition

Imaging was performed on a 7 T small animal MRI system (Magnex Scientific, Yarnton, Oxford, UK) equipped with 670 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 custom 30 mm diameter $\times$ 50 mm long solenoid coil for p80 specimens and a custom 15 mm diameter by 30 mm long solenoid coil for p0 specimens. High-resolution T2*-weighted gradient recalled echo (GRE) images were acquired using a 3D GRE sequence (TR = 50 ms, TE = 8.3 ms, NEX = 2, $\alpha=60^\circ$). The acquisition matrix was $1600 \times 800 \times 800$ over a $40 \times 20 \times 20$ mm field of view (FOV) for p80 specimens and $1024 \times 512 \times 512$ over a $25.6 \times 12.8 \times 12.8$ mm for the p0 specimens. In both cases, the Nyquist isotropic spatial resolution was 25 μm (voxel volume = 15.6 picoliter).

Diffusion-weighted images were acquired using a spin-echo diffusion-weighted pulse sequence (TR = 100 ms, TE = 16.2 ms, NEX = 1). Diffusion preparation was accomplished using a modified Tanner-Stejskal diffusion-encoding scheme 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 b_0 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]. The acquisition matrix was $800 \times 400 \times 400$ over a $40 \times 20 \times 20$ mm field of view (FOV) for p80 specimens and $512 \times 256 \times 256$ over a $25.6 \times 12.8 \times 12.8$ mm for the p0 specimens. The Nyquist isotropic spatial resolution was 50 μ m; however, diffusion weighted data were zero-filled in k -space to twice the original matrix size in each dimension so that the resulting voxel dimensions matched the GRE data (25 μ m final isotropic voxel size).

2.3 Image registration and averaging

GRE and diffusion-weighted images (DWI) from each set of specimens (5 per time point) were acquired sequentially without moving the specimen, and we manually ensured that the two datasets were aligned to each other. Inter-specimen image registration was accomplished with the ANTs software package (<http://www.picsl.upenn.edu/ANTs/>) (Avants et al., 2008). Data were aligned using a six-parameter rigid 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 3, 4000 iterations at a down-sampling factor of 2, and 200 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 convergence threshold was reached at each step of the registration, usually with fewer than 500 iterations.

2.4 Alignment of image data to histologic atlases

After registration and averaging, MR data were aligned to conventional histology atlas stereotaxic coordinates to facilitate and validate MR segmentation. The adult (p80) rat data were manually aligned to the Paxinos and Watson atlas (Paxinos and Watson, 2007) as part of another study (Johnson et al., 2012). Alignment and validation of the p0 data proceeded in a similar fashion. We used the Ashwell and Paxinos atlas of the developing rat brain (Ashwell and Paxinos, 2008) as a histologic reference for the p0 data. First, the isotropic MR data were reoriented to match the coronal sectioning plan outlined on page 359 of the Ashwell and Paxinos atlas. We then manually compared 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.

2.5 Selection of structures and image segmentation

Our segmentation scheme is based on the three major embryonic subdivisions of the brain—the hindbrain, midbrain, and forebrain. We parcellated each major subdivision into smaller, constituent parts based on the borders that can be rapidly and reliably identified on T2* and diffusion-weighted MRI. We focused on relatively large structures that are likely to be useful for quantitation of postnatal changes and omitted some smaller regions that we felt

would not contribute significantly to the utility of the segmentation as a screening tool (e.g. the individual lobules of the cerebellum). T2* and diffusion-weighted contrasts were chosen because they are both commonly used in MRH studies and they rely on two very different sources of contrast (spin-spin relaxation and diffusivity, respectively). In addition, several previous studies have noted that T2* and diffusion-weighted contrasts highlight different, and somewhat complementary brain anatomy (Kovacevic, 2004; Ma et al., 2005; Chuang et al., 2011; Johnson et al., 2012). All 3D digital segmentation of MRI volumes was accomplished using Avizo 3D analysis software (Visualization Sciences Group, Burlington, MA, USA). The resulting label volumes and the 3D datasets used for segmentation are provided in the supplementary material (www.civm.duhs.duke.edu/neuro2012ontology). A detailed guide to MRH-based segmentation of all 26 structures is provided in the supplement and is available on-line on the Neuroscience Lexicon wiki (http://neurolex.org/wiki/Category:CIVM_postnatal_rat_brain_atlas_parcellation_scheme).

3. Results

3.1 Alignment of p0 image data to the Ashwell and Paxinos histology atlas

As described in the Methods section, the p0 MRH volumes were aligned to the p0 images in the Ashwell and Paxinos (2008) histology atlas using iterative manual rotation followed by comparisons of six coronal planes. An example of a coronal comparison is presented in Figure 1. In the dorsal portion of Figure 1, the letter A marks the rostral-most crossing of the ventral hippocampal commissure (vhc), which is not present in adjacent histology sections. B denotes the posterior limb of the anterior commissure (acp), which makes its first contact with the external capsule (ec) in this section. Rostral to this plane, the acp and ec are not in contact with each other and caudally they cannot be distinguished as two separate structures. Finally, C marks the rostral-most crossing of the anterior commissure (ac), which has a distinctive oval shape across the midline that is not present in more rostral sections. The Ashwell and Paxinos atlas does not provide rostral/caudal coordinates, so we could not directly compare histologic coordinates to MR coordinates. However, the histology sectioning interval was 200 μ m, so we can compare inter-slice distance on MR and histologic sections as a metric for alignment of the two datasets. These data are presented in Table 1. The mean discrepancy between MR coordinates and histologic coordinates was 1.06 mm, which is comparable to similar attempts at aligning MRI volumes with histology (Johnson et al., 2012). The remaining 5 comparison planes are provided in supplementary material (www.civm.duhs.duke.edu/neuro2012ontology).

3.2 Selection of structures for segmenting MRH volumes

We identified 26 developmentally defined structures that collectively represent the entire brain. Importantly, each of these structures is visible in both the early postnatal (p0) and adult (p80) rat brain, allowing a consistent segmentation scheme to be used throughout postnatal neurodevelopment. A complete hierarchical list of these structures is presented in Table 2. The MAIN ONTOLOGY section presents structures that are clearly derived from one of the three major embryonic subdivisions of the brain: hindbrain, midbrain, and forebrain. The SPECIAL CASES section of Table 1 contains two groups of structures that do not comfortably fit into the main ontology framework. One group includes developmentally diverse structures, which arise from two or more major embryonic subdivisions of the brain, and the other includes major white matter structures, which course through two or more discrete brain structures. Table 3 lists the 26 structures in alphabetical order along with their color code, which will be used to identify these structures throughout this document.

3.3 Segmentation of p0 and p80 rat brain MRH volumes

We manually segmented early postnatal (p0) and adult (p80) rat brain image volumes into the 26 structures listed in Table 3. The segmented images were an average of 5 different specimens, which were registered together using a minimum deformation template strategy to reduce individual specimen bias. The results of this segmentation are presented in Figures 2-3. Figure 2 shows three coronal sections from both the p0 (Figure 2:A-C) and p80 (Figure 2:D-F) rat brain with the segmentation presented as a color overlay. Corresponding sections (i.e. Figure 2:A and Figure 2:D) are roughly analogous; however, a perfect match is not possible due to significant bending of the neuraxis that occurs during postnatal neurodevelopment in the rat. Figure 2:A and 2:D are sections through the decussation of the anterior commissure, Figure 2:B and 2:E through the rostral portion of the hippocampal formation, and Figure 2:C and 2:F through the caudal portion of the hippocampal formation. Images were scaled to the same size to show detail. A 1-cm reference is provided for scale.

Figure 3 shows the p0 and p80 segmentation as rendered 3D surfaces. The isocortex and corpus callosum/deep cerebral white matter complex are displayed in transparency to show underlying structures. Once again, p0 (top) and p80 (bottom) images are scaled to the same size to show detail. Figure 3:A and 3:D are profile views with the olfactory bulb angled to the bottom right of the Figure and the brainstem angled to the top left. Figure 3:B and 3:E are dorsal views and Figure 3:C and 3:F are ventral views, with the olfactory bulb on the right in each case.

Figure 4 demonstrates the contrast differences between gradient recalled echo (GRE) images (Figure 4:A) and isotropic diffusion weighted images (DWI) (Figure 4:B) in the p80 rat brain. Both images (Figure 4:A-B) are taken from the same coronal plane at the level of the posterior commissure. The magnified insets show the caudal amygdala and ventral hippocampal formation in the two image contrasts. The most striking anatomical differences between the two contrasts are indicated with arrows and/or structure abbreviations. Abbreviations are consistent with the standard rat brain nomenclature used in the Paxinos and Watson adult rat brain atlas (Paxinos and Watson, 2007).

3.4 Volume changes in the postnatal rat brain

We measured volumes for all 26 structures listed in Figure 3 in manually segmented p0 and p80 MRI volumes. These data are presented in Table 4. Mean volume and standard error are listed for each structure. We have also included percentage of total brain volume, as well as the change in absolute volume and the change in percent of total brain volume for each structure between p0 and p80. Finally, we have calculated percent growth for each structure, where 100% means no apparent growth between p0 and p80.

Figure 5 is a graphical representation of regional postnatal brain growth in the rat. Displayed are four different coronal planes from the p80 average diffusion-weighted image. Figure 5:A-C match the coronal planes displayed in Figure 2:D-F. Figure 5:D is a slice through the hindbrain at the level of the exit of the facial nerve. Each of the 26 segmented structures is colorized based on its percent growth during the postnatal period. Note that the pituitary and cerebellum grew by ~8000% and ~4000% respectively, both of which fall well outside of the displayed scale.

4. Discussion

Previous studies have attempted manual, whole brain segmentation of MRI volumes with widely varying results. Even in the relatively small field of rodent brain MRI, a majority of published whole brain segmentation attempts use dramatically different ontologies and nomenclature sets (Kovacevic, 2004; Ma et al., 2005; Johnson et al., 2010; Chuang et al.,

2011). This problem is compounded in the case of postnatal neurodevelopment where even fewer structures are visible on MRI (Zhang et al., 2005; Chuang et al., 2011). The general lack of consensus on which structures can and should be routinely segmented in mammalian brain MRI highlights the need for a robust and relevant ontology designed for this purpose. Such an ontology would not only provide a guideline for future segmentation attempts, but would also allow comparison of results between studies. A unified ontology and nomenclature is essential for effective communication of brain segmentation results, but its usefulness can be extended by focusing on the developmental origins of the brain (Puelles et al., 2007). There has been growing interest in the importance of postnatal neurodevelopment and in the use of MRI to study neurodevelopmental changes both in normal and disease states (Mori et al., 2001; Knickmeyer et al., 2008; Giedd and Rapoport, 2010). An ideal brain MRI segmentation ontology would, therefore, be consistent and tractable throughout postnatal life, allowing developmentally relevant comparisons of MR biomarkers in brain sub-regions throughout neurodevelopment. It is with this in mind that we have created the developmental ontology presented in Table 2.

4.1 Alignment to histology atlases

We chose to align our data to conventional histology atlases for two reasons. First, we wanted a reliable method to validate our segmentations and ensure that borders visible on MRI corresponded with true cytological borders. Second, we wanted to incorporate our work in to the large body of existing neuroanatomical research on the rat brain. By doing so, we hope to facilitate intermodal research in future studies on rat neuroanatomy. There is, for example, considerable value in producing finer MRI subdivisions of certain structures based on a priori anatomic knowledge (Richards et al., 2011; Ullmann et al., 2012).

Although the goal of this study was to segment only those structures that are clearly visible on MRI, we would like to facilitate future work on this and similar data by using an established coordinate system.

4.2 The developmental ontology

Several features of this ontology warrant explanation. First, the list of structures that we have selected (see Table 3) is not intended to be exhaustive; any one of the structures in Table 3 could be further subdivided in to several different parts based on a priori anatomic knowledge or comparison with conventional histology (Richards et al., 2011). However this level of detail is impractical for brain-wide quantitation of postnatal development. Our goal was to great a logical, tractable list of structures that could be used to screen for and quantify regional postnatal changes in volume and other MRI metrics. The list in Table 3 was designed to: 1) cover the entire brain volume, 2) use only those structures with borders that are readily apparent using the MRH imaging strategies we have employed, and 3) focus on structures that are of particular interest in current brain research, particularly neurodevelopmental investigations. The MAIN ONTOLOGY section of Table 2 is a hierarchical list of those brain regions that clearly originate from, and are part of, only one of the three major embryonic subdivisions of the brain: the hindbrain, the midbrain, and the forebrain.

4.2.2 Hindbrain

The hindbrain is divided into the cerebellum and the axial hindbrain. Although many older neuroanatomy texts divide the hindbrain into the metencephalon (consisting of the cerebellum and the pons) and the myelencephalon (consisting of the medulla oblongata), this division is based on the external appearance of the human brain rather than on the developmental origins of the mammalian hindbrain (Watson, 2012). The rostral part of the hindbrain, consisting of the isthmus and rhombomere 1, gives rise to the cerebellum (Puelles

et al., 2007). The term “axial hindbrain” refers to the remainder of the hindbrain consisting of rhombomeres 2-11.

4.2.3 Midbrain

The midbrain is not further subdivided for this segmentation. The reason for this is two-fold: 1) the midbrain consists largely of the tectum and tegmentum, the border between which is difficult to identify, particularly in the neonatal rat brain, and 2) the boundary between the two segmental components (mesomeres) (Puelles et al., 2012a) is also difficult to see on rodent MRI.

4.2.4 Forebrain

The forebrain is the most extensively subdivided of the three major embryonic divisions in our ontology for a number of reasons. First, the forebrain is the largest of the three divisions in virtually all vertebrate species (Puelles et al., 2007; Watson et al., 2012). Second, the forebrain is the focus of a majority of research on human neurologic diseases including Alzheimer’s disease, Parkinson’s disease, schizophrenia, autism, and many others. Finally, the forebrain is quite heterogenous in appearance on MRI, and several major borders are readily apparent. The first major subdivision of the forebrain yields the hypothalamus (including the eye), diencephalon, and telencephalon (Puelles et al., 2007). Many neuroanatomy texts list the hypothalamus as part of the diencephalon; however, recent gene expression studies have provided substantial evidence that these two brain regions are both genetically and developmentally distinct (Puelles and Rubenstein, 2003). The telencephalon is further divided into the pallium and subpallium.

Within the pallium are the major cortical structures including the hippocampal formation (the dentate gyrus, subiculum, and the hippocampus proper—the medial pallium), the olfactory structures (the olfactory bulb, olfactory tubercle, lateral olfactory tract, and the piriform cortex—the ventral pallium), and the lateral and dorsal pallium (the last of which is represented by the neocortex in mammals) (Puelles et al., 2007).

The subpallium is further divided into eight structures, two of which require further explanation. First, the accumbens nucleus, although generally considered to be part of the striatum, is segmented separately because of its clinically relevant role in addiction and reward circuitry, and because it has quite distinctive borders on diffusion weighted MRI (Kelly et al., 1975; Sturm et al., 2003). Second, the bed nucleus of the stria terminalis is segmented separately because it is developmentally distinct from the adjacent septum and because it is believed to play an important role in fear responses and the development of sex differences in mammals (Hines et al., 1992; Walker et al., 2003).

4.2.5 Special cases

The SPECIAL CASES section of the ontology is reserved for two classes of structures that do not comfortably fit into the main ontology hierarchy: the developmentally diverse structures and the major white matter tracts. The term “developmentally diverse structures” refers to those structures that develop from two or more distinct embryonic domains. This group includes the pituitary gland, which is derived from the hypothalamus and oral ectoderm; the substantia nigra, which has both midbrain and forebrain components; and the ventricles, whose walls are formed by all three major embryonic divisions of the brain (Watson et al., 2012). An additional, though accidental, benefit in listing these structures separately is the unique role that they play in human diseases. For example, the pituitary is involved in a variety of endocrine disorders, the substantia nigra in Parkinson’s disease, and ventricle volume is a surrogate marker for the cortical volume loss seen in Alzheimer’s disease (Silbert et al., 2003).

4.2.6 Major white matter structures

The “major white matter structures” section contains several major white matter tracts that are not completely contained within another structure. These white matter structures are segmented separately because: 1) they may contain projections from multiple different brain regions, 2) they pass through or along multiple different structures, and 3) there is considerable interest in measuring normal and/or pathologic changes in white matter structures throughout neurodevelopment (Mukherjee and McKinsty, 2006; Asato et al., 2010; Chuang et al., 2011). In most cases, the listed white matter structures are a composite of two or more white matter structures as defined by the Paxinos and Watson atlas of the adult rat brain (Paxinos and Watson, 2007). For example, the corpus callosum, external capsule, and deep cerebellar white matter are combined into a single structure, as are the internal capsule, the cerebral peduncles and the pyramids. This grouping is done because these pathways are actually continuous with each other and separating them would require drawing an arbitrary line based on surrounding landmarks. Such dividing lines could easily be added, but we believe that they do not considerably increase the utility of the resulting label set.

4.3 Challenges to segmentation of the developing rodent brain

Despite substantial changes that occur in both the neuraxis and in total brain volume during development, segmentation of the p0 and p80 rat brain using our ontology was remarkably similar. The only major difference that complicated segmentation was that the p0 brain contains a significant amount of neuroepithelium surrounding the lateral ventricles, which is absent in the adult rat brain. The neuroepithelium contains neural precursor cells that differentiate into the surrounding brain structures during postnatal neurodevelopment (Frederiksen and McKay, 1988). Because of this, we chose to allocate parts of the neuroepithelium to the nearest neighbor. Practically speaking this involves dividing the neuroepithelium between the adjoining striatum and septum based on proximity.

Due in part to the limited spatial resolution and contrast resolution provided by MR microscopy, some brain regions can be quite difficult to reliably segment. One of the most valuable assets to the would-be segmenter is the use of multiple different MR contrasts. Just as conventional neurohistologists rely on multiple tissue stains, such as Nissl, acetyl cholinesterase, and calbindin, so too should the MR histologist rely on multiple “proton stains” such as T2*-weighting and diffusion-weighting (Delnomdedieu et al., 1996). A particular brain structure border may be nearly invisible on one MR contrast, and readily apparent on another. By using multiple MR contrasts for segmentation, one can circumvent many of the limitations of MR microscopy as compared to conventional histology.

The rat amygdala highlights the utility of using multiple MR contrasts for brain segmentation. Figure 4 shows a coronal section of the adult rat brain at the caudal border of the amygdala displayed with T2*-weighted contrast (Figure 4:A) and diffusion-weighted contrast (Figure 4:B). The T2*-weighted image shows excellent white matter contrast, which reveals the full extent of the deep cerebral white matter (dcw) both laterally and ventrally. Additionally, the optic tract (opt) is visible as it runs between the diencephalon and the dentate gyrus. Despite this exquisite white matter detail, the ventral cortical region of the T2*-weighted image is virtually devoid of image contrast. In comparison, the diffusion-weighted image (Figure 4:B) quite clearly reveals the caudal amygdaloid nuclei: the posteromedial cortical amygdaloid nucleus (PMCo) and the amygdalopiriform transition area (APir). Further, the caudal extent of the piriform cortex can clearly be identified based on the high level of diffusion attenuation present in layer 2 (Pir2). In this example, and in many others, these different image contrasts each contributed unique and essential information for identifying the structures listed in Table 3. Just as no rational histologist

would attempt to segment an entire brain based on one tissue stain, we suggest that no accurate whole brain segmentation can be achieved with only a single MR contrast.

4.4 Volume changes in the rat brain during postnatal neurodevelopment

To demonstrate the utility of our ontology for tracking regional, postnatal neurodevelopmental changes in the brain, we compared brain sub-structure volumes from manually segmented p0 and p80 rat brain MR microscopy volumes. The aggregate of these results is presented in Table 4. These data show a nearly 9-fold increase in total brain volume from 0.26 mL at p0 to 2.29 mL at p80. Interestingly this growth is not uniform throughout the brain, with some structures growing much faster relative to others during the postnatal period. For example the cerebellum grows substantially, both in absolute and relative terms, experiencing a nearly 40-fold increase in volume and increasing from just 3.44% of total brain volume at p0 to 15.20% at p80. This growth can be appreciated in Figure 3, where the cerebellum (teal) is quite small relative to the p0 brain (Figure 3:A-C), and much larger relative to the p80 brain (Figure 3:D-F). This observation is consistent with reports that a majority of rodent cerebellar growth and development occurs in the postnatal period (Altman, 1972). In contrast, both the midbrain and hindbrain grow, on average, more slowly than the brain as a whole during this period.

Examination of Figure 5 reveals a general trend towards slower postnatal growth for axial structures (hindbrain, midbrain, diencephalon, hypothalamus) and more rapid growth for paraxial structures (cerebellum, isocortex, hippocampus, striatum). This trend likely relates to the functional roles of these brain regions; axial, subtelencephalic regions tend to be involved in basic life support and homeostatic functions (i.e. breathing, heart rate, temperature control) and therefore must be fully functional at birth, whereas paraxial and, in particular, telencephalic structures tend to be involved in higher-order functions that can develop during postnatal life. While it is certainly true that increased structure volume is not an ideal surrogate marker for increasing complexity or functionality, it nonetheless suggests some degree of developmental differentiation. Importantly, many of the volumes listed here are considerably larger than previously published values. This is likely because many previous studies on rat brain volume are derived from conventional histology methods that cause considerable brain shrinkage and distortion (Rosen and Harry, 1990; Bailey et al., 2004). We believe that our results more closely represent native, in-vivo brain volumes due to our tissue fixation methods, and the fact that brains are imaged inside the intact neurocranium.

5. Conclusions

The ontology presented here is based on a vast body of past research on brain structure and embryology, well summarized by Puelles et al. (2007). We have adapted and refined this developmental ontology specifically for tracking postnatal changes in the rodent brain. Rodent model systems have revolutionized our understanding of brain structure and function both in health and in disease. It seems inevitable that rodent model systems will continue to play a pivotal role in helping the medical research community understand the importance of postnatal neurodevelopment and its role in disease. We believe that adaptation of conventional anatomical knowledge for new and emerging technologies like MRH has the potential to greatly accelerate our understanding of neurodevelopment. With this in mind, we have created what we believe to be the first simplified ontology specifically designed for tracking MR biomarkers in the postnatal rodent brain. We have confirmed that all 26 structures in our ontology are identifiable throughout postnatal life by segmenting high-resolution MRI volumes of the p0 and p80 rat brain. Further, we have shown that multiple proton contrasts are helpful, if not essential, for complete segmentation of MR volumes, just

as multiple histology stains are required to fully segment a histology volume. Finally, we have used these labels to measure regional postnatal brain growth in the rat and correlated these results with previous observations.

We have provided our label volumes for use in future studies on rodent neurodevelopment, but this approach certainly not limited to Order Rodentia. In fact our ontology and the use of MRH can be readily adapted for virtually any mammalian species, including humans, because it is based on a shared mammalian neuroembryology. This brings up the enticing possibility of direct regional brain comparisons between different studies, and even different species, all using a shared developmental ontology. Even if this ontology is expanded to include any number of smaller sub-structures, the hierarchical arrangement allows several labels to be combined to create a single higher order structure. In this study, we have demonstrated that conventional neuroanatomical organization has an important role to play in the growing field of MRH-based neuroscience research. We hope that future studies will attempt to further integrate MR into the neuroscience community so that it can continue to complement our understanding of the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

An ontology-based segmentation scheme for tracking postnatal changes in the developing rodent brain with MRI

- The first ontology-based segmentation for tracking postnatal changes in the rodent brain with MRI
- Population averaged, p0 and p80 rat brain images at 25 μm isotropic resolution
- Full 3D segmentation of p0 and p80 average brains using our ontology
- Comparison of regional brain volume changes between birth (p0) and adulthood (p80)

Figure 1.

A comparison of a plate from the Ashwell and Paxinos p0 rat brain histology atlas (Ashwell and Paxinos, 2008) (left) with the oriented p0 GRE data (right). The displayed section is at the level of the caudal crossing of the anterior commissure. Letter A marks the ventral hippocampal commissure (vhc). B marks the intersection of the posterior limb of the anterior commissure (acp) with the external capsule (ec). C marks the caudal extent of the crossing of the anterior commissure.

Figure 2.

The complete p0 (A-C) and p80 (D-F) segmentation presented as 2D color-overlays on coronal, isotropic diffusion-weighted images. The colors used are consistent with those shown in Table 3. A and D are sections through the decussation of the anterior commissure, B and E through the rostral portion of the hippocampal formation, and C and F through the caudal portion of the hippocampal formation.

Figure 3.

The complete p0 (A-C) and p80 (D-F) segmentation presented as 3D rendered surfaces. The colors used are consistent with those shown in Table 3. A and D are profile views with the olfactory bulb pointed to the bottom right; B and E are dorsal views with the olfactory bulb to the right; and C and F are ventral views with the olfactory bulb to the right.

Figure 4.

A comparison of the two MR contrasts used for brain segmentation—T2*-weighted contrast (A) and isotropic diffusion-weighted contrast (B). Magnified insets show a coronal section through the caudal amygdala. The T2*-weighted image shows excellent white matter contrast including the deep cerebral white matter (dcw) and the optic tract (opt). In contrast, the diffusion-weighted image shows excellent contrast in the posteromedial cortical amygdaloid nucleus (PMCo), the amygdalopiriform transition area (APir), and layer 2 of the piriform cortex (Pir2). Also labeled is the CA3 layer of the hippocampus.

Figure 5.

A graphic representation of regional brain growth between p0 and p80. Percent growth for each of the 26 brain regions listed in Figure 3 is displayed as a heat-map color overlay on coronal diffusion-weighted images of the p80 rat brain. A-C correspond to Figure 3:D-F. D is a section through the hindbrain at the level of the exit of the facial nerve. The pituitary and cerebellum grew by ~8000% and ~4000% respectively, both of which fall well outside of the displayed scale.

Table 1

Comparison of interstice distance between corresponding coronal planes in the p0 rat brain as measured by MRI and the Ashwell and Paxinos p0 rat histology atlas (Ashwell & Paxinos, 2008).

Histology Plate #s	MR Slice #s	Histology Interslice Dist. (mm)	MR Interslice Dist. (mm)	Discrepancy (mm)
217-228	536-485	2.2	1.275	0.925
228-235	485-442	1.4	1.075	0.325
235-245	442-403	2	0.975	1.025
245-251	403-381	1.2	0.55	0.65
251-279	381-251	5.6	3.25	2.35
Mean Discrepancy (mm)				1.06

Table 2

Hierarchical organization of the developmentally defined ontology for segmenting rodent MRH brain volumes. In the MAIN ONTOLOGY section, parent structures are subdivided to constituent parts at each level of the bulleted tree (i.e. axial hindbrain is part of hindbrain).

MAIN ONTOLOGY	
○ Hindbrain	
○ Axial Hindbrain	
○ Cerebellum	
○ Midbrain	
○ Forebrain	
○ Diencephalon	
■ Pineal gland	
○ Hypothalamus	
○ Telencephalon	
■ Pallium	<ul style="list-style-type: none"> • Hippocampal Formation • Isocortex • Olfactory Structures
■ Subpallium	<ul style="list-style-type: none"> • Amygdala • Bed Nucleus of the Stria Terminalis • Diagonal Domain • Pallidum • Preoptic Area • Septum • Striatum
	○ Accumbens Nucleus
SPECIAL CASES	
○ Developmentally diverse structures	
○ Pituitary	
○ Substantial Nigra	
○ Ventricles	
○ Major white matter structures	
○ Anterior Commissure	
○ Cingulum	
○ Corpus Callosum/Deep Cerebral White Matter	
○ Fimbria/Fornix	
○ Internal Capsule/Cerebral Peduncle/Pyramids	
○ Optic Pathways	

Table 3

Color code for brain structures used throughout this document.

Structure	Color
Accumbens nucleus	Red
Amygdala	Green
Anterior Commissure	Light Green
Axial Hindbrain	Light Green
Bed Nucleus of the Stria Terminalis	Pink
Cerebellum	Teal
Cingulum	Red
Corpus Callosum/Deep Cerebral White Matter	Yellow
Diagonal Domain	Cyan
Diencephalon	Blue
Fimbria/Fornix	Brown
Hippocampal Formation	Pink
Hypothalamus	Cyan
Internal Capsule/Cerebral Peduncle/Pyramids	Purple
Isocortex	Green
Mesencephalon	Orange
Olfactory Structures	Teal
Optic Pathways	Purple
Pallidum	Pink
Pineal Gland	Pink
Pituitary	Blue
Preoptic Area	Green
Septum	Light Green
Striatum	Blue
Substantia Nigra	Yellow
Ventricles	Blue

Table 4

Sub-structure volume and standard error derived from whole brain segmentation of the p0 and p80 rat brain. All volumes are in microliters. “% of total volume” refers to the volume percent of each structure mean

Structure	p0 structure volume	p80 structure volume	p0 % of total volume	p80 % of total volume	Δ volume	Δ % of total volume	% growth
Cingulum	0.56±0.07 μL	6.01±0.18 μL	0.21%	0.26%	5.45 μL	0.05%	1073.8%
Midbrain	24.27±1.58 μL	97.58±3.72 μL	9.26%	4.26%	73.31 μL	-5.00%	402.0%
Substantia nigra	0.65±0.05 μL	7.59±0.19 μL	0.25%	0.33%	6.94 μL	0.08%	1173.2%
Anterior commissure	0.56±0.07 μL	4.13±0.18 μL	0.22%	0.18%	3.57 μL	-0.03%	732.4%
Hindbrain	43.39±1.45 μL	314.31±5.84 μL	16.56%	13.71%	270.92 μL	-2.84%	724.4%
Septum	3.68±0.26 μL	15.14±0.69 μL	1.40%	0.66%	11.47 μL	-0.74%	411.9%
Diagonal domain	1.42±0.09 μL	5.58±0.36 μL	0.54%	0.24%	4.16 μL	-0.30%	391.8%
Hypothalamus	11.11±0.70 μL	41.87±1.36 μL	4.24%	1.83%	30.76 μL	-2.41%	377.0%
Striatum	13.59±1.76 μL	94.25±3.46 μL	5.19%	4.11%	80.65 μL	-1.08%	693.3%
Diencephalon	20.69±1.66 μL	112.40±3.61 μL	7.89%	4.90%	91.71 μL	-2.99%	543.2%
Internal capsule	3.59±0.32 μL	36.58±0.74 μL	1.37%	1.60%	32.99 μL	0.22%	1017.6%
Hippocampus	10.41±0.90 μL	111.57±3.89 μL	3.97%	4.87%	101.16 μL	0.89%	1071.6%
Pallidum	2.70±0.24 μL	19.12±0.62 μL	1.03%	0.83%	16.42 μL	-0.20%	708.3%
Accumbens nucleus	2.38±0.27 μL	14.19±0.61 μL	0.91%	0.62%	11.82 μL	-0.29%	597.3%
Fimbria/fornix	2.11±0.20 μL	19.32±0.51 μL	0.80%	0.84%	17.22 μL	0.04%	917.4%
Corpus callosum	15.56±2.10 μL	79.94±3.61 μL	5.94%	3.49%	64.38 μL	-2.45%	513.7%
Amygdala	10.16±0.74 μL	48.43±1.41 μL	3.88%	2.11%	38.27 μL	-1.76%	476.8%
Preoptic area	3.47±0.20 μL	9.94±0.30 μL	1.32%	0.43%	6.47 μL	-0.89%	286.6%
Isocortex	56.59±7.05 μL	640.22±20.32 μL	21.59%	27.93%	583.63 μL	6.34%	1131.3%
Cerebellum	9.02±0.95 μL	348.34±10.74 μL	3.44%	15.20%	339.32 μL	11.76%	3862.3%
Olfactory structures	21.46±1.92 μL	232.54±7.25 μL	8.19%	10.15%	211.08 μL	1.96%	1083.8%
Ventricles	2.20±0.06 μL	9.27±1.05 μL	0.84%	0.40%	7.07 μL	-0.44%	420.7%
Pituitary	0.16±0.02 μL	12.74±0.87 μL	0.06%	0.56%	12.59 μL	0.50%	8104.1%
Optic pathways	0.58±0.03 μL	5.72±0.21 μL	0.22%	0.25%	5.14 μL	0.03%	989.3%
Bed nuc. str. term.	1.52±0.13 μL	3.42±0.18 μL	0.58%	0.15%	1.90 μL	-0.43%	225.2%

Structure	p0 structure volume	p80 structure volume	p0 % of total volume	p80 % of total volume	Δ volume	Δ % of total volume	% growth
Pineal gland	0.26 $\pm$ 0.03 μ L	1.87 $\pm$ 0.20 μ L	0.10%	0.08%	1.61 μ L	-0.02%	724.9%
Whole brain	262.09 $\pm$ 22.81 μ L	2,292.09 $\pm$ 63.85 μ L	100.00%	100.00%	2,030.01 μ L	0.00%	874.6%