***Abstract***

The postnatal period of neurodevelopment has been implicated in a number of brain disorders including autism and schizophrenia (Courchesne et al., 2001; Karlsgodt et al., 2008; Knickmeyer et al., 2008). Rodent model systems 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 propose and demonstrate the utility of a developmental neuro-ontology designed specifically for tracking regional changes in MR biomarkers throughout postnatal neurodevelopment. Using this ontological classification we track regional changes in brain volume between the p0 and p80 rat brain and demonstrate differential postnatal growth rates in axial versus paraxial brain regions. Both the ontology and the associated label volumes are provided in the hopes that they will be used to investigate postnatal neurodevelopment in normal and disease states.

***Introduction and Background***

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 InvestigatorsCenters for Disease Control and Prevention (CDC), 2009; Centers for Disease Control and Prevention (CDC), 2010). Despite the emergence of many prominent theories, the root causes of these disorders remain largely unknown. These and other neurodevelopmental disorders are difficult to study because mammalian neurodevelopment is a complex process that spans a large time period, from early organogenesis to late adolescence. Although the majority of neurodevelopment occurs in-utero, virtually all mammals exhibit significant postnatal brain growth and differentiation (Clancy, Finlay, & Darlington, 2007). Surprisingly little is known about the time-course and nature of postnatal neurodevelopmental changes. 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, Lenroot, Greenstein, & Tran, 2010; White, 2012). For this reason, rodent postnatal neurodevelopment can serve as a model for human neurodevelopment from late gestation through to 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, King, & Weitzman, 2008; Jaaro-Peled et al., 2009). 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 Microscopy (MRM) 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 (Badea, Ali-Sharief, & Johnson, 2007; Chuang et al., 2011; Johnson et al., 2007; 2002a; Petiet et al., 2008). Typically, comparisons between quantitative image sets are done using whole-brain, voxel-wise, or region of interest (ROI) based approaches (Giuliani, Calhoun, & Pearlson, 2005). Large postnatal changes is brain shape and volume make whole-brain and voxel-wise approaches problematic, leaving the ROI-based approach the most straightforward type of comparison. The major advantage of the ROI-based approach is that it allows direct comparison of specific brain regions throughout neurodevelopment, however ROIs must be chosen carefully to avoid artificially creating or obscuring significant differences. In the case of postnatal brain development, ROI selection is particularly important because many brain regions undergo significant changes in size and shape.

In order to appropriately characterize post-natal 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 known commonly as the "basal ganglia" when in fact this arbitrarily 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 (E. Puelles, Puelles, & Watson, 2012; L. Puelles & Watson, 2012; Watson, 2012). 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 one must move away from an arbitrary classification 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, the most important example of which is the chick brain ontology (L. Puelles, 2007). There have been a number of attempts to create a mammalian brain ontology in the recent past, the most notable of which include the NeuroNames ontology (Bowden, 1995), BAMS (Mihail Bota, 2008), BIRN (Bug et al., 2008), and the Allan Brain Atlas ontology (Dong, 2008). These four ontologies are limited in the fact 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 MRM has limited resolution (10-25 μm) and a variety of contrast mechanisms, none of which exactly recapitulates conventional histology stains, the range of structures that can be readily identified in MRM volumes of the mammalian brain is limited. Even fewer structures can be identified throughout postnatal neurodevelopment owing to large changes in brain volume and apparent MR contrast.

An ideal developmental ontology for segmenting MRM volumes would be comprehensive, developmentally accurate, technically realizable, and flexible enough to accommodate further advances in MR contrast and spatial resolution. Here we introduce a developmental ontology 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 developmentally accurate 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 the ontology throughout post-natal neurodevelopment by successfully segmenting MRM volumes of the rat brain immediately after birth (p0) and at adulthood (p80). Finally, we discuss the flexibility of this ontology in accommodating new imaging technologies and brain images of other mammalian species.

## *Methods*

### *Specimen Preparation*

All experiments and procedures were done with the approval of the Duke University Institutional Animal Care and Use Committee. Five postnatal day zero (p0) and five postnatal day 80 (p80) male, Wistar rats were selected for imaging studies. All experimental animals were from litters of 10-12 pups (average = 11) and had body weight within one standard deviation of mean weight for age. Animals were perfusion fixed using the active staining technique (Johnson, Cofer, Gewalt, & Hedlund, 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-situ* in the neurocranium to preserve native spatial relationships.

### *Image Acquisition*

All imaging experiments were 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 × 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 echo (GRE) images were acquired using a custom-designed 3D gradient recalled echo (GRE) sequence (TR = 50 ms, TE = 8.3 ms, NEX = 2, $\alpha$=60°). The acquisition matrix was 1600 × 800 × 800 over a 40 × 20 × 20 mm field of view (FOV) for p80 specimens and 1024 × 512 × 512 over a 25.6 × 12.8 × 12.8 mm for the p0 specimens. In both cases the native isotropic voxel size was 25 μm (voxel volume = 15.625 picoliter).

Diffusion weighted images were acquired using a custom-designed 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 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]. The acquisition matrix was 800 x 400 x 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 native Nyquist limited isotropic voxel size for diffusion images was 50 $\mu$m, however diffusion weighted data were zerofilled in k-space to twice the original matrix size in each dimension so that the resulting voxel dimensions matched the GRE data (25 $\mu$m final isotropic voxel size).

*Image Registration and Averaging*

Inter-specimen registration of MR data was accomplished with the ANTs software package (Avants, Epstein, Grossman, & Gee, 2008). Skull-stripped GRE and mean diffusion weighted images (DWI) from each set of specimens (5 per time point) 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 downsampling factor of 3, 4000 iterations at a downsampling 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.

*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 & Watson, 2007) as part of another study (Johnson, Calabrese, Badea, & Paxinos, 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 & 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.

*Selection of Structures and Image Segmentation*

In order to determine which structures we would be able to reliably segment from the rat brain we started with the three major embryonic subdivisions of the brain: the hindbrain, midbrain and forebrain. We then proceeded to subdivide each major subdivision into smaller constituent parts based on which borders we could reliably identify on T2* and diffusion weighted MRI. T2* and diffusion weighted contrasts was chosen because they are two of the most commonly used contrasts for high-resolution small animal brain imaging. In addition, several previous studies have noted that T2* and diffusion weighted contrasts highlight different, and somewhat complementary brain anatomy (Johnson et al., 2010) (Johnson et al., 2012; Kovacevic, 2004; Ma, Hof, Grant, Blackband, & Bennett, 2005). All 3D digital segmentation of MRI volumes was accomplished using Avizo 3D analysis software (Visualization Sciences Group, Burlington, MA, USA).

## *Results*

### *Alignment of p0 Image Data to the Ashwell and Paxinos Hitology Atlas*

As described in the methods section, the p0 MRM volumes were aligned to the Ashwell and Paxinos p0 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 rostralmost 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 rostralmost crossing of the anterior commissure (ac), which has a distinctive oval shape across the midline that is not present in more rostral sections. Unfortunately the Ashwell and Paxinos atlas does not provide rostral/caudal coordinates so we could not directly compare histologic coordinates to MR coordinates. However, the p0 histology atlas states that the mean sectioning interval is 200 μm, so we can compare interslice 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 the supplementary material.

### *Selection of structures for segmenting MRM 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.

*Segmentation of p0 and p80 rat brain MRM volumes*

We manually segmented early post-natal (p0) and adult (p80) rat brain image volumes into the 26 structures described above. 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 2:1-3a) and p80 (Figure 2:1-3b) rat brain with the segmentation presented as a color overlay. Corresponding sections (i.e. Figure 2:1a and Figure 2:1b) 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 2:1a and 2:1b are sections through the decussation of the anterior commissure, Figure 2:2a and 2:2b through the rostral portion of the hippocampal formation, and Figure 2:3a and 2:3b 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 3:1a and 3:1b are profile views with the olfactory bulb angled to the bottom right of the Figure and the brainstem angled to the top left. Figure 3:2a and 3:2b are dorsal views and Figure 3:3a and 3:3b 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 & Watson, 2007).

*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 Figure 4. All volumes are in microliters. Listed volumes are average structure volumes from 5 different specimens to reduce individual bias. 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 2:1-3b. 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.

## *Discussion*

A number of 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 (Chuang et al., 2011; Johnson et al., 2010; Kovacevic, 2004; Ma et al., 2005). 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, however its usefulness can be extended by focusing on the developmental origins of the brain (L. Puelles, 2007). Recently 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 (Giedd, 2010; Knickmeyer et al., 2008; Mori et al., 2001). 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.

### *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 but rather is designed to: 1) cover the entire brain volume, 2) use only those structures whose borders are readily apparent on MRI 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 only one of the three major embryonic subdivisions of the brain: the hindbrain (rhombencephalon), the midbrain (mesencephalon) and the forebrain (prosencephalon). 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

considered somewhat artificial by some, as it is based on the external appearance of the human brain rather than on developmental origins (Watson, 2012). In fact, the rostral part of the hindbrain, consisting of the isthmus and rhombomere 1, gives rise to the cerebellum (L. Puelles, 2007).

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

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 (L. Puelles, 2007; Watson, Paxinos, & Puelles, 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, diencephalon and telencephalon (L. Puelles, 2007). Although the pineal gland is derived from the diencephalon, it is presented here as a separate structure because it is spatially detached from the rest of the diencephalon during neurodevelopment (L. Puelles & Watson, 2012). 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 olfactory structures (the olfactory bulb, olfactory tubercle, lateral olfactory tract and the piriform cortex) and the isocortex (the remaining portion of the neocortex) (L. Puelles, 2007). The subpallium is further divided into 8 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 & Seviour, 1975; Sturm, Lenartz, Koulousakis, & Treuer, 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 & Allen, 1992; Walker & Toufexis, 2003). It is also worth mentioning that the diencephalon, although segmented as a single structure here, could reasonably be sub-divided into prosomeres 1-3 (L. Puelles & Watson, 2012).

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 (Paxinos, 2011). 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).

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) with the recognition that white matter plays an important role in a variety of human neurological conditions and the growing prevalence of white matter imaging modalities such as diffusion tensor imaging (DTI), there is considerable interest in measuring normal and/or pathologic changes in white matter structures throughout neurodevelopment (Asato, Terwilliger, Woo, & Luna, 2010; Chuang et al., 2011; Mukherjee & McKinstry, 2006). 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 & Watson, 2007). For example, the corpus callosum, external capsule and deep cerebellar white matter are combined into a single structure, as are the internal capsulae, 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.

*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 & 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, Hedlund, Johnson, & Maronpot, 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. One can also appreciate the optic tract (opt) running between the lateral border of the diencephalon and the medial border of 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 (column 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 that were encountered during the segmentation of the p0 and p80 rat brain, 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 and reproducible whole brain segmentation can be achieved with only a single MR contrast.

*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 0.262 mL at p0 to 2.292 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 3:1-3a), and much larger relative to the p80 brain (Figure 3:1-3b). This observation is consistent with reports that a majority of cerebellar growth and development in rodents 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 and therefore become a smaller percent of total brain volume in the adult rat brain.

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.

### *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 MR microscopy 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 carefully and thoroughly confirmed that all 26 structures in our ontology are clearly 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 necessary 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 hope that this developmental ontology will be used in future studies on rodent neurodevelopment, but this approach certainly not limited to Order Rodentia. In fact our ontology can be readily adapted for virtually any mammalian species, including humans, because of the fact that 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 MRI-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.

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