Characterization of the pig brain as a neuroimaging model for early human brain development: a combined structural MRI and DTI study

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Introduction: MRI is an important tool for understanding human brain development and the studying the long-term effects of injury to the immature brain. In humans, previous studies characterized morphological aspects of human brain development, including regional and global increases in brain volume, changes in cortical thickness and the development of complex cortical folds[1]. More recent investigations have assessed the microstructural changes in the developing brain using diffusion tensor imaging (DTI), and demonstrated log-linear increases in the fractional anisotropy (FA) and concomitant decreases in mean diffusivity (MD) during maturation[2]. To gain further insight into pathology and its effects on development, there is a need for suitable translational animal models of the immature human brain. Despite limited use in neuroimaging research, the swine brain offers an appropriate model to investigate pathology because it is relatively large, gyrencephalic and exhibits a grey matter (GM) -to-white matter (WM) ratio similar to humans[3]. The aim of the current study was to further explore the swine brain as a potential developmental model for neuroimaging studies by characterizing the structural and microstructural changes with combined high-resolution structural MRI and DTI.

Methods: We imaged 11 Yorkshire pigs, one to three months old (5.2 – 43.8 kg), on a 1.5 Tesla GE MRI system. Animals were induced, intubated and mechanically ventilated. Anesthesia was maintained with constant infusion of ketamine, midazolam and pancuronium. We acquired high-resolution 3D T_1-weighted anatomical images using an inversion-recovery fast spoiled gradient-recalled echo sequence with the following imaging parameters: TE = 4.2 ms, TR = 8.46 ms, flip angle = 20°, FOV = 180 mm, slab thickness = 180 mm, matrix size = 192 × 192 × 120. For the DTI acquisition, we collected diffusion-weighted images from 25 unique diffusion-encoded directions (b = 700 s/mm^2), as well as one image without diffusion-weighting. DTI data was acquired with a spin echo EPI sequence with parallel imaging (R = 2); imaging parameters included: TE = 80 ms, TR = 5.4 s, FOV = 160 mm, matrix size = 128 × 128, slices = 16, slice thickness = 4.5 mm.

All images were transferred to an independent work station for post-processing and analysis. For the 3D anatomical images, we isolated the brain and then employed a combination of automatic (FAST, FSL, FMRIB Software Library, http://www.fmrib.ox.ac.uk/fsl) and manual segmentation to indentify the following tissue regions: cortical WM, cortical GM, deep WM and deep GM. In addition, we outlined the cortex to calculate the surface folding index (SFI = Length^2/4π·Area), a measure of the degree of cortical folding[4]. DTI processing included correction for eddy current related geometric distortions and computation of the diffusion tensor (FDT, FSL), from which MD and FA scalars were generated. Next, scalar DTI parametric images were transformed to the 3D anatomical volume via a set of low resolution T1-weighted images collected at the exact slice locations for DTI (FLIRT, FSL). Mean values for each DTI parameter were extracted for each region interrogated. Although we scanned pigs from different age groups, we opted to use the body weight as a continuous variable to assess the changes with development. Data were analyzed using curve estimation (logarithmic) with body weight as the independent variable and the following measures as dependent variables: brain volume, SFI, MD, and FA. Statistical analyses were performed in SPSS v 11.0 (SPSS Inc., Chicago, IL). We considered $p < 0.05$ statistically significant.

Results: The logarithmic regression analysis revealed a significant log-linear relationship between body weight and the GM, WM and total brain volume (Figure 1, Table 1). The surface folding index, a measure of cortical folding, also exhibited a logarithmic increase with body weight (Figure 2, Table1). The FA exhibited a log-linear increase with body weight for all regions investigated (Figure 3, Table 1). No MD changes existed.

	Region	R^2	P value
Volume	GM	0.95	<0.001
	WM	0.68	<0.005
	Total	0.92	<0.001
SFI	-	0.86	<0.001
FA	Cor GM	0.67	<0.005
	Cor WM	0.79	<0.005
	Deep GM	0.72	<0.005
	Deep WM	0.85	<0.001

Table 1. Results of the statistically significant log-linear regressions observed for brain volume, SFI and FA versus body weight.

Discussion: The brain volume increases observed in the 1 wk – 3 month old pigs qualitatively match the range of human infancy to pre-pubertal adolescence[3]. Over this same period of swine brain development we also observed a progression of complex cortical folding, which is present in human brain development. The DTI characteristics of the pig brain were similar to the developing human brain in terms of the FA changes[2], which is believed to mirror processes of myelination in the developing mammalian brain[5]. However, in contrast to human development[2], we failed to observe a decreaseding MD with development. This MD discrepancy is most likely attributed to water content, as rapid water content changes observed in the developing human brain[1] are not observed in the postnatal pig brain[6]. Moreover, MD in the one day old pig is similar to adult humans, which suggests that most MD changes in the pig brain occur before birth[7]. Results suggest the swine brain may provide an informative model of early human brain development that could be useful for translational studies.

References: 1. Dobbing J *et al.*, Arch Dis Child, **48**:757–767 (1973); 2. Hermoye L *et al.*, Neuroimage, **29**:493-504 (2006); 3. Dobbing J *et al.*, Pediatrics, **53**:2-6(1974); 4. Dieni S *et al.*, J Neuropathol Exp Neurol **63**:1297-1309 (2004); 5. Kinney HC *et al.*, J Neuropathol Exp Neurol, **47**:217:34 (1988); 6. Dickerson JWT *et al.*, Proc R Soc Lond B,**166**:384-395 (1967); 7. Winter JD *et al.*, Pediatr Res, **65**:181-7 (2009).

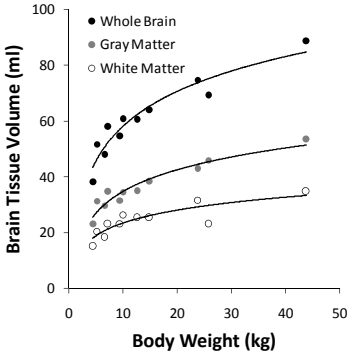

Figure 1. Brain tissue volume versus total body weight in the pig. The solid line represents the log-linear regression.

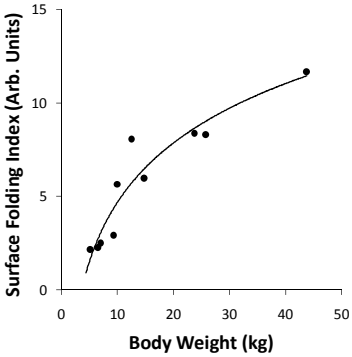

Figure 2. Surface folding index versus total body weight in the pig. The solid line represents the log-linear fit to the data.

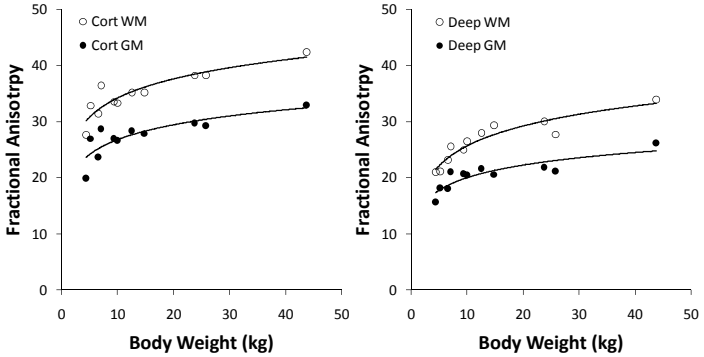

Figure 3. Fractional anisotropy versus total body weight in the pig for the cortical (a) and deep brain regions (b). The solid line represents the log-linear fit to the data.