Brain Growth of the Domestic Pig (*Sus scrofa*) from 2 to 24 Weeks of Age: A Longitudinal MRI Study

Matthew S. Conrad^a Ryan N. Dilger^{b, c} Rodney W. Johnson^{a–c}^aNeuroscience Program, ^bDivision of Nutritional Sciences and ^cDepartment of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Ill., USA

Key Words

Neonatology • Pig • Magnetic resonance imaging • Hippocampus • Development • Brain

Abstract

An animal model with brain growth similar to humans, that can be used in MRI studies to investigate brain development, would be valuable. Our laboratory has developed and validated MRI methods for regional brain volume quantification in the neonatal piglet. The aim of this study was to utilize the MRI-based volume quantification technique in a longitudinal study to determine brain growth in domestic pigs from 2 to 24 weeks of age. MRI data were acquired from pigs 2–24 weeks of age using a 3-dimensional magnetization-prepared gradient echo sequence on a Magnetom Trio 3-tesla imager. Manual segmentation was performed for volume estimates of total brain, cortical, diencephalon, brainstem, cerebellar and hippocampal regions. Logistic modeling procedures were used to characterize brain growth. Total brain volume increased 130% ($\pm 12\%$) and 121% ($\pm 7\%$) from 2 to 24 weeks in males and females, respectively. The maximum increase in total brain volume occurred about the age of 4 weeks and 95% of whole brain growth occurred by the age of 21–23 weeks. Logistical modeling suggests there are sexu-

ally dimorphic effects on brain growth. For example, in females, the cortex was smaller ($p = 0.04$). Furthermore, the maximum growth of the hippocampus occurred about 5 weeks earlier in females than males, and the window for hippocampal growth was significantly shorter in females than males ($p = 0.02$, $p = 0.002$ respectively). These sexual dimorphisms are similar to what is seen in humans. In addition to providing important data on brain growth for pigs, this study shows pigs can be used to obtain longitudinal MRI data. The large increase in brain volume in the postnatal period is similar to that of human neonates and suggests pigs can be used to investigate brain development.

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Introduction

At birth the human infant brain is just 25% of adult size. It undergoes massive postnatal growth so by the age of 2 years overall brain size reaches about 85% of adult volume [1]. This period of accelerated brain growth may be the most important phase of postnatal brain development in humans, as it is the result of synaptogenesis, gliosis and myelination [2]. The rapid growth and development of the brain are accompanied by an equally rapid

development of cognitive and motor function [3, 4]. Thus, this early postnatal phase is recognized as a period of increased vulnerability to injury, and disruption of neurodevelopment by environmental insults may have long-lasting or permanent effects on brain structure and function. The idea that environmental insults in an early sensitive period affect behavior later is supported by studies showing that early-life stress increases the likelihood for several neuropsychological disturbances later in life [5], and that iron deficiency between the ages of 6 months and 2 years leads to long-term deficits in learning and memory [6–8]. Nonetheless, very little is known about how human brain growth and development proceed during this period let alone how insults interfere.

Quantitative magnetic resonance imaging (MRI) has emerged as a powerful tool for assessing human brain development. However, most large-scale MRI studies of brain development have been done with older children (>4 years of age), and use of MRI in infants and younger children has mostly been restricted to those born before term [9, 10]. Only recently was MRI used to assess brain growth in healthy full-term infants from birth to the age of 2 years [1]. This study has provided an impressive view of the massive brain growth and development that occur postnatally in healthy full-term infants and underscores this as being an influential period. Although this application of MRI in human infants is impressive, the ability to address mechanistic questions related to early-life insults cannot be readily done due to practical or ethical concerns. Therefore, a preclinical animal model with brain growth and development similar to humans that can be used in MRI studies to investigate how different insults at critical periods of rapid brain growth affect development and function would be valuable.

Due to its anatomical and physiological similarities with humans, the domestic pig (*Sus scrofa*) is a preferred preclinical model in several areas including pediatric nutrition and organ transplant surgery [11, 12]. Because pigs and humans are thought to share similar brain growth and development patterns, appeal for the pig as a model for human infant brain development has increased. The major brain growth spurt extends from the late prenatal to early postnatal period in both the pig and human, which is different from other common animal models [13]. Additionally, gross anatomical features including gyral pattern and distribution of grey and white matter of the neonatal porcine brain are similar to that of human infants [14–16]. The physical size of piglets also allows for quantitative structural MRI of the brain using clinical scanners. Structural MRI scanning and analysis proto-

cols have been developed for neonatal and adult domestic pigs [17, 18] and for adult Göttingen pigs [19]. More advanced techniques including functional MRI and diffusion tensor imaging have also been used [20, 21]. However, we are unaware of previous studies where the brain growth trajectory was assessed in pigs using MRI in a longitudinal study. Previously, our laboratory has developed MRI methods for quantifying brain region volumes in the neonatal piglet [17]. The purpose here was to use these techniques to determine total brain and brain region volumes in a cohort of male and female domestic pigs on 7 occasions from the age of 2 weeks to near sexual maturity at the age of 24 weeks. The results using this noninvasive longitudinal approach confirm substantial postnatal brain growth in domestic pigs. Furthermore, using logistic modeling procedures to determine the maximum volume and the age of maximum growth rate, we revealed a number of attributes of pig brain growth that are similar to humans, including evidence for sexual dimorphic effects.

Material and Methods

Subjects

A total of 15 pigs, 6 intact males and 9 females (*Sus scrofa domestica*, York breed), from 7 litters, were obtained from the University of Illinois swine herd at 2 days of age and placed into an artificial rearing system that was previously described [22]. Briefly, each piglet was housed individually in an acrylic-sided caging unit with a 12-hour light/dark cycle. The ambient temperature was maintained at 27°C with intracage temperatures maintained at 32°C using overhead radiant heaters. Piglets were provided a commercially available liquid milk replacer (Milk Specialties Global Animal Nutrition, Carpentersville, Ill., USA) until 3 weeks of age at which time they were moved to individual floor pens (4.65 m²) and provided ad libitum access to water and a corn-soybean meal-based diet formulated to provide recommended levels of all essential nutrients [23]. Pigs were weighed weekly to determine weight gain. All procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of Illinois Institutional Animal Care and Use Committee.

Image Acquisition

At 2 weeks of age pigs were subjected to MRI scanning procedures to obtain brain images for in vivo estimation of volume. Before scanning, pigs were anesthetized by intramuscular injection of a telazol:ketamine:xylazine solution (4.4 mg/kg body weight), and placed in a prone position in the MRI scanner. Pigs remained anesthetized during the MRI scanning procedure (approx. 20 min) and were returned to their pen afterwards where they were monitored until recovering from anesthesia. All pigs underwent additional MRI scans at 4, 8, 12, 16, 20 and 24 weeks of age. At the conclusion of the 24-week MRI scan, pigs were eu-

thanized by intracardiac administration of an overdose of sodium pentobarbital (390 mg/ml Fatal Plus – administered at 1 ml/5 kg body weight).

Magnetic resonance scanning was conducted using a 3-dimensional T_1 -weighted magnetization-prepared gradient echo sequence. A 32-channel head coil was used for pigs up to 8 weeks of age, and a flexible 6-channel coil was used thereafter. The parameters used were: repetition time = 1,900 ms; echo time = 2.48 ms; inversion time = 900 ms; flip angle = 9° ; matrix = 256×256 (interpolated to 512×512); slice thickness = 1.0 mm. The final voxel size was $0.35 \text{ mm} \times 0.35 \text{ mm} \times 1.0 \text{ mm}$.

Brain Region Volume Estimation

Digital Imaging and Communications in Medicine (DICOM) images were imported and analyzed using 3-dimensional visualization software (Amira®, Visage Imaging Inc., San Diego, Calif., USA). The whole brain, cortex, diencephalon, cerebellum, hippocampus and brainstem were manually segmented using a Wacom Cintiq 21UX graphic input screen and pen (Wacom, Vancouver, Wash., USA) and criteria previously described [17]. Each region was manually segmented in the three anatomical planes using the atlas by Felix et al. [24], and regional volume estimates were calculated using the Amira software. The reliability of this technique in pigs has been validated [17].

Statistics

All data analysis was done using SAS software (SAS, Cary, N.C., USA). Weight gain data were subjected to a repeated 2-way mixed-model ANOVA (age $\times$ sex). Brain region growth curves were constructed for each brain area in each individual pig using the logistic growth model shown below [25] and the PROC NLIN function of SAS:

$$\text{brain region volume} = \frac{A}{1 + e^{-(\text{age} - C)/B}}$$

Parameter estimations were computed for maximum brain region volume (A), ‘duration’ of growth (B) and age of maximum growth rate (C) for each pig. The ‘duration’ of the growth term (B) is defined as $B = (\sqrt{3}/\pi)\sigma$ where sigma is the standard deviation of the logistic function [26]. Individual parameter estimations were then analyzed using a mixed model including sex, litter and the interaction. For analysis of hippocampal asymmetries, hippocampal data were subjected to a 2-way mixed model Anova (sex $\times$ hemisphere). There were no significant differences due to hemisphere (data not shown); therefore left and right hippocampal volumes were combined to create a total hippocampal volume measure. The logistic growth model was further used to estimate the age when total brain and brain region volume was 50, 75 and 95% of maximum volume. The significance level was set at $p < 0.05$.

Results

Body Weight

Whole body weight for male and female pigs from 2 to 24 weeks of age is shown in figure 1. There were differences in body weight due to age ($F_{6,78} = 702.88, p < 0.0001$),

Fig. 1. Body weight of pigs from 2 to 24 weeks of age. Body weight of male pigs higher than female pigs starting at 20 weeks of age. The error bars indicate SEM. * $p < 0.01$, ** $p < 0.001$.

sex ($F_{1,13} = 14.25, p = 0.0023$), and an age $\times$ sex interaction ($F_{6,78} = 2.51, p = 0.0283$), with males weighing more than females beginning at 20 weeks of age.

Total Brain and Brain Region Volumes

A representative set of DICOM images from an individual female pig across all ages is shown in figure 2a, and DICOM images in figure 2b show the brain of a 12-week-old female pig in three planes. Total brain volume and volume of cortex, hippocampus, diencephalon, cerebellum and brainstem were calculated after manual segmentation in three planes and the changes from 2 to 24 weeks of age are shown in figure 3 as are the growth curves estimated by logistic modeling. The estimations for maximum volume, ‘duration’ of growth and the age when the maximum growth rate occurred are presented for both males and females in table 1.

Total brain volume increased 130% ($\pm 12\%$) and 121% ($\pm 7\%$) from 2 to 24 weeks in males and females, respectively. The maximum increase in total brain volume occurred when pigs were about 4 weeks old and 95% of whole brain growth occurred by 21–23 weeks of age. Cortical volumes changed similarly although the estimated maximum volume was greater for males than females ($F_{1,2} = 21.21, p = 0.04$). In 2-week-old piglets, hippocampal volume (right and left hemispheres combined) was similar in males and females. However, the growth trajectory of the hippocampus was sex dependent and at 24 weeks, the hippocampus was larger in males than females ($F_{1,2} = 141.60, p = 0.007$). The maximum increase in hip-

Fig. 2. Representative MRI images displaying brain growth from 2 to 24 weeks of age. The top images are T1-weighted axial sections (skull stripped) from a female pig at each age (**a**). The bottom images show the coronal, axial, and sagittal views left to right of a 12 week old female pig (**b**). Segmentation is shown for cortex (white), diencephalon (green), hippocampi (blue), cerebellum (red), and brainstem (yellow). Colors refer to the online version only.

hippocampal volume occurred earlier in females than males (3 weeks of age vs. 8 weeks of age; $F_{1,2} = 51.68$, $p = 0.02$). Based on logistic modeling, 95% of hippocampal growth occurred in females by 24 weeks of age whereas in males this milestone was not expected until after 39 weeks of age ($F_{1,2} = 551.99$, $p = 0.002$). In the diencephalon, there were no significant differences in maximum volume, duration of growth, and age of maximum growth rate due to sex. For cerebellar growth, males showed a significantly larger maximum volume than females ($F_{1,2} = 35.98$, $p = 0.03$), but there were no sex differences in duration of growth. In males the age of maximum growth rate occurred roughly 1 week later than in females ($F_{1,2} = 40.14$, $p = 0.02$). The maximum volume of the brainstem was similar for males and females, but the age of maximum growth rate occurred about a week later in males ($F_{1,2} = 75.53$, $p = 0.01$).

From the logistic growth model we further estimated the age when total brain and brain region volumes were 50, 75 and 95% of maximum volumes (table 2). For example, the total brain volume of males was estimated to be 50 and 75% of maximum volume at 4 and 11 weeks of age, respectively. On the other hand, the volume of the hippocampus in males was estimated to be 50 and 75% of maximum volume at about 8 and 20 weeks of age, respectively.

Discussion

In the present study, each piglet was subjected to an MRI scan on 7 occasions from 2 to 24 weeks of age to determine brain growth throughout the neonatal period to near sexual maturity. The results using this noninvasive longitudinal approach confirm substantial postnatal brain growth, with the most rapid growth occurring at about the age of 4 weeks when the brain is approximately 50% of maximum volume. The period of rapid brain growth continued to about the age of 12 weeks, when the growth rate began to slow. Results from two recent cross-sectional studies where MRI was used to estimate brain volumes of pigs at different ages and weights, also suggest rapid brain growth in the postnatal period [17, 27]. The large increase in total brain volume in the postnatal period is similar to that of human neonates and suggests this is a critical period where disruption of developmental processes by environmental insults can have long-lasting or permanent effects on brain structure and function. Therefore, the domestic pig may serve as a preclinical model for postnatal human brain development.

Quantitative MRI has yielded important information on brain development in early childhood and adolescence [9], but because of potential health concerns for infants,

Fig. 3. Brain region growth curves and logistic models. The graphs show total brain (**a**) and brain region volumes (cortex, **b**; whole hippocampus, **c**; diencephalon, **d**; cerebellum, **e** and brainstem, **f**) for male and female pigs from 2 to 24 weeks of age, as well as logistic growth models for male and female pigs. Error bars indicated SEM.

Table 1. Average logistic growth parameter estimates by sex for each brain region

	Maximum volume, mm ³			'Duration' of growth, weeks			Age of maximum growth rate, weeks		
	mean	SEM	significance (by sex)	mean	SEM	significance (by sex)	mean	SEM	significance (by sex)
Total brain volume									
Male	111,087	5,434	0.1054	6.40	0.41	0.4096	4.08	0.30	0.8239
Female	103,150	4,174		5.90	0.45		3.81	0.40	
Cortex									
Male	77,847	4,324	0.0441	6.67	0.71	0.2349	4.01	0.53	0.8829
Female	71,807	3,476		5.93	0.57		3.70	0.50	
Whole hippocampus									
Male	2,602	277	0.0070	10.51	2.68	0.0018	8.28	3.03	0.0188
Female	2,039	100		7.35	0.57		3.28	0.65	
Diencephalon									
Male	13,099	713	0.9779	5.81	0.57	0.4415	4.36	0.53	0.3926
Female	12,710	580		6.23	0.69		4.42	0.81	
Cerebellum									
Male	10,216	593	0.0267	5.84	0.62	0.2041	5.54	0.38	0.0240
Female	9,302	260		5.27	0.32		4.89	0.22	
Brainstem									
Male	9,197	933	0.1530	7.72	1.07	0.0561	5.95	1.12	0.0133
Female	8,101	388		6.65	0.67		4.61	0.51	

The averages by sex for maximum volume, 'duration' of growth and age of maximum growth rate are indicated. Mixed model analysis was used to determine the main effect of sex on each parameter.

Table 2. Age when total brain volume reaches 50, 75 and 95% of maximum volume

	50%		75%		95%	
	age weeks	SEM	age weeks	SEM	age weeks	SEM
Total brain volume						
Male	4.08	0.12	11.12	0.30	22.94	0.61
Female	3.81	0.14	10.30	0.30	21.19	0.57
Cortex						
Male	4.01	0.22	11.34	0.53	23.66	1.07
Female	3.70	0.19	10.22	0.39	21.19	0.74
Whole hippocampus						
Male	8.27	1.24	19.82	2.37	39.21	4.36
Female	3.27	0.16	11.35	0.25	24.92	0.43
Diencephalon						
Male	4.36	0.22	10.74	0.45	21.47	0.87
Female	4.42	0.07	11.26	0.12	22.75	0.26
Cerebellum						
Male	5.54	0.16	11.97	0.43	22.76	0.89
Female	4.89	0.05	10.68	0.13	20.41	0.29
Brainstem						
Male	5.95	0.46	14.44	0.92	28.69	1.72
Female	4.61	0.18	11.92	0.46	24.20	0.93

Using the logistic models, estimates with standard errors were computed for the ages when different brain regions of each sex reach 50, 75 and 95% of adult brain volume for each measure. These data can be compared to human data to determine comparable growth timelines for designing experimental time windows.

few studies have focused on the period from birth to 4 years of age when dramatic brain development occurs. Most MRI studies of infants have been with those born prematurely or with significant health complications. Only recently was MRI used to assess structural brain development of healthy full-term infants from birth to 2 years of age [1]. The first year after birth, total brain volume more than doubled and hemispheric gray and white matter increased by 149 and 11%, respectively. With total brain volume estimates for healthy infants and healthy adults [28], it was determined that total brain volume at 2–4 weeks of age is approximately 36% of adult volume; and at 1 year and 2 years of age, total brain volume is approximately 72 and 83% of adult volume, respectively [1]. Thus, in healthy human infants there is enormous brain growth the first year after birth, making this a period of increased vulnerability to injury. Investigating questions related to early-life environmental insults and brain development and function is difficult in human infants due to obvious practical and ethical concerns. Thus, in addition

to providing important data on brain growth trajectory for pigs, the present study shows pigs can be used to obtain longitudinal data in MRI studies. This indicates pigs can be used in MRI studies to investigate how different insults at critical periods of rapid brain growth affect development and function. This is particularly useful because unlike some common animal models, piglets can be tested in learning and memory tasks at an early age when brain growth is maximal [22, 29].

Despite substantial brain growth the first 2 years after birth, in humans total brain volume continues to increase until about puberty, with the total brain volume peaking at 10.5 years of age in females and 14.5 years of age in males [30]; thereafter, total brain volume diminishes with age so total cerebral volume follows an inverted U-shaped trajectory [31]. Maximum total brain volume in human males is 8–10% larger than females [32]. In the present study, total brain volume data obtained from pigs aged 2–24 weeks were best described using a logistic (sigmoidal) model. Consistent with what has been reported for humans, the logistic model-suggested maximum total brain volume for male pigs was approximately 8% larger than for females. The final MRI scan occurred near puberty, and how total brain volume for pigs might change further into adulthood is not known.

From the present study, we cannot determine composition of brain growth. In humans, most neurogenesis and migration occur early in the prenatal period (starting in the first month of gestation) with astrocyte and oligodendrocyte proliferation extending from the late prenatal period into the postnatal period [2]. The increase in gray matter the first few years of life is likely due to glial cell proliferation, dendritic and axonal arborization and synapse formation, while white matter increases are due to myelination [33, 34]. Similar to humans, the domestic pig does not have substantial neocortical neurogenesis in the postnatal period [35]. This is different from the Göttingen minipig, which has significant neuronal and glial cell development in the postnatal period [35]. Therefore, the substantial increase in brain volume seen in the current study is likely due to changes in the neuropil (dendrites, axons, glia) and myelination, but not neurogenesis. A limitation to our study is that gray and white matter volumes could not be determined due to the use of manual segmentation. However, a recent cross-sectional study of domestic pigs suggests gray and white matter volume continues to increase at least through the first 12 weeks of age [27].

In humans, the cerebellum is 8–13% larger in males than females [9, 36]. Here we show that the cerebellum in adult male pigs is roughly 10% larger than in females.

Also in humans, males show peak cerebellar volume later in life at 15.6 years of age compared to 11.8 in females [36]. We did not find any difference in the age range of cerebellar growth in pigs, but we did find that the peak growth rate occurred earlier in life for females. Developmental growth patterns of subcortical structures depend on the age range being studied. During early postnatal development, newborn through 2 years of age, subcortical structures develop similar in males and females [1]. Later in development, from 5 to 18 years of age, subcortical structures including the putamen and globus pallidus have been shown to be larger in males [9]. In this study, we found that the diencephalon and brainstem have similar growth patterns in male and female pigs.

Studies on human hippocampal development have shown significant growth from birth through 2 years of age [37]. At the age of 2 years, the hippocampus is about 85% of adult size but data for males and females have not been reported separately so whether sexual dimorphic effects are present early on is not clear. Sexual dimorphic effects are, however, present later. In females, e.g., maximum volume is smaller and achieved at a younger age compared to males [37, 38]. Our data suggest a similar sex-dependent growth trajectory for the pig hippocampus. For example, the period of maximum growth occurred about 5 weeks earlier in females than males, and the window for hippocampal growth was significantly shorter in females than males. One feature of hippocampal development that is different between pigs and humans relates to left/right asymmetries. Multiple studies of human subjects have determined that the right hippocampus is significantly larger in both males and females, and this difference is present from birth [37–40]. In our study, no difference was found between the left and right hippocampal formation. The importance of hippocampal asymmetry is unknown, but asymmetries can be affected by environmental insults, and changes in asymmetries have been seen in patients with psychiatric illness [41, 42]. Although we did not find differences, it may be an important measure to include in future studies.

The present study has several other limitations. First, the study included a small sample size, meaning the sexual dimorphisms in brain growth and development observed should be cautiously interpreted. However, our confidence is bolstered because variation was minimized by: (1) using a longitudinal design where each pig served as its own control, (2) including littermates of each sex when possible and (3) maintaining pigs individually in an identical environment throughout the study. A second limitation was the use of manual segmentation for quan-

tifying brain region volume. Technical challenges arise when imaging the neonatal brain including poor spatial resolution and low tissue contrast [43]. In addition, there is no MRI-based atlas available for the neonatal piglet. Construction of an atlas set across multiple ages in the pig would allow for more complex automated segmentation protocols. Nonetheless, this manual segmentation protocol has been used previously and is a reliable method for determining brain region volume changes in the pig [17]. A third limitation is that pigs were only scanned through 24 weeks of age, which is near sexual maturity [14]. Beyond this age, most domestic pigs are too large to fit in a standard MRI machine bore. This limitation precluded us from extending the study. Thus, use of an open bore magnet may be useful to track changes past this age in order to determine if there are brain volume losses after puberty as seen in many brain areas in the human [30]. Fourth, the data in this study suggests there are a few differences in brain growth in pigs compared to humans. For example, we did not find any sexual dimorphisms in maximum volumes of the diencephalon and brainstem regions, nor did we observe asymmetries in the hippocampus. These differences present a limitation of the pig as an animal model for human neurodevelopment.

In summary, the present study shows the normal brain growth pattern in the domestic pig from 2 to 24 weeks of age. The brain undergoes significant growth during this period, and several sexual dimorphisms affecting growth, including maximum volume, appear to be similar for humans and pigs. The study shows that domestic pigs can be used in longitudinal MRI-based studies to investigate brain growth and development, and suggests the pig can serve as a preclinical model for human neurodevelopment.

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