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Miniature pig magnetic resonance spectroscopy model of normal adolescent brain development

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ABSTRACT

Background: We are developing the miniature pig (*Sus scrofa domestica*), an *in-vivo* translational, gyrencephalic model for brain development, as an alternative to laboratory rodents/non-human primates. We analyzed longitudinal changes in adolescent pigs using proton magnetic resonance spectroscopy (^{1}H-MRS) and examined the relationship with white matter (WM) integrity derived from diffusion weighted imaging (DWI).

New method: Twelve female Sinclair™ pigs underwent three imaging/spectroscopy sessions every 23.95 ± 3.73 days beginning at three months of age using a clinical 3 T scanner. ^{1}H-MRS data were collected using 1.2 × 1.0 × 3.0 cm voxels placed in left and right hemisphere WM using a Point Resolved Spectroscopy sequence (TR = 2000 ms, TE = 30 ms). Concentrations of *N*-acetylaspartate, myo-inositol (MI), glutamate + glutamine, choline, creatine, and macromolecules (MM) 09 and 14 were averaged from both hemispheres. DWI data were collected using 15 shells of b-values (b = 0–3500 s/mm2) with 32 directions/shell and fit using the WM Tract Integrity model to calculate fractional anisotropy (FA), kurtosis anisotropy (KA) and permeability-diffusivity index.

Results: MI and MM09 significantly declined with age. Increased FA and KA significantly correlated with decline in MI and MM09. Correlations lost significance once corrected for age.

Comparison with existing methods: MRI scanners/protocols can be used to collect 1H-MRS and DWI data in pigs. Pigs have a larger, more complex, gyrencephalic brain than laboratory rodents but are less complex than non-human primates, thus satisfying the "replacement" principle of animal research.

Conclusions: Longitudinal effects in MRS measurements were similar to those reported in adolescent humans. MRS changes correlated with diffusion measurements indicating ongoing WM myelination/maturation.

1. Introduction

Adolescent human brain development is a critical period associated with continued myelination of cerebral white matter (WM), pruning of cerebral gray matter, and formation of functional networks that serve higher cognitive functions (Toga et al., 2006; Tau and Peterson, 2010; Grayson and Fair, 2017; Sowell et al., 2004; Shaw et al., 2008; O'Donnell et al., 2005; Lenroot and Giedd, 2006; Hua et al., 2009; Gogtay et al., 2004; Lebel et al., 2008; Eluvathingal et al., 2007; Bonekamp et al., 2007; Barnea-Goraly et al., 2005; Schmithorst et al., 2002; Mukherjee et al., 2001; Kochunov et al., 2010a; Bastos Leite et al., 2004; Bartzokis et al., 2010). Adolescence is also a crucial period wheredevelopmental alterations can lead to the emergence of life-long neuropsychiatric disorders including schizophrenia and depression (Weinberger and Lipska, 1995; Lewis and Levitt, 2002; Rapoport et al., 2012; Rapoport et al., 2005; Murray et al., 1992; McGorry et al., 2011). Human brain development is primarily studied using magnetic resonance imaging and spectroscopy, and development of an animal

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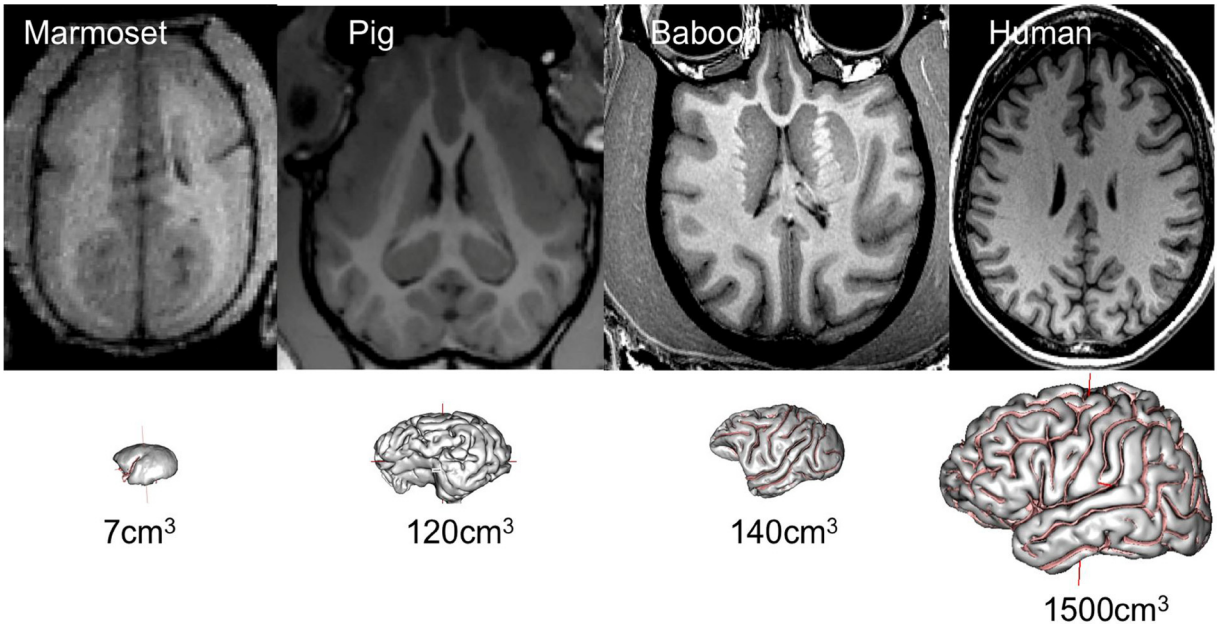

Fig. 1. Axial slices and 3D rendering of cortical surface for Marmoset, Miniature Pig, Baboon and Human Brains.

model that is more reflective of trends observed in the human brain would benefit neuroscience. We are investigating the miniature pig (*Sus scrofa domestica*), as an alternative to laboratory rodents and ferrets (Jacob, 1999; Bryda, 2013; Rosenthal and Brown, 2007; Hardouin and Nagy, 2000; Armstrong et al., 1991; Welker, 1990) and non-human primates (Goodman and Check, 2002; Bontrop, 2001; Torres et al., 2010; Patterson and Carrion, 2005; VandeBerg and Williams-Blangero, 1997). Our previous work demonstrated that unlike rodents and ferrets, miniature pigs could be studied using human MRI protocols and scanners (Ryan et al., 2018). Their gyrencephalic cortex and similar brain myelination and white matter development patterns (Sauleau et al., 2009) provides improved translational potential compared to the lissencephalic rodent cortex (Jacob, 1999; Bryda, 2013; Rosenthal and Brown, 2007; Hardouin and Nagy, 2000; Armstrong et al., 1991; Welker, 1990). The brain volumes and gyrification pattern of miniature pigs are intermediate between old world and new world primates (Fig. 1). However, miniature pigs are less ethically constrained compared to non-human primates (Goodman and Check, 2002; Bontrop, 2001; Torres et al., 2010; Patterson and Carrion, 2005; VandeBerg and Williams-Blangero, 1997) thus allowing for imaging research to be complemented with more invasive neuroscience techniques. For this study, we used proton magnetic resonance spectroscopy (1H-MRS) to quantify longitudinal changes in metabolites during the adolescent brain development of miniature pigs and to link developmental changes in metabolites to WM integrity measurements derived from diffusion weighted imaging (DWI) (Bluml et al., 2013; Cohen-Gilbert et al., 2014).

Humans and miniature pigs have fully gyrencephalic brains, and the primary gyrification process occurs *in utero* (Swindle et al., 2012; Schomberg et al., 2016; Conrad et al., 2012; Ostergaard et al., 1998). During the third trimester (Kochunov et al., 2009a, b, a), cortical folding (Kroenke and Bayly, 2018; Bayly et al., 2013), surface area expansion (Garcia et al., 2018), and the differentiation/branching of axons and dendrites create the gyrencephalic cortex (Xu et al., 2010; Bayly et al., 2014). We previously showed comparable whole-brain and regional heterochronic development trajectories during adolescence in miniature pigs (Ryan et al., 2018). Heterochronic (Dubois et al., 2012; Kulikova et al., 2015; Dean et al., 2016, 2015; Dean et al., 2017; Lebel and Beaulieu, 2011; Lebel et al., 2012; Kinney et al., 1988; Barkovich et al., 1988) cerebral myelination begins shortly after birth and continues through adulthood (Flechsig, 1901). In humans, the motor and sensory WM bundles myelinate during childhood (Lebel et al., 2008; Eluvathingal et al., 2007; Bonekamp et al., 2007; Barnea-Goraly et al., 2005; Schmithorst et al., 2002; Mukherjee et al., 2001; Ashtari et al., 2007), while the fronto-cortical and fronto-striatal circuits do not fully myelinate until adolescence (Bastos Leite et al., 2004; Bartzokis, 2004; Bartzokis et al., 1999, 2008; Kochunov et al., 2009b, c; Prins et al., 2004; Schiavone et al., 2009).

In this study, we compared adolescent longitudinal MRS measurement trends in miniature pigs to those reported in humans. We focused on age-related changes in *N*-acetylaspartate (NAA) and myo-inositol (MI), neuronal (axonal) and glia markers respectively. Both metabolites are hypothesized to change (rise NAA and decline MI) with the myelination of cerebral WM (Bluml et al., 2013; Mehta and Namboodiri, 1995; Chakraborty et al., 2001; Francis et al., 2016; Kinney et al., 1994; Moffett et al., 2007). We also explored changes in glutamate plus glutamine (Glx), the major excitatory neurotransmitter (glutamate) and its metabolite, and macromolecules (MM), which comprise proteins, lipids, and nucleic acids found in the cytoplasm of neurons and glial cells (Pardon et al., 2016; Brand et al., 1993). The two MM investigated in this project, MM09 and MM14, reportedly may reflect thymosin B4 levels, a protein expressed by microglia (Zhou et al., 2015; Kauppinen et al., 1992; Paulussen et al., 2009).

This study combined diffusion and ^{1}H-MRS measurements, such that changes in DWI measurements can be interpreted based on their association with concentrations of neurochemicals with known biological functions. Cross-sectional human data show that a significant proportion of the variability in diffusion properties in cerebral white matter can be explained by the variability of specific neurochemicals (Kochunov et al., 2009b; Acheson et al., 2014; Wijtenburg et al., 2012; Hao et al., 2013). However, the conclusions drawn from these results are limited by the cross-sectional nature of these studies because findings from longitudinal studies often fail to confirm the trends from cross-sectional data (Royall et al., 2005).We previously demonstrated significant longitudinal effects of cerebral development in miniature pigs during adolescent development for Fractional Anisotropy (FA), Kurtosis Anisotropy (KA), and Permeability Diffusivity Index (PDI). Here, we hypothesized that several neurochemicals, including NAA and MI, would serve as significant predictors for longitudinal FA, KA, and PDI.