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DEVELOPMENTAL TRAJECTORIES OF MACROANATOMICAL STRUCTURES IN COMMON MARMOSET BRAIN

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Abstract—Morphometry studies of human brain development have revealed characteristics of some growth patterns, such as gray matter (GM) and white matter (WM), but the features that make human neurodevelopment distinct from that in other species remain unclear. Studies of the common marmoset (*Callithrix jacchus*), a small New World primate, can provide insights into unique features such as cooperative behaviors complementary to those from comparative analyses using mouse and rhesus monkey. In the present study, we analyzed developmental patterns of GM, WM, and cortical regions with volume measurements using longitudinal sample (23 marmosets; 11 male, 12 female) between the ages of one and 30 months. Regional analysis using a total of 164 magnetic resonance imaging datasets revealed that GM volume increased before puberty (5.4 months), but subsequently declined until adulthood, whereas WM volume increased rapidly before stabilizing around puberty (9.9 months). Cortical regions showed similar patterns of increase and decrease, patterns with global GM but differed in the timing of volume peak and degree of decline across regions. The progressive–regressive pattern detected in both global and cortical GM was well correlated to phases of synaptogenesis and synaptic pruning reported in previous marmoset studies. A rapid increase in WM in early development may represent a distinctive aspect of human neurodevelopment. These findings suggest that studies of marmoset brain development can provide valuable comparative information that will facilitate a deeper understanding of human brain growth and neurodevelopmental disorders.

Key words: brain development, common marmoset, cortex, growth trajectory, longitudinal MRI, non-human primate.

INTRODUCTION

Characterization of neuroanatomical substrates using multilevel neurobiological approaches, from the macroanatomic to the cellular or even molecular scale, are essential for gaining a better understanding of changes in the brain during postnatal development (Stiles and Jernigan, 2010). Structural magnetic resonance imaging (MRI) provides a macroanatomical view capable of capturing multiple developmental processes that occur in synchronized manner by visualization of tissues in living subjects (Thompson et al., 2005; Lenroot and Giedd, 2006; Toga et al., 2006). Human longitudinal volumetric studies have revealed a number of tissue-specific developmental patterns; e.g., the volume of gray matter (GM) increases until preadolescence, but decreases thereafter, whereas the volume of white matter (WM) increases linearly until adulthood (Giedd et al., 1999; Lenroot et al., 2007; Aubert-Broche et al., 2013). Whether and how these growth trajectories are unique to human development, however, remains unclear due to difficulties in interspecies comparison.

The use of MRI approaches in experimental animal models makes it possible to obtain equivalent measurements, enabling smoother and more relevant comparisons across species. Longitudinal volumetric studies in rhesus monkey (Malkova et al., 2006; Liu et al., 2015; Scott et al., 2015) and mouse (Chuang et al., 2011; Nieman et al., 2015; Hammelrath et al., 2016) have reported growth pattern characteristics of both species. In rhesus monkey, the WM volume increases until adulthood, while the GM volume, such as cerebral cortex, stabilizes after an initial increase (Malkova et al., 2006). Mouse studies have shown in contrast that the volume of both GM (Chuang et al., 2011; Hammelrath et al., 2016) and WM (Chuang et al., 2011; Nieman et al., 2015) remains unchanged after an initial increase. These findings suggest that humans and commonly used animal models exhibit both conserved and species-specific features, and that thus careful comparisons of growth trajectories across multiple species may

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Abbreviations: GM, gray matter; mPFC, medial prefrontal cortex; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; T1WI, T1-weighted images; V1, primary visual cortex; WM, white matter.

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reveal important aspects of human-specific characteristics in brain development.

MRI studies of brain development in the common marmoset (*Callithrix jacchus*), a small New-World primate, can provide complementary insights into human brain development (Hikishima et al., 2011, 2013, 2015). As these animals mature by the age of two years (Mano et al., 1987), longitudinal assessment is achievable at shorter time scales than in other non-human primates. Unique behaviors such as imitation (Bugnyar and Huber, 1997; Voelkl and Huber, 2000, 2007; Miller et al., 2016) and vocal usage learning (Takahashi et al., 2013, 2015, 2016; Chow et al., 2015; Gultekin and Hage, 2017) via interactive communication, which few other non-human primates exhibit, are comparable to those in human (Borjon and Ghazanfar, 2014); these may shed light on distinctive developmental characteristics. In addition, marmosets share a number of neuroanatomical structures with human: its central nervous system displays structural features, including subdivided cortical areas, resembling those in human (Paxinos, 2012), and studies of prenatal brain development in marmoset MRI have shown that global patterns of prenatal brain development in marmoset are comparable to those in human and other species (Hikishima et al., 2013; Sawada et al., 2014).

In the present study, we sought to longitudinally investigate morphological changes during postnatal development, focusing on the global brain and cerebral cortex using widely used volume measurement (Lenroot and Giedd, 2006), which enables straightforward interspecies comparisons. We performed multiscalar measurements of global GM and WM and some regions of the cerebral cortex to observe region-specific developmental patterns in marmosets.

EXPERIMENTAL PROCEDURES

Marmosets

Twenty-three marmosets (male 11, female 12) from the Central Institute for Experimental Animals (CIEA) were used in this study; animals were measured at ages between one and 30 months. All procedures were conducted in accordance with the Laboratory Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). All experiments were approved by the local Animal Experiment Committee of CIEA of Japan (Approval number 14040A, 16017A).

All subjects were born from five families reared at this institute. Details of composition are as follows: three subjects in family A (one pair of twins, one singleton); six subjects in family B (one pair of twins, one group of triplets, and one singleton); seven subjects in family C (one group of triplets, two pairs of twins); five subjects in family D (one group of triplets, one pair of twins), and two subjects in family E (one pair of twins). Offspring were raised by their parents; all family members were kept in the same cage until the age of 12 months (cage size for a family: W 820 × D 610 × H 1600 mm), and then kept with siblings or alone thereafter (cage size for a single animal, W 4100 × D 610 × H 730 mm). Typically, onset of the puberty occurred at the age of approximately 7–8 months, and animals reached maturity at 21 months (Abbott and Barnett, 2003).

MRI was performed at ages 1–30 months, specifically at the following time points: 1, 2, 3, 4.5, 6, 9, 12, 15, 18, 21, 24, 27, and 30 months, yielding a total of 164 datasets (Table 1). Measurement schedules varied among individuals, but measurements were taken a minimum of three times and a maximum of 10 times per subject. Data obtained at ages of 1, 3, 6, 9, 12, 18, 24, and 30 months (±1 month) were used for cross-sectional comparisons by age. To determine whether brain changes stabilized in adulthood, data were taken until the age of 30 months; in marmoset, ages of ≥24 months are considered adulthood (Abbott and Barnett, 2003; Tardif et al., 2011; Ross et al., 2012).

MRI acquisition

All MRI data were obtained on 7.0 T Biospec 70/16 scanner system (Bruker BioSpin GmbH; Ettlingen, Germany) equipped with actively shielded gradients at a maximum strength of 700 mT/m, using a bespoke 4-channel phased array surface coil developed for acquisition of a marmoset head data (Takashima Seisakusho Ltd., Tokyo, Japan) as the receiver coil, and a conventional linear polarized birdcage resonator with inner diameter 72 mm (Bruker BioSpin) as the transmit coil.

All animals were intramuscularly administered 0.1 mg/kg of atropine sulfate (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) and then 12 mg/kg of alfaxalone (Jurox Pty. Ltd., Rutherford, NSW, Australia). The anesthetized state was maintained throughout each experiment by administration of mixture of oxygen and 1.0–2.0% isoflurane (Wako, Osaka, Japan) using an artificial respirator, SN-480-7 (Shinano, Tokyo, Japan). Intratracheal intubation was performed for subjects ≥4 months old, while a ventilation mask was used for smaller subjects aged 1–3 months. Physiological condition during imaging was monitored by transcutaneous pulse oximetry estimates of O_2 saturation, and skin and rectal temperature.

T1-weighted images (T1WI) were acquired using an optimized magnetization-prepared rapidly acquired gradient-echo sequence for marmoset brain

Table 1. All data used in this study divided by age

Age	1	2	3	4.5	6	9	12	15	18	21	24	27	30
Male	7	3	6	7	9	8	8	3	7	5	5	4	9
Female	5	4	8	5	7	9	8	3	10	4	8	4	8
Total	12	7	14	12	16	17	16	6	17	9	13	8	17