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Mapping orbitofrontal-limbic maturation in non-human primates: A longitudinal magnetic resonance imaging study

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ABSTRACT

Brain development involves spatiotemporally complex microstructural changes. A number of neuropsychiatric disorders are linked to the neural processes of development and aging. Thus, it is important to understanding the typical developmental patterns of various brain structures, which will help to define critical periods of vulnerability for neural maturation, as well as anatomical mechanisms of brain structure-related neuropathology. In this study, we used magnetic resonance imaging to assess development of the orbitofrontal cortex, cingulate cortex, amygdala, and hippocampus in a non-human primate species, the common marmoset (*Callithrix jacchus*). We collected a total of 114 T2-weighted and 91 diffusion-weighted scans from 23 animals from infancy to early adulthood. Quantitative and qualitative evaluation of age-related brain growth patterns showed non-linear structural developmental changes in all measured brain regions, consistent with reported human data. Overall, robust volumetric growth was observed from 1 to 3 months of age (from infancy to the early juvenile period). This rapid brain growth was associated with the largest decrease in mean, axial, and radial diffusivities of diffusion tensor imaging in all brain regions, suggesting an increase in the number and size of cells, dendrites, and spines during this period. After this developmental period, the volume of various brain regions steadily increased until adolescence (7–13 months of age, depending on the region). Further, structural connectivity derived from tractography data in various brain regions continuously changed from infancy to adolescence, suggesting that the increase in brain volume was related to continued axonal myelination during adolescence. Thereafter, the volume of the cortical regions decreased considerably, while there was no change in subcortical regions. Familial factors, rather than sex, contributed the development of the front-limbic brain regions. Overall, this study provides further data on the factors and timing important for normal brain development, and suggest that the common marmoset is a useful animal model for human neural development.

1. Introduction

Brain development is accompanied by a range of spatiotemporally complex neural events (Bakken et al., 2016; Giedd et al., 1999; Lebel et al., 2008; Wierenga et al., 2014). Such complexity may result in vulnerability to abnormal structural and functional maturation of the brain, and associated neuropathological changes. Indeed, abnormalities in the fronto-limbic regions of the brain have been reported in a number of neuropsychiatric disorders and diseases (Kujawa et al., 2016; Saitoh et al., 2001; Schumann et al., 2004; Vai et al., 2015). These brain regions include the orbitofrontal cortex, cingulate cortex, amygdala, and hippocampus, which are important for construction and maintenance of smooth social relationships between individuals by inducing and regulating emotion, self-monitoring, and control, associating memories, expecting outcomes from actions, and enhancing memories for emotionally-arousing events (Clarke et al., 2015; Law et al., 2009a, 2009b; Jackson et al., 2016; Kujawa et al., 2016; Rolls, 2015; Shenhav et al., 2013; Yu et al., 2014).

A detailed understanding of the normal developmental patterns and connectivity of these front-limbic regions may help to clarify the anatomical mechanisms underlying their functions. Proper structural maturation in the front-limbic regions is likely important for optimal

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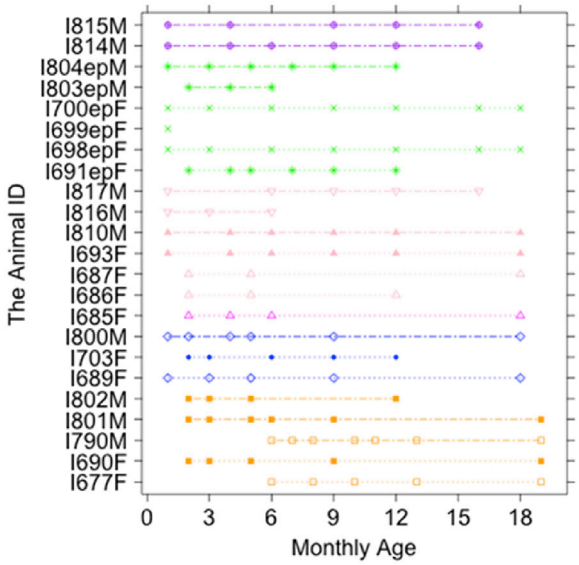

Fig. 1. Animal age (months) at scan, colored by consanguineous family. Each littermate has the same plot symbol. The dotted lines represent females, and the double-dashed lines represent males. Animal ID: I685F (triangle with magenta color) was raised in a different cage from her pink-colored consanguineous family.

development of these functions. However, the deep locations of the orbitofrontal-limbic regions in the brain and their marked individual variability limits *in vivo* physiological studies, such as those using electrodes and tracer injection, when compared with neocortical areas. Further, previous studies on orbitofrontal-limbic regions have produced inconsistent results in terms of gender and hemispheric effects (Dennison et al., 2013; Giedd et al., 1996; Goddings et al., 2014; Marwha et al., 2017; Pedraza et al., 2004; Wierenga et al., 2014). Thus, the detailed patterns of development of the orbitofrontal-limbic regions structures remain elusive.

Magnetic resonance imaging (MRI) is an ideal, noninvasive method for studying longitudinal development of deep brain regions *in vivo*, and can minimize confounding factors such as individual differences in growth. MRI can also provide multiple types of image contrast by using different scanning acquisition sequences. In particular, diffusion-weighted MRI data provides information through a set of mathematical operations, including diffusion tensor imaging (DTI) data sets such as fractional anisotropy (FA), apparent mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD). These variables are calculated from the eigenvectors and eigenvalues of apparent water diffusivity, which is influenced by the structure and characteristics of tissues (Hüppi and Dubois, 2006; Mori and Zhang, 2006). Hence, DTI data can be used to assess developmental changes at the microstructural level, including local axonal myelination (Deoni et al., 2012; deIpolyi et al., 2005; Hüppi and Dubois, 2006; Oishi et al., 2013; Tournier et al., 2004). Diffusion tractography can also be used to delineate development of structural neural networks (Aggarwal et al., 2015; Hüppi and Dubois, 2006; Oishi et al., 2013).

However, it is difficult to assess longitudinal brain development in humans using MRI or other techniques, as it is time-consuming, costly, and limited to a few time points. Further, it is difficult to control for genetic and environmental factors that are likely to cause individual variability. Thus, in the present study, we examined front-limbic region development in a non-human primate, the common marmoset (*Callithrix jachus*), which provides an excellent model for studying human-like traits (Abbott et al., 2003; Miller et al., 2016; Okano et al., 2016; t'Hart et al., 2012). Despite having smooth surface, the marmoset brain shares many anatomical features and neural growth patterns found in other primates including humans, especially in the temporal lobes and internal structures (Newman et al., 2009; Ichinohe, 2015; Oga et al., 2013; Sasaki et al., 2015). A particular strength of marmosets over other non-human primates used in research (e.g., rhesus macaque) is their colony structure. Marmoset colonies are organized into a family unit (Rothe and Darms, 1993). The presence of both maternal and paternal care, and even elder siblings' care, allows the assessment of parenting and familial effects on brain development. Human imaging studies of psychiatric disorders/disease have reported a relationship among anatomical structures in the fronto-limbic regions and familial factors (Vai et al., 2015; Whittle et al., 2008). These regions are also affected by parenting in marmosets (Law et al., 2009a, 2009b; Schultz-Darken et al., 2016). Importantly, marmosets mature faster than other primates, reaching puberty at approximately 9 months, and sexually maturity at 2 years (de Castro Leão et al., 2009; Schultz-Darken et al., 2016; Yamamoto, 1993). This makes the common marmoset an ideal primate species to collect longitudinal data over a relatively short time period. Nevertheless, the degree to which the developmental patterns in the marmoset are similar to those in humans remain unclear.

In the present study, we acquired longitudinal MRI data in common marmosets from infancy to early adulthood to assess the anatomical development of orbitofrontal-limbic regions, including the orbitofrontal cortex, cingulate cortex, amygdala, and hippocampus. Specifically, we tested the hypothesis that the orbitofrontal-limbic regions in common marmosets grow non-linearly with age, and with sex and hemispheric influences, as observed in humans (Giedd et al., 1996; Goddings et al., 2014; Sussman et al., 2016). As these regions are functionally related in common marmosets (Clarke et al., 2015), we also hypothesized that their developmental patterns would be synchronized.

2. Materials and methods

2.1. Animals

This study was approved by the local Animal Experiment Committee and was conducted in accordance with the Guidelines for Conducting Animal Experiments of Central Institute for Experimental Animals (Approval number 14040A, 16017A). Ongoing longitudinal brain MRI data were obtained from 23 normal developing common marmosets from five families (11 males, 12 females). All except one animal grew in the same size cages with their parents, littermates, and elder and/or younger siblings until at least 9 months after birth. Each cage (820 mm wide, 610 mm deep and 1600 mm high) contained play equipment such as a swing and a box. If the number of family members living together was >6, then two cages were connected using a handmade tunnel. A total of 114 time points for T2-weighted MRI data and 91 time points for diffusion-weighted MRI data were collected over 1–19 months. The individual scan points at each age are shown in Fig. 1. Each animal was scanned at 1, 3, 6, 9, 12, and 18 months of age, although some data points were missed because health conditions, death, and MRI scanner unavailability. Note that the animal ID: I685F in Fig. 1 belongs consanguineously to the family plotted in pink. However, this animal was reared by a pair of unrelated animals in a different cage, as her original parents had difficulty in rearing triplets.

2.2. Image acquisition

Before scanning, anesthesia was induced with alfaxalone (1.2 ml/kg; Alfaxan®; Jurox Pty Ltd., Rutherford, NSW, Australia), and each animal was intubated to maintain constant respiration under anesthesia with a mixture of oxygen and isoflurane (Abbott Laboratories, Abbott Park, IL, USA) using an artificial respirator (SN-480-7; Shinano, Tokyo, Japan). Animals <3 months of age were laid in a prone or side position during scanning, and the physiological conditions were continuously monitored for all animals.

MRI scans were obtained on a 7.0 T Biospec 70/16 MRI (Bruker BioSpin; Ettlingen, Germany) with a conventional linear polarized birdcage resonator transmitter coil (Bruker BioSpin; inner diameter 72 mm)