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FETAL SULCATION AND GYRIFICATION IN COMMON MARMOSETS (*CALLITHRIX JACCHUS*) OBTAINED BY *EX VIVO* MAGNETIC RESONANCE IMAGING

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Abstract—The present study characterized fetal sulcation patterns and gyrification in the cerebrum of the New World monkey group, common marmosets, using a 3D T_2-weighted high-resolution anatomical magnetic resonance imaging (MRI) sequence from the fixed brain at 7-tesla *ex vivo*. Fetal sulcation in the marmoset cerebrum began to indent the lateral fissure and hippocampal sulcus in gestational week (GW) 12, and then the following sulci emerged: the callosal and calcarine sulci on GW 15; the superior temporal sulcus on GW 17; and the circular and occipitotemporal sulci on GW 18. The degree of cortical convolution was evaluated quantitatively based on 2D MRI slices by the gyrification index (GI) and based on 3D MRI data by sulcation index (SI). Both the mean GI and SI increased from GW 16, and were closely correlated with the cortical volume and the cortical surface area during fetal periods (their correlation coefficients marked more than 0.95). After birth, both the mean GI and SI decreased slightly by 2 years of age, whereas the cortical volume and surface area continuously increased. Notably, histological analysis showed that the outer subventricular zone (oSVZ) in non-sulcal regions was thicker than that in the presumptive calcarine sulcal region on GW 13, preceding the infolding of the calcarine sulcus. The present results showed definite sulcal infolding on the cerebral cortical surface of the marmosets, with similar pattern and sequence of their emergences to other higher-order primates such as macaques and humans. Differential expansion of the oSVZ may be involved in gyral convolution and sulcal infolding in the developing cerebrum.

Key words: basal radial glia, common marmoset, gyrification, non-human primates, outer subventricular zone.

INTRODUCTION

The common marmoset (*Callithrix jacchus*) is a small laboratory primate belonging to the New World monkey group, and exhibits a high sociality with abundant vocal communication, making the use of this animal appropriate for studies of higher cognitive function (Epple, 1968) and modeling human behaviors (Yamazaki et al., 2011a,b). In fact, several studies have been reported with regard to models of cognitive and neurological disorders in marmosets (Iwanami et al., 2005a,b; Fujiyoshi et al., 2007; Yamada et al., 2008; Kobayashi et al., 2012).

A great majority of primate species have a cerebral cortex with a convoluted surface, sulci and gyri. The development of the cerebral sulci has been reported in humans and nonhuman primates by anatomic (Chi et al., 1977; Naidich et al., 1994; Fukunishi et al., 2006; Kashima et al., 2008), sonographic (Naidich et al., 1994), and magnetic resonance imaging (MRI) (Naidich et al., 1994; Prayer et al., 2006; Sawada et al., 2009) studies and three-dimensional (3D) reconstruction of the cortical surface based on MRI (Dubois et al., 2008). Such a fetal sulcation pattern is phylogenetically conserved among primate species (Sawada et al., 2012a). The New World monkey group including the marmosets also has a gyrencephalic cerebrum, while the degree of their cortical convolution is less developed than that in higher order primates, i.e., the Old World monkey group, apes and human (Zilles et al., 1988, 1989). However, the developmental pattern of cerebral sulci in the New World monkey group has not yet been addressed. The aim of this study was to characterize the fetal sulcation pattern of common marmosets. We used the 3D T_2-weighted high-resolution anatomical

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Abbreviations: bRG, basal radial glia; cal, calcarine sulcus; cas, callosal sulcus; cc, corpus callosum; cgs, cingulate sulcus; cirs, circular sulcus; CP, cortical plate; cs, central sulcus; E, embryonic day; GI, gyrification index; GW, gestational week; HE, hematoxylin and eosin; his, hippocampal fissure; lf, lateral fissure; MRI, magnetic resonance imaging; oSVZ, outer subventricular zone; ots, occipitotemporal sulcus; PBS, phosphate-buffered saline; PD, postnatal day; Pe, petrous portion of the temporal bone; PH3, phosphohistone H3; pos, parietooccipital sulcus; SI, sulcation index; SP, subplate; VZ, ventricular zone.

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MRI sequence from the fixed brain of the common marmoset fetuses at 7-tesla, which had been acquired in our recent report (Hikishima et al., 2013).

To correlate the anatomical MRI sequence on fetal sulcation and gyrification with histological findings, we paid close attention to the structure, called the outer subventricular zone (oSVZ). The oSVZ was first identified in the human fetal brain as the second proliferative zone in the cerebral cortex and contains many self-renewable progenitor cells called basal radial glia (bRG) (Hansen et al., 2010; Fietz et al., 2010), which is likely to serve to thicken the cerebral cortex in gyrencephalic mammals (Lui et al., 2011). We recently found that oSVZ appeared between embryonic day (E) 85 and E92 (Kelava et al., 2012) in the marmoset, the gestation period of which is 21 weeks. By histological analysis, we showed that the oSVZ in the non-sulcal (convoluted) region was found to be thicker than that in the presumptive calcarine sulcal region on GW 13 of marmoset. Though the common marmosets are generally considered to have a near-lissencephalic cerebrum, we obtained intriguing findings related to the gyrification mechanism by investigating the morphological changes in the neocortex in the early stages of sulcal development in this primate.

EXPERIMENTAL PROCEDURES

MRI procedures

The present study used the 4% paraformaldehyde (PFA)-fixed brains of common marmosets at each gestational week (GW) 11 (n = 1), 12 (n = 1), 13 (n = 1), 14 (n = 1), 15 (n = 1), 16 (n = 1), 17 (n = 1), 18 (n = 1), 19 (n = 1), and postnatal day (PD) 0 (n = 2), and adult marmosets (2 years of age) (n = 1) for evaluating sulcation and gyrification. These were the same samples that had been used previously (Hikishima et al., 2013). Marmoset fetuses were obtained from six breeding pairs of animals purchased from CLEA Japan (Tokyo, Japan) by Cesarean section. For Cesarean section, the mothers were anesthetized by intramuscular administration of 70 mg/kg ketamine (Sankyo Lifetech Co. Ltd., Tokyo, Japan) and 0.1 mg/kg atropine sulfate (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan). The animals were then anesthetized with 1.0–3.0% isoflurane (Abbott Japan, Tokyo, Japan) via a ventilation mask. During the operation, anesthesia was maintained by spontaneous respiration, and both heart rate and arterial oxygen saturation were monitored. Twenty-four embryonic and fetal marmosets ranging in age from GW8–19 (two embryos per each GW) were obtained and immediately euthanized in a humane manner via deep anesthesia. Adult and neonatal (PD 0) animals were euthanized via deep anesthesia (intravenous sodium pentobarbital, 100 mg/kg) for high resolution *ex vivo* MRI. All specimens were then immersed in 4% PFA/phosphate-buffered (PBS) saline for 2 weeks. After fixation specimens were stored for 2 weeks in PBS containing 0.5% sodium azide and the contrast agent of gadopentetate dimeglumine, 1 mM Magnevist (Schering, Berlin, Germany) (Newman et al., 2009). *Ex vivo* MRI scans were performed using a 7-tesla Biospec 70/16 MRI scanner (Bruker Biospin GmbH; Ettlingen, Germany) equipped with actively shielded gradients at a maximum strength of 700 mT/m. 3D T_2-weighted MRI was acquired by rapid acquisition with relaxation enhancement (RARE) using the following parameters: effective echo time (TE), 18 ms; repetition time (TR), 250 ms; number of averages (NA), 6; RARE factor, 4 (Hikishima et al., 2013). Specimen and data acquisition specifications are detailed in Table 1.

3D volume-rendered images

All MR images were incorporated into the 3D reconstruction. The cerebrum was semi-automatically segmented on MR slices using ‘‘Morpho’’ tool of SliceOmatic software ver 4.3 (TomoVision, Montreal, Canada) based on image contrast as well as user knowledge of anatomy. The segmented images were then analyzed using the 3D-rendering module of the same software, and cerebral images were rendered in 3D using a surface projection algorithm, which best visualized the surface and sulci of the cerebrum. The 3D-rendered images were then rotated and manipulated in a manner that best visualized the brain morphology by a linear registration method using SliceOmatic software.

Identification of sulci

The criteria for identifying a sulcus were referenced with the Stereotaxic Atlas of the marmoset Brain (Yuasa

Table 1. Specimen and imaging specifications

Gestational weeks/postnatal ages	CRL [mm]	RF coil	Isotropic resolution [lm]
GW8	6.2	CryoProbe	30
GW9	7.8	CryoProbe	30
GW10	10.8	CryoProbe	30
GW11	15.8	22 mm I.D. volume coil	40
GW12	26.6	22 mm I.D. volume coil	55
GW13	36.7	22 mm I.D. volume coil	55
GW14	44.5	38 mm I.D. volume coil	55
GW15	52.8	38 mm I.D. volume coil	75
GW16	61.8	38 mm I.D. volume coil	75
GW17	64.5	38 mm I.D. volume coil	100
GW18	68.7	38 mm I.D. volume coil	100
GW19	86.3	38 mm I.D. volume coil	100
PD0	–	38 mm I.D. volume coil	100
Adult			

This table was reproduced from our previous study (Hikishima et al., 2013).