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BIPHASIC ASPECT OF SEXUALLY DIMORPHIC ONTOGENETIC TRAJECTORY OF GYRIFICATION IN THE FERRET CEREBRAL CORTEX

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Abstract—The present study characterized quantitatively sexual dimorphic development of gyrification by MRI-based morphometry. High spatial-resolution 3D MR images (using RARE sequence with short TR and minimum TE setting) were acquired from fixed brain of male and female ferrets at postnatal days (PDs) 4–90 using 7-tesla preclinical MRI system. The gyrification index was evaluated either throughout the cerebral cortex (global GI) or in representative primary sulci (sulcal GI). The global GI increased linearly from PD 4, and reached a peak at PD 42, marking 1.486 ± 0.018 in males and 1.460 ± 0.010 in females, respectively. Sexual difference was obtained by greater global GI in males than in females on PD 21 and thereafter. Rostrocaudal GI distribution revealed an overall male-over-female sulcal infolding throughout the cortex on PD 21. Then, an adult pattern of sexually dimorphic cortical convolution was achieved so that gyrification in the temporo-parieto-occipital region was more progressive in males than in females on PD 42, and slightly extended posteriorly in males until PD 90. In the sulcal GI, sulcus-specific male-over-female GI was revealed in the rhinal fissure, and presylvian sulcus on PD 42, and additionally in the coronal, splenial, lateral, and caudal suprasylvian sulci on PD 90. The current results suggest that age-related sexual dimorphism of the gyrification was biphasic in the ferret cortex. A male-over-female gyrification was allometric by PD 21, and was thereafter specific to primary sulci located on phylogenetically newer multimodal cortical regions.

Key words: cerebrum, sulcus, gyrification, carnivores, sex difference.

INTRODUCTION

The cerebral cortex expands remarkably within the narrow space of the cranial cavity, allowing formation of species-associated characteristic features of the cortical convolution (gyrification) in mammals including primates and carnivores (Chi et al., 1977; Ferrer et al., 1988; Wosinski et al., 1996; Sawada and Watanabe, 2012; Sawada et al., 2012a,b, 2014). The cortical convolution is considered to be implicated in several genetic, ontogenetic and hormonal factors. Sex is one of the factors affecting the cortical convolution. Sexual dimorphism of the gyrification has been documented in humans (Liu et al., 2010) and ferrets (Sawada et al., 2015). Gyrification abnormalities reportedly appeared in some neurodevelopmental disorders, whose onset and incidences are known to be sexually different (Kulynych et al., 1997; Libero et al., 2014; Palaniyappan et al., 2015). Therefore, knowledge of sex-associated changes in global and/or local gyrification during the development and maturation of the cortex will be helpful for elucidating the cause of those human neurodevelopmental disorders as well as their gyrification mechanisms.

The sulcal morphology, a mediator of local gyrification, reflects the local expansion of the cerebral cortex, which has a linkage with functional development of particular cortical regions. For example, handedness affects morphological asymmetry of the inferior frontal sulcus in humans (Powell et al., 2012). Cognitive performance including language, executive function, and attention/processing speed, are correlated with the complexity of superior temporal sulcal morphology (Liu et al., 2011). On the other hand, a wider sulcal span, which reflects reduced volumes of adjacent gyri, is associated with poor cognitive performance (Liu et al., 2011) and Alzheimer's disease (Liu et al., 2012). Dimorphic development of the sulcal morphology/local gyrification between sexes may indicate structural changes in the cortex with sex-related specification. In humans, leftward asymmetry of the length of the paracingulate sulcus is reportedly enhanced in males but not in females during adolescence (Clark et al., 2010). However, inconsistent results were obtained in infants: local gyrification was larger in the left calcarine sulcal region in males at age 0 and in the left paracentral and precuneus regions at 2 years of age, but there was no gender difference in the paracingulate sulcal region at either age (Li et al., 2014). The results of those two studies led us to consider that the developmental courses of sulcal morphology/local gyrification are different between sexes.

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Abbreviations: ac, anterior commissure; cns, coronal sulcus; crs, cruciate sulcus; csss, caudal suprasylvian; FO, frontoocciptal; gcc, genu of corpus callosum; GI, gyrification index; ls, lateral sulcus; pc, posterior commissure; prs, presylvian sulcus; pss, psuedosylvian sulcus; rf, rhinal fissure; rs, rhinal sulcus; rsss, rostral suprasylvian sulcus; ss, splenial sulcus.

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Recently, we quantitatively characterized sex-associated changes of the gyrification and sulcal infolding in young adult ferrets (Sawada et al., 2015). Although ferrets have sulcal patterns distinct from primates, there is male-enhanced infolding of primary sulci in the prefrontal, perisylvian and parietal neocortical regions with no left/right side difference (Sawada et al., 2015). Those regions are known to contain a large proportion of multisensory neurons (Ramsay and Meredith, 2004; Bizley et al., 2007; Foxworthy et al., 2013). The current study attempted to elucidate developmental courses of gyrification in male and female ferrets during postnatal days (PDs) 4–90 using MRI-based morphometry. The results will provide advantageous information regarding the cortical development linked with the sex-related specification, and the cause of human neurodevelopmental disorders involving altered gyrification and gender vulnerability, i.e., schizophrenia and autism.

EXPERIMENTAL PROCEDURES

Samples

A total of 15 male and 15 female ferrets consisting of three animals each at five different postnatal ages (i.e., PDs 4, 10, 21, 42 and 90) were purchased from SLC (Hamamatsu, Japan). All ferrets were perfused intracardially with 4% paraformaldehyde in 10 mM phosphate buffer, pH 7.4 under a deep anesthetization by injecting 400 μg/g body weight of chloral hydrate, intraperitoneally. Then, the brains were separated from the spinal cord at C1 levels and removed from the skull. Those brain samples were the same that had been used for gross-anatomical chronologies of sulcal and gyral formations of ferrets in our report (Sawada and Watanabe, 2012).

MRI measurements

MRI measurements were performed according to our procedure described in the previous report (Sawada et al., 2013). Three-dimensional (3D) MR images (using RARE sequence with short TR and minimum TE setting) were obtained using a 7.0-T MRI system (Magnet; 400 mm inner diameter bore, Kobelco and Jastec, Kobe, Japan) (Console; AVANCE-I, Bruker BioSpin, Ettlingen, Germany). A birdcage RF coil for transmission and reception (70 mm inner diameter, Rapid Biomedical, Rimpar, Germany; or 60-mm inner diameter, Bruker BioSpin) was selected depending on the dimensions of the brain samples. The transaxial slice orientation was defined horizontally in relation to the cerebral base. 3D MR images were acquired entirely covering the brain samples using the rapid acquisition with relaxation enhancement (RARE) sequence, while arranging other imaging settings as follows: repetition time (TR) = 300 ms, echo time (TE) = 9.6 ms (effective TE = 19.2 ms), RARE factor = 4, acquisition matrix = 256 × 256 × 256, number of acquisitions (NEX) = 2, and total scan time = 2 h 43 min 50 s. The field of view (FOV) was determined in the rage from 13.2 × 13.2 × 25.6 to 32 × 32 × 40 mm^3, depending on the size of brain samples. Thus, the voxel resolution was 100–125 × 100–125 × 100–156 μm^3.

Cortical volume, fronto-occipital (FO) length, and cortical surface area

All 3D MR images of the cerebral cortex were used for the analysis. On a basis of the contrast of MR images, the cerebral cortex was segmented semi-automatically using the SliceOmatic software ver 4.3 (TomoVision, Montreal, Canada), along with the user's knowledge of the anatomy in reference to MR images obtained previously (Sawada et al., 2013, 2015). 3D rendered images of the cerebral cortex were then reconstructed on a basis of segmented images using the 3D-rendering module of the same software. Then, the length of the cerebral cortex from the frontal pole through the occipital pole on the 3D-rendered images was measured as the frontooccipital (FO) length.

The volumetric analysis was carried out, according to our procedure described in the previous report (Sawada et al., 2013). Areas of the segmented cortex were computed on MR images at the coronal plane with the equal Z-axis interval (125 or 156 μm) using the SliceOmatic-software. Then, the cortical volumes were obtained by a multiplication of the summed areas of the cortex by the Z-axis interval.

Cortical contours including sulcal grooves (inner contour; Ref. Fig. 1A) on MR images at the coronal plane with the equal Z-axis interval (125 or 156 μm) were semi-automatically delineated using the "Morpho" tool of SliceOmatic software. The cortical surface area was calculated by multiplying all measurements of the inner contours by the Z-axis interval.

Cortical thickness

3D MR images of the cerebral cortex were used for the analysis. The mean cortical thickness throughout the cerebral hemisphere was computed from the 3D cortical area reconstructed by the segmented cortex semi-automatically on MR images using the Amira ver. 5.2 (Visage Imaging, Inc., San Diego, CA, USA). Furthermore, 3D color maps of the cerebral cortical thicknesses were obtained by computing the segmented images of the cortex using Amira software.

Gyrification index

3D MR images of the cerebrum were used for calculating the gyrification index. The indication of a sulcus was defined optically by the cortical surface indentation, where the underlying white matter made a similar curvature, according to our criteria (Sawada et al., 2014). Using such criteria, small indentations of the cerebral sulci could be defined, whereas those indentations were undistinguishable from hollows by blood vessels on the cerebral surface by the curvature analysis of digital cortical surface, one of the procedures for defining the cerebral sulci as having a curvature above a certain threshold (Sawada et al., 2014). Identifications of cerebral sulci and gyri were referred to our previous studies