

1 **Primary sulci formation in human cerebral cortex development**

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Abstract

We aimed to determine the timing of appearance and the morphologic and morphometric features of the initial human cerebral sulcal formation. Using high-resolution magnetic resonance images obtained from 33 samples between 11 and 16 weeks (w) of gestation (crown-rump length <130 mm), the cerebral surface and internal structures on serial two-dimensional planes and all possible sulci on three-dimensional reconstructions were marked, allowing comparison of the positions of the sulci in the samples and inter-samples. Our method provided accurate conclusions regarding the timing of sulcal formation. Detection timing was as early as and earlier than those in previous studies using anatomical dissection and magnetic resonance imaging (MRI), respectively: <12 w for the callosum, <13 w for the hippocampal, calcarine, and parieto-occipital sulci, and <15 w for the lateral sulcus. Occasionally, an olfactory sulcus was detected. However, the cingulate sulcus could not be definitely identified. The lateral sulcus gradually appeared and changed shape. The lengths of the left and right sides of the olfactory sulci and the left side of the hippocampal sulcus increased linearly with the CRL. The length of the right side of the hippocampal sulcus and the left and right sides of the calcarine, parieto-occipital, and not determined sulci did not increase with the CRL. The depth of the all sulci, except for the parieto-occipital sulci, increased linearly with the CRL. The sulci might not arise as if they elongate gradually but arise simultaneously over some distance. We determined the timing of the initial sulcal formation using high-resolution MRI. Our findings may significantly impact prenatal diagnosis and research on neurodevelopmental disorders.

Keywords: primary sulci formation, human cerebral cortex, magnetic resonance imaging

Introduction

Most information on fetal cortex development is obtained from descriptive anatomical studies,¹⁻⁸ fetal magnetic resonance imaging (MRI),⁹⁻¹³ and sonography.¹⁴⁻²¹ Developmental patterns of the appearance of human sulci and gyri have been qualitatively studied using gross dissections.¹⁻⁸ In these studies, sulci and gyri formation chronology was described using samples from a relatively remarkable period until birth. Samples corresponding to the timing of the initial detection of primary sulci were limited in these studies. Thus, reinforcement of available data, especially for fetuses at <17 weeks (w) of gestational age (crown-rump length [CRL] <130 mm), is required. Moreover, most studies using MRI and sonography have examined clinical patients at different gestational ages to demonstrate the standard cortical structure at a particular gestational age.⁹⁻²¹ Comparison of the timing of cerebral sulci detection between their appearance in anatomical studies and studies using MRI and ultrasonography reveals approximately a 1-w delay using MRI and a 2- to 4-w delay using sonography.^{5,14,15,18} This implies that sulci are scarcely detected in samples from young fetuses of <17 w of gestation using MRI and sonography.⁹⁻²¹ Using sonography, Cohen et al.¹⁵ concluded that the first sulcus can be seen as early as at 18 w of gestation. Detailed morphological and quantitative characterizations of sulci and gyri formations are limited in the previous studies using sonography and MRI.^{12,17,18}

All primary sulci are formed during fetal life, and the appearance of each sulcus follows a characteristic timing pattern.²² This pattern can potentially be used as a reliable guide for determining gestational age and normal fetal development. The timing of primary and secondary sulci formation is considered an accurate marker of brain development in the clinical radiological investigation of MRI slices.¹³ Furthermore, the quantitative characterization of gyrification during development may help reveal the mechanism of neurodevelopmental disorders.^{23,24} Therefore, our study aimed to determine the timing of initial sulcal formation and reveal each sulcus's initial morphologic and morphometric features using high-resolution images.

Materials and Methods

Human fetal specimens

The Ethics Committees of the Graduate School of Medicine, Kyoto University Faculty and Shimane University approved this study (approval numbers: R2224 and 02150226-2, respectively).

Thirty-three human fetal specimens (CRL: 50–129.3 mm, 11–16 weeks of gestational age, 16 females and 17 males) were selected for this study and stored at Shimane University. Most specimens were obtained at the termination of pregnancy for socioeconomic reasons under the Maternity Protection Law of Japan.²⁵ Specimens were collected from 1963 to 1995, according to the relevant regulations. Written informed consent was not required from the parents. Instead, the parents provided oral informed consent to deposit the specimens, and the permission obtained from each participant was documented in their medical records. Some of the specimens were undamaged in well-preserved fetuses. The aborted specimens were taken to the laboratory, measured, and examined for external features. Only fetuses without external abnormalities and those that were well preserved without deformity owing to fixation were used in the present study. Gestational age was estimated using data indicating the relationship between the CRL and gestational age obtained from fetal specimens.²⁶

Image acquisition

T1-weighted (T1W) MRI of human fetal specimens was performed, as previously reported.²⁷ Briefly, MRI was performed using two 7-T preclinical MRI systems: BioSpec 70/20 USR (Bruker BioSpin MRI GmbH) at the Medical Research Support Center, Graduate School of Medicine, Kyoto University and BioSpec 70/30 USR (Bruker BioSpin MRI GmbH) at the National Cerebral and Cardiovascular Center. The 7-T MRI system was equipped with ¹H quadrature transmit-receive volume coils with diameters of 72 and 86 mm (T9562 and T12053, respectively; Bruker BioSpin MRI GmbH) and 75 mm (Takashima Seisakusyo Co., Ltd.).²⁸ The T1W MRI datasets were acquired using spoiled gradient-echo sequence. Based on the specimen size, appropriate data acquisition settings were selected from three conditions in which different MRI coils and acquisition parameters were prepared.²⁸

Image analysis and length and depth measurements

The MRI data of the selected specimens were analyzed in detail using reconstructed serial two-dimensional (2D) and three-dimensional (3D) images. 3D images of the cerebrum were manually reconstructed using the Amira software, version 5.5.0 (Visage Imaging GmbH, Berlin, Germany).

All grooves were marked with dots on the serial coronal planes (Figure 1). Yellow dots indicated the grooves with width (mm) > depth (mm), and red dots indicated the grooves with depth (mm) > width (mm). The dots were interposed on the reconstructed brain surfaces, and the lining of the dots was identified. The sulcus was determined from the position of each dotted line on the external and internal brain structures. Regarding the sulci at the medial cerebral surface, the presence of the layered structure around the groove and relative changes in the thickness of the cerebral cortex between the groove and adjacent region were required for a definitive sulcus.²⁹ The lateral sulcus was categorized as smooth (ambiguous) or angular (conspicuous) according to previous studies, with modifications.^{6,14} The lengths of the sulci were measured using reconstructed 3D images. The intersection line between the upper border of the callosum and the medial surface of the cerebrum was recognized as a substitute for the callosal sulcus and measured as the callosal length.

The depth of the definitive sulci was measured at both ends and the center of the red dots in each sulcus. The mean of the three measured values was used as the depth of each sulcus.

Statistical analysis

The association between the CRL and the presence of the sulcus was assessed using logistic regression analysis. In addition, the relationships between the CRL and sulci length and depth were assessed using linear regression analysis. The relationship between the left and right sulci and sulci length and depth were quantified using Pearson correlation coefficients. All statistical analyses were performed using JMP 17.0.0 (JMP Statistical Discovery LLC Inc., NC, USA), with a significance level of 0.05.

Results

Superolateral cerebral surface

Longitudinal cerebral fissure (longitudinal invagination of the telencephalic vesicle) and transverse fissure, which separates the telencephalic vesicles from those of the diencephalon, were observed in all samples, including the most miniature fetus (11 w; CRL, 50–58.7 mm).

The lateral cerebral surface was initially smooth (11–12w) (Figures 2 and 3, Table 1). The lateral sulcus initially appeared as a smooth and ambiguous marginal indentation (13–14 w). After approximately 15 w of gestation, a smooth indentation developed at angular margins at the site of the developing lateral sulcus, with the development of the parietal and temporal lobes. The angular margin was not acute in any of the samples (15–16 w). No definitive central sulci were detected in all the included samples.

Sulci of the inferior cerebral surface

The preliminary hippocampal sulcus was detected from 11 w (Figures 2 and 4; Table 1). This sulcus was shallow but was almost constantly detected in the samples from 13 to 16 w (Figure 5).

The olfactory sulcus occasionally appeared as a superficial depression on the medial aspect of the cerebral orbital surface, running underneath the olfactory bulb and tract (Figures 2, 4, and 5). The sulcus was shallow, faint, and difficult to detect without olfactory bulbs as landmarks.

Sulci of the medial cerebral surface

On the MRI sagittal plane, the callosum was detected as convex shaped with a high intensity in four of seven samples at 12 w of gestational age and all older samples (Figures 2 and 4B). The callosum developed from the anterior to the posterior direction (Figure 5B). On coronal sections, the callosum and medial surface of the cerebrum formed a right angle, although no apparent sulci were detected in any of the samples. Thus, the intersection line of the callosum and medial surface of the cerebrum was recognized as a substitute for the callosal sulcus and measured morphometrically (black line on the callosum in Figures 4C and 5C). The parietal-occipital sulcus separates the parietal lobe from the occipital lobe along the medial cerebral surface and separates the precuneus of the parietal lobe from the cuneus of the occipital lobe. The parietal-occipital and calcarine sulci were almost definitely observed in samples older than 13 w of

gestational age (CRL 85 mm) (Figures 2 and 5).

Several potential sulci were observed anterior to the parietal-occipital sulcus in samples at 11 w of gestation or older (Figure 5A). Of these, a common sulcus (designated as the ND_a, candidate of the cingulate sulcus) was detected in the anterior part parallel to the olfactory sulcus (Figures 2 and 5A). In addition, several sulci, which varied among the samples, were detected (ND_b, _c, _d, and _e in Figure 5A).

The presence of sulci and gestational age

The presence of the primary sulci was related to gestational age, except for the olfactory sulcus (Supplementary Table 1). Logistic regression analysis showed that the unit odds for the presence of the sulcus to the CRL increased was significant (i.e., unit odds were significantly large compared to 1.00) in the hippocampal sulcus (unit odds ratio [95% confidence interval, CI]: 1.10 [1.03, 1.18], $P=0.007$), calcarine sulcus (1.07 [1.04, 1.11], $P<0.001$), parieto-occipital sulcus (1.05 [1.02, 1.08], $P=0.001$), and sulcus ND_a (1.05 [1.01, 1.09], $P=0.012$); however, it was not significant in the olfactory sulcus (1.01 [0.98, 1.03], $P=0.490$) (Table 2).

Length and depth measurements

Inter-hemisphere and callosal lengths increased linearly with the CRL ($p<0.001$ for all lengths; Figure 6A, Supplementary Table 2).

The lengths of the left and right sides of the olfactory sulci and the left side of the hippocampal sulcus increased linearly with the CRL ($p=0.013$, 0.030 , $R^2=0.61$, 0.36 for olfactory sulci and $p=0.049$, $R^2=0.13$ for the left side of the hippocampal sulcus, respectively; Figure 6B, Supplementary Table 2). The length of the other sulci (the right side of the hippocampal sulcus and the left and right sides of the calcarine, parieto-occipital, and ND_a sulci) did not increase with the CRL (Figure 7). The left and right sulci lengths were highly correlated in callosal length ($r=0.88$) and the hippocampal ($r=0.93$) and olfactory sulci ($r=0.92$); moderately correlated in the calcarine ($r=0.64$) and ND_a sulci ($r=0.67$); and weakly correlated in the parieto-occipital sulcus ($r=0.29$) (Supplementary Figures 1, 2).

The depth of the all sulci, except for the parieto-occipital sulci, increased linearly with the CRL (Figure 6B, 7, Supplementary Table 3). The left and right sulci

depths were highly correlated in the hippocampal sulcus ($r = 0.94$) and moderately correlated in the parieto-occipital ($r = 0.67$), ND_a ($r = 0.68$), and calcarine sulci ($r = 0.55$) (Supplementary Figures 1, 2).

Discussion

We qualitatively and quantitatively assessed the dynamics of brain gyrification during human fetal development. Qualitatively, we determined the age at which the sulcus was visible in 50% or more of the fetuses (Table 1): <12 w for the callosum, <13 w for the hippocampal, calcarine, and parieto-occipital sulci, and <15 w for the lateral sulcus. The olfactory sulcus was occasionally detected but the timing of appearance was not determined. The cingulate sulcus could not be definitively identified. Regarding the lateral sulcus, the timing of appearance may depend on the definition, as the sulcus gradually appears and changes its form. Several studies might not have distinguished between the lateral sulcus and the Sylvian fissure.^{15,18} Different definitions between each study may be a cause of the discrepancy in the timing of appearance observed in previous studies^{2,5,9,14} and the present study.

We acquired high-resolution MR images, observed the cerebral surface and internal structures on serial 2D planes, and marked all probable sulci on 3D reconstructions to compare the positions of the sulci in the samples and inter-samples. This process identified the sulci. As a result, the detection timing was as early as those in previous studies using anatomical dissection^{2,4,5} and earlier than those in studies using MRI (Table 2).^{9-13, 30}

MR images in the present study can observe the formation of internal structures in the hippocampus, olfactory bulb and tracts, and callosum.³¹ Therefore, the positions of the hippocampal, olfactory, and callosal sulci can be predicted by their association with such internal structures. Notably, surface observations alone may lead to inaccurate estimations of brain development because these sulci are not always detected.

The hippocampal sulcus corresponds to the first phylogenetic sulcus because it separates the archicortex of the hippocampus from the paleocortex of the subiculum of the parahippocampal gyrus.^{2,5,22,30-32} This separation occurs owing to inferior and lateral displacement of the hippocampus, which occurs evolutionarily and embryologically.⁸ In anatomical dissections, the hippocampal sulcus gradually narrows, whereas the medial portion of the temporal lobe develops. Chi et al. and Nishikuni et al. detected this in fetuses as young as 10 and 15 w of gestation, respectively.^{2,5} We detected this sulcus at a time consistent with that of the anatomical dissection. However, previous studies have revealed that it is difficult to detect this sulcus in young fetuses using MRI and sonography.^{9-13, 30}

The olfactory sulcus initially appears as a superficial depression on the medial aspect of the cerebral orbital surface, which develops underneath the olfactory bulb and tract and extends, delimiting the rectal gyrus laterally. Dooling et al.⁷ reported that the olfactory sulcus is correlated with the formation of the olfactory bulb and tract. Previous studies have found it difficult to detect this sulcus using MRI and sonography in young fetuses.⁹⁻²⁰ Our study revealed that the superficial depression on the medial aspect was extremely shallow and scarce, making the detection of the sulcus difficult. Furthermore, the increased CRL had no effect on the presence of this sulcus.

The callosal sulcus separates the callosum from the cingulate gyrus^{22,32} and is the first sulcus to appear on the medial surface of the brain during anatomical dissection.^{2,5} The apparent features of the callosum and cingulate gyrus such as stiffness, color, and fiber orientation, based on gross findings, may aid in the detection of the borderline (sulcus). Chi et al. and Nishikuni et al. detected this in fetuses as young as 14 and 12 w of gestation, respectively.^{2, 5} In the MRI sagittal plane, the callosum was detected as convex shaped with high intensity from the samples at 12 w of gestation and older almost constantly, as described in our previous study.³³ However, in our coronal sections, the intersection of the callosum and medial surface of the cerebrum formed a rectangle; moreover, no apparent sulci were detected in any of the samples. Therefore, we provided the measurement value as the callosal length and not as callosal sulcus.

The parietal-occipital and calcarine sulci were observed in most of the samples older than 13 w of gestation. No definite cingulate sulci were detected in this

study. Instead, several possible sulci were observed anterior to the parietal-occipital sulcus in samples 11 w and older. A common sulcus among the samples (ND_a in Figures 2, 5A, and 5C) was detected in the anterior part, parallel to the olfactory sulcus. Chi (1977)² described that the cingulate sulcus initially forms at the anterior part and elongates posteriorly. Based on its position and running course, the sulcus may be the definitive cingulate sulcus. In addition to this ND_a, several possible sulci, including the cingulate sulcus, were detected (ND_b, _c, _d in Figure 5A). In our previous study³³, the cingulate sulcus was described at the occipital regions in the fetus at 14 w of gestation, which may correspond to the ND_c in Figure 5A. Those sulci, however, varied in number and position among the samples. Previous studies have indicated the presence of transitory furrows, which are unrelated to any defined sulci that develop subsequently.^{5,34} Several sulci in the present study may have been transitory furrows not related to the cingulate sulcus. Another possibility is that these were artificial furrows owing to sample conditions such as long-term preservation in formaldehyde solutions. Many furrows were judged as artifacts in the atlas of Bayer and Altman.³⁵ Cortical layered structures and changes in the thickness at the groove and adjacent regions may aid in distinguishing the results from those of artificial grooves, which are imperfect.²⁹

The callosum and intercerebral hemisphere lengths increased linearly with the CRL. However, a few sulci lengths on the inferior cerebral surface increased linearly with the CRL, whereas most sulci depths increased linearly with the CRL. One possible explanation for these results is that we used rough methods in the length measurements of sulci. Linear measurements were performed from one end of the sulci to another. The sulci ran along the medial and inferior surfaces, which were curved, increasing the measurement error. Another possibility is that the endpoints may be challenging to determine because they are shallow and widely open. Another possibility is that the sulcus length at initial appearance did not correlate with the CRL. The timing of the appearance of sulci sometimes varied significantly between samples, and no sulci was occasionally observed even in the samples of older gestational ages. Several studies have described sulci formation differentiated from small dots and V- and Y-shape.^{2,11,14,17} Our findings of yellow and red dots may be consistent with

changes from a U-shape to a Y-shape in previous studies; however, our findings revealed no initial small dots at the expected site of the sulcus. We speculate that sulci may not arise as if they elongate gradually but simultaneously over some distance.

Limitations

The present study had some limitations. First, the samples were preserved in formaldehyde solution for an extended period, which might have affected the measurements. Shrinkage of the sample may have caused an underestimation of the CRL and the calculated gestational age. It is sometimes difficult to distinguish sulci formations from artificial depressions. Second, the detection timing and measurement values varied between samples, which led to difficulty in evaluating the left-right symmetry and sex differences. Limited studies have indicated left-right differences.^{1,8,18} Regarding sex differences, male and female brains appear to develop similarly in anatomical, autopsy, and neonatal cerebral ultrasound studies.^{2,14,21,36} Research using a large sample size will be required to know whether the left-right symmetry and sex differences.

Conclusion

We determined the timing of initial sulcal formation using high-resolution MR images. Our findings may significantly impact prenatal diagnosis and research on neurodevelopmental disorders.

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STATEMENT OF ETHICS

The Ethics Committees of the Graduate School of Medicine, Kyoto University Faculty and Shimane University approved this study (approval numbers: R2224 and 02150226-2, respectively).

AUTHOR CONTRIBUTIONS

Miyu Kumagai performed data analysis/interpretation and drafting of the manuscript.

Toru Kanahashi performed data analysis.

Hirohiko Imai acquired the MR images.

Hiroki Otani contributed to data acquisition and critical revision of the manuscript.

Tetsuya Takakuwa contributed to the manuscript's concept/design and critical revision.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Figure legends

Figure 1. Representative images indicating definitive sulci with depth > width (red dot), preliminary sulci with width > depth (yellow dot), and uncertain (gray dot). For the sulci at the medial and inferior cerebral surface, the presence of a layered structure around the groove and relative changes in the thickness of the cerebral cortex between the groove and adjacent region on the magnetic resonance image were required for the definitive sulci (red dots). Definitions of the lateral sulcus are presented in the Materials and Methods section.

Figure 2. Detection of the primary sulci in fetal samples of 11 to 16 w of gestational age. O highlighted with red and ++ highlighted with orange; definitive sulci, O with yellow; preliminary sulci

LateralS; lateral sulcus, CalS; calcarine sulcus, POS; parieto-occipital sulcus, ND_a; sulcus not determined_a (candidate of the cingulate sulcus), HipS; hippocampal sulcus, OlfS; olfactory sulcus, GA; gestational age, L; left, R; Right, F; female, M; male, CRL; crown-rump length

Figure 3. The superolateral cerebral surface.

A) Three-dimensional reconstructions indicating the superolateral cerebral surface of the samples from 11 to 14 w. ID and CRL of the samples were shown.

B) Coronal plane indicating the lateral sulci and hypophysis. The lateral sulcus angular margin was not acute in the samples (14 w) (asterisks).

Figure 4. Cerebral surface of the samples at 12 w of gestation.

A) Representative 3D reconstructions (medial view) showing the callosum and hippocampus sulcus (F2240_CRL 70.5 mm). Purple dots indicate the callosum. Red line on the callosum indicate the intersection line between the upper border of the callosum and medial surface of the cerebrum. Yellow and red dots

483 indicate the hippocampal sulcus.

484 B) MRI of the mid-sagittal plane. Note that the callosum was detected as a high-
485 intensity convex.

486 aq: Aqueduct of the cerebrum, cal: callosum, ce: cerebellum, hy: hypophysis,
487 op: optic chiasm, pg: pineal gland, po: pons, v3: third ventricle, v4: fourth
488 ventricle

489 C) Coronal plane images indicating the callosum and hippocampal sulcus
490 (asterisks). The location of planes for the callosum and hippocampal sulcus is
491 indicated in the illustration.

492 D) Coronal plane (F2373_CRL 71.0 mm) indicating the olfactory and
493 hippocampal sulci (asterisks). The olfactory bulb is indicated by yellow
494 arrowheads.

495

496 Figure 5. Cerebral surface of the samples at 14 w of gestation (F1915, CRL 112
497 mm).

498 A) Representative 3D reconstructions (medial view) indicating definitive four
499 sulci and several undetermined sulci (ND_a, b, c, and d). Red and yellow dots
500 indicate sulci candidates. Purple dots indicate callosum. Red line on the
501 callosum indicate the intersection line between the upper border of the callosum
502 and medial surface of the cerebrum.

503 CalS; calcarine sulcus, POS; parieto-occipital sulcus, ND_a; sulcus not
504 determined_a (candidate of the cingulate sulcus), HipS; hippocampal sulcus,
505 OlfS; olfactory sulcus

506 B) MRI of the mid-sagittal plane. Note that the callosum is elongated anterior
507 posteriorly as a high-intensity convex.

508 aq: Aqueduct of the cerebrum, cal: callosum, ce: cerebellum, hy: hypophysis,
509 op: optic chiasm, pg: pineal gland, po: pons, v3: third ventricle, v4: fourth
510 ventricle

511 C) Representative coronal plane images indicating definitive sulci (orange

512 asterisks and crossing).

513 ND_a; sulcus not determined_a (candidate of the cingulate sulcus), eb; eyeball,
514 OlfS; olfactory sulcus, ob; olfactory bulb, Callosum (yellow triangle), ND_c ;
515 sulci not determined_c (asterisk), HipS; hippocampal sulcus, POS; parieto-
516 occipital sulcus (*), CalS; calcarine sulcus (+)

517 The coronal plane was perpendicular to the line connecting the anterior and
518 posterior commissures.

519

520 Figure 6. Sulci length and depth, according to the increase in CRL.

521 A) Interhemispheric length and callosal length.

522 The callosal length was measured as the intersection line between the upper
523 border of the callosum and medial surface of the cerebrum for the substitute of
524 the callosal sulcus.

525 B) Length and depth of the sulci located on the inferior cerebral surface.

526 The depth of the definitive sulcus was measured at both ends and the center of
527 the red dots in each definitive sulcus. The mean of the three measured values
528 was used as the depth of each sulcus.

529 Circular and triangular dots indicate data values for the left-sided and right-
530 sided sulci. Approximate straight lines and R2 values in black and blue indicate
531 left-sided and right-sided data values, respectively. For the calculation of the
532 approximate straight line, the data in which the sulcus was not present was
533 removed, and the p-value for the slope was indicated.

534

535 Figure 7. Length and depth of the sulci located on the medial cerebral surface.

536 The depth of the definitive sulcus was measured at both ends and the center of
537 the red dots in each definitive sulcus. The mean of the three measured values
538 was used as the depth of each sulcus.

539 Circular and triangular dots indicate data values for the left- and right-sided

540 sulci. Approximate straight lines and R2 values in black and blue indicate left-
541 and right-sided data values, respectively.

542 For the calculation of the approximate straight line, the data in which the sulcus
543 was not present was removed, and the p-value for the slope was indicated.

546 Supplementary Figure 1. Comparison between left- and right-sided sulci lengths
547 and depth located on the inferior cerebral surface

548 Red dots indicate that either the left or right sulcus is definitive.

549 Yellow dots indicate that the left and right sulci are preliminary.

551 Supplementary Figure 2. Comparison between left- and right-sided sulci lengths
552 and depth located on the medial cerebral surface

553 Red dots indicate that either the left or right sulcus is definitive.

554 Yellow dots indicate that the left and right sulci are preliminary.

564 Table 1. Chronology of sulcation in the present study

565

566 Table 2. The unit odds ratio of the crown-rump length to the presence of the
567 sulcus

568

569 Table 3. Comparing the timing of the appearance of the cerebral sulci between
570 anatomical and neuroimaging studies

571

572 Supplementary Table 1 Summary of the primary sulci formation in the fetal
573 samples from 11 to 16 gestational age

574

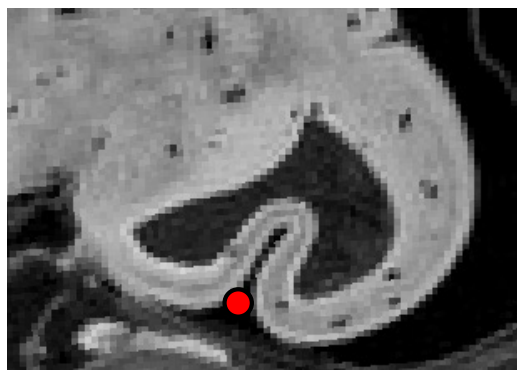
575 Supplementary Table 2 Summary of the Length measurements

576

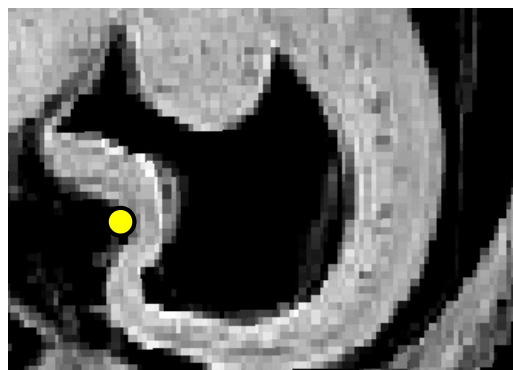
577 Supplementary Table 3 Summary of the Depth measurements

578

● Definitive (depth > width)



● Preliminary (width > depth)



○ uncertain

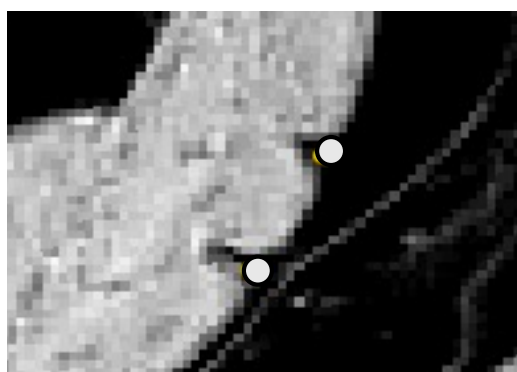


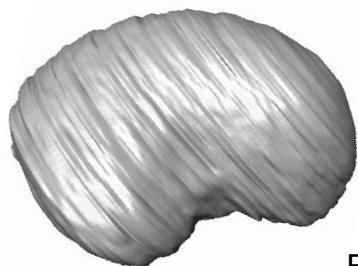
Figure 1

Gestational weeks	ID	Sex	CRL	Lateral		Medial								Inferior			
				LateralS		Callosum		CalS		POS		ND_a		HipS		OlfS	
				L	R	L	R	L	R	L	R	L	R	L	R	L	R
11	F1485	F	50.0	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	F2396	M	51.5	×	×	×	×	×	×	×	×	○	○	○	○	×	×
	33087	F	58.7	×	×	×	×	×	×	●	×	●	●	●	●	○	○
12	F2983	F	67.3	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	71030	M	67.3	×	×	×	×	×	×	×	×	●	●	○	○	×	×
	F2133	M	69.5	×	×	○	○	○	○	●	×	●	●	○	○	○	○
	F2240	F	70.5	×	×	○	○	×	×	×	×	×	×	○	○	×	×
	F2373	M	71.0	×	×	○	○	○	●	●	●	●	●	●	●	○	○
	F2248	F	71.0	×	×	×	×	×	○	○	○	×	○	○	○	×	×
	F2149	M	74.0	×	×	○	○	×	×	●	×	●	●	×	×	×	×
13	F1877	F	85.0	+	+	○	○	●	●	●	●	●	●	○	○	×	○
	37304	F	87.5	×	×	○	○	×	×	●	●	●	●	○	○	×	×
	F2286	F	88.0	×	×	○	○	●	●	×	●	●	●	○	○	×	○
	F2230	M	92.5	+	+	○	○	●	●	●	×	●	●	●	●	○	○
	F15	F	93.7	+	+	○	○	●	●	●	●	●	●	○	○	×	○
	F1874	M	95.0	×	×	○	○	●	●	×	×	●	●	●	●	×	×
	37729	F	95.0	++	++	○	○	●	●	●	●	●	●	●	●	○	○
	F1927	F	96.5	+	+	○	○	●	●	○	●	●	●	●	●	×	×
14	37827	M	100.2	+	+	○	○	●	●	●	●	●	●	●	●	×	×
	F1879	M	101.0	+	+	○	○	●	●	●	●	●	●	○	●	×	×
	F1780	M	102.0	+	+	○	○	○	○	○	×	●	●	○	●	×	×
	F2070	F	102.5	+	+	○	○	●	●	●	●	×	●	●	●	×	×
	F2245	M	103.0	+	+	○	○	●	●	○	○	●	●	●	●	×	×
	70323	M	103.0	+	+	○	○	●	○	×	×	●	●	○	○	×	×
	F2139	F	106.5	+	+	○	○	●	●	×	×	○	●	●	●	×	×
	F1925	F	109.0	++	++	○	○	●	●	●	○	●	●	●	●	×	×
	F1915	M	112.0	++	++	○	○	●	●	○	●	●	●	●	●	○	○
15	F2011	F	115.0	++	++	○	○	●	●	●	●	○	○	●	●	○	○
	F1892	M	117.0	++	+	○	○	●	●	●	●	●	●	●	●	○	○
	F2275	F	120.0	++	++	○	○	×	×	○	○	×	●	●	●	○	○
16	F2225	M	126.0	++	++	○	○	●	●	●	●	●	●	●	●	×	×
	37626	M	128.1	++	++	○	○	●	●	●	●	●	●	●	●	×	○
	37866	M	129.3	++	++	○	○	×	○	×	●	●	●	●	●	×	×

Figure 2

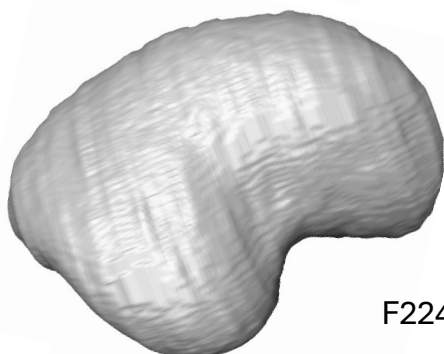
A

11w



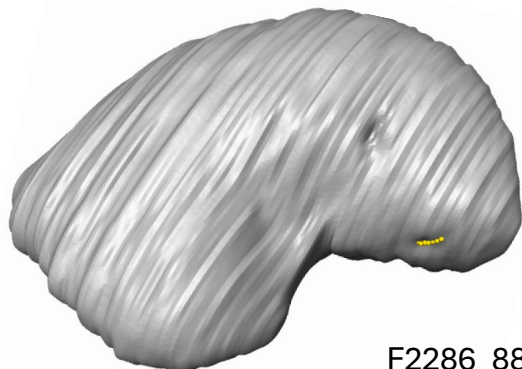
F1485_50 mm

12w



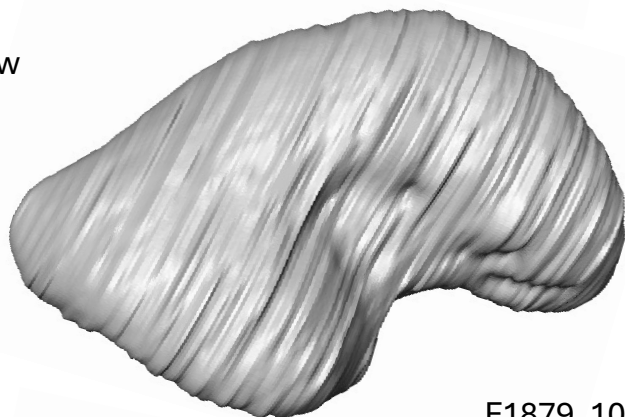
F2240_70.5 mm

13w



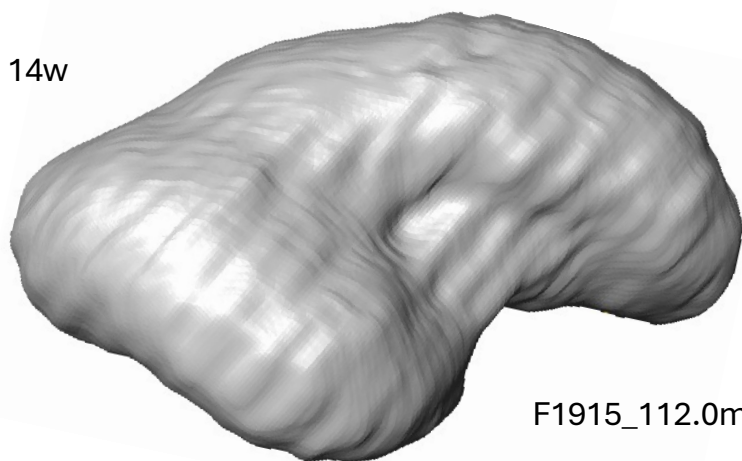
F2286_88.0mm

14w



F1879_101.0mm

14w



F1915_112.0mm

B

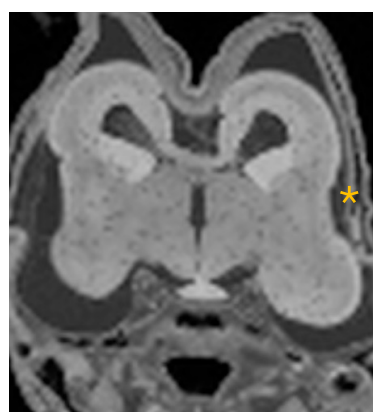
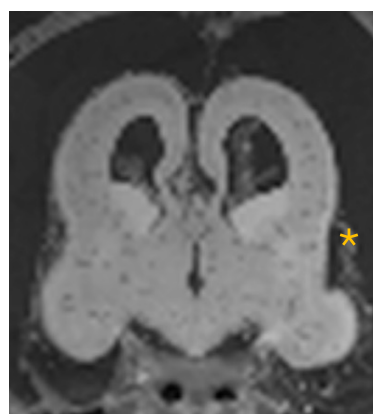
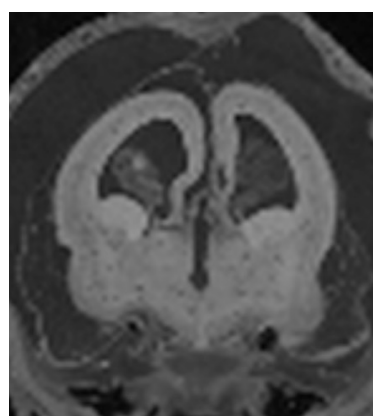
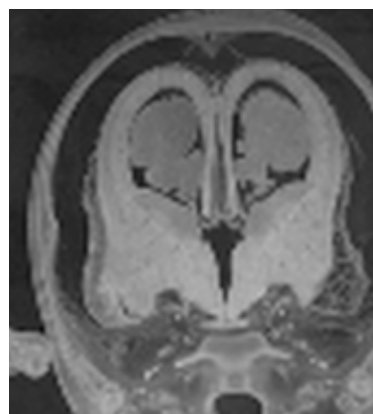
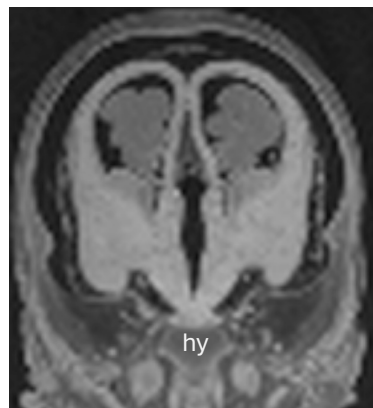
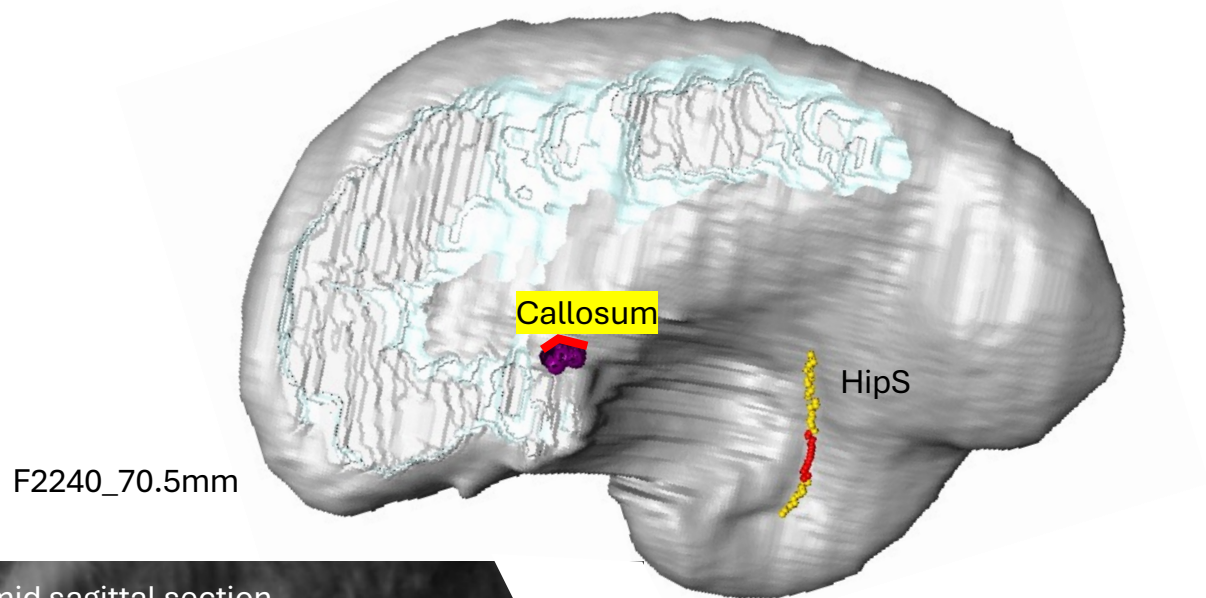
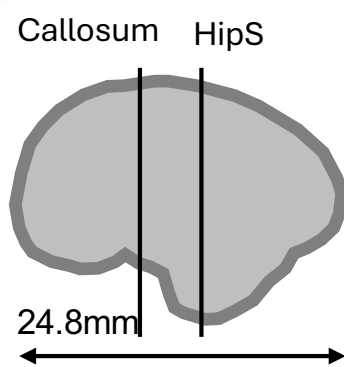
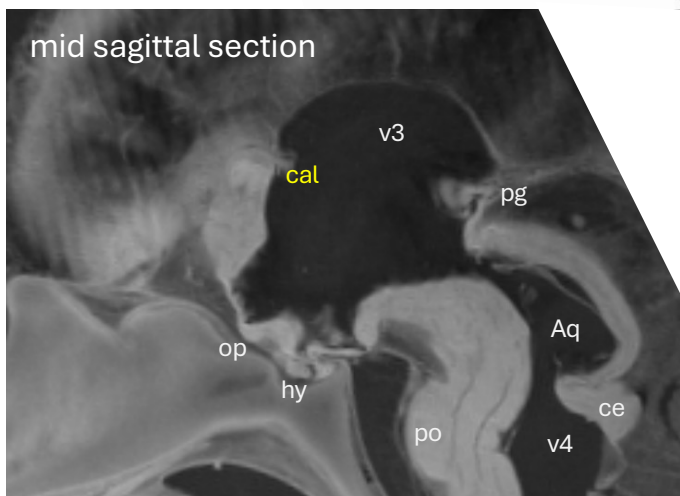


Figure 3

A



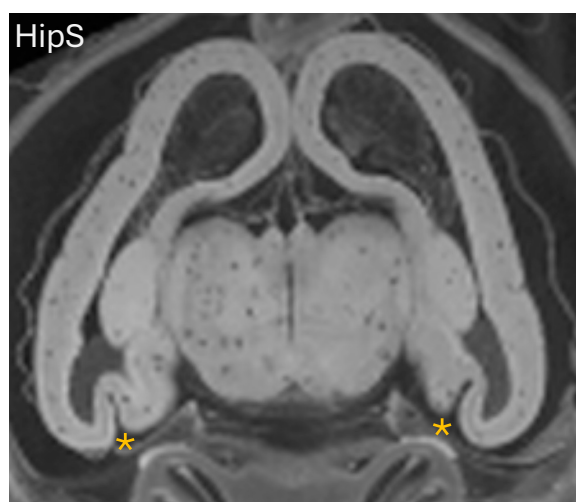
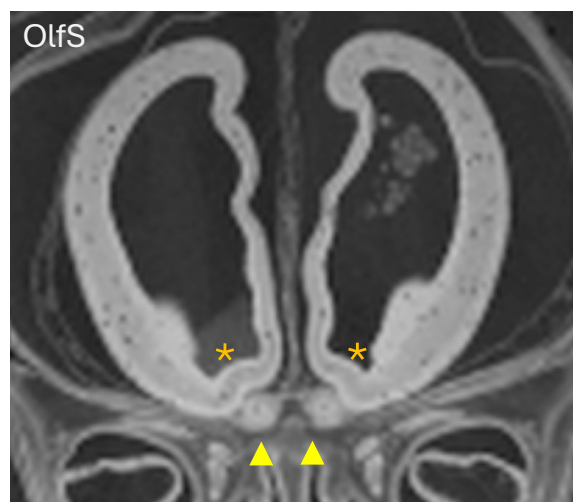
B



C



D



F2373_71.0mm

Figure 4

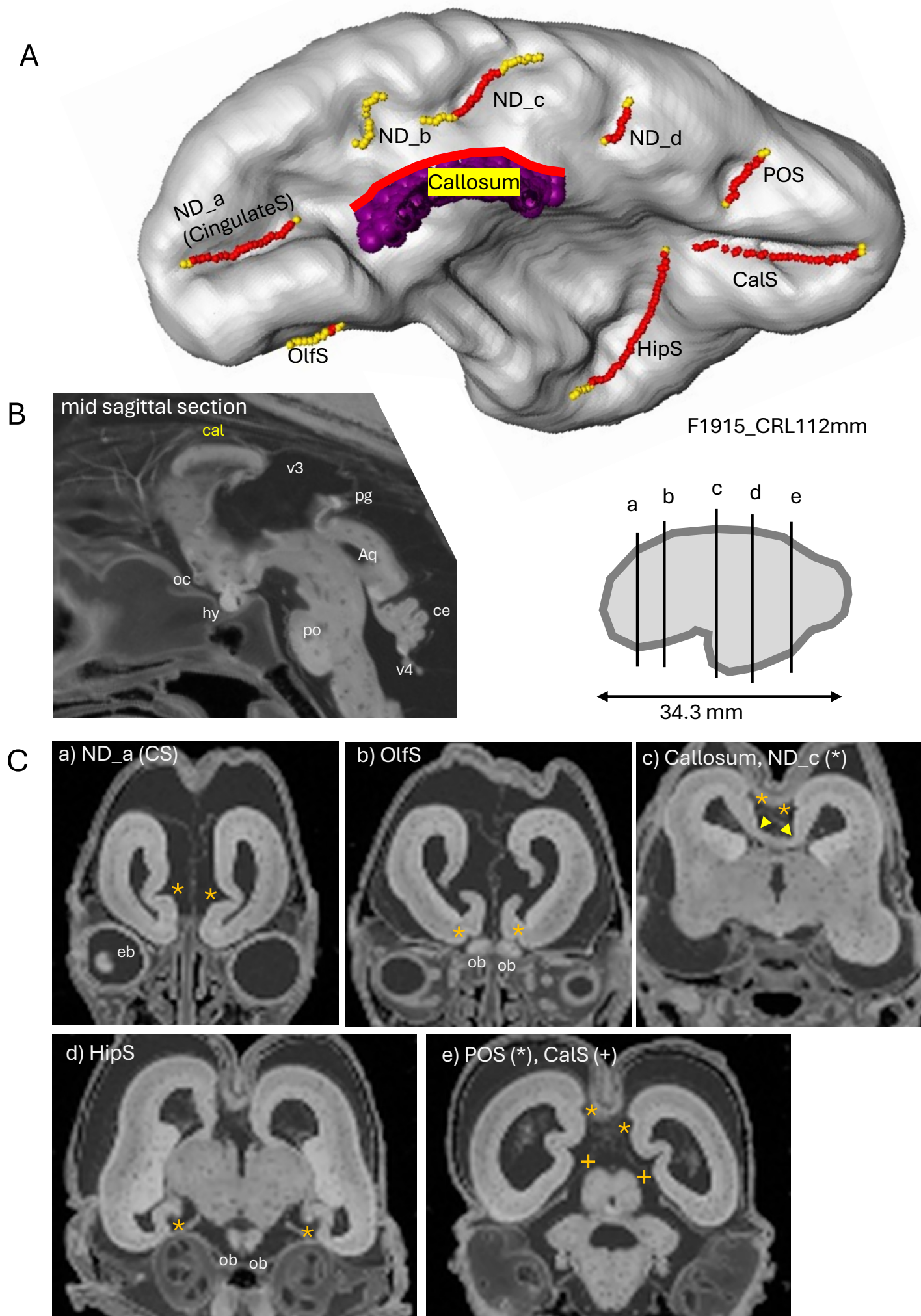


Figure 5

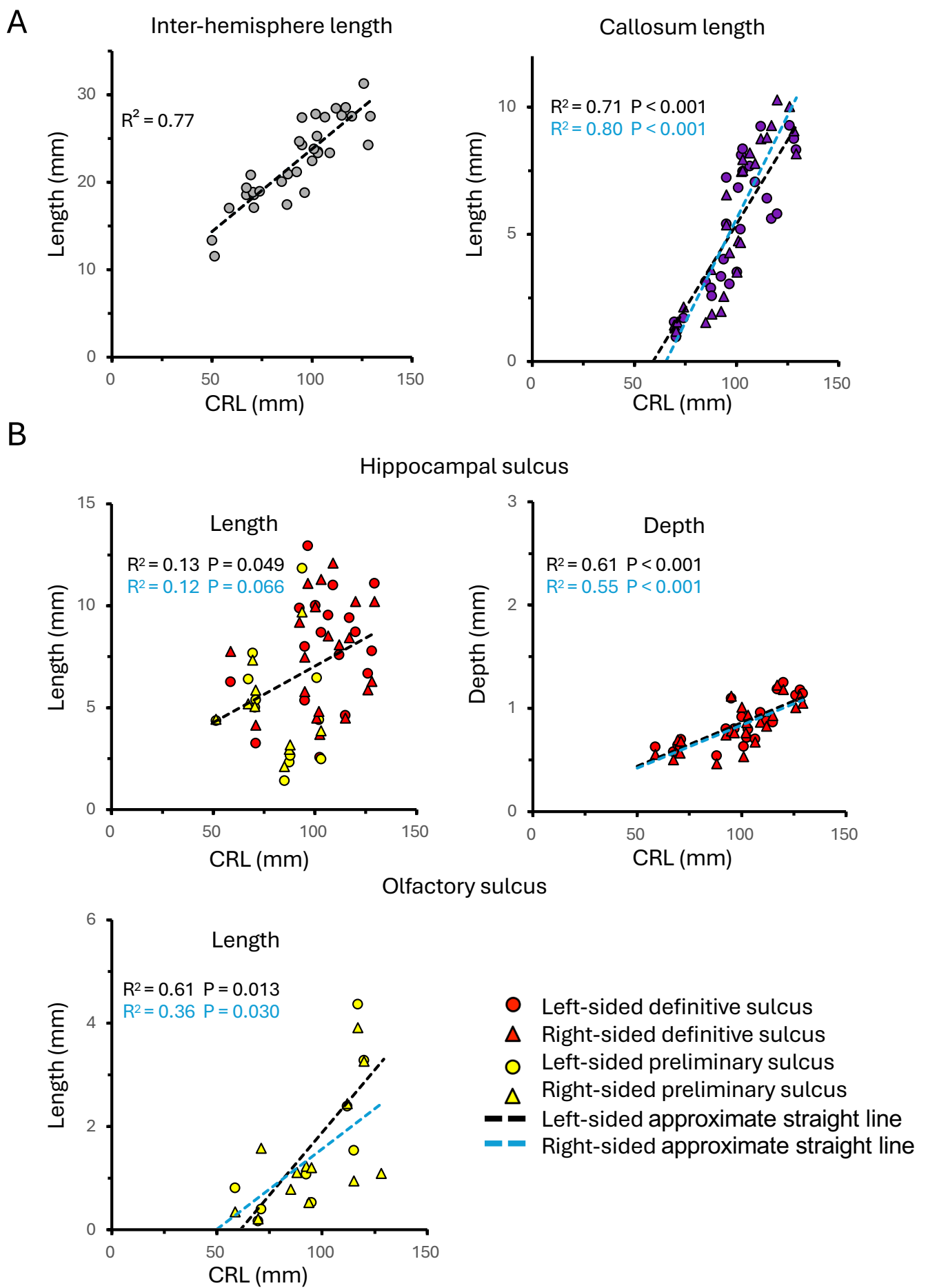


Figure 6

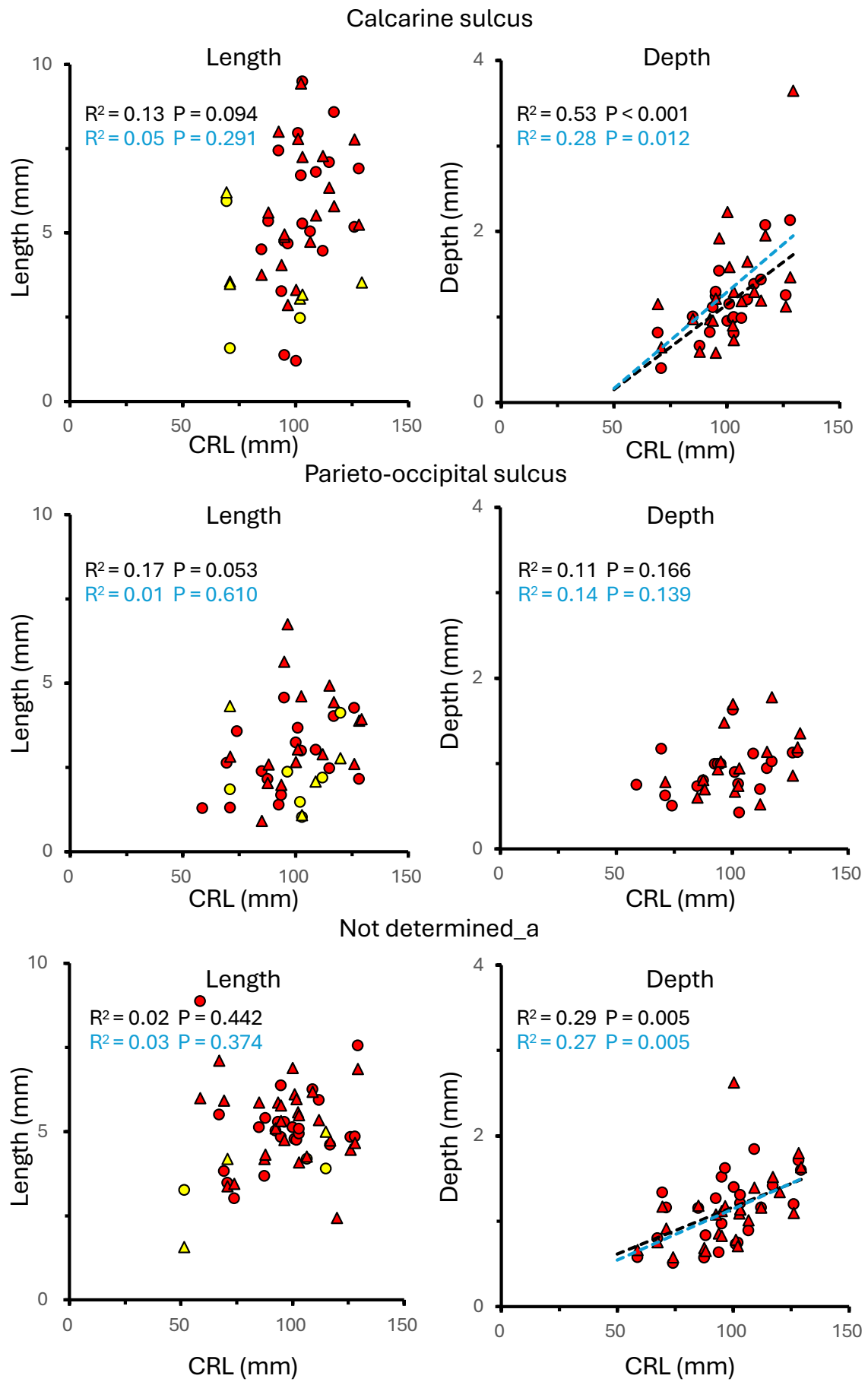
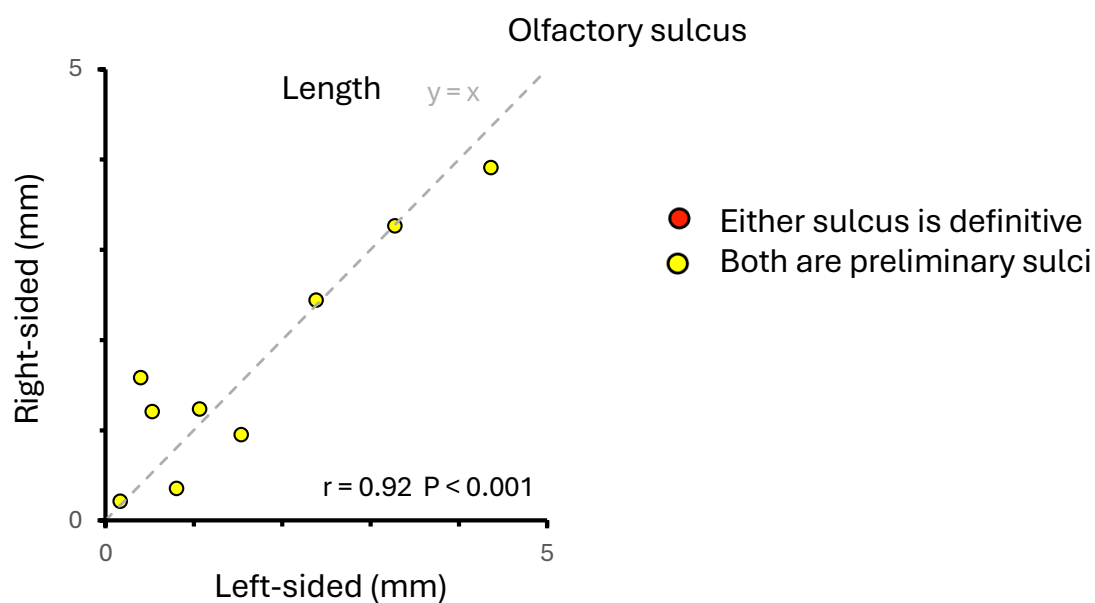
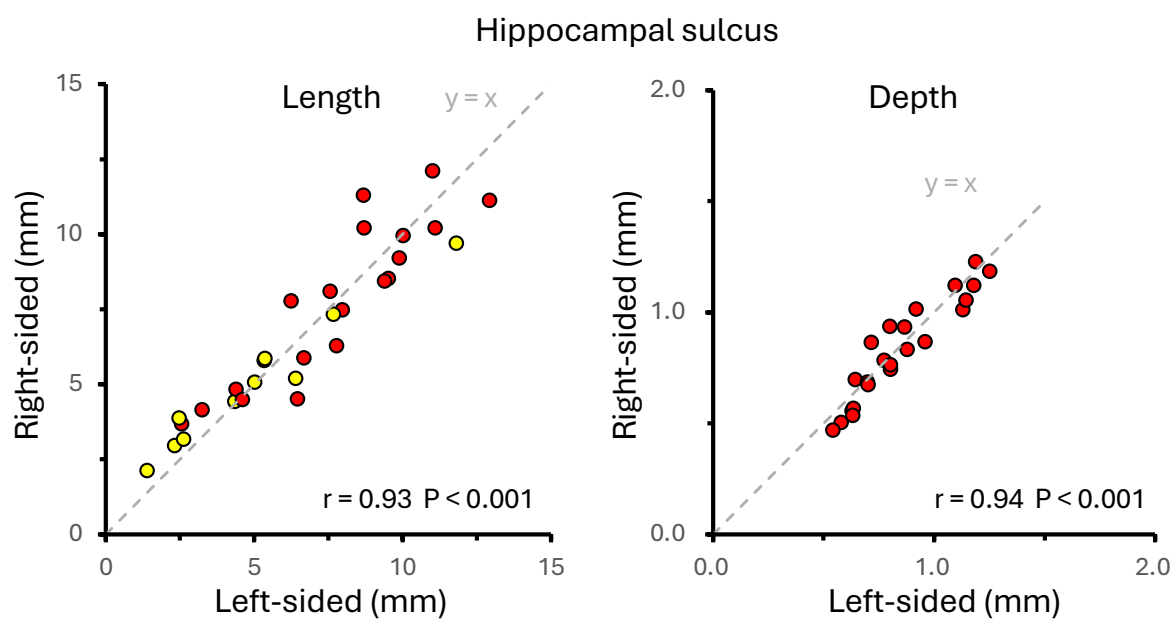
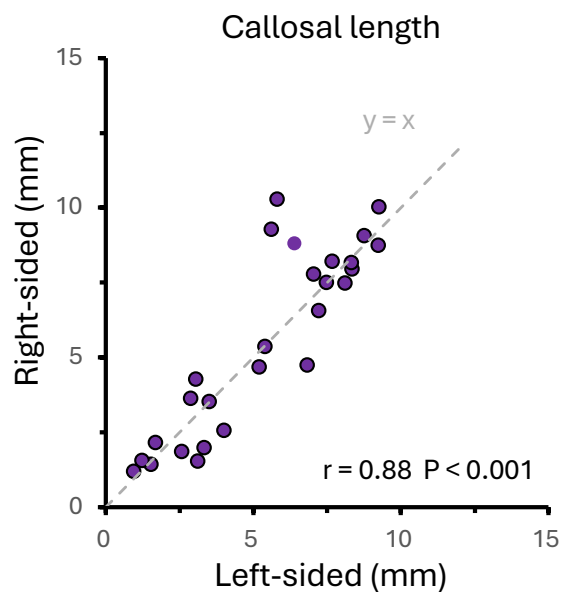
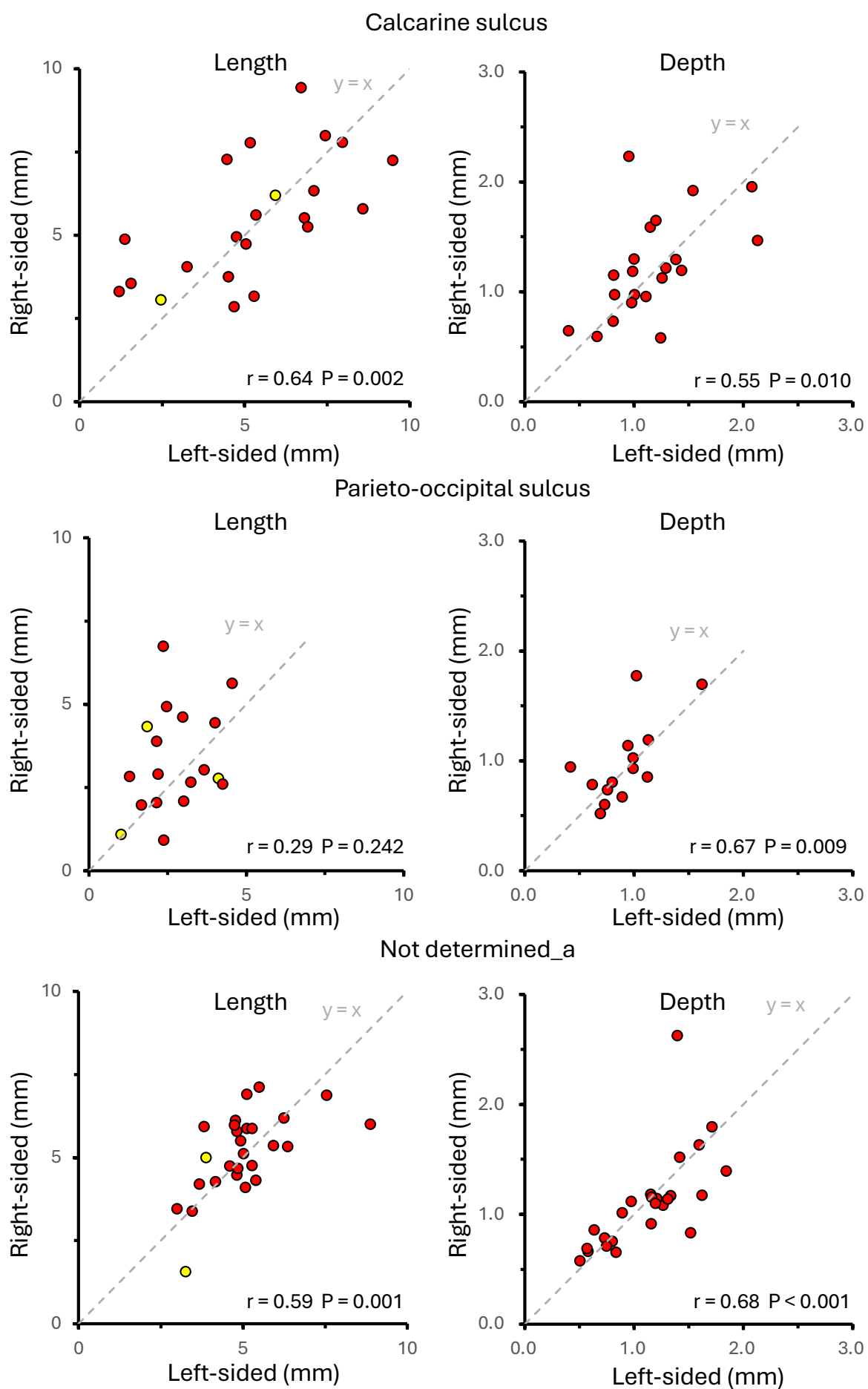


Figure 7





Sup. Figure 2

Table 1. Chronology of sulcation in the present study

Gestational weeks	<i>n</i>	CRL range (mm)	Lateral	Medial				Inferior	
			LateralS	Callosum	CaIS	POS	ND_a	HipS	OlfS
11	3	50.0-58.7	0	0	0	1	1	1	(1)
12	7	67.3-74	0	4	1	3	4	1	(2)
13	8	85-96.5	1	8	7	7	8	4	(5)
14	9	100.2-112	2	9	8	5	9	8	(1)
15	3	115.0-120.0	3	3	2	2	2	3	(3)
16	3	126.0-129.3	3	3	2	2	3	3	(1)

LateralS; lateral sulcus, CaIS; calcarine sulcus, POS; parieto-occipital sulcus, ND_a; not determined_a, HipS; hippocampal sulcus, OlfS; olfactory sulcus, CRL; crown-rump length

The number in brackets for OlfS includes the preliminary sulci.

Table 2 Unit odds ratio of the crown-rump length to the presence of the sulcus

	Unit odds ratio	95% confidence interval			<i>P</i> value
Hippocampal sulcus	1.10	1.03	-	1.18	0.007
Olfactory sulcus	1.01	0.98	-	1.03	0.490
Calcarine sulcus	1.07	1.04	-	1.11	< 0.001
Parieto-occipital sulcus	1.05	1.02	-	1.08	0.001
Not determined_a	1.05	1.01	-	1.09	0.012

Table 3: Comparing the timing of the appearance of the cerebral sulci between anatomical and neuroimaging studies

	Anatomical studies			Ultrasonography studies			MRI studies		
	Chi et al., 1977	Larroche, 1981	Nishikuni, 2006	Monteagudo & Timor-Tritsch, 1997	Bernard et al., 1988	Toi et al., 2004	Levine & Barnes, 1999	Lan et al., 2000	Present study
No. of fetuses	207	30	107	262	70	50	40	25	33
Age range (weeks)	10–44		12–40	14–40	10–37	15–30	14–38	12–38	11–16
Longitudinal cerebral fissure	10		12		10		14–15	12	11–
Lateral sulcus	14		17	18			16–17	15	15–
Circular insular sulcus	18		17			17	18–19		
Central sulcus	20	20	21				26–27	24–26	
Olfactory sulcus	16		17						(13,15)
Hippocampal sulcus	10		15						13–
Callosal sulcus	14	18	12	14	19				12–
Cingulate sulcus	18		19	26	22–24	23	24–25		(12–)
Calcarine sulcus	16	18	17	18	23	19	18–19		13–
Parieto-occipital sulcus	16	18	17	18	23	19	18–19		13–