ORIGINAL ARTICLE

Genes, Brain and Behavior

Heritability in corpus callosum morphology and its association with tool use skill in chimpanzees (*Pan troglodytes*): Reproducibility in two genetically isolated populations

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Abstract

The corpus callosum (CC) is the major white matter tract connecting the left and right cerebral hemispheres. It has been hypothesized that individual variation in CC morphology is negatively associated with forebrain volume (FBV) and this accounts for variation in behavioral and brain asymmetries as well as sex differences. To test this hypothesis, CC surface area and thickness as well as FBV was quantified in 221 chimpanzees with known pedigrees. CC surface area, thickness and FBV were significantly heritable and phenotypically associated with each other; however, no significant genetic association was found between FBV, CC surface area and thickness. The CC surface area and thickness measures were also found to be significantly heritable in both chimpanzee cohorts as were phenotypic associations with variation in asymmetries in tool use skill, suggesting that these findings are reproducible. Finally, significant phenotypic and genetic associations were found between hand use skill and region-specific variation in CC surface area and thickness. These findings suggest that common genes may underlie individual differences in chimpanzee tool use skill and interhemispheric connectivity as manifest by variation in surface area and thickness within the anterior region of the CC.

KEYWORDS
asymmetry, brain evolution, chimpanzees, cognition, corpus callosum, forebrain volume, genetic correlation, heritability, reproducible, tool use skill

1 | INTRODUCTION

Chimpanzees are one of the closest living relatives of humans. Humans and chimpanzees have >98% overlap in DNA coding sequence and diverged from a common ancestor approximately 6 million years ago. Additionally, though humans and chimpanzees differ neuroanatomically in a number of important ways, they also share many features of the brain that are absent in more distantly related species including other nonhuman primates.[1] For instance, recent studies have reported that like humans, in chimpanzees, and indeed all great apes, there is a common sulcal variant on the medial wall of the frontal lobe called the paracingulate sulcus, which is absent in rhesus macaques and baboons.[2,3] Similarly, cortical folding within the inferior frontal gyrus is more similar between humans and chimpanzees when

compared to more distantly related primate species,[4] findings consistent with previous studies on differences in whole brain gyrification.[5–7] Comparative studies of brain and behavior between primate species can offer insights into the evolutionary mechanism that may underlie the emergence of cognitive and neurobiological specializations in humans. Within this framework, of specific interest to this study are previous reports of species differences among primates in the relative size of the corpus callosum (CC).[8]

The CC is comprised of the major homotopic and heterotopic white matter tracts that connect the left and right cerebral hemispheres.[9–11] Numerous studies, particularly in split-brain patients and monkeys, have shown that the CC plays a central role in interhemispheric connectivity and transmission of sensory, motor and cognitive functions.[12–18] From an evolutionary perspective, Rilling and Insel[8] compared the surface area of the CC in eight diverse anthropoid primate species and found that as brain size increased during primate brain evolution, the CC did not allometrically keep pace. Thus, humans have a proportionally small CC for species of their brain size compared to apes, ceropithecoid and platyrrhine monkeys. One interpretation of these findings is that humans have more disconnected hemispheres compared to other nonhuman primates and this may account for phylogenetic variation in behavioral and brain asymmetries between species. That is to say, as brain size increased across primates, the two cerebral hemispheres become more disconnected which resulted in increasing specializations of functions within each half. Though the Rilling and Insel (1999) study focused on phylogenetic differences in brain size in relation to CC surface area, it is important to acknowledge that there are also individual differences in brain size within species. This has led some to postulate that there is a more basic law that governs the relationship between brain size and CC surface area;[19–21] that is, for both within and between species variation, larger brains are associated with a smaller CC surface area.

One aim of this study was to examine the role of genetic and nongenetic factors on individual variation in CC morphology in chimpanzees. In this study, the available pedigree of the chimpanzees was used to evaluate heritability in CC surface area and thickness. There are several recent studies demonstrating significantly heritability in CC size in human and nonhuman primates[22–26] and therefore it was hypothesized that chimpanzees would similarly show significant heritability in CC surface area and thickness. Additionally, heritability in the raw CC surface area and thickness measures (and adjusted for total forebrain volume [FBV]) were evaluated in this study. It has been proposed that CC surface area is inversely associated with brain size and, it has previously been reported that chimpanzee brain size is significantly heritable ($h^2 = 0.525$, $p < 0.001$).[27,28] Thus, these analyses tested whether heritability in the CC surface area and thickness was independent of or largely attributable to genetic influences on brain size. This was accomplished by (1) comparing the heritability estimates in surface area for both the raw and adjusted values and (2) performing a genetic correlation between FBV and total CC surface area and average thickness.

With respect to nongenetic factors influencing individual variation in CC morphology, this study also examined the contribution of early social rearing experiences. In rodents, there is an abundance of data demonstrating that early handling can influence surface area and thickness of the CC.[29] There is least one report that rhesus monkeys experiencing maternal neglect differed in CC surface area compared to non-neglected individuals.[30] Within the chimpanzee sample, there were individuals with a wide range of early rearing experiences that have previously been shown to influence some aspects of brain morphology including gray and white matter volume and gray matter thickness within the cortical sulci.[31,32] Therefore, early rearing experience (defined below) was included as an additional covariate in the heritability analyses.

In addition, phenotypic and genetic associations between CC surface area and thickness and motor performance on a tool use task were assessed in this study. Phenotypically, in humans, there is a plethora of studies on the association between handedness, CC surface area and connectivity, which have produced largely mixed results.[33–39] Previous studies in chimpanzees and other nonhuman primates have also reported some evidence that right and left-handed individuals differ in CC surface area.[40–43] Here we sought to test for phenotypic and genetic associations between CC surface area and thickness with performance asymmetries on a tool use task in the chimpanzees for three reasons. First, chimpanzees are well known for their diversity in tool manufacture and use in both captivity and the wild[44–46] and there some evidence that hand preferences and skill for different forms of tool use are strongly lateralized at the individual and, in some cases, population-level.[47–59] Thus, if there is an association between individual variation in lateralization and CC surface area and thickness, as has been previously hypothesized, then tool use tasks are ideal measures. Second, it has been reported that asymmetries in tool use performance are associated with white matter integrity, as measured by fractional anisotropic values obtained in DTI scans, within the CC of chimpanzees.[42] Third, in two previous studies in chimpanzees, performance on a simulated termite fishing task was found to be significantly heritable and reproducible in the same cohort of chimpanzees used in this study.[60,61] These findings allowed for testing both phenotypic and genetic correlations between tool use performance and variation in CC surface area and thickness. If increasing selection for manual specialization in tool use selected for increasing brain size and potential interhemispheric connectivity, then significant phenotypic and genetic associations should be evident in the chimpanzees.

Finally, because of the composition of our chimpanzee sample, there was a unique opportunity to examine the reproducibility of heritability in CC surface area and thickness and its phenotypic association with tool use. The issue of reproducibility and replication of scientific findings is a topic of increasing interest in the social, biological and medical fields.[62,63] Specifically, the chimpanzee sample in this study consisted of two cohorts of animals that were housed at separate facilities with distinct founder animals. No interbreeding took place between chimpanzees from these two populations of apes; thus, they were genetically isolated from each other and therefore this provided the opportunity to evaluate heritability in CC surface area and thickness for the entire sample and within each cohort. If CC surface

area and thickness are heritable and reproducible, then it was hypothesized that both cohorts would show similar patterns of heritability. Similarly, if phenotypic associations between tool use skill and CC morphology are reproducible, then it was hypothesized that similar patterns of findings would be evident between the two chimpanzee cohorts.

2 | METHOD

2.1 | Chimpanzee sample

The neuroimaging data was retrieved from the National Chimpanzee Brain Resource (NCBR, www.chimpanzeebrain.org) and included all *in vivo* magnetic resonance image (MRI) scans. The entire study sample consisted of 221 captive chimpanzees (*Pan troglodytes)* including 135 females, 86 males) covering an age range from 9 to 54 years (mean age ± SD: 26.6 ± 10.3). MRI scans and behavioral data were obtained from two distinct and genetically isolated cohorts of chimpanzees. One cohort consisted of 83 chimpanzees housed at the Yerkes National Primate Research Center (YNPRC) of Emory University while the second cohort consisted of 138 chimpanzees from the National Center for Chimpanzee Care (NCCC), which is part of the University of Texas MD Anderson Cancer Center. According to existing records, the NCCC and YNPRC chimpanzees had different founder animals and no interbreeding took place between the facilities, thereby making each population genetically isolated. A breakdown of the composition of sexes and rearing histories of the chimpanzee sample is shown in Table 1.

Within the entire sample, retrospective review of the animal care records indicated that there were 112 mother-reared (MR), 70 nursery-reared (NR) and 39 wild-born chimpanzees. With the exception of two individuals, NR chimpanzees were separated from their mothers within the first 30 days of life, due to unresponsive care, sickness or injury.[64,65] The NR chimpanzees were placed in incubators, fed standard human infant formula and cared for by humans until they could sufficiently care for themselves, at which time they were placed with other infants of the same age until they were 3 years of age.[64,65] At or about 3 years of age, NR chimpanzees were integrated into larger social groups of adult and sub-adult chimpanzees. MR chimpanzees were not separated from their mother for at least 2.5 years of life and were raised in nuclear family groups ranging from 4 to 20 individuals. Exceptions were two chimpanzees born at the YNPRC but subsequently transferred and raised in a rich social-linguistic environment by humans as part of an initiative to investigate the linguistic skills of apes at the Language Research Center of Georgia State University.[66-68] Wild-born (WB) chimpanzees were individuals who had been captured in the wild and subsequently brought to research facilities within the United States prior to 1974, when the importation of chimpanzees was banned. With respect to the measures of tool use skill, behavioral data were available in 177 chimpanzees including 108 females and 69 males. Based on the data reported in Hopkins, Mareno, and Schapiro,[60] 100 chimpanzees performed the task better with their right hand and 77 did better with their left.

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2.2 | MRI scanning procedure and image acquisition

Collection of the MRI scans was coordinated with each chimpanzees' annual physical examination to minimize stress and the number of anesthesia events. In brief, the chimpanzees were first sedated using either ketamine (10 mg/kg) or telazol (3–5 mg/kg) before being anesthetized with propofol (40–60 mg/[kg/h]). Then, the animals were transported to the MR imaging facility. After completion of the MRI acquisition, the animals were returned to their home facility and temporarily monitored in single housing to ensure a safe recovery from the anesthesia before returning to their social group. T1-weighted images of 144 chimpanzees (6 from YNPRC, 138 from NCCC) were acquired with a 1.5 T G.E. echo-speed Horizon LX MR scanner (GE Medical Systems). Data was collected in transverse plane using a gradient echo protocol (repetition time, TR = 19.0 ms; echo time, TE = 8.5 ms; number of signals averaged = 8; scan matrix: of 256×256) with a reconstructed image resolution of $0.7 \times 0.7 \times 1.2$ mm^3. The remaining 77 chimpanzees (all from YNPRC) were scanned on a 3.0-T Siemens Trio platform (Siemens

TABLE 1 Distribution of sexes and rearing histories of chimpanzees used in this study

	#F	#M	#MR	#NR	#WB	Age range	#Tool use data
YNPRC	55	28	26	49	8	11–53 years	69
NCCC	80	58	86	21	31	9–51 years	108
Total	135	86	112	70	39	9–53 years	177

Abbreviations: Age range, range in age of subjects in each cohort and for entire sample; #F, number of females; #M, number of males; #MR, number of mother-reared; #NR, number of nursery-reared; NCCC, National Center for Chimpanzee Care; #Tool Use Data, number of subjects for which tool use performance data were available for analysis; #WB, number of wild-caught; YNPRC, Yerkes National Primate Research Center.

Medical Solutions USA, Inc.). T1-weighted images were acquired using a 3D gradient echo sequence (pulse repetition ranged from 2300 to 2500 ms; echo time, TE = 3.0–4.4 ms; number of signals averaged = 1–3; scan matrix of 320 × 320 × 192 or 256 × 256 × 128) yielding a range of 0.6 × 0.6 × 0.6 mm^3 to 0.9 × 0.9 × 0.9 mm^3 voxel resolution). All methods of data collection were approved by the Institutional Animal Care and Use Committees at YNPRC and NCCC and followed the guidelines of the Institute of Medicine on the use of chimpanzees in research.

2.3 | CC segmentation and measurements

Midsagittal callosal surface area and thickness were determined based on the T1-weighted images in native space using methods that have been described in detail elsewhere.[69,70] First, to obtain a non-tilted midsagittal slice, individual images were co-registered to a template using rigid-body transformation (i.e., preserving size and shape of the CC) in SPM12 routines (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK) and resampled to a 0.5 × 0.5 × 0.5 mm^3 resolution. All resampled images were visually inspected to confirm a straight midsagittal plane as indicate by the longitudinal fissure forming a vertical line in coronal and axial views of the images.

The midsagittal slice was identified using the criterion of minimal appearance of cerebral gray/white matter (lowest intensity) from regions adjacent to the longitudinal fissure. The cross-section of the CC was then manually traced on the midsagittal slice using MRIcron software.[71] Slides adjacent to the midline were used to inform the segmentation in cases were a delineation of callosal voxels from the fornix was required or where high intensity blood vessels were located close to the CC. Next, the tip of the rostrum (defined as the inferior- or posterior-most voxel of the in-bend anterior callosal half) and the base of the splenium (ventral-most voxel in the posterior half) were identified on the callosal mask. Then, the mask was rotated so that an imagined line connecting rostrum tip and splenium base were horizontally orientated. The size of the callosal mask was determined as measure of the midsagittal surface area for each individual. As reported previously, these manual segmentation steps yield interrater reliability estimates of r_{ICC} = 0.86 and 0.96 (intra-class correlations calculated as two-way random effects, considering absolute agreement for a single measure and) for midsagittal surface area.[72] To obtain subregions, the midsagittal callosal surface was subdivided using the schema suggested by Jancke, Staiger, Schlaug, Huang, Steinmetz.[73] That is, relative to the rostro-dorsal extend of the CC, the anterior third defined the "genu" and the middle third the "truncus" subregion. The posterior third was additionally subdivided into splenium (i.e., the posterior fifth) and the isthmus (i.e., the remaining area of the posterior third after subtracting the splenium).

To determine regional thickness, the outline of callosal mask was automatically created and divided into a ventral and dorsal outline using the tip of the rostrum and the base of the splenium as dividing point. A midline between ventral and dorsal outline was determined as a reference line for the thickness measurements. That is, 100 support points spaced equidistantly on the two outlines were created and the midline coordinates were calculated as the average coordinate of two corresponding support points. The resulting midline was resampled into 60 equidistant sampling points, which marked the location of the thickness measurement. Callosal thickness was defined as the distance between the ventral and dorsal outline orthogonal to the midline at these points. The number of 60 measurement points was chosen as it provides a sufficiently high density of sampling points to capture the structure of CC while not inflating the number of statistical tests excessively.[72,74]

2.4 | Brain-size extraction

FBV was selected to account for brain size differences. FBV was preferred over measures of total intracranial volume as the CC is formed from axons originating from the two cerebral hemispheres,[75] and brain structures irrelevant for the CC (e.g., brain stem, cerebellum) are excluded. For this purpose, a custom mask was created covering the supra-tentorial brain in standard space defined by the chimpanzee template. FBV was then determined for each data set in three steps. First, using SPM12 brain segmentation routines gray- and white-matter maps were created in native space.[76] Then, the standard FBV mask was transferred to the individual brain by using the same transformation parameters used when creating the tissue segmentations in native space. Finally, FBV was determined as the sum of gray- and white-matter probabilities within the mask in native space. Thus, the resulting FBV estimate did not include cerebral spinal fluid compartments.

2.5 | Tool use hand preference and performance asymmetries

Performance asymmetry data from the previous studies by Hopkins, Mareno, and Schapiro[60] were used in this study. To measure performance asymmetries, the average latency to insert a small stick into a hole to extract food was recorded for 30 responses each with the left and right hand. Overall performance in tool use skill was computed by averaging the data between the left and right hands. Asymmetries in tool use motor skill were computed by subtracting the average left hand latency from average right hand value. Based on these data, Hopkins, Mareno, and Schapiro[60] reported that average performance was significantly heritable (h^2 = 0.544, p = 0.0001) as were the individual values for the left (h^2 = 0.344, p = 0.011) and right (h^2 = 0.625, p = 0.000009) hands; however, asymmetries in hand skill were not significantly heritable (h^2 = 0.172, p = 0.071). The patterns of heritability were further evident within both the NCCC and YNPRC chimpanzees.

2.6 | Data analysis

In addition to the raw surface area and thickness measures, the CC surface area measures were adjusted for FBV. As suggested by Smith,[77] we converted FBV (volume) before the division so that it had the same

unit of geometric dimensional unit with respect to the callosal area and thickness measures. Thus, we raised FBV to the power of 2/3 (i.e., $FBV^{0.666}$) to calculate the ratio with area and to the power of 1/3 (i.e., $FBV^{0.333}$) to calculate the ratio with thickness. The resulting ratios are hereafter referred to as relative callosal area and relative callosal thickness. As in with previous studies, heritability and genetic correlations for overall and region-specific CC regions were performed using SOLAR.[78] SOLAR uses a variance component approach to estimate the polygenic component of variance when considering the sire and dam information for focal subjects from an entire pedigree of animals see.[24,79–81] Total additive genetic variance (h^2) is the amount of total phenotypic variance that is attributable to all genetic sources. Total phenotypic variance attributable to genetic and nongenetic variables is constrained to a value of 1; therefore, all nongenetic contributions to the phenotype are equal to $1–h^2$. We used SOLAR to determine heritability in the raw and adjusted CC surface area and thickness measures. The overall and region-specific raw and adjusted CC surface area measures were the outcome measures while scanner magnet, sex, rearing and age were covariates. After completing the heritability analyses, to evaluate the effect of sex and rearing, and their potential interaction on overall and region-specific CC morphology, we performed mixed-model analyses of covariance. In the analyses on CC surface area and thickness, region was the repeated measure while sex (F, M) and rearing (WB, MR, NR) were the between group factors. Scanner magnet, age and relatedness coefficients for each individual served as covariates.

3 | RESULTS

3.1 | Overall and repeatability heritability analyses in CC morphology

3.1.1 | Surface area

We initially examined heritability in the raw and adjusted surface area measures of the total and each CC region for the entire sample. For these analyses, sex, age, rearing history and scanner magnet were covariates and the results are shown in Table 2. Significant heritability was found for the total CC and each region and these findings were evident for both the raw and adjusted measures. In terms of the covariates, for both the raw and adjusted CC measures, age was found to consistently account for a small but significant portion of the variance. In contrast, scanner magnet and sex accounted for a significant proportion of variance for one region within the raw data and two regions for the adjusted CC values. Rearing history failed to account for individual variability in either the raw or adjusted CC values. Because the heritability results for the raw and adjusted CC values were largely comparable, we only considered the adjusted data in all subsequent analyses involving the CC area measures and removed rearing history a covariate.

We next evaluated the consistency in heritability of the CC surface area measures by performing separate quantitative genetic analyses within the NCCC and YNPRC samples and these results are

TABLE 2 Heritability in raw and adjusted CC surface area measures

Region	h^2	SE	p	Covariates	Variance
Raw					
Genu	0.684	0.141	0.000001	Age	0.071
Truncus	0.369	0.151	0.003	Age, scan	0.106
Post3rd	0.534	0.150	0.00006	Age	0.019
Isthmus	0.576	0.181	0.004	Sex	0.003
Splenium	0.524	0.140	0.00003	Age	0.023
Total	0.579	0.140	0.000004	Age	0.051
Adjusted					
Genu	0.638	0.152	0.00001	Age	0.101
Truncus	0.443	0.160	0.002	Age, sex	0.099
Post3rd	0.681	0.145	0.000002	Age, scan	0.056
Isthmus	0.593	0.176	0.0003	None	
Splenium	0.667	0.135	0.0000007	Age, scan	0.068
Total	0.638	0.151	0.00001	Age	0.079

shown in Table 3. Within the NCCC population, significant heritability was found for all five CC regions and the total area. For the YNPRC sample, the overall CC surface area was significantly heritable as were the genu, post3rd and splenium. The surface area of truncus and isthmus within the YNPRC were not significantly heritable, though the values approached conventional levels of statistical significance. Within the NCCC sample, age was a significant covariate for all CC regions whereas this was only the case for the genu within the YNPRC cohort. Further, sex was a significant covariate for the truncus within the YNPRC sample but did not account for a significant proportion of variance for any of the CC regions within the NCCC cohort.

3.1.2 | CC thickness

Rather than estimate heritability for all 60 CC thickness measures, we initially performed a principal component analysis (PCA) with varimax rotation to reduce the number of CC measures into factors reflecting regions with high covariation. The PCA on the 60 CC thickness measures yielded nine factors that had eigenvalues >1.0. Shown in Table 4 are the individual CC thickness measures that loaded on each factor with item-component correlation coefficients of 0.60 or greater. Figure 1 depicts the regions within the CC that corresponded to each factor component. Note that factor 9 was omitted from Figure 1 and all subsequent analyses because none of the item loadings on this factor exceeded a value of 0.60, which was the cut-point we adopted for determining those CC regions contributing to a factor.

We next calculated the heritability in thickness corresponding to the CC regions that loaded on each factor. To do this, we took the average of the CC thickness measures for the individual CC regions that loaded on each factor and these values were subjected to quantitative genetic analyses using SOLAR. We excluded factor 9 because

none of the 60 individual CC thickness measures loaded at greater than 0.60 and the variance accounted for by this factor was very low. We also computed a mean overall CC thickness measure by averaging the values from all 60 regions. The heritability results are also shown in Table 4. Significant heritability was found for factors 1–6 while components 7 and 8 were not. As with the CC surface area measure, we also performed separate heritability analyses within the NCCC and YNPRC chimpanzee populations and these findings are shown in Table 5. Within the NCCC population, significant heritability was found for factor components 1–6. By contrast, significantly heritability was found for components 1, 2, 4, 6 and 8 within the YNPRC population. Both the NCCC and YNPRC chimpanzee populations showed significant heritability in CC thickness when averaged across the 60 measures.

TABLE 3   Heritability in adjusted CC surface area measures within two genetically isolated chimpanzee populations

Region	h^2	SE	p	Covariates	Variance
NCCC ($N = 138$)					
Genu	0.716	0.167	0.00002	Age	0.136
Truncus	0.448	0.199	0.008	Age	0.059
Post3rd	0.706	0.157	0.00005	Age	0.056
Isthmus	0.770	0.190	0.0002	Age	0.012
Splenium	0.658	0.151	0.00004	Age	0.061
Total	0.729	0.149	0.000006	Age	0.117
YNPRC ($N = 83$)					
Genu	0.503	0.274	0.030	None	
Truncus	0.344	0.301	0.112	Sex	0.082
Post3rd	0.653	0.294	0.004	None	
Isthmus	0.327	0.298	0.105	None	
Splenium	0.713	0.286	0.001	None	
Total	0.557	0.279	0.012	None	

Abbreviations: NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center.

3.2 | Rearing and sex effects on adjusted CC surface area and thickness

To examine the influence of rearing and sex on CC adjusted surface area and thickness, analysis of covariance was performed on each CC outcome measure. CC region was the repeated measure whiles sex and rearing were the between group factors. Age, scanner magnet and relatedness coefficients were covariates. There were no significant main effects for sex or rearing nor a significant interaction between these two variables for either CC adjusted surface area or thickness.

3.3 | Phenotypic and genetic associations between FBV and CC surface area and thickness

Phenotypic and genetic associations between FBV and the raw measures for both total CC surface area and mean thickness were tested using SOLAR. Phenotypically, FBV was significantly and positively associated with total CC area ($r = 0.474$, df $= 220$, $p < 0.001$) and mean thickness ($r = 0.305$ df $= 220$, $p < 0.001$). Moreover, FBV was found to be significantly heritable ($h^2 = 0.531$, SE $= 0.149$, $p < 0.0003$). However, there was no significant genetic association between FBV and either total CC surface area (rhoG $= 0.263$, SE $= 0.213$, $p = 0.373$) or mean thickness (rhoG $= 0.393$, SE $= 0.236$, $p = 0.129$). Thus, larger FBVs were phenotypically but not genetically associated with variation in overall CC surface area or mean thickness.

3.4 | Phenotypic and genetic associations between average tool use skill, CC surface area and thickness

For these analyses, to determine phenotypic associations, partial correlation coefficients were performed between tool use performance (averaged between the left and right hands) and each of the CC regions while controlling for sex, age, relatedness and scanner

Factor	CC regions	Variance	h^2	SE	p	Covariate	Variance
1	14–27	24.26	0.414	0.164	0.003	None	
2	29–39	8.43	0.394	0.167	0.006	Sex	0.069
3	40–49	6.14	0.669	0.161	0.00001	None	
4	50–57	3.92	0.618	0.146	0.000004	None	
5	7–13	3.33	0.701	0.127	0.0000001	Age	0.040
6	1–4	2.30	0.000	–	0.500	Scanner	0.043
7	58–60	2.01	0.283	0.139	0.009	Scanner	0.074
8	5–6	1.56	0.037	0.183	0.417	Scanner	0.078
9	None >0.60	1.18					
Mean (1–60)			0.497	0.156	0.0002	Sex, Age	0.053

TABLE 4   Factor analysis and heritability of adjusted CC thickness scores

Abbreviation: CC, corpus callosum.

magnet. For the entire sample, significant negative associations were found between tool use latencies scores, the genu, and truncus (see Table 6) and FAC2 for the thickness measures while no significant associations were found for either the mean or any region-specific CC thickness measures. Within the NCCC cohort, no significant associations were found between tool use performance and any of the CC surface area or thickness measures. Within the YNPRC cohort, significant negative associations were found between tool use latencies scores and the truncus surface area and FAC2 for the thickness measures (see Table 6). In short, associations between overall tool use skill and some of the CC measures were relatively weak and they were not reproducible between the NCCC and YNPRC cohorts.
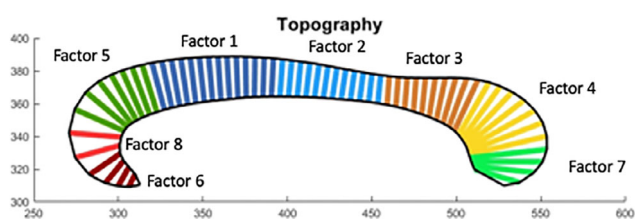

FIGURE 1 Corpus callosum image with the labeled regions in thickness showed by the PCA factor analysis of the 60 measures. PCA, principal component analysis

3.5 | Phenotypic associations between asymmetries in tool use skill, CC surface area and thickness

3.5.1 | Surface area

We initially performed a repeated measure analysis of co-variance (ANCOVA) with CC region as the repeated measure while sex (M, F), tool use performance asymmetry (right, left) and colony (NCCC, YNPRC) were the between group factors. Covariates included relatedness and age. We found a significant main effect for tool use performance asymmetry $F(1, 166) = 6.432$, $p = 0.015$ and a significant two-way interactions between tool use performance asymmetry and region $F(4, 664) = 3.023$, $p = 0.017$. The mean CC surface area measures as a function tool use performance asymmetry within each chimpanzee cohort are shown in Figure 2. Post-hoc analysis indicated that chimpanzees who performed better with their right-hand had larger CC areas than individuals who performed better with their left hand for all CC regions with the exception of the truncus. Further, as can be seen in Figure 2, this pattern was consistent between the two chimpanzee cohorts.

3.5.2 | Thickness

As with the surface area measures, we initially performed a repeated measure analysis of co-variance (ANCOVA) with CC region (i.e., the

TABLE 5 Heritability in adjusted CC thickness measures within two genetically isolated chimpanzee populations

	h^2	SE	p	Covariates	Variance
NCCC (N = 138)					
Factor 1	0.365	0.191	0.016	Age	0.040
Factor 2	0.556	0.234	0.009	None	
Factor 3	0.825	0.167	0.00005	Age	0.017
Factor 4	0.592	0.174	0.0002	None	
Factor 5	0.704	0.176	0.0001	Age	0.064
Factor 6	0.000	–	0.500	None	
Factor 7	0.264	0.167	0.037	None	
Factor 8	0.000	–	0.500	None	
Mean	0.507	0.199	0.003	Age	0.057
YNPRC (N = 83)					
Factor 1	0.458	0.287	0.050	None	
Factor 2	0.231	0.234	0.144	Sex	0.073
Factor 3	0.518	0.258	0.009	None	
Factor 4	0.515	0.267	0.018	None	
Factor 5	0.694	0.180	0.00007	None	
Factor 6	0.217	0.266	0.182	Sex	0.061
Factor 7	0.328	0.242	0.051	None	
Factor 8	0.425	0.280	0.058	Sex	0.098
Mean	0.453	0.275	0.043	None	

Abbreviations: NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center.

eight subregions determined by PCA) as the repeated measure while sex, tool use performance asymmetry and colony were the between group factors. Covariates included relatedness and age. This analysis showed a significant main effect for hand skill $F_{(1, 166)} = 6.758$, $p = 0.023$, a three-way interaction between sex, colony and CC region $F_{(7, 1162)} = 4.276$, $p = 0.001$. As with the surface area measures, chimpanzees that perform better with their right compared with left hand have a thicker CC and this pattern was consistent between the NCCC and YNPRC cohorts (see Figure 3). Regarding the colony, sex by CC region interaction, post-hoc analysis indicated that, within the NCCC, males (mean = 6.098, SE = 0.422) had a thicker CC for regions within factor 8 compared to females (mean = 5.745, SE = 0.422). In contrast, within the YNPRC cohort, females (mean = 4.655, SE = 0.653) had a thicker CC for regions within factor 8 compared to males (mean = 3.940, SE = 0.693). No other significant differences in thickness were found between males and females within each colony for the remaining 7 CC factors (see Figure 4A,B).

3.6 | Genetic correlations between tool use skill and CC surface area and thickness

Finally, recall from Table 6 that small but significant phenotypic associations were found between average performance on the tool use task and the CC surface area and thickness measures. Shown in Table 7 are the genetic and environmental correlations between

TABLE 6 Phenotypic associations between tool use skill, CC surface area and thickness for the overall sample, NCCC and YNPRC cohorts

	Mean	NCCC	YNPRC
Surface area			
Genu	**−0.149**	−0.106	−0.180
Truncus	**−0.167**	−0.042	**−0.269**
Post3rd	−0.130	−0.055	−0.210
Isthmus	−0.126	−0.072	−0.205
Splenium	−0.116	−0.040	−0.192
Total	**−0.168**	−0.084	−0.234
Thickness			
FAC1	−0.106	−0.041	−0.160
FAC2	−0.139	−0.011	**−0.258**
FAC3	−0.125	−0.041	−0.220
FAC4	−0.083	+0.007	−0.174
FAC5	−0.060	+0.005	−0.120
FAC6	+0.038	+0.110	+0.001
FAC7	−0.082	−0.107	−0.092
FAC8	−0.057	+0.085	−0.104
Mean	−0.101	+0.016	−0.179

Note: Bolded values are significant.
Abbreviations: NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center.

average performance scores on the tool use task and the CC surface area and thickness measures. As can be seen, significant negative genetic associations were found between tool use performance and surface area of the genu and truncus. Significant negative genetic and significant positive environmental associations were found between tool use performance and the thickness measures within FAC1. Because asymmetries in tool use skill were not significantly heritable,[60] it was not possible to test for genetic associations with any of the CC surface area and thickness measures.

4 | DISCUSSION

Several findings were showed in this study. First, chimpanzee CC morphology is significantly heritable and this is not accounted for by overall FBV. Second, CC morphology, particularly in the genu and truncus, is phenotypically and genetically associated with tool use skill. Moreover, chimpanzees that perform a tool use task better with their right compared with left hand have larger and thicker CC. Third, the heritability in CC morphology and the phenotypic relationship between CC morphology and asymmetries in tool use skill are reproducible between two genetically isolated populations of captive chimpanzees.

Consistent with previous reports in humans and other nonhuman primate species, chimpanzees showed modest heritability in CC morphology and these results were evident for the raw measures for each region as well as for the values after adjustment for total FBV. Further, for both overall CC morphology and for some regions, heritability was consistent between two genetically isolated populations of apes. This shows that the results are largely reproducible despite the fact that the two chimpanzee cohorts differed in sample size and were scanned on machines with different magnet strengths. Regarding the contribution of subject factors, like in other nonhuman primates,[70,82,83] age appears to be a significant covariate and this is not surprising as previous studies in this same chimpanzee sample using this same method of quantifying CC morphology reported associations with age.[69]

Sex also accounted for a small portion of variance in raw (isthmus) and adjusted (truncus) CC morphology with females having higher values than males (see Figure 4). In humans, like in nonhuman primates, evidence of sex differences in CC morphology are somewhat inconsistent and the findings appear to be influenced subject age, brain size and how differences in overall brain and body size are adjusted for in these analyses.[34,84–89] Nonetheless, the small but significant evidence for increased size of the truncus in females is consistent with at least some previous findings in human and nonhuman primates.[43,90]

Early social rearing experiences of the chimpanzees failed to account for a significant amount of variability in measures of CC surface area and thickness. This finding is somewhat at odds with previous research on the influence of early adversity on brain morphology in rhesus monkeys[30,91] and developing children.[92] In humans, differences in CC surface area have been reported primarily in the posterior CC regions. In rhesus monkeys, Sanchez et[30] reported that NR infants
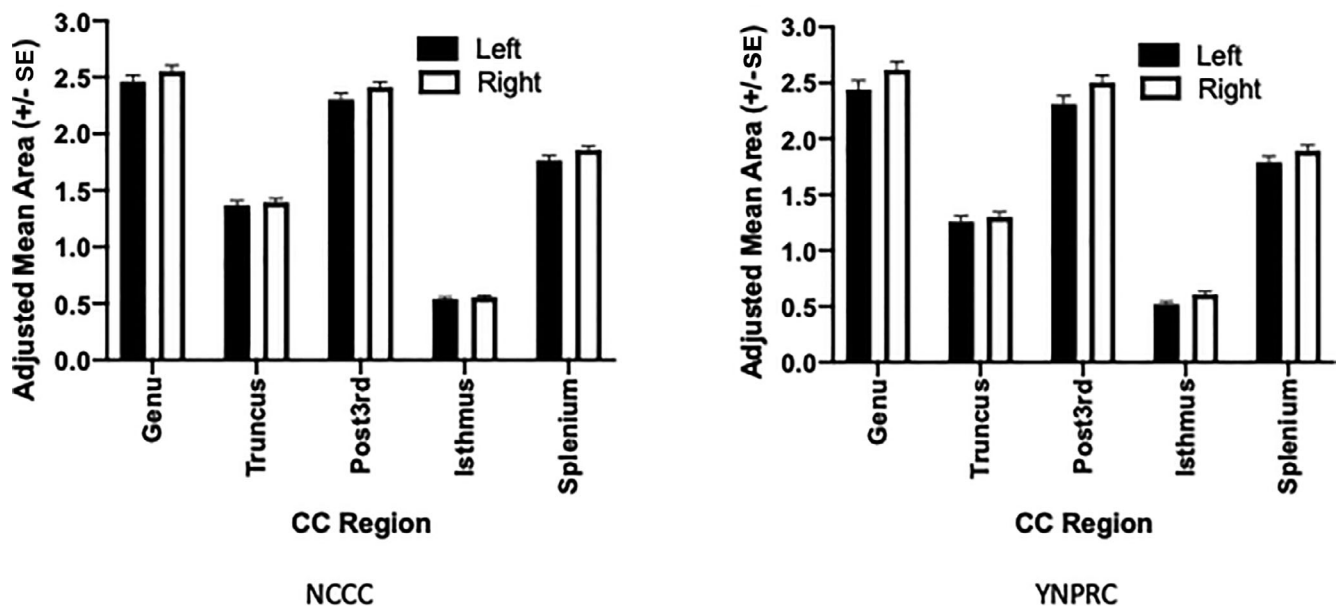
FIGURE 2 Mean (+/− SE) adjusted region-specific CC surface area for chimpanzees from the NCCC and YNPRC that performed the tool use task better with their left or right hand. CC, corpus callosum; NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center
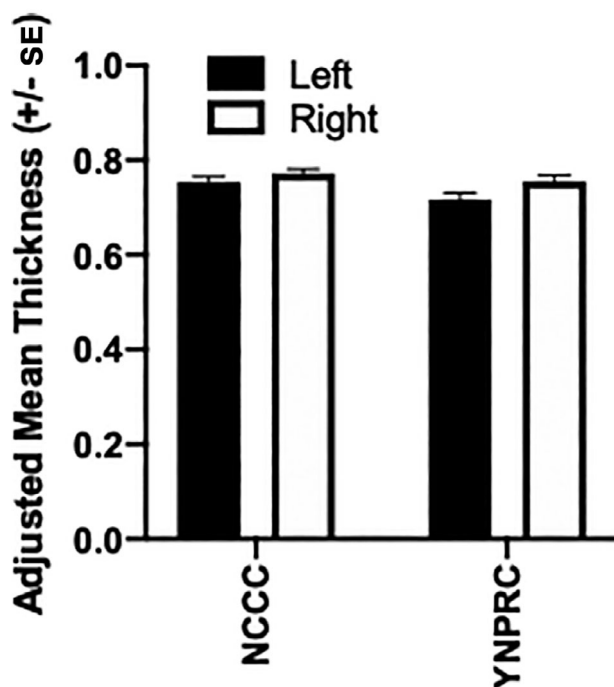

FIGURE 3 Mean (+/− SE) adjusted mean CC thickness measure for chimpanzees from the NCCC and YNPRC that performed the tool use task better with their left or right hand. CC, corpus callosum; NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center

reduced CC surface area compared to MR individuals, a finding that was not replicated by Spinelli et al.[91] in a slightly larger and older sample of monkeys. These discrepancies might be attributable to subtle

differences between the NR and MR cohorts in these two papers. The NR cohort within the Sanchez et al.[30] paper was not separated from their biological mothers until 7 weeks of age whereas the NR cohort in the Spinelli et al.[91] report were separated at birth. Recall that the findings (or lack thereof) reported here are more similar to the results from the Spinelli et al.[86] compared with Sanchez et al.[30] papers and the NR cohort of chimpanzees was similarly separated at birth. More broadly, the role that rearing potentially plays on CC morphology in chimpanzees, at least within this sample, is further complicated by the fact that rearing is confounded with age. As noted above, WB chimpanzees were individuals who were captured from the wild and brought to captive settings prior to 1974 when the importation of chimpanzees was banned. Thus, WB chimpanzees were predominantly the oldest chimpanzees in the two cohorts. Therefore, it is difficult to isolate the influence of age, independent of rearing history, within the WB chimpanzees.

Small but significant differences in CC surface area and thickness were also found between chimpanzees that performed a tool use task better with their right compared with left hand. Moreover, like the heritability results, these findings were consistent between the two chimpanzee cohorts (see Figures 2 and 3). However, in humans, previous reports on CC morphology and asymmetries in hand skill have failed to find significant results.[88,93] The difference between species may be attributed to the larger sample size in this study compared with the previous studies in humans (N = 104 and 120, respectively), particularly given the small effect size. It is also possible that task used to measure asymmetries in skill may account for the differences. The studies in humans used simple paper-and-pencil tasks such as dot filling or line tracing. Overall performance was also negatively associated with CC surface area within the genu and truncus and for FAC1 for
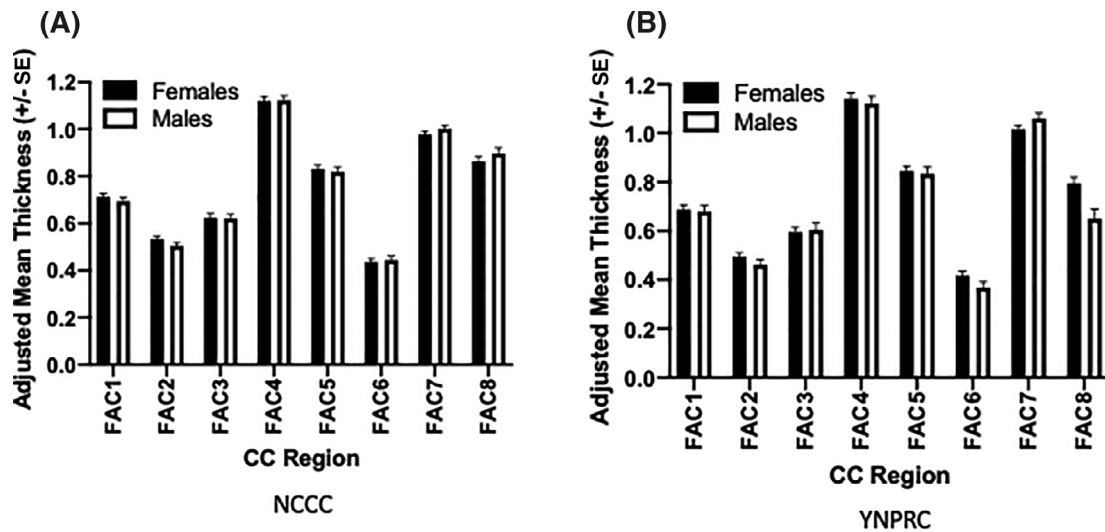
FIGURE 4 Mean ($+/-$ SE) adjusted region-specific thickness scores for males and females within the NCCC and YNPRC chimpanzee cohorts. CC, corpus callosum; NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center

TABLE 7 Genetic and environmental correlations between average adjusted tool use skill, CC surface area and thickness

	rhoG	SE	*p*	rhoE	SE	*p*
Surface area						
Genu	−0.441	0.281	0.093	+0.548	0.501	0.254
Truncus	−**0.773**	0.488	0.044	+0.500	0.536	0.247
Post3rd	+0.029	0.211	0.887	−0.829	1.372	0.334
Isthmus	−0.213	0.315	0.503	−0.007	0.545	0.989
Splenium	+0.065	0.161	0.681	−0.104	0.192	0.593
Total	−0.338	0.255	0.182	+0.271	0.559	0.619
Thickness						
FAC1	−**0.778**	0.348	0.019	+**0.718**	0.484	0.030
FAC2	−0.146	0.224	0.527	−0.019	0.478	0.967
FAC3	−0.109	0.256	0.668	−0.085	0.472	0.855
FAC4	−0.149	0.247	0.548	+0.044	0.506	0.930
FAC5	−0.301	0.257	0.206	+0.587	0.372	0.136
FAC6	–	–	–	–	–	–
FAC7	+0.008	0.282	0.976	−0.253	0.331	0.464
FAC8	+0.397	0.329	0.200	−0.544	0.341	0.087
Mean	−0.571	0.324	0.069	+0.686	0.588	0.131

Note: Bolded values are significant.

the thickness measure, a region largely corresponding to the truncus. Thus, chimpanzees that had slower latencies on the tool use task had smaller CC surface areas and thickness. Average tool use skill was also genetically associated with truncus and FAC1 and borderline significant with the genu. This suggests that common genes appear to underlie individual variation in tool use skill and interhemispheric connectivity within the genu and truncus regions of the CC. Based on tractography results in chimpanzees, the genu and truncus connect premotor regions between the left and right hemisphere.[11,42] Because of the high-motor planning demands of the tool use task used in this

study, the association found between performance and the genu and truncus makes sense and suggest that fibers connecting these regions possibly facilitate interhemispheric communication of motor information between the left and right hemisphere premotor and primary motor cortex.

In the YNPRC chimpanzees, it has previously been reported that right-handed and ambiguous-handed chimpanzees have larger CC surface areas compared to left-handed apes for a task requiring bimanual coordination called the TUBE task.[40,41] Though not the main focus of this study, shown in Figure 5A,B are the mean CC surface area and
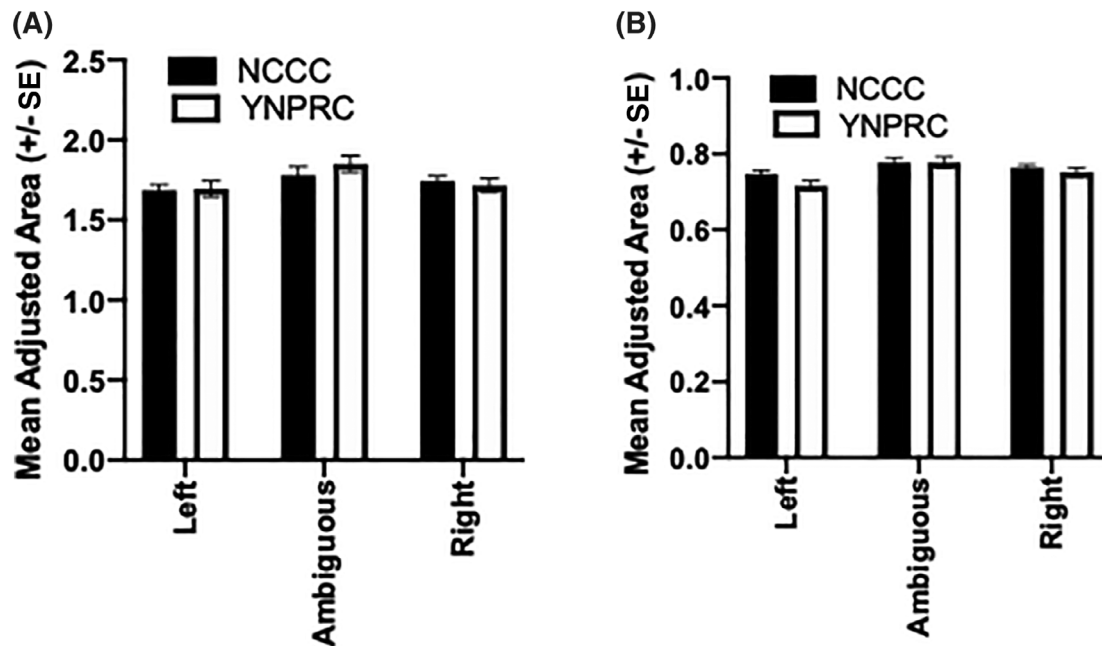
FIGURE 5 (A) Mean ($+/-$ SE) adjusted CC surface area and (B) thickness scores (B) for right-, ambiguous- and left-handed chimpanzees based on the TUBE task in the NCCC (black square) and YNPRC (white square) cohorts. CC, corpus callosum; NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center

thickness measures for YNPRC and the NCCC chimpanzees used in this study for the TUBE task. As can be seen, as with the tool use task used in this study, the NCCC and YNPRC showed consistent results with right- and ambiguously handed chimpanzees showing larger CC morphology measures than left-handed apes. Thus, the reproducibility in phenotypic association between tool use skill asymmetries and CC morphology are not limited to this measure of performance asymmetry but also includes at least one other measure of hand preference in this population of chimpanzees.

The observed association between tool use skill and CC morphology also appears to specific to this measure in performance asymmetries. Within a subset of the chimpanzee sample in this study ($n = 194$), we have previously reported data on left–right differences in errors made when grasping small food items.[94–96] As a follow up analysis, we compared the adjusted CC surface area and thickness between chimpanzees that made fewer errors with their left hand ($n = 72$), right hand ($n = 89$) or made an equal number of errors between the right and left hand ($n = 33$). We found no significant main effects or interactions for either the adjusted CC areas or thickness measures (see Figure 6A,B). Further, in terms of overall grasping skill, there was no significant association between performance based on the proportion of errors made on 40 trials (20 trials each for the left and right hands) and any of the CC morphology measures.

Though measures of CC morphology and FBV were both significantly heritable and positively correlated with each other, there was no significant genetic association between these two dimensions of brain organization. Some have hypothesized that there is a fundamental inverse relationship between brain volume and CC surface area.[19,20] If this were the case, then arguably one might expect to find not only a significant phenotypic but also a genetic relationship. This finding suggests that selection pressure for interhemispheric connectivity in primate brain evolution may have occurred in part, or entirely, independent of those linked to increasing brain size. One selection pressure might have been for increasing specialization in function between the left and right hemispheres. Evolutionary models of hemispheric specialization among primates are often framed within the context of selection for increasing brain size.[97] For example, it has been suggested that as brain size increases across primates, there is concomitant selection for increasing specialization in function between the two halves of the brain, thereby potentially doubling its information processing capabilities.[98] One possibility is that differential specializations of the left and right hemispheres were evident far earlier in the primate tree and long before the split between the common ancestor of humans and chimpanzees. Evolutionary increases in brain size were accompanied by increasing numbers and complexity in motor and cognitive functions.[99] In support of this argument is the evidence of functional and behavioral asymmetries, such as handedness, in a variety of nonhuman primate species from different taxa.[100–105] Further, the degree to which different functions between the two hemispheres became increasingly consistent across most individuals within a species may have also been an elaboration in hemispheric specialization associated with increasing brain size.

In summary, this is the first report of finding on heritability in CC morphology in chimpanzees and its phenotypic and genetic association with tool use skill. Future studies should focus on identifying those shared genes that implicated in the expression of CC morphology and tool use skill. Additional further studies should attempt to tease apart the interrelationship between CC morphology and the
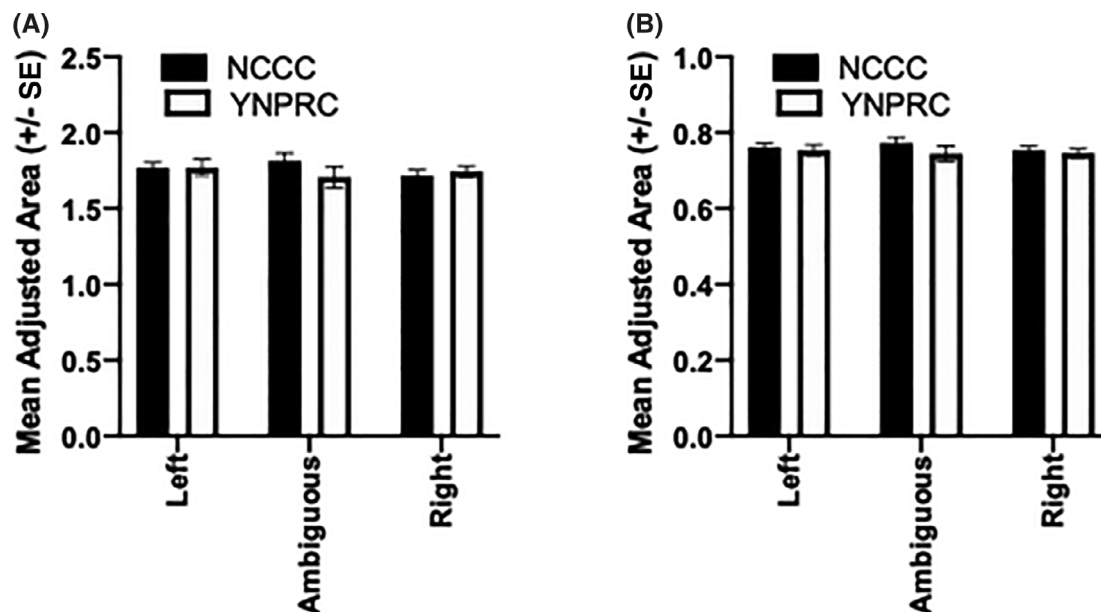
FIGURE 6 (A) Mean ($+/-$ SE) adjusted CC surface area and (B) thickness scores for right-, ambiguous- and left-handed chimpanzees based on performance on grasping errors in the NCCC (black square) and YNPRC (white square) cohorts. CC, corpus callosum; NCCC, National Center for Chimpanzee Care; YNPRC, Yerkes National Primate Research Center

evolution of hemispheric specialization as it relates to individual and phylogenetic variation in hemispheric specialization in primates, including humans.

DATA AVAILABILITY STATEMENT

The data reported in this manuscript are available from the corresponding author upon request. Alternatively, the data can be found at the National Chimpanzee Brain Resource (www.chimpanzeebrain.org).

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