

## ORIGINAL ARTICLE

# Heritability of Gray Matter Structural Covariation and Tool Use Skills in Chimpanzees (*Pan troglodytes*): A Source-Based Morphometry and Quantitative Genetic Analysis

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## Abstract

Nonhuman primates, and great apes in particular, possess a variety of cognitive abilities thought to underlie human brain and cognitive evolution, most notably, the manufacture and use of tools. In a relatively large sample ( $N = 226$ ) of captive chimpanzees (*Pan troglodytes*) for whom pedigrees are well known, the overarching aim of the current study was to investigate the source of heritable variation in brain structure underlying tool use skills. Specifically, using source-based morphometry (SBM), a multivariate analysis of naturally occurring patterns of covariation in gray matter across the brain, we investigated (1) the genetic contributions to variation in SBM components, (2) sex and age effects for each component, and (3) phenotypic and genetic associations between SBM components and tool use skill. Results revealed important sex- and age-related differences across largely heritable SBM components and associations between structural covariation and tool use skill. Further, shared genetic mechanisms appear to account for a heritable link between variation in both the capacity to use tools and variation in morphology of the superior limb of the superior temporal sulcus and adjacent parietal cortex. Findings represent the first evidence of heritability of structural covariation in gray matter among nonhuman primates.

**Key words:** chimpanzee, gray matter covariation, heritability, source-based morphometry, tool use

Primates, in general, and great apes specifically have been particularly important species in comparative neuroscience studies because of their phylogenetic similarity to humans. Furthermore, compared with more distantly related primate

species, great apes display a variety of behavioral and cognitive abilities that are thought to underlie human brain and cognitive evolution, such as rudimentary linguistic skills, delay of gratification, complex social cognition, and with specific

reference to this study, the manufacture and use of tools (Savage-Rumbaugh 1986; Savage-Rumbaugh and Lewin 1994; de Waal 1996; Shumaker et al. 2011; Vaesen 2012; Beran 2015). Indeed, save humans, the complexity and scope of tool manufacture and use in chimpanzees are unmatched among primates. For instance, a variety of forms of tool manufacture and use have been described across different geographical regions of Africa as well as in different captive settings (Whiten et al. 1999, 2001; Shumaker et al. 2011). Within communities of wild chimpanzees, there is evidence of intergenerational transmission of local forms of tool use expression suggesting that social learning plays an important role in the acquisition and maintenance of these specific traditions. Thus, the manufacture and use of tools in chimpanzees is highly adaptive skill and was likely strongly selected for in human evolution after the split from the last common ancestor with chimpanzees.

Despite the significance of tool manufacture and use in primate evolution, there are relatively few studies on their genetic and neural basis in nonhuman primates, and particularly chimpanzees. In humans, meta-analyses of functional brain imaging data have identified a set of connected regions within the frontal, parietal, and temporal cortices, particularly in the left hemisphere, that are implicated in planned tool use actions (Johnson-Frey 2004; Frey et al. 2005). There is also evidence that lesions to regions within this circuit can result in deficits in the representation and execution of planned motor actions, including language and speech (Goldenberg and Randerath 2015; Weiss et al. 2015). Studies in captive chimpanzees have previously found that variation in skill and hand use are linked to variation in gray matter volume and asymmetry, particularly within premotor, parietal, and primary motor cortices, as well as the cerebellum (Hopkins et al. 2007, 2017; Cantalupo et al. 2008; Gilissen and Hopkins 2013).

Here, instead of using an a priori region-of-interest approach and method, we assessed phenotypic associations in tool use skill with structural covariation in gray matter measured from magnetic resonance images (MRIs). Specifically, we used source-based morphometry (SBM), a relatively new method used to characterize gray matter structural covariation in a sample of MRI scans of chimpanzees (Alexander-Bloch et al. 2013; Bard and Hopkins 2018). Unlike univariate analytic methods, such as voxel-based morphometry (VBM), SBM is a multivariate, data-driven analytic approach that utilizes information about relationships among voxels to group voxels carrying similar information across the brain. Without requiring prior determination of regions of interest, the resulting components or sources are identified based on the spatial information between voxels grouped in a natural manner and represent similar covariation networks between subjects; thus, this approach has been described as a multivariate version of VBM (Xu et al. 2009). Previous studies in humans have identified roughly 30 distinct gray matter sources that encompass a variety of different cortical regions that are presumably involved in different behavioral and cognitive functions and may be disrupted in certain clinical populations (Xu et al. 2009; Kasperek et al. 2010; Caprihan et al. 2011; Rektorova et al. 2014; Grecucci et al. 2016).

Based on the components derived from the SBM analysis, we subsequently correlated individual variation in the weighted scores for each subject and component with a measure of tool use skill previously measured in the chimpanzees (Hopkins et al. 2009). Of specific interest was whether performance measures of tool use skill were associated with source-based component scores that reflected structural

covariation in gray matter in regions within the frontal, parietal, and temporal cortices.

In addition, we also tested for genetic associations between individual differences in gray matter structural covariation and tool use skill in the chimpanzee sample using quantitative genetic analyses. Notably, following methods we and others have previously used in humans (Eylar et al. 2012; Jansen et al. 2015; Strike et al. 2015), chimpanzees, and other nonhuman primates (Rogers et al. 2007; Fears et al. 2009; Kochunov et al. 2010; Fears et al. 2011; Gomez-Robles et al. 2015; Gomez-Robles et al. 2016), we initially estimated heritability for (1) each component derived from the SBM analysis and (2) tool use performance measures (Hopkins et al. 2015). For those SBM components that showed significantly heritability and phenotypically correlated with tool use performance, we then performed genetic correlations to test whether common genes underlie their expression (i.e., pleiotropy). Evidence of significant genetic association would suggest that potentially common genes underlie individual variation in both tool use skill and gray matter structural covariation.

## Materials and Method

### Subjects

This study includes data from 226 captive chimpanzees (136 females, 85 males), comprising 88 chimpanzees housed at the Yerkes National Primate Research Center (YNPRC) and 138 chimpanzees housed at the National Center for Chimpanzee Care (NCCC). Ages at the time of their in vivo MRI scans ranged from 8 to 53 years (Mean = 27.04, SD = 6.74). Of the 226 chimpanzees for which MRI scans were obtained, measures of tool use skill were available for 204 individuals, including 123 females and 81 males. Of these 204 apes, 134 were housed at NCCC and 70 at the YNPRC. These subjects were included in all analyses pertaining to phenotypic associations between tool use skill and the SBM components. All tool use data were collected within 3 years of the acquisition of the MRI scans. We note here that the NCCC and YNPRC are genetically isolated populations of captive chimpanzees. That is to say, these populations were created from separate founder chimpanzees and there was no interbreeding between chimpanzees living in these 2 facilities. We took advantage of this opportunity to evaluate consistency and reproducibility in the estimates of heritability in SBM components and their association with tool use skill measures in our analyses (see Baker 2016).

### Tool Use Skill

The apparatus and procedure used to quantify tool use skill, as well as heritability, have been described in detail elsewhere (Hopkins et al. 2009, 2015). Briefly, to assess tool use skill, we recorded the latency to insert a small stick into a hole to extract food, averaged across a total of 50 trials in each chimpanzee. The average latency scores were converted to standardized z-scores within the NCCC and YNPRC to account for differences in the duration of experience that chimpanzees at each colony had with the tool use device. In previously published studies (Hopkins et al. 2015), we found average tool latency to be significantly heritable ( $h^2 = 0.395$ , s.e. = 0.129,  $P < 0.001$ ) and this was the case for chimpanzees at both the NCCC ( $h^2 = 0.356$ , s.e. = 0.155,  $P < 0.007$ ) and YNPRC ( $h^2 = 0.463$ , s.e. = 0.190,  $P < 0.007$ ) when analyzed separately.

## MRI Collection

All chimpanzees were scanned during one of their annual physical examinations. MRI scans followed standard procedures at the YNPRC and NCCC and were designed to minimize stress. Thus, the animals were first sedated with ketamine (10 mg/kg) or telazol (3–5 mg/kg) and were subsequently anesthetized with propofol (40–60 mg/(kg/h)). They were then transported to the MRI scanning facility and placed in a supine position in the scanner with their head in a human head coil. Upon completion of the MRI, chimpanzees were briefly singly housed for 2–24 h to permit close monitoring and safe recovery from the anesthesia prior to return to the home social group. All procedures were approved by the Institutional Animal Care and Use Committees at YNPRC and NCCC and also followed the guidelines of the Institute of Medicine on the use of chimpanzees in research. Seventy-seven chimpanzees (all from YNPRC) were scanned using a 3.0-T scanner (Siemens Trio, Siemens Medical Solutions USA, Inc.). T1-weighted images were collected using a 3D gradient echo sequence (pulse repetition = 2300 ms, echo time = 4.4 ms, number of signals averaged = 3, matrix size = 320 × 320, with 0.6 × 0.6 × 0.6 resolution). The remaining 149 chimpanzees (11 from YNPRC, 138 from NCCC) were scanned using a 1.5 T.G.E. echo-speed Horizon LX MR scanner (GE Medical Systems). T1-weighted images were collected in the transverse plane using a gradient echo protocol (pulse repetition = 19.0 ms, echo time = 8.5 ms, number of signals averaged = 8, matrix size = 256 × 256, with 0.7 × 0.7 × 1.2 resolution).

## Image Processing and SBM Analysis

All T1-weighted MRI scans were realigned in the AC-PC plane and skull-stripped using the BET function in FSL (Zhang et al. 2001; Smith et al. 2004) and resampled at 0.7-mm isotropic voxels. Following this initial preprocessing step, the images were analyzed following the steps used for voxel-based morphometry analyses using FSL (Analysis Group, FMRIB) (Smith et al. 2004). Specifically, images were registered to a chimpanzee template brain, then segmented into gray and white matters as well as CSF. Subsequently, a study-specific gray matter template brain was created and each subject's segmented scan was nonlinearly registered to the template brain and the Jacobian warping matrix was saved for each subject. The gray matter intensity values were then multiplied by the Jacobian warp to estimate the modulated gray matter volume within each voxel.

For the SBM, the individual modulated gray matter volumes were analyzed using the software program Group ICA of fMRI Toolbox (GIFT) (<http://mialab.mrn.org/software/gift/index.html>). In SBM, the images are concatenated into a 2D array or matrix with the number of subjects and voxels as the matrix. Subsequently, principal component analysis (PCA) is performed on the matrix to reduce dimensionality using the Minimum Description Length algorithm, which was estimated to be 24 for the combined chimpanzee sample. Consistent with other SBM studies in humans (Xu et al. 2009; Grecucci et al. 2016), the data were then subjected to spatial PCA using the Infomax algorithm, which produces a source and mixing matrix. The source matrix is a subject X PCA array with each value presenting the relative contributions of each subject's data to the composition of each PCA. The source matrix values were the primary dependent measure of interest. To visualize the component structures, we used the mixing matrix which is a 3D volume that depicts the characteristics of the spatial characteristics and

covariation in gray matter for each PCA. Values within the mixing matrices are represented as standardized scores and can therefore take on both negative and positive values. Consistent with previous studies, we thresholded each PCA component at an absolute value of 3.00 and included only those clusters that survived this threshold as significant.

## Heritability Analyses

From the SBM analysis, one outcome measure is the individual subject's weighted score in deriving each independent component. Much like in factor or PCA, each subject's weighted score can vary on a continuous scale from negative to positive with the absolute indicating the magnitude of their score. To estimate heritability in our chimpanzee sample, the outcome measures for all identified SBM components were subjected to a quantitative genetic analysis to estimate heritability using the software program SOLAR (Almasy and Blangero 1998). SOLAR uses a variance components approach to estimate the polygenic component of variance when considering the entire pedigree (see Rogers et al. 2007; Fears et al. 2009; Fears et al. 2011; Hopkins 2013; Hopkins, Keebaugh, et al. 2014; Hopkins, Russell, et al. 2014). We used SOLAR in 2 ways in this study. First, we used it to estimate and statistically determine whether the weighted component scores were significantly heritable in the entire chimpanzee sample as well as within each population to assess the reproducibility. Second, we used SOLAR to calculate genetic correlations between the tool use performance data and the SBM component scores. Covariates included sex, age, scanner magnet, and rearing history of the subjects (i.e., wild-caught, mother-reared, or human-reared).

## Results

### Descriptive SBM Results

From the SBM analysis, there were 24 components identified that were distributed throughout the cortex and cerebellum. An anatomical description of the 24 components and their volumes are provided in Table 1. 3D renderings of each component are shown in Supplementary Figure 1.

### Heritability of SBM Component Scores

For the SOLAR analyses, we estimated the heritability for the standardized SBM z-scores for each component. Age, sex, rearing history, and scanner magnet served as covariates in these analyses. The proportion of variability attributed to genetic factors and the covariates are shown in Table 2. Significant heritability estimates were found for 18 of the 24 components with significant  $h^2$  values ranging from 0.246 to 0.886, suggesting moderate to strong effects. Significant covariate effects of scanner magnet were found for 20 components which was not surprising given that the gray and white matter contrast is influenced by the scanner magnet. Age accounted for a significant proportion of variance in components 8, 11, 14, 19, and 21, respectively. Sex accounted for a significant proportion of variance in components 10, 12, 13, 15, 19, and 23, while the rearing history variable was not significant for any components.

### Sex and Age Covariate Effects

To further evaluate the contributions of the factors sex and age, we performed several follow-up analyses. For the SBM components in which age was a significant effect, we fit

**Table 1** Anatomical description and volume of each SBM component

Component	Volume
Component 1	
Precuneus (L), precentral gyrus (L), medulla oblongata	4264.18
Component 2	
Lateral cerebellar hemispheres (inferior), bilateral	8019.00
Component 3	
Superior parietal cortex, bilateral	6313.60
Component 4	
Anterior cingulate cortex (R), dorsolateral prefrontal cortex (R), supplemental motor area (R)	5621.77
Component 5	
Primary visual cortex (L)	7319.62
Component 6	
Frontopolar cortex (B)	9232.19
Component 7	
Primary motor and premotor cortex (dorsal) (B)	8512.57
Component 8	
Cuneus (L), lateral cerebellar hemisphere (R)	5761.37
Component 9	
Cuneus (B), hippocampal formation (R)	5140.88
Component 10	
Lateral cerebellar hemispheres (B)	7903.75
Component 11	
Anterior cingulate cortex (B), frontopolar cortex (B), dorsolateral prefrontal cortex (B)	9430.10
Component 12	
Primary visual cortex (R)	5181.70
Component 13	
Primary visual cortex (B), cuneus (B)	9880.11
Component 14	
Anterior temporal cortex (B), anterior insular cortex (B), inferior temporal cortex (R)	4870.26
Component 15	
Anterior temporal cortex (B)	9169.76
Component 16	
Basal forebrain (B)	5470.51
Component 17	
Primary motor and somatosensory cortex (dorsal) (B)	8646.34
Component 18	
Lateral cerebellar hemispheres (B)	10 408.68
Component 19	
Frontopolar cortex (B)	5212.23
Component 20	
Lateral cerebellar hemispheres (B)	8719.75
Component 21	
Cerebellar vermis (B)	9140.95
Component 22	
Primary visual cortex (B)	9248.31
Component 23	
Cerebellar vermis and medial hemisphere (B)	8076.21
Component 24	
Superior parietal cortex (B)	8040.95

Note: Volumes are in mm<sup>3</sup>. (R) = right hemisphere, (L) = left hemisphere, (B) = bilateral.

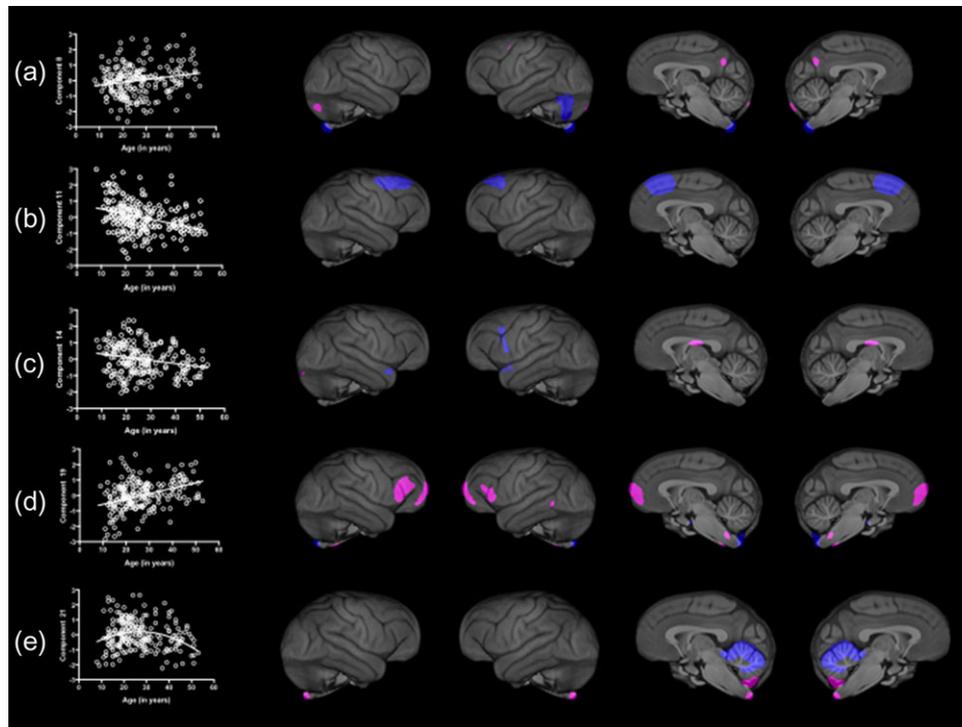
polynomial lines between age and weighted scores for components 8, 11, 14, 19, and 21 using stepwise multiple regression. The outcome measures were the component scores, while the predictor variables were sex, scanner strength, the linear age, then curvilinear age variables. We calculated the significance in change in  $R^2$  to determine which age distribution best explained the variability in the SBM component score. The

**Table 2** Heritability and covariate effects for each SBM component

Component	$h^2$	s.e.	P	Covariates	Variance
1	<b>0.378</b>	0.156	0.004	Scanner	0.290
2	<b>0.886</b>	0.115	<b>0.0000001</b>	Scanner	0.127
3	<b>0.854</b>	0.101	<b>0.0000001</b>	None	
4	<b>0.314</b>	0.132	<b>0.003</b>	Scanner	0.218
5	<b>0.341</b>	0.162	<b>0.01</b>	Scanner	0.131
6	0.216	0.112	0.065	None	
7	0.184	0.161	0.107	Scanner	0.395
8	<b>0.260</b>	<b>0.141</b>	<b>0.025</b>	Age	0.035
9	<b>0.374</b>	<b>0.126</b>	<b>0.0007</b>	Scanner	0.197
10	<b>0.497</b>	<b>0.171</b>	<b>0.001</b>	Scanner, sex	0.252
11	<b>0.658</b>	<b>0.171</b>	<b>0.0003</b>	Scanner, age	0.189
12	<b>0.366</b>	<b>0.159</b>	<b>0.006</b>	Scanner, sex	0.103
13	<b>0.565</b>	<b>0.157</b>	<b>0.0003</b>	Scanner, sex	0.433
14	<b>0.304</b>	<b>0.153</b>	<b>0.016</b>	Scanner, age	0.151
15	0.146	0.147	0.139	Scanner, sex	0.215
16	0.038	0.111	0.363	Scanner	0.292
17	<b>0.465</b>	<b>0.137</b>	<b>0.00001</b>	Scanner	0.305
18	0.154	0.148	0.127	None	
19	<b>0.531</b>	<b>0.149</b>	<b>0.00005</b>	Scanner, sex, age	0.252
20	<b>0.830</b>	<b>0.121</b>	<b>0.0000001</b>	Scanner	0.076
21	<b>0.579</b>	<b>0.166</b>	<b>0.00003</b>	Scanner, age	0.008
22	0.000	0.000	0.5000	Scanner	0.034
23	<b>0.246</b>	<b>0.153</b>	<b>0.039</b>	Scanner, sex	0.136
24	<b>0.252</b>	<b>0.129</b>	<b>0.014</b>	Scanner	0.219

Note:  $h^2$  = heritability coefficient, s.e. = standard error. Covariates indicate those variables that accounted for a significant proportion of variance in the SBM scores and proportion of variance accounted for them. Bolded values are those heritability estimates that are significant at  $P < .05$ .

scatterplots between age and the SBM-weighted scores, as well as the best fit line are shown in Figure 1a-e. For component 8, the overall model was significant;  $R = 0.239$   $F(4, 219) = 3.313$ ,  $P = 0.012$ . Significant changes in  $R^2$  (0.170 to 0.235) were found for the linear;  $F(1, 220) = 6.179$ ,  $P = 0.012$  but not the curvilinear (0.235 to 0.239);  $F(1, 219) = 0.379$ ,  $P = 0.539$  age variable. Component 8 is comprised of the right cerebellum and left cuneus and the association was positive with older individuals having higher values compared with younger individuals. For component 11, the overall model was significant;  $R = 0.487$   $F(4, 219) = 17.012$ ,  $P = 0.001$ . Significant changes in  $R^2$  (0.149 to 0.219) were found for the linear;  $F(1, 220) = 20.876$ ,  $P = 0.001$ , but not the curvilinear (0.219 to 0.223);  $F(1, 219) = 2.153$ ,  $P = 0.144$  age variable. Component 11 included the dorsal lateral prefrontal cortex, frontopolar and anterior cingulate cortex, and the associations were negative with older individuals having lower values compared with younger apes. For component 14, the overall model was significant;  $R = 0.405$   $F(4, 219) = 10.751$ ,  $P = 0.001$ . Significant changes in  $R^2$  (0.373 to 0.402) were found for the linear;  $F(1, 220) = 5.656$ ,  $P = 0.018$ , but not the curvilinear (0.401 to 0.405);  $F(1, 219) = 0.825$ ,  $P = 0.365$  age variable. Component 14 was comprised of the anterior temporal, inferior temporal, and anterior insular cortices. The linear association was negative, suggesting that older subjects have lower values. For component 19, the overall model was significant;  $R = 0.543$   $F(4, 219) = 22.885$ ,  $P = 0.001$ . Significant changes in  $R^2$  (0.439 to 0.541) were found for the linear;  $F(1, 220) = 31.043$ ,  $P = 0.001$  but not the and curvilinear (0.541–0.543);  $F(1, 219) = 0.724$ ,  $P = 0.396$  age variables. Component 19 was comprised of frontopolar cortex, and older chimpanzees had relatively higher weighted scores compared to middle-aged and younger individuals. Finally, for component 21, the overall model was significant;



**Figure 1.** Scatterplots showing significant associations between age and SBM components (a) 8, (b) 11, (c) 14, (d) 19, and (e) 21. Left panel shows the scatterplot between age and the weighted SBM component scores and the right panel shows regions comprising each component.

$R = 0.255$   $F(4, 219) = 3.803$ ,  $P = 0.005$ . Significant changes in  $R^2$  (0.148 to 0.197) were found for the linear;  $F(1, 220) = 3.824$ ,  $P = 0.052$  and the curvilinear (0.197–0.255);  $F(1, 219) = 6.153$ ,  $P = 0.014$  age variables. Component 21 was comprised of vermis of the cerebellum. Older and younger chimpanzees had relatively lower weighted scores compared to middle-aged individuals. The mean weighted z-scores for components 10, 12, 13, 15, 19, and 23 in male and female chimpanzees are shown in Figure 2. Males had significantly higher weighted scores compared with females on all components with the exception of 13 (see Table 1 for descriptions of the regions).

### Phenotypic and Genetic Associations between Tool Use Skill and SBM Components

For this analysis, we used partial correlation coefficients between the standardized z-scores of the tool use latency measures and each SBM component while statistically controlling for sex, scanner magnet, rearing history, and age of the subjects. Three subjects (all females) were removed from this analysis because they were identified as outliers on their tool use performance measure based on boxplots of the standardized z-scores. Significant positive associations were found between tool use latency scores and 2 SBM regions, including components 3 ( $r = -0.211$ ,  $P = 0.003$ ) and 13 ( $r = -0.168$ ,  $P = 0.019$ ) (see Fig. 3). Component 3 consisted of the posterior superior temporal sulcus (STS) and superior parietal cortex, while component 13 was comprised of primary visual cortex and cuneus. The associations were negative, thus subjects with slower average latency scores contributed less to the component scores within each of these regions. Finally, we calculated genetic correlations between the tool use skill measures and each of the SBM components (see Table 3). Significant and large genetic correlations

were found between tool use skill and components 3 ( $r_{hog} = 0.519$ ,  $P = 0.03$ ) and 13 ( $r_{hog} = 0.717$ ,  $P = 0.02$ ).

### Reproducibility Between Chimpanzee Populations

Recall that we tested 2 colonies of genetically unrelated chimpanzees that were scanned on different platforms. Thus, to assess the consistency in results between the 2 colonies, we performed several additional analyses. First, we performed separate heritability analyses in the NCCC ( $n = 138$ ) and YNPRC ( $N = 88$ ) chimpanzees for each SBM component derived from the entire sample (see Fig. 4). Within the NCCC samples, 17 of the 24 components were significantly heritable compared with only 7 within the YNPRC sample. Further, the average heritability across all 24 components was significantly higher in the NCCC ( $h^2 = 0.575$ ) compared with YNPRC ( $h^2 = 0.233$ ) sample  $t(23) = 3.434$ ,  $P = 0.002$ . Ten of the 24 SBM components showed consistently significant or nonsignificant heritability in both the NCCC and YNPRC samples (Components 1, 2, 3, 4, 15, 16, 19, 20, 22, and 24, respectively). In addition, we assessed the phenotypic correlations between the tool use performance measures and the SBM components' scores within the NCCC and YNPRC samples. These data are shown in Table 3. As can be seen, for component 3 significant negative associations were found between tool use skill and the SBM-weighted component scores for the entire sample as well as within both the NCCC and YNPRC samples. A similar pattern was observed for component 13, although the YNPRC did not reach conventional levels of statistical significance ( $P < 0.05$ ). Indeed, although the estimate did not reach the  $P < 0.05$  level of significance, the magnitude of the correlation within the YNPRC sample ( $-0.150$ ) was very similar to the significant ( $P < 0.05$ ) association in the full combined sample ( $-0.168$ ).

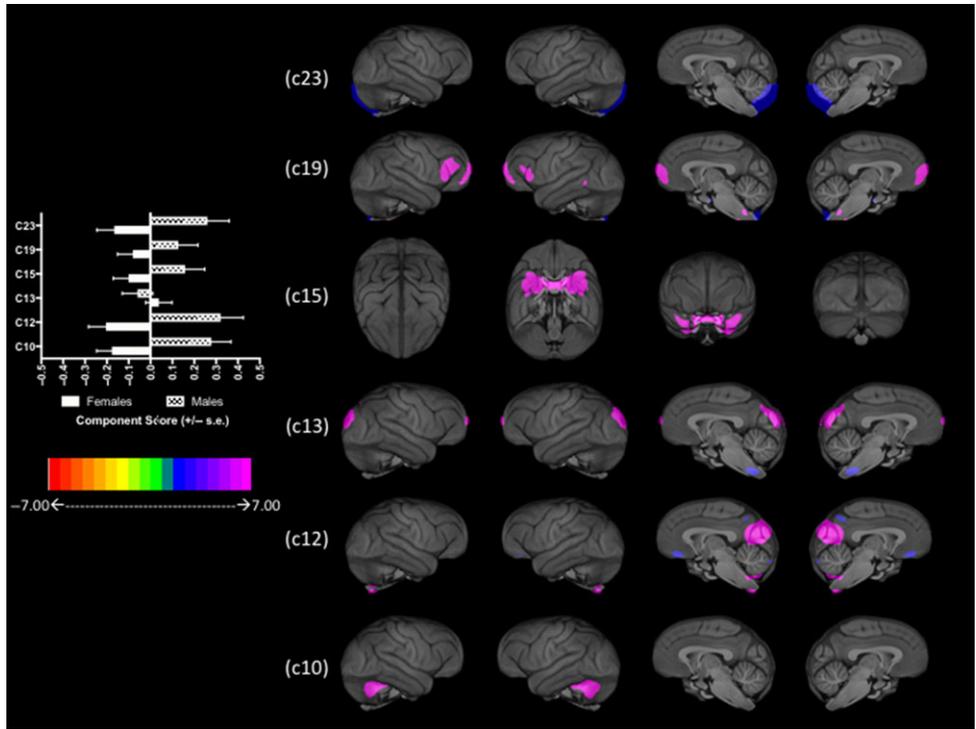


Figure 2. Left panel: Mean SBM-weighted scores for males and females for components 10, 12, 13, 15, 19, and 23. Right panel: Brain regions comprising each component.

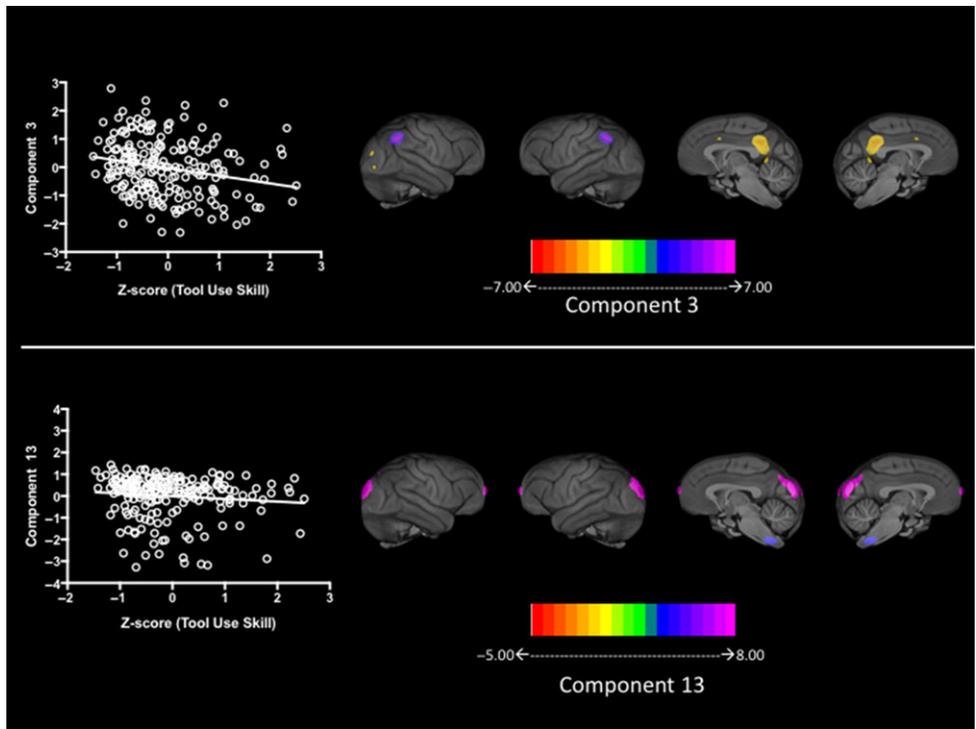


Figure 3. Upper and lower left panel = scatterplot between tool use performance measures and weighted scores for SBM components 3 (left) and 13 (right). Upper and lower bottom panel shows brain regions in SBM components 3 and 13.

**Discussion**

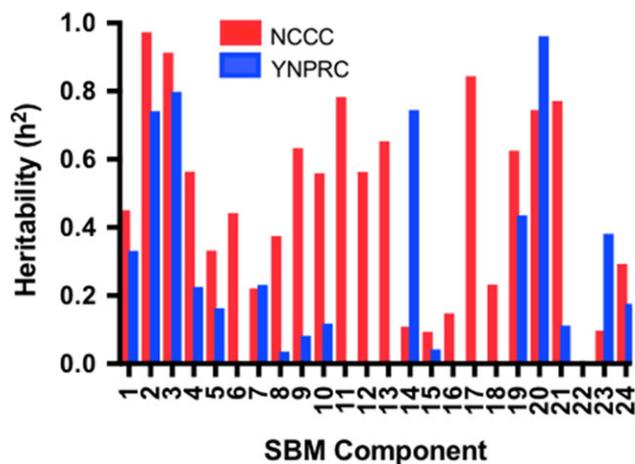
There were 5 main findings in this study. First, we found 24 gray matter SBM components in chimpanzees. Second, gray

matter structural covariation was influenced by sex and age. Third, a majority of the SBM components were significantly heritable, suggesting that genetic factors may influence their

**Table 3** Phenotypic correlations between tool use skill and SBM component scores for the entire sample and within the NCCC and YNPRC chimpanzee colonies

	Overall	NCCC	YNPRC
1	+0.018	+0.103	-0.125
2	-0.035	-0.046	-0.030
3	<b>-0.211</b>	<b>-0.202</b>	<b>-0.248</b>
4	+0.057	+0.106	-0.021
5	+0.026	-0.023	+0.187
6	-0.053	-0.104	-0.010
7	-0.111	-0.061	-0.213
8	-0.110	-0.127	-0.079
9	-0.058	+0.004	-0.165
10	-0.055	-0.041	-0.068
11	+0.028	+0.104	-0.115
12	-0.017	-0.027	-0.032
13	<b>-0.168</b>	<b>-0.296</b>	-0.150
14	+0.031	+0.065	-0.052
15	-0.030	+0.057	-0.053
16	+0.078	-0.003	-0.180
17	+0.126	+0.091	+0.190
18	+0.079	+0.146	-0.141
19	-0.002	-0.017	+0.028
20	+0.030	-0.005	+0.110
21	+0.075	+0.100	+0.033
22	+0.008	-0.038	+0.194
23	+0.022	+0.015	+0.067
24	-0.027	-0.017	-0.069

Note: Bolded values are significant at  $P < 0.05$ .



**Figure 4.** Heritability for each SBM component in the NCCC (red) and YNPRC (blue) chimpanzee populations.

expression across subjects. Fourth, heritability of the SBM components were modestly consistent between 2 genetically isolated populations of captive chimpanzees. Finally, we found significant phenotypic and genetic correlations between tool use skill and 2 SBM components. These latter findings have several important implications for primate brain evolution and the emergence of tool manufacture and use.

With respect to the 24 component revealed by the SBM analysis, this is fewer than the number reported in at least some previous reports in human brains (Xu et al. 2009). The differing numbers of components may reflect inherent differences in the

covariation of gray matter between humans and chimpanzees; however, we cannot rule out that the potential differences in SBM organization between humans and chimpanzees may be a result of different sample size, scanner parameters, voxel resolution, or other methodological factors. Notwithstanding, many of the components identified in our chimpanzee sample have been similarly described in human SBM analyses (Grecucci et al. 2016).

Second, gray matter structural covariation in the chimpanzee brain was influenced by age and sex. Males and females differed significantly on 6 of the 24 components and these differences presumably underlie behavioral, affective, motor, or cognitive functions that distinguish the 2 sexes. Certainly male and female chimpanzees differ with respect to social behavior, such as aggression and grooming partners, as well as in their role within the community where, for example, males typically patrol the home range and females do not (Goodall 1986; Boesch and Boesch-Achermann 2000; Mitani and Watts 2005; Lehmann and Boesch 2008). Further, there is some evidence of sex differences in learning, hand use, and performance on tool use tasks in chimpanzees (Pandolfi et al. 2003; Lonsdorf et al. 2004; Gruber et al. 2010; Bogart et al. 2012; Sanz et al. 2016). While it is tempting to speculate that the observed sex-dependent gray matter covariation differences reported here underlie male–female behavioral differences, we have no direct evidence to support this assertion. This will require additional studies beyond the scope of this report.

Age significantly and linearly correlated with 4 components and showed a significant quadratic association for one component. For components 8 and 19, we found positive associations between age and the weighted scores, suggesting that older individuals are contributing more to the generation of these components than younger individuals. These 2 sources largely comprised prefrontal, premotor, and portion of the cerebellum, and the most parsimonious explanation is that maturational factors contribute to the increased covariation in gray matter density within these regions (Terribilli et al. 2011; Lemaire et al. 2012). Age was negatively correlated with components 11 and 14 which included superior frontal, supplementary motor and anterior temporal cortex suggesting a reduction in covariation with increasing age. The associations between age and component 21 is slightly more difficult to interpret because it exhibited curvilinear relationship. Older and younger chimpanzees had relatively lower weighted scores than middle-aged apes for this component, which was comprised entirely of the cerebellum.

It is worth noting that, within the larger context of studies on age-related changes in the great ape brain (Gearing et al. 1994, 1997; Rosen et al. 2008; Perez et al. 2013; Edler et al. 2017), the results reported here are somewhat novel. For instance, Sherwood et al. (2011) failed to find any significant age-related changes in overall gray and white matter volume in a sample of 99 chimpanzees. More recently, Autrey et al. (2014), in a sample of 219 chimpanzee MRI scans, reported that chimpanzees show (1) increasing gyrification with age, (2) a cubic association between age and white matter volume, and (3) a negative association between age and the depth and width of the fronto-orbital sulcus. Recall that here, we found significant linear and quadratic associations between gray matter covariation and age, a finding not previously reported in the chimpanzee brain at least with respect to gray matter variation.

Regarding heritability, there are some reports of the genetic contributions to individual differences in cortical organization in nonhuman primates, including chimpanzees (Rogers et al.

2007, 2010; Fears et al. 2009; Kochunov et al. 2010). For instance, Gomez-Robles et al. (2015) have previously reported modest heritability of cortical shape and for different linear measures of sulci in chimpanzees. Our findings similarly reveal moderate heritability in most (18 of the 24 components, see Table 2), but not all structurally covarying gray matter regions in the chimpanzee brain. We also found modest consistency in heritability between the NCCC and YNPRC chimpanzee populations. Ten of the 24 components showed consistent heritability (or lack thereof) between the 2 populations. One limitation in our effort to replicate the heritability results between the 2 chimpanzee populations were (1) differences in the sample sizes, (2) variation in the scanner platform and magnet strength, and (3) the composition of the number of differentially reared chimpanzees. There were 138 NCCC chimpanzees and 88 YNPRC and all the NCCC chimpanzees were scanned on a 1.5-T machine, while 77 of the YNPRC apes were scanned on a 3-T machine and remaining on a 1.5-T magnet. Additionally, the proportion of nursery-reared chimpanzees was higher in the YNPRC compared to NCCC chimpanzees. Previous studies have shown that differences in early rearing can influence gray matter structural covariation in chimpanzees (Bard and Hopkins 2018) and therefore these experiences may have altered the genetic basis of development as manifest by reduced heritability.

Finally, we found that individual variation in tool use motor skill was associated with structural covariation in 2 SBM components that were largely comprised of superior temporal, parietal, and cerebellar cortices. The phenotypic associations between tool use performance and components 3 and 13 were consistent and significant within each chimpanzee population. Further, we found significant genetic correlations between tool use skill and components 3 and 13, which include areas within the posterior STS, posterior cingulate, visual cortex, and the brainstem, suggesting that common genetic mechanisms may underlie their expression.

As noted above, component 3 is comprised of the cuneus and the superior portion of the STS that projects dorsally into the parietal lobe, while component 13 includes occipital regions. Clinical and functional neuroimaging studies in humans have clearly implicated portions of the parietal lobe as playing an important role in providing visual feedback during planned visuomotor actions, such as grasping an object or in the use of tools (Johnson-Frey 2004; Stout and Chaminade 2012; Gilissen and Hopkins 2013; Caminiti et al. 2015; Bruner and Iriki 2016). Furthermore, some have suggested that expansion of the parietal lobe and cuneus was associated with the emergence of increasing complex motor, cognitive and linguistic functions during primate brain evolution (Gannon et al. 2005; LeRoy et al. 2015; Bruner and Iriki 2016; Bruner et al. 2017). Our results suggest that these as yet unknown genetic mechanisms, may account for a heritable link between variation in the capacity to use tools and variation in the morphology of the inferior and superior parietal lobe. Such heritable covariation is a key for natural selection as an explanation for the coevolution of tool skill and cortical structure in humans and apes. Indeed, our results suggest that increased selection for tool use skill may have resulted selective changes in the size, connectivity, or organization of the parietal cortex in humans after that split form the last common ancestor.

In summary, the findings reported here are the first evidence of heritability in structural covariation in gray matter among nonhuman primates. Though this study focused on associations between tool use skill and gray matter structural covariation, future studies should expand this analytic

approach to additional behavioral and cognitive phenotypes. This approach could potentially identify brain regions in chimpanzees that exhibit heritable variation associated with particular behavioral or cognitive abilities, providing insight into neuroanatomical targets that could have been selected for expansion in hominins after the split from a last common ancestor. Additionally, this approach could be used to identify key brain regions as foci for subsequent gene expression analyses that could lead to the discovery of candidate genes linked to typical and atypical praxic functions.

## Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

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## Notes

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