

Behavioral and neuroanatomical correlates of white matter asymmetries in chimpanzees (*Pan troglodytes*)

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Abstract

Although behavioral and brain asymmetries have been documented in non-human primates, lateralization in cortical connectivity as reflected in white matter has not been described in any species, despite the intrinsic theoretical interest in white matter expansion during primate brain evolution. Here we report evidence of population-level leftward asymmetries in the white matter of chimpanzees. We further report that lateralization in white matter correlates with their handedness as well as neuroanatomical asymmetries in the precentral gyrus. These findings suggest that chimpanzees show asymmetries in cortical connectivity and these may serve as the foundation for morphological and behavioral laterality in primates, including humans.

Introduction

Historically, the link between functional and neuroanatomical asymmetries in humans has focused on comparisons of volumetric or linear measurements of specific regions of interest (ROI) or sulci between the left and right hemispheres. For example, it has been well documented that the planum temporale and inferior frontal gyrus are larger in the left compared with right hemisphere, and that this pattern of results is somewhat modulated by subjects' characteristics, including sex and handedness (Beaton, 1997; Foundas *et al.*, 1998). More recently, rather than focus on left–right differences in morphology, investigators have begun to examine asymmetries in the density, distribution and connectivity of white and gray matter between hemispheres using either manual tracing or thresholding procedures from magnetic resonance images (MRIs), voxel-based morphology or diffusion tensor imaging (DTI) (Good *et al.*, 2001; Watkins *et al.*, 2001; Pujol *et al.*, 2002; Buchel *et al.*, 2004). Like ROI approaches, significant region-specific differences in white and gray matter have been found between hemispheres.

As with studies in human subjects, historical and contemporary research has documented population-level neuroanatomical asymmetries in non-human primates, notably great apes, using ROI approaches including linear measurements of sulci (e.g. sylvian fissure length) or volumetric measures of specific brain areas, such as the planum temporale or inferior frontal lobe (Gannon *et al.*, 1998; Cantalupo & Hopkins, 2001; Cantalupo *et al.*, 2003). However, whether non-human primates show asymmetries in cortical connectivity as reflected in relative white matter rather than morphology has yet to be examined. Nonetheless, this is of

considerable theoretical interest given the importance some have placed on white matter expansion in primate brain evolution (Schoenemann *et al.*, 2005), particularly as it relates to the emergence of lateralization in the CNS (Rilling & Insel, 1999; Partadiredja *et al.*, 2003). Specifically, it has been hypothesized that phylogenetic and individual differences in asymmetries are a consequence of intrahemispheric connectivity as reflected in white matter density (Rilling & Insel, 1999) rather than interhemispheric connectivity (Ringo *et al.*, 1994; Aboitiz *et al.*, 2003). If white matter expansion in part explains individual and species differences in laterality, then based on the ROI findings in chimpanzees it can be hypothesized that asymmetries in connectivity should also be present in these apes. Alternatively, humans have a disproportionately larger amount of white matter compared with other primates (Schoenemann *et al.*, 2005). Despite a large body of data to the contrary (Hopkins *et al.*, 2003), some continue to argue that population-level behavioral and brain asymmetries are unique to human evolution (Crow, 2004). If white matter expansion underlies the development of behavioral and brain asymmetries, and brain asymmetries are uniquely human, then it can be hypothesized that asymmetries in connectivity will not be present in chimpanzees.

The aim of this study was to examine if chimpanzees show population-level asymmetries in the relative amounts of white matter between the left and right hemispheres. In addition, we sought to test the hypothesis that behavioral and neuroanatomical asymmetries are associated with interhemispheric differences in white matter asymmetries. In previous studies in chimpanzees using ROI approaches, hand preferences for a coordinated bimanual task have been linked to a neuroanatomical asymmetry in the precentral gyrus (Hopkins & Cantalupo, 2004). If white matter asymmetries explain individual differences in lateralization, then significant associations should be found between white matter asymmetries, handedness and laterality in the precentral gyrus.

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Materials and methods

Subjects

MRI scans were obtained from a sample of 56 captive chimpanzees, including 23 males and 33 females. All the chimpanzees were members of a captive colony housed at Yerkes National Primate Research Center (YNPRC) in Atlanta, Georgia, USA. The subjects ranged in age from 6 to 42 years (mean = 22.31, SD = 11.45). Within the sample there were 12 mother-reared, 38 nursery-reared and six wild-caught chimpanzees. Mother-reared chimpanzees were those born in captivity and raised by their conspecific mother. Nursery-reared chimpanzees were those, due to medical complications or maternal neglect, taken from their biological mother before 30 days of life and raised by humans in a nursery setting. The remaining wild-caught subjects were captured and brought from their native home in Africa prior to 1973 and had remained in captivity since that time. This study was approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Image collection and procedure

Subjects were first immobilized by telazol injection (2–6 mg/kg) and subsequently anesthetized with propofol (10 mg/kg/h) following standard procedures at the YNPRC. Subjects were then transported to the MRI facility. The subjects remained anesthetized for the duration of the scans as well as the time needed to transport them between their home cage and the imaging facility (total time ~2 h). Subjects were placed in the scanner chamber in a supine position with their head fitted inside the human-head coil. Scan duration ranged between 40 and 80 min as a function of brain size. The majority of the subjects ($n = 38$) were scanned using a 1.5-T scanner (Phillips, Model 51). The remaining chimpanzees ($n = 18$) were scanned using a 3.0-T scanner (Siemens Trio, Siemens Medical Solutions USA, Malvern, PA, USA) at the YNPRC.

For all chimpanzees scanned *in vivo* using the 1.5-T machine, 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 and a 256×256 matrix). Eighteen of the chimpanzees were scanned using a 3.0-T scanner (Siemens Trio). T1-weighted images were collected using a three-dimensional gradient echo sequence (pulse repetition = 2300 ms, echo time = 4.4 ms, number of signals averaged = 3, matrix size = 320×320).

After completing MRI procedures, the subjects were returned to the YNPRC and temporarily housed in a single cage for 6–12 h to allow the effects of the anesthesia to wear off, after which they were returned to their home cage. The archived MRI data were transferred to a PC running Analyse 6.0 (Mayo Clinic, Mayo Foundation, Rochester, Minnesota, USA) software for post-image processing.

Handedness measurement

Handedness was measured for a task measuring coordinated bimanual actions, referred to as the TUBE task (Hopkins, 1995). The initial data were collected in 1994, and several different methods of assessment and replications have occurred in the past 12 years (Hopkins *et al.*, 2001; Hopkins & Cantalupo, 2003). Individual hand preferences were based on the cumulative data available for each chimpanzee in the study. For the TUBE task, peanut butter is smeared on the inside edges of polyvinyl chloride (PVC) tubes approximately 15 cm in length and 2.5 cm in diameter. Peanut butter is smeared on both ends of the PVC pipe, and is placed far enough down the tube that the subjects cannot lick the

contents completely off with their mouths but rather must use one hand to hold the tube and the other hand to remove the substrate. The PVC tubes were handed to the subjects in their home cages, and a focal sampling technique was used to collect individual data from each subject. The hand of the finger used to extract the peanut butter was recorded as either right or left by the experimenter. Each time the subjects reached into the tube with their finger, extracted peanut butter and brought it to their mouth, the hand used was recorded as left or right. All the chimpanzees were tested in the outdoor portion of their home cages. The number of responses obtained from each subject differed depending on their efficiency in removing the food, motivation and availability for testing. Notwithstanding, a minimum of 30 responses were obtained for each individual for each task. Six year test–retest correlations on handedness for the TUBE task have revealed reliable and consistent hand preferences in the chimpanzees (Hopkins *et al.*, 2001).

Motor-hand area (knob)

The knob was localized in serial 1-mm slices in the axial plane following procedures similarly used in human and non-human brain specimens (Yousry *et al.*, 1997; Hopkins & Pilcher, 2001). The horizontal epsilon or inverted omega that projected into the postcentral gyrus was traced on each image (Fig. 1). The dorsal and ventral edges of the knob served as the markers for defining the boundaries of the area. For each slice and hemisphere, an area measurement of the region was calculated by use of a mouse-driven pointer that traced the omega-shaped ROI. The total areas from all slices in which the knob was present were summed and used to derive a volume of the knob for each hemisphere (ranging from 5 to 13 slices in the sample). Intra- and inter-rater reliability for the knob have been previously reported from a random selection of five subjects. The intra- and inter-rater reliability estimates for the knob were 0.868 and 0.843, respectively (see Hopkins & Pilcher, 2001).

White matter

The MRI scans were aligned in the coronal planes and cut into 1-mm slices. Measurement of the relative amount of white matter was performed following procedures that have been previously applied to MRI scans in non-human primates (Rilling & Insel, 1999; Malkova *et al.*, 2006). Consistent with previous studies, prior to measurement, the maximum length of the left and right hemisphere from the frontal to occipital pole was identified. Once the length was determined, the value was divided by 11, which resulted in 10 equally spaced regions along the anterior to posterior plane. Subsequently, on each slice, two measurements were obtained (Fig. 2A and B). First, the entire contour of the region, including the sulci, was traced with freehand tool. Second, the perimeter of the entire white matter region was traced by using the auto-trace function to threshold on the white matter in each hemisphere. This was done manually by the individual tracing the brain, and the thresholding function could be manipulated so as to segment as much white matter from non-white matter as possible within a slice. The white matter area measures were subtracted from the perimeter area measure to derive an estimate of non-white matter on each slice. The white matter area measures were then divided by the non-white matter area values to compute a ratio of white to non-white matter (WMR) for each of the 10 slices within each hemisphere. Larger ratio values represented a relatively greater amount of white matter.

Individuals tracing the brains were blind to the sex and handedness of the chimpanzees. Raters were also blind to the asymmetries in the knob of each chimpanzee. To assess inter-rater reliability, two

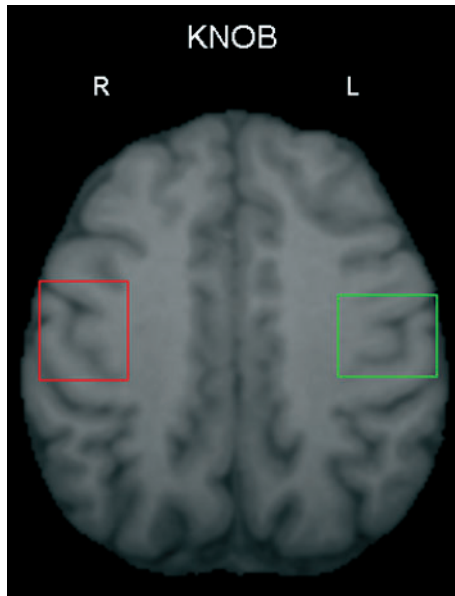


FIG. 1. Outline and tracing of the motor-hand area (knob) in the axial plane of an MRI scan of a chimpanzee brain.

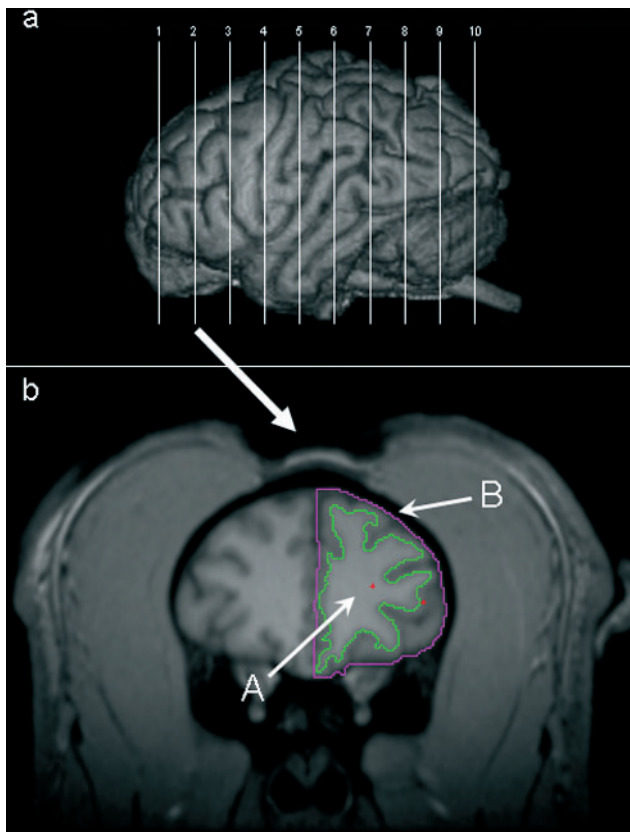


FIG. 2. (A) Three-dimensional reconstruction of a chimpanzee brain from MRI. The numbered lines indicate the 10 evenly distributed slices through the brain. (B) Coronal view of a T1-weighted *in vivo* chimpanzee brain through slice 2. The white matter tissue is outlined in green (A) and the entire area of the hemisphere is outlined in purple (B).

individuals measured all 10 brain regions for 10 individual chimpanzees. The area measures of the left and right hemispheres for the white matter were correlated within each individual chimpanzee between the

two raters. The average correlation coefficients for the right and left hemispheres were 0.85 and 0.92, respectively. When summed across all slices, the inter-rater reliabilities for the left ($r = 0.96$, d.f. = 8, $P < 0.01$) and right hemispheres ($r = 0.98$, d.f. = 8, $P < 0.01$) were positive and significant.

Data analysis

For the TUBE task, binomial z -scores were calculated for each subject based on the frequency of left and right hand use. Subjects with z -scores greater than 1.95 or less than -1.95 were classified as right- and left-handed, respectively. All other subjects ($n = 8$) were classified as ambiguously handed. Because there were so few ambiguously handed apes, these individuals were combined with the left-handed subjects to form one group referred to as non-right-handed.

The total areas from all slices in which the knob was present were summed and used to derive a volume of the knob for each hemisphere. Asymmetry quotients (AQ) for the knob were calculated using the formula $[AQ = (R - L) / ((R + L) * 0.5)]$. Using well-established criteria from the existing literature in humans and non-humans, subjects with AQ values ≥ 0.025 or ≤ -0.025 were classified as being rightward or leftward biased. Two apes had AQ values between -0.025 and 0.025 , and they were added to the rightward asymmetry group and these individuals were classified as non-leftward biased. For the WMR measures from the left and right hemisphere, individual AQ values were derived for each region following the same formula as used for the knob. Positive AQ values reflected right hemisphere biases and negative values reflected left hemisphere biases.

Results

Descriptive statistics

Because different magnets were used in this study, we initially performed some preliminary analyses on the total relative white matter values for each region to assess whether the use of these different magnets influenced the results. For each region and hemisphere, we summed the relative white values for the left and right hemispheres to derive a total relative white matter value. We then compared the 10 regions in relation to the subject's sex and magnet type (1.5, 3.0) using a mixed model analysis of covariance (ANCOVA). Region was the repeated measure, while sex and magnet type served as between-group factors. The age of the subjects was the covariate. Age was used as a covariate because there were a disproportionately larger proportion of older subjects in the wild-caught group, an expected finding due to the fact that importation ceased in 1973. This analysis revealed a significant main effect for region $F_{9,459} = 8.68$, $P < 0.001$, but no significant main effects for sex or magnet type. Moreover, none of the interaction terms approached statistical significance. The mean relative white matter values for each region and magnet are shown in Table 1. Because magnet strength did not have a significant influence on the relative white matter values, we combined all the data in subsequent analyses.

Laterality effects

The individual AQ values for each subject and region are shown in Table 2. In this analysis, a mixed model ANCOVA was performed with the AQ values for each region (1–10) serving as repeated measures, while sex served as the between-group factor. Age served as the covariate. A significant main effect for region was found

TABLE 1. Mean white matter ratio (WMR) values for each region as a function of magnet strength

Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10
Magnet strength 1.5T									
1.28 ± 0.071	1.50 ± 0.088	2.08 ± 0.182	2.76 ± 0.203	3.95 ± 0.214	3.97 ± 0.276	3.07 ± 0.197	2.41 ± 0.155	2.40 ± 0.103	2.38 ± 0.133
Magnet strength 3.0 T									
0.96 ± 0.100	1.59 ± 0.124	2.37 ± 0.256	3.03 ± 0.285	3.97 ± 0.300	3.65 ± 0.387	3.40 ± 0.277	2.16 ± 0.217	2.02 ± 0.144	2.19 ± 0.187

Regions 1–10 correspond to the anterior to posterior plane (see Fig. 2A). WMR, white matter ratio.

TABLE 2. Individual AQ values for each chimpanzee and brain region

Subject	Sex	Age	AQ1	AQ2	AQ3	AQ4	AQ5	AQ6	AQ7	AQ8	AQ9	AQ10
Agatha	F	9	-0.134	0.095	-0.048	-0.240	-0.187	0.446	0.098	-0.231	0.410	0.292
Amanda	F	11	-0.132	0.210	0.042	-0.114	0.317	-0.235	0.224	0.036	-0.028	0.336
Artifee	F	26	-0.277	-0.092	0.109	-0.422	-0.365	0.351	0.071	-0.233	0.029	0.449
Augusta	F	27	-0.648	0.306	-0.014	0.179	0.088	-0.285	-0.058	0.165	-0.093	0.249
Beleka	F	40	-0.229	-0.469	0.073	-0.084	-0.218	0.396	0.150	-0.311	0.073	-0.193
Bo	F	35	-0.002	-0.117	0.112	0.017	-0.149	-0.008	-0.419	-0.027	0.157	-0.655
Boka	F	44	-0.186	-0.352	-0.161	-0.667	-0.252	0.098	-0.094	0.341	0.798	-0.009
Brandy	F	17	0.092	-0.113	-0.112	-0.355	-0.192	-0.003	0.177	-0.012	-0.376	0.255
Callie	F	15	0.068	0.047	0.004	-0.022	-0.092	-0.218	-0.045	0.415	0.191	0.033
Cheeta	F	42	-0.419	-0.037	0.066	-0.711	-0.109	-0.107	0.072	0.044	0.197	0.042
Christa	F	8	-0.192	0.401	0.146	0.039	-0.069	-0.121	-0.365	-0.192	-0.216	0.609
Dara	F	15	-0.161	0.341	0.485	0.006	0.068	-0.234	0.129	0.211	0.215	-0.006
Elvira	F	14	0.136	0.050	-0.015	-0.158	-0.175	0.124	-0.067	0.182	-0.302	0.265
Evelyne	F	15	-0.102	0.027	-0.098	-0.146	-0.133	0.082	-0.286	0.041	0.060	-0.478
Faye	F	12	-0.535	-0.021	-0.158	-0.286	0.202	-0.133	0.096	0.094	0.158	-0.190
Fiona	F	8	0.504	-0.223	0.172	-0.588	-0.075	-0.032	-0.135	-0.110	-0.779	-0.390
Foxy	F	39	0.100	0.252	0.017	0.080	-0.033	0.132	-0.484	-0.104	0.454	0.111
Frannie	F	14	0.001	-0.260	-0.054	-0.235	-0.162	0.121	-0.498	0.043	0.091	0.443
Jacqueline	F	30	-0.309	-0.112	-0.348	-0.023	0.063	-0.143	-0.165	-0.186	-0.173	-0.774
Julie	F	11	0.000	-0.121	-0.045	-0.295	-0.062	-0.158	-0.370	0.243	-0.389	0.200
Katrina	F	17	-0.839	-0.174	0.009	-0.060	-0.026	0.197	0.111	-0.335	-0.272	0.641
Kengee	F	8	0.386	0.349	-0.157	0.149	-0.304	0.108	0.017	-0.366	-0.180	0.131
Lana	F	27	-0.026	0.000	-0.431	-0.817	0.345	-0.409	-0.134	0.352	-0.417	-0.350
Lulu	F	39	0.280	0.134	-0.131	0.119	0.247	0.037	-0.029	0.185	-0.594	-0.348
Marietta	F	28	0.271	-0.284	-0.144	0.095	-0.084	-0.164	-0.266	0.121	-0.021	0.428
Mary	F	36	0.092	-0.049	0.119	0.241	0.017	-0.046	-0.081	-0.206	0.045	0.102
Melissa	F	25	-0.157	-0.488	-0.209	-0.361	-0.316	0.001	-0.343	0.353	0.236	0.250
Panzee	F	14	-0.143	0.200	-0.269	0.098	0.207	0.165	0.350	-0.012	-0.307	-0.257
Rebecca	F	13	0.272	0.250	0.145	-0.173	-0.538	-0.067	0.028	-0.202	0.211	-0.244
Shirley	F	18	-0.156	0.134	0.059	0.135	0.086	-0.410	-0.297	-0.401	-0.195	-0.050
Suwanee	F	18	0.486	0.554	-0.191	-0.252	0.130	-0.235	0.006	0.156	-0.070	0.438
Suzanna	F	25	-0.471	0.093	-0.380	0.128	-0.146	0.007	0.406	-0.106	-0.185	-0.646
Sylvia	F	7	0.556	0.496	0.345	-0.193	0.276	-0.011	0.144	0.606	-0.313	0.334
Artemus	M	12	0.255	-0.011	-0.470	-0.038	-0.333	0.036	-0.272	-0.241	0.194	0.338
Arthur	M	14	-0.674	-0.120	-0.324	-0.225	-0.406	-0.176	0.210	-0.064	-0.296	0.290
Barney	M	16	0.141	0.268	-0.148	-0.090	0.223	-0.145	0.017	-0.232	0.202	-0.005
Carl	M	16	0.048	0.325	-0.470	-0.201	0.166	0.206	0.022	0.686	0.399	0.139
David	M	25	0.015	-0.101	-0.123	0.534	-0.315	-0.066	-0.459	-0.274	0.527	-0.256
Duff	M	13	0.189	0.142	-0.198	-0.141	-0.182	-0.263	-0.396	-0.091	-0.291	0.644
Elwood	M	14	0.531	-0.017	0.181	0.185	-0.154	-0.147	-0.390	-0.207	-0.486	-0.310
Fritz	M	14	-0.201	0.069	0.205	-0.133	-0.247	0.251	0.295	0.889	0.389	-0.257
Iyk	M	44	0.090	0.210	-0.164	-0.442	0.166	0.107	-0.448	0.180	-0.418	-0.235
Jarred	M	15	0.279	0.602	-0.189	-0.256	-0.116	-0.056	-0.064	-0.052	0.387	-0.699
Jcarter	M	19	-0.093	-0.092	-0.267	-0.574	-0.091	0.053	-0.370	-0.274	-0.489	-0.022
Jolson	M	13	-0.152	0.099	-0.001	0.126	-0.242	-0.190	-0.013	0.336	0.297	-0.323
Justin	M	19	0.047	0.029	-0.030	-0.221	-0.066	-0.038	-0.185	0.041	-0.251	-0.282
Lamar	M	16	0.178	0.131	-0.292	-0.074	0.100	-0.255	0.193	0.170	0.473	0.433
Lazarus	M	20	0.135	-0.089	-0.371	-0.193	-0.112	0.083	-0.003	0.196	-0.136	-0.044
Lux	M	22	-0.212	0.050	-0.161	0.264	-0.084	-0.306	0.144	0.090	-0.684	0.047
Merv	M	20	0.167	0.187	0.122	-0.125	-0.038	0.005	0.066	0.103	0.458	-0.504
Patrick	M	11	0.006	-0.060	0.364	0.059	-0.420	0.591	0.181	-0.104	0.297	0.721
Rogger	M	34	0.426	-0.038	0.151	0.020	0.213	-0.259	-0.379	0.008	0.195	-0.085
Rowena	M	15	-0.221	0.358	0.373	0.526	-0.463	-0.084	0.044	0.068	-0.594	0.412
Scott	M	17	-0.352	0.256	-0.196	0.159	-0.341	0.098	-0.018	-0.007	0.130	-0.470
Winston	M	15	-0.383	-0.124	-0.322	0.043	-0.228	-0.455	-0.494	0.491	-0.082	-0.386
Woodruff	M	27	0.087	-0.258	-0.075	-0.204	-0.292	-0.379	-0.123	-0.287	0.259	0.313

Values represent AQ values. AQ1 is the most anterior and region 10 is the most posterior section through the brain (see Fig. 2A). AQ, asymmetry quotient; F, female; M, male. Ages are rounded to the closest year.

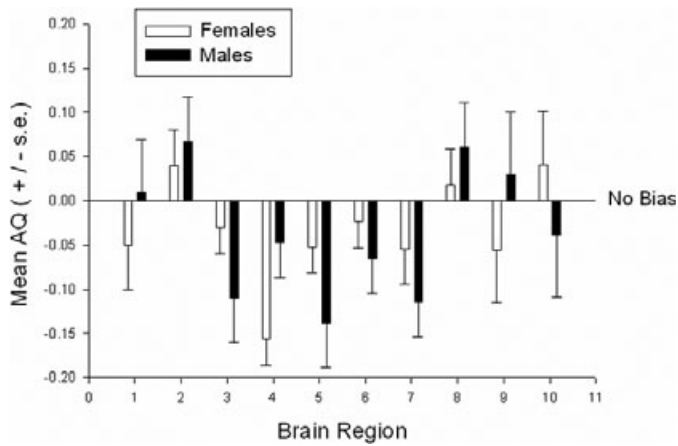


FIG. 3. Mean asymmetry quotient (AQ) values (\pm SEM) for males and females for each of the 10 slices. Significant leftward asymmetries were found for regions 3, 4, 5 and 7, respectively (see text for details).

$F_{9,477} = 2.13$, $P < 0.03$ (Fig. 3). One-sample t -tests for each set of AQ values indicated significant leftward asymmetries for regions 3 $t_{55} = 2.15$, $P < 0.05$, 4 $t_{55} = 2.91$, $P < 0.05$, 5 $t_{55} = 3.12$, $P < 0.05$ and 7 $t_{55} = 2.54$, $P < 0.05$, respectively. No other significant main effects or interactions were found.

Associations between relative white matter and behavioral and brain asymmetries

In the next analysis, we tested whether variation in white matter asymmetries was associated with neuroanatomical and behavioral asymmetries. Both handedness and knob asymmetry data were available in all 56 chimpanzees, respectively. Recall that the chimpanzees were classified as being either right- or non-right-handed, with respect to handedness, and leftward or non-leftward for their knob asymmetry. Based on these classifications of asymmetry, a mixed model ANCOVA was performed with brain asymmetry (leftward, non-leftward), handedness (right, non-right) and sex serving as between-group variables, while the 10 AQ values served as the repeated measures. As with previous analyses, age was the covariate. A significant two-way interaction was found between brain asymmetry and sex ($F_{1,46} = 7.99$, $P < 0.003$), and a significant three-way interaction was found between sex, handedness and region ($F_{9,414} = 2.60$, $P < 0.03$) for the two-way interaction between brain asymmetry and sex. No significant differences were found between the overall AQ values for leftward (mean = -0.035) and non-leftward (mean = -0.029) females, but leftward males (mean = -0.109) had significantly lower AQ values compared with non-leftward (mean = 0.042) males.

For the three-way interaction between handedness, sex and region, the significant between-hand differences as a function of sex were found in regions 4, 5 and 8, respectively. Shown in Fig. 4A and B are the mean AQ values for non-right- and right-handed female and male chimpanzees for each region. For females, significant differences between non-right- and right-handed individuals were evident only in region 4. For males, significant differences between non-right- and right-handed individuals were found for regions 5 and 8, respectively. Where differences were found, right-handed chimpanzees had significantly greater leftward asymmetries than non-right-handed individuals.

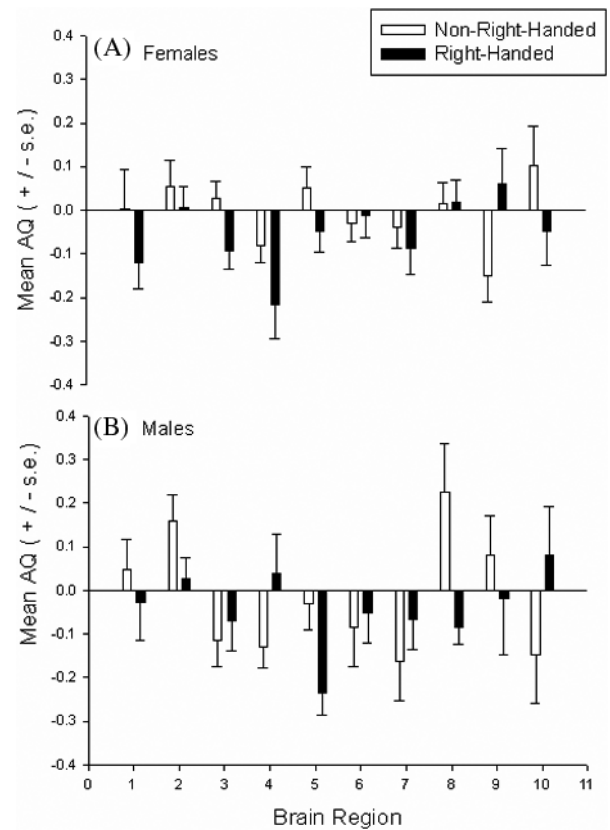


FIG. 4. Mean asymmetry quotient (AQ) values (\pm SEM) for each region in right- and non-right-handed (A) female and (B) male chimpanzees.

Discussion

Our results indicate that chimpanzees showed leftward asymmetries in regions 3, 4, 5 and 7, which roughly correspond to regions from the middle frontal lobe to posterior temporal lobes (see Fig. 2A). Region 3 corresponds to the premotor cortex, including the superior, middle and inferior frontal lobe. Regions 4 and 5 principally bifurcated the primary motor and somatosensory cortex and the anterior portion of the temporal lobe. Region 7 roughly corresponds to the anterior parietal lobe and posterior temporal lobe. One interpretation of these results is that the leftward asymmetries seen in these regions reflect the morphological leftward asymmetries that have been found using ROI approaches in chimpanzees. For example, population-level leftward asymmetries have been found for the inferior frontal gyrus and planum temporale in chimpanzees. It may be that the ROI asymmetries are a consequence of white matter expansion in these regions.

Alternatively, the evidence of increased left hemisphere white matter in these regions reflects greater connectivity between the language homologs of the chimpanzee brain. The arcuate fasciculus is a well-known pathway connecting Wernicke's and Broca's area in the human brain, and recent DTI studies in humans have shown that this pathway is larger in the left compared with the right hemisphere (Nucifora *et al.*, 2005). The arcuate fasciculus would roughly connect regions 3–7 in the brain, as quantified in these chimpanzee brains. Thus, our analysis of white matter may be indirectly measuring asymmetries in this same pathway of the chimpanzee brain, but more definitive data are needed to address this possibility.

Previously established associations between handedness and a morphological asymmetry in the precentral gyrus were similarly found when considering relative white matter asymmetries. Specifically,

differences in overall or regional variation in relative white matter were found as a function of hand use and asymmetries in the knob, although for both measures the effects were more robust in males compared with females. Apes with larger leftward asymmetries in the knob had more leftward asymmetries in relative white matter. Right-handed apes had relatively larger white matter asymmetries in region 5, the area that bifurcated the primary motor cortex. These overall findings suggest that asymmetries in specific cortical brain areas that are associated with both handedness and morphological asymmetries (e.g. the knob) may reflect greater white matter concentrations. This interpretation is consistent with at least one report in humans (Buchel *et al.*, 2004). Buchel *et al.* (2004) examined white matter asymmetries using DTI in a sample of 43 human subjects. Using a measure of fractional anisotropy (FA), they found greater FA in the dorsal portion of the left premotor cortex in right-handed individuals and the opposite pattern among left-handed individuals.

The association between brain asymmetry, handedness and white matter laterality was more pronounced in males compared with females. The evidence for a stronger association between behavioral and brain asymmetries in males compared with females is consistent with at least one previous report in chimpanzees (Hopkins *et al.*, 2007). In a recent study examining the association between handedness, brain asymmetries and corpus callosum morphology, Hopkins *et al.* found that right-handed males had greater leftward asymmetries in the inferior frontal gyrus compared with females, after adjusting for differences in the relative size of the corpus callosum. Thus, when considering overall brain lateralization in chimpanzees, either through intrahemispheric connectivity as reflected in white matter or via interhemispheric connectivity (i.e. relative corpus callosum size), the findings reveal consistent sex differences in brain and behavior associations.

Collectively, our results represent the first evidence of asymmetries in white matter and their association with handedness and morphological asymmetries in non-human primates. The findings further support the hypothesis that individual differences in behavioral and neuroanatomical asymmetries may arise from greater intrahemispheric connectivity between different brain regions. Lastly, the findings present yet further evidence that chimpanzees exhibit population-level lateralization in the nervous system, suggesting that this neurological trait was present prior to, rather than after, the split between humans and apes approximately 5–6 million years ago as suggested by some (Crow, 2004).

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Abbreviations

AQ, asymmetry quotient; DTI, diffusion tensor imaging; FA, fractional anisotropy; MRI, magnetic resonance imaging; PVC, polyvinyl chloride; ROI, region of interest; WMR, white matter ratio.

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