

Corpus Callosum Anatomy in Right-Handed Homosexual and Heterosexual Men

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Received: 26 May 2007 / Revised: 31 August 2007 / Accepted: 15 September 2007 / Published online: 2 November 2007
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Abstract The results of several studies have shown that homosexual men have an increased prevalence of non-right-handedness and atypical patterns of hemispheric functional asymmetry. Non-right-handedness in men has been associated with increased size of the corpus callosum (CC), particularly of the isthmus, which is the posterior region of the callosal body connecting parietotemporal cortical regions. We hypothesized that isthmal area would be greater in homosexual men, even among right handers. Twelve homosexual and ten heterosexual healthy young men, all consistently right-handed, underwent a research-designed magnetic resonance imaging scan. We found that the isthmal area was larger in the homosexual group, adding to the body

of findings of structural brain differences between homosexual and heterosexual men. This result suggests that right-handed homosexual men have less marked functional asymmetry compared to right-handed heterosexual men. The results also indicate that callosal anatomy and laterality for motoric functions are dissociated in homosexual men. A logistic regression analysis to predict sexual orientation category correctly classified 21 of the 22 men (96% correct classification) based on area of the callosal isthmus, a left-hand performance measure, water level test score, and a measure of abstraction ability. Our findings indicate that neuroanatomical structure and cognition are associated with sexual orientation in men and support the hypothesis of a neurobiological basis in the origin of sexual orientation.

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Keywords Corpus callosum ·
Magnetic resonance imaging · Sexual orientation ·
Homosexuality · Functional asymmetry · Hand preference ·
Cognition

Introduction

Numerous neurobiological correlates of sexual orientation have been observed or hypothesized (for review, see Mustanski, Chivers, & Bailey, 2002). These include genetic factors, as evidenced in family and twin studies (Pillard & Bailey, 1998) and in DNA linkage studies (Hamer, Hu, Magnuson, Hu, & Pattatucci, 1993; but see Rice, Anderson, Risch, & Ebers, 1999); hormonal factors (Hines, Brook, & Conway, 2004; van Anders & Hampson, 2005); neurophysiological factors including otoacoustic emissions (McFadden & Pasanen, 1998) and pheromone responses (Savic, Berglund, & Lindstrom, 2005); and hypothesized immunological factors, such as maternal antibodies (Blanchard, 2004). A few

postmortem studies have reported neuroanatomical correlates of sexual orientation, including size of the suprachiasmatic nucleus of the hypothalamus (Swaab & Hofman, 1990), size of the third interstitial nucleus of the anterior hypothalamus (INAH-III) (Byne et al., 2001; LeVay, 1991), and cross-sectional area of the anterior commissure (Allen & Gorski, 1992; but see Lasco, Jordan, Edgar, Petito, & Byne, 2002). Of the ~200 reports listed on Medline since 1990 on neuroimaging studies of homosexual people, all have focused either on brain health in HIV-positive individuals or on brain activity associated with exposure to sexual stimuli, but none on brain anatomy per se.

Behavioral and cognitive variables have also been studied in relation to sexual orientation. Numerous studies have found that homosexual men performed less well than heterosexual men on some tests of spatial ability (e.g., Gladue, Beatty, Larson, & Staton, 1990; Rahman & Wilson, 2003) and a few have shown that homosexual men perform better on some verbal tests (e.g., McCormick & Witelson, 1991; Sanders & Wright, 1997). However, as in the genetic and neuroanatomical studies, there is inconsistency in the evidence (see review in Zucker & Bradley, 1995). One of the most reliable findings to date is the increased prevalence of non-right-handedness among homosexual men and women compared to the general population: In some studies as high as 50% in men and 90% in women compared to 35% in the general population (see meta-analysis of 29 studies by Lalumière, Blanchard, & Zucker, 2000). Each of the studies which reported a greater incidence of left-handedness among homosexual people in the Lalumiere et al. review (e.g., Lindesay, 1987; McCormick, Witelson, & Kingstone, 1990) used stringent definitions of right-hand preference and thus broad definitions of left-hand preference, determined by hand-preference testing on extensive lists of manual tasks. Most studies which used broader measures of right-hand preference (e.g., Bogaert & Blanchard, 1996) found a tendency for similar results but the difference was not statistically significant. Some of the inconsistency among these studies may be related to the methodological difference in hand-preference classification.

Since hand preference is associated with the pattern of hemispheric functional asymmetry for language functions (Kolb & Whishaw, 2003), with left handers showing greater bihemispheric or more frequent right-sided representation of language functions compared to right handers, we suggested that the increased prevalence of non-right-handedness among homosexual individuals indicated less strong left-hemisphere specialization for language among groups of homosexual men and women (McCormick et al., 1990). The question arises whether there are two subgroups of homosexual individuals: Left handers with an atypical pattern of

functional asymmetry and right handers with the typical pattern; or whether even homosexual people with phenotypic right-hand preference have less hemispheric functional asymmetry compared to right-handed heterosexual people.

Hand preference has been shown to vary with the mid-sagittal area of the corpus callosum (CC) in men, but not in women. In postmortem study, non-consistent-right-handed (nonCRH) men were found to have a larger CC than CRH men. This was particularly true for the isthmus (the posterior region of the body of the CC), which showed a 60% difference between hand-preference groups (Witelson, 1989) and a significant correlation with degree of hand preference ($r = -.67$) (Witelson & Nowakowski, 1991). This association between handedness and CC anatomy has been replicated in numerous subsequent MRI studies (e.g., Cowell, Kertesz, & Denenberg, 1993) and especially between handedness and callosal isthmus (e.g., Tuncer, Hatipoglu, & Ozates, 2005), although some studies which did not use the consistent-nonconsistent handedness dichotomy found no evidence for a structure-function association (Luders et al., 2003; Steinmetz et al., 1992). An MRI study using diffusion tensor imaging (DTI) found greater molecular diffusion, indexed by fractional anisotropy, in left handers, interpreted to indicate a different microstructure, such as a greater number or density of axons in the CC of left- than right-handed men (Westerhausen et al., 2003).

The isthmus contains axons connecting right and left parietotemporal cortical regions (Hofer & Frahm, 2006), regions which mediate functions of language and spatial cognition which are asymmetrically represented (Kolb & Whishaw, 2003) and for which test scores have been found to differ between homosexuals and heterosexuals. Witelson (1989) hypothesized that the greater area of the callosal isthmus in nonCRH than CRH men may provide greater interhemispheric communication which may underlie the greater bihemispheric representation of language and spatial functions in left handers. The hypothesis that variation in callosal size is associated with variation in functional asymmetry is supported by findings that the CC was larger in subjects with right-hemisphere representation of language or with low laterality-scores on a verbal dichotic listening task compared to those with high laterality-scores indicative of left-hemisphere dominance (Morton & Rafto, 2006). In addition, Clarke, Lufkin, and Zaidel (1993) demonstrated that greater right-ear accuracy on verbal dichotic tests (indicative of left-hemisphere language representation) was negatively correlated with callosal size. It remains to be determined whether callosal size and particularly isthmus size which are associated with hand preference in men show differences between heterosexual and homosexual men, particularly between CRH groups.

Since sexual orientation is associated with hand preference and with measures of hemispheric functional asymmetry, and since hand preference is associated with anatomy of the CC in men, it was hypothesized that sexual orientation, at least in men, is associated with callosal anatomy, specifically the isthmus. To control the confound caused by the increased prevalence of non-right-handedness among homosexual men, our study focussed on the association between callosal anatomy and sexual orientation in strongly right-handed men. Specifically, it was predicted that homosexual men, even those who present phenotypically with strong right-hand preference, would have a greater callosal isthmus compared to CRH heterosexual men.

Method

Participants

A sample of 12 homosexual men who met the following specific criteria was recruited through advertisements on a university campus and in community-based organizations: (1) exclusive or nearly exclusive homosexual orientation with regard to current sexual activity or fantasy (scoring 6 or 5, respectively, on the Kinsey Scale, which ranges from 0 to 6) (Kinsey, Pomery, & Martin, 1948); (2) aged from 18 to 35 years; (3) no history of any neurologic or psychiatric disorder, nor known HIV-positive status based on detailed individual medical history interviews administered by a psychiatrist (AS). None of the potential participants interviewed had to be excluded on the basis of the medical interview; (4) consistent-right-hand (CRH) preference (see definition under the Hand-Edness section below).

The control group consisted of ten heterosexual men, who were recruited via advertisements on a university campus, scored 0 or 1 on the Kinsey scale, were CRH, and were free of medical illness. Cases were selected so that the homosexual and heterosexual groups were comparable for age (M age = 23 and 25 years, respectively) and levels of personal and parental education. Number of years of education was determined by interview. Two control cases of the original 12 had to be excluded due to problems with movement error in their MRI scans. All participants were Caucasian. All participants gave fully informed consent and the study was conducted in accordance with the Declaration of Helsinki and approved by the McMaster University Research Advisory Group. Table 1 provides descriptive data for age, educational levels, and hand-preference scores for the homosexual and heterosexual groups. The groups did not differ on any measure.

Table 1 Mean and SD for age, educational levels, and hand preference scores for heterosexual and homosexual men

	Heterosexual (<i>n</i> = 10)		Homosexual (<i>n</i> = 12)		<i>p</i>
	M	SD	M	SD	
Age (years)	23.3	4.9	25.4	5.0	ns
Education (years) ^a	14.5	2.0	14.7	1.9	ns
Father's education (years) ^a	14.7	2.8	13.4	3.4	ns
Mother's education (years) ^a	13.7	2.2	14.1	2.5	ns
Hand preference score ^b	8.9	0.3	8.7	0.8	ns

^a Years of education were determined by individual interviews

^b Range of hand preference score is 0–9

Measures and Procedure

MR Imaging

Each participant underwent an MR imaging research protocol. The MRI technician was blind to group identity of the participants. Scans were performed at Sunnybrook Health Sciences Centre with a General Electric Signa 1.5 T MR system. Using the standard head coil, an initial sagittal T₁-weighted scout image was done to obtain landmarks. This was followed by an axial T₂-weighted series to screen for any unsuspected neuropathology. From this T₂ series, the actual mid-sagittal axis of the brain was defined and a series of sagittal T₁-weighted slices through the central part of the brain was taken to view the CC for measurement. Superior and inferior saturation bands were used and the sequence parameters were TR/TE 400/20 ms, 20 cm FOV, seven 4-mm slices with a 1.5 mm interslice gap, 256 × 256 acquisition matrix and four NEX, giving an imaging time of 5.1 min. This method provided one slice on the mid-sagittal axis of the brain regardless of the tilt of the head in the imager or the position of the brain in the skull.

Corpus Callosum (CC)

Area measures of the total mid-sagittal surface of the hemisphere, the CC, and four CC subregions, defined to approximate major cortical regions, were made following the procedure developed previously with postmortem specimens (Witelson, 1989) and used in numerous subsequent studies (e.g., Hofer & Frahm, 2006) (see Fig. 1). The mid-sagittal area of the hemisphere was measured on the true mid-sagittal slice using the inferior border of the CC as the inferior boundary. To determine areas of the CC, hard

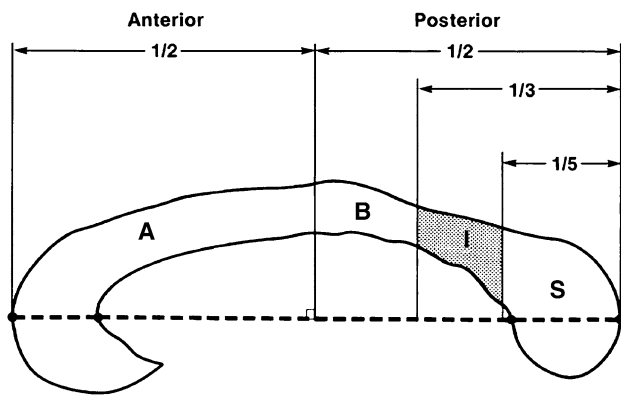


Fig. 1 The anterior–posterior maximal length of the CC was used as the linear axis to subdivide the CC into halves, posterior third (I + S) and posterior fifth (S) regions. The isthmus (*stippled* region), was defined as the posterior third minus the posterior fifth. A anterior half, B posterior mid-body, I isthmus, S splenium. Subdivisions adapted from Witelson (1989)

copies of the mid-sagittal view were printed along with a dimensional calibration scale and the magnification factor was used to obtain CC measurements. Outline of the CC was traced by hand twice by a technician blind as to the group identity of each case. Each tracing was then subdivided.

Area measurements were made for each subregion on each tracing with the use of a digitizer (Houston Instruments DT11BL Hipad having 0.125 mm resolution) and areas were calculated with a software package (Bioquant II Digitizing Morphometry Program A5-1A2; Bioquant Image Analysis Corporation, Nashville, TN, USA). On each tracing, each subregion was measured twice (until <2% difference between two consecutive measurements was obtained) and the mean values were obtained. The final value for each callosal subregion was the mean of the two mean values obtained from the two CC tracings.

Intra-rater reliability for area measures of each callosal region was consistently <1% and the intraclass correlation coefficients (ICC) were 0.99. To assess inter-rater reliability, a sample of scans was measured by a second rater. The mean difference between the two raters was 4.4 mm² (0.6%) for total CC area and 1.0 mm² (1.4%) for the isthmus, and the ICCs were 0.96 and 0.97, respectively.

Handedness

Hand-preference scores were based on demonstrated preferences on a nine-item test adapted from Annett's (1967) questionnaire in which the broom, shovel, and jar items were excluded. Scores for each item were +1, 0, and -1, for right hand, either hand or left hand, respectively. CRH preference was defined as in previous work as all right or right with

some "either" preferences, but no left-hand preferences (Witelson, 1989).

Since there is little variation in hand-preference scores among CRH individuals and even multiple-item tests of hand preference may not discriminate motoric laterality among strong right handers at the end of the continuum of right-handedness, the Hand Performance Test was given as a continuous measure of asymmetry in manual performance, adapted from Tapley and Bryden (1985). It involves filling as many circles with dots as quickly as possible within a time limit. Scores were the number of correctly filled circles for each hand across two 20-s trials. A laterality score was also calculated as $(R - L)/(R + L)$.

Cognitive Tests

A small battery of tests was given to each participant to document their cognitive status. The Shipley Institute of Living Scale (Shipley, 1940) was given as a test of general intellectual function and includes Vocabulary and Abstraction subtests. Accuracy scores were obtained. Three tests of spatial cognition were given: (1) Primary Mental Abilities-Spatial Relations (Thurstone & Thurstone, 1962), a measure of mental rotation ability (score was the number of correct responses minus any incorrect answers completed in a 7-min time limit); (2) Water Level Test (Thomas, Jamison, & Hummel, 1973), a measure of spatial perception (score was number of correct responses); and (3) Hidden Patterns (Ekstrom, French, Harman, & Dermen, 1976), a measure of spatial visualization (score was total number of correct responses in two 12-min test segments).

The Making Sentences Test was selected as a measure of written word fluency (score was total number of correct sentences in two 2.5-min test segments), and the Number Comparison Test was given as a test of perceptual speed (score was number of correct responses minus any incorrect answers, completed in two 1.5-min test segments) (Ekstrom et al., 1976).

Data Analysis

Independent sample *t*-tests were used to compare group differences with alpha level $p = .05$. Because of our selection criteria, scores on the Kinsey scale were at the extreme ends of the scale, resulting essentially in a dichotomous variable and preventing correlational analyses. Consequently, a multiple logistic regression analysis was used (SPSS, Version 12) to test prediction of sexual orientation category using anatomic and cognitive variables.

Table 2 Mean and SD for anatomical variables for homosexual and heterosexual men (area in mm²)

	Heterosexual (<i>n</i> = 10)		Homosexual (<i>n</i> = 12)		<i>p</i>	Cohen's <i>d</i>
	M	SD	M	SD		
Anterior half	383.84	41.47	401.37	43.14	Ns	0.41
Posterior mid-body	79.84	9.87	85.11	13.70	ns	0.44
Isthmus	71.89	12.29	82.48	13.07	0.06*	0.83
Splenium	209.94	24.06	226.30	32.62	ns	0.57
Total CC	745.51	55.77	795.26	91.10	ns	0.66
Mid-sagittal area	7,996.64	451.65	8,379.65	653.18	ns	0.68

* With one-tailed test, *p* = .03

Results

Table 2 presents area measures for the total CC and subdivisions for homosexual and heterosexual men. Since we had the directional hypothesis for the isthmus that it would be greater in the homosexual group, a one-tailed *t*-test for the isthmal region was used (*p* = .03). No other CC subregions reached statistical significance. Effect sizes were of medium to large magnitude, with the isthmus having the largest value (Table 2).

Table 3 presents the scores for the cognitive tests for the homosexual and heterosexual groups. Since all participants were CRH, right-hand performance was greater than left-hand performance within each group (*p* < .001). There were no significant differences between groups on any of these cognitive tests. However, left Hand Performance score was lower in the homosexual than heterosexual group and this difference approached significance (*p* = .06), as did the laterality score (*p* = .09).

Multiple logistic regression analysis was done to predict group membership (homosexual or heterosexual) using the four CC regions and the ten psychological test measures (see Tables 2, 3). A stepwise (forward selection) regression (*p* = .15 and .20 for entry and removal) provided a model including four variables: Left Hand Performance, isthmus, Shipley Abstraction and Water Level, which was significant (*p* < .001, Nagelkerke *r*² = 85%) and had a 96% correct classification rate, with one male heterosexual misclassified. In the model, each variable had a *p* < .10 (two-tailed *t*-tests). There was no evidence for unequal variance for any of these variables, or for multicollinearity or interaction among the variables.

Discussion

A difference was obtained in the size of the callosal isthmus between right-handed homosexual and heterosexual men. As predicted, callosal isthmus size was greater in the homosexual men. The larger isthmal area in even strongly

right-handed homosexual men suggests that they may have a brain structure and organization more typical of left handers, regardless of their manifest right-hand preference. Based on these findings, even strongly right-handed homosexual men have some evidence of atypical functional asymmetry. These anatomical results are consistent with previous results from neuropsychological studies indicating that right-handed homosexual men showed less functional asymmetry compared to right-handed heterosexual men based on verbal

Table 3 Mean accuracy scores and SD of cognitive variables for homosexual and heterosexual men

	Heterosexual (<i>n</i> = 10)		Homosexual (<i>n</i> = 12)	
	M	SD	M	SD
Hand performance				
Right ^a	97.50	15.28	93.92	19.36
Left ^a	71.90	11.00	61.75	12.37*
Laterality ^b	0.15	0.08	0.21	0.07**
Shipley				
Vocabulary ^c	32.60	3.27	32.58	3.26
Abstraction ^d	37.00	2.36	35.00	4.05
Primary mental abilities ^e	44.40	6.98	42.50	11.41
Water level ^f	5.10	1.66	4.33	1.61
Hidden patterns ^g	90.80	20.75	90.83	16.55
Making sentences ^h	12.60	4.17	12.00	3.95
Number comparison ⁱ	46.20	9.36	48.92	5.49

* *p* = .06, two-tailed *t*-test

** *p* = .09, two-tailed *t*-test

^a Maximum score for each hand is 208

^b $(R - L)/(R + L)$

^c Maximum score is 40

^d Maximum score is 40

^e Maximum score is 70

^f Maximum score is 6

^g Maximum score is 201

^h Maximum score is 20

ⁱ Maximum score is 96

dichotic tasks (McCormick & Witelson, 1994), less functional asymmetry on divided visual-field tasks (Sanders & Wright, 1997), and on neurophysiological EEG measures (Wegesin, 1998).

To our knowledge, this is the first report of an association between callosal anatomy and sexual orientation and it is consistent with a report of greater cross-sectional area of the anterior commissure in homosexual than heterosexual men (Allen & Gorski, 1992). A recent study of men and women with gender identity disorder and transsexualism studied shape of the CC and found that it was atypical for genetic sex and more similar to the opposite sex (Yokota, Kawamura, & Kameya, 2005). Our findings add to the body of literature showing a relationship between brain structure and sexual orientation. Using twin studies, cross-sectional area of the CC has been shown to have a strong genetic basis (94% heritability, Scamvougeras, Kigar, Jones, Weinberger, & Witelson, 2003; 82% heritability, Hulshoff Pol et al., 2006), indicating that experiential factors have a relatively minor role compared to heredity for variation in callosal size. The association of sexual orientation with a brain structure having high heritability supports a genetic component to sexual orientation.

The Hand Performance Test was selected to examine the possibility that a continuous measure of manual motoric laterality might reveal some left-hand dominance not observable via hand preference in the homosexual men. The Hand Performance Test results were consistent with the hand-preference scores, indicating that both groups were strongly right-handed. Thus, in right-handed homosexual men, laterality of motor functions is not related to callosal anatomy as it is in heterosexual men. By inference, the pattern of functional asymmetry for cognitive functions of language and spatial cognition appear dissociated from the representation of motoric laterality. Consistent with this speculation is our previous finding that right- and non-right-handed homosexual men showed no statistically significant difference on verbal dichotic laterality scores, in contrast to the difference observed between right- and non-right-handed heterosexual men, leading us to suggest a lack of association between motoric and verbal cerebral lateralization in homosexual men (McCormick & Witelson, 1994).

In this small sample, no reliable group differences were observed on the cognitive measures. The similarity between groups on the Shipley test attested to the comparable general intelligence between groups. Homosexual men tended to perform less well on the Water Level Test as consistently reported in the literature (McCormick & Witelson, 1991; Sanders & Ross-Field, 1986). On two verbal tasks, Shipley Vocabulary subtest and Making Sentences, the two groups were almost identical, again as in some previous studies (Gladue et al., 1990).

A logistic regression analysis was used as a combinatorial analysis to detect a possible reliable result from several

weak findings. A model using four scores (isthmal area, left Hand Performance, Water Level Test score, and the Shipley Abstraction score) predicted 96% correct classification of sexual orientation in our sample of 22 men. These factors, of course, are only one possible set among those variables documented to date and those likely to be delineated associated with sexual orientation in men. It is noted that because our ratio of number of observations to predictors was low, there is increased likelihood of chance findings with this analysis (Peng, So, Stage, & St. John, 2002).

The participants in the present study were stringently selected and the groups were carefully matched, but the results are limited due to low power. Our specific results require replication in larger samples and should be considered hypothesis generating, as for example in further studies using DTI. This kind of technology has already demonstrated the association of handedness and sex of the individual with microstructure of the CC (Westerhausen et al., 2003). Future work may be able to assess the relationship between sexual orientation and the microstructure of the callosal isthmus using these techniques and help elucidate what histologic differences may exist. In summary, the present results provide some evidence that sexual orientation is associated with callosal anatomy and, by inference, with the pattern of functional asymmetry, and that in homosexual men motoric laterality may be dissociated from other cognitive functional asymmetries.

Acknowledgments This work was supported by research grants NIH NS18954 and MRC 10610 to S.F.W. A.S. held an Ontario Mental Health Foundation fellowship and D.M.K. held an Ontario Graduate Scholarship, while at McMaster University. We thank Dr. Charles Goldsmith, Department of Epidemiology and Biostatistics, McMaster University, for statistical advice.

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TITLE: Corpus Callosum Anatomy in Right-Handed Homosexual
and Heterosexual Men

SOURCE: Arch Sex Behav 37 no6 D 2008

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