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Handedness and the corpus callosum: A review and further analyses of discordant twins.

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Abstract

Handedness effects with respect to regional corpus callosum (CC) anatomy remain open to question. Midsagittal CC tracings were obtained from structural MRIs in 21 female monozygotic twin pairs with discordant handedness (MZHd). The CC was divided into 99 percentile widths which were grouped into seven regions based on Denenberg's (1989, 1991b) factor analysis. Results showed that left handed (LH) twins had significantly larger regional widths in CC region W22-39 compared to right handed (RH) twins, an effect present in 19/21 MZHd pairs. Cross-study analyses revealed CC W22-39 to have the highest variation across female singletons (Cowell et al., 1992, Cowell et al., 1993). In the adjacent genu region (W3-18), CC size did not differ between RH and LH twins. However, when twins were reclassified according to handedness direction and consistency to form consistent RH, non-consistent RH, consistent LH and non-consistent LH groups, patterns of CC size in W3-18 closely matched those of singleton women (Cowell et al., 1993). Namely, CC W3-18 was larger in consistent compared to non-consistent RHs. Results support a claim that CC region W22-39, interconnecting premotor cortex in females, provides for environmentally influenced components of handedness, given the difference within MZHd twin pairs. By contrast, CC W3-18, connecting prefrontal cortex, was sensitive to direction and consistency of handedness, both in twins and singletons, a result consistent with combined genetic and environmental effects. Findings highlight the significance of MZHd twin studies in elucidating the developmental mechanisms underpinning structure-function asymmetry, cortical interconnectivity and neurodevelopmental bases of left hand preference.

Key words: monozygotic twins, corpus callosum, cortical white matter, laterality, handedness.

Introduction

“What does it mean to be left handed?” The answer to this enduring question requires consideration of diverse factors and multiple neurocognitive systems within developmental gradients. There is neither a unique behavioural phenotype (Levy and Reid, 1976), nor a single underlying causal agent. However, understanding the structure and function of neural systems that underpin fundamental differences between right and left handers is one way to address this key question about human asymmetry. Our study focuses on the regional anatomy of the corpus callosum (CC), a structure with an extensive history of research findings related to hand preference, typically in singleton samples. To improve the matching between handedness groups, we used a twin based model to help control for the multiple variables which could serve as confounding factors in such research. Thus, the regional features of the CC are described and considered, within a naturally controlled paradigm to examine handedness effects. Specifically, we describe results from a sample of healthy adult monozygotic twins strongly discordant for writing hand (MZHd).

Mapping structure-function relationships in the callosum is not straightforward. Challenges arise with respect to anatomical features of the structure, and its role in mediating a range of complex behavioural and cognitive systems. In anatomical terms, the CC interconnects left and right cerebral cortex via networks of fibres that transfer sensory-motor information, support interhemispheric cognitive function, and serve a range of excitatory and inhibitory functions in the mediation of lateralised behaviours (Aboitiz et al., 1992, Clarke and Zaidel, 1994, Fabri and Polonara, 2013). Starting with research in the rhesus monkey (Pandya and Seltzer, 1986), followed by decades of fibre tracking studies (De Lacoste et al., 1985), including *in vivo* neuroimaging reports in humans (Huang et al., 2005, Hofer and Frahm, 2006), the detailed topography of cortical connectivity in terms of anterior-posterior and overlapping dorso-ventral gradients has been documented. Yet, efforts to subdivide the

gross anatomy of the structure in humans, as visualised in its midsagittal aspect, have only partially succeeded at mapping neurofunctionally relevant zones of interest. Callosal fibers can connect homotopic or homologous versus heteropic or non-homologous cortical regions (Di Virgilio and Clarke, 1997, Clarke, 2003, Ruddy et al., 2017). This complicates the task of identifying functionally specific interhemispheric fibre bundles in studies using planimetric methods to measure gross morphometry. Thus, at a macroscopic level, structure-function relationships may be obscured by the overlapping topographical gradients between antero-posterior and dorsal-ventral organisation of cortical fibre tracts. There is also heterogeneity in the composition of anatomical fiber types (Aboitiz et al., 1992, Hofer and Frahm, 2006), and their functions at a microscopic level.

Neuropsychological challenges exist in pinpointing the behavioural specificity required to measure discrete task-structure relationships. Despite this, correlates have successfully been demonstrated between regional CC anatomy and behaviouro-cognitive measures linked to lateralised verbal and manual systems such as hand preference (Witelson, 1989, Denenberg et al., 1991b, Cowell et al., 1993), speech perception (Clarke et al., 1993, Clarke and Zaidel, 1994, Gadea et al., 2009), verbal fluency (Hines et al., 1992) and verbal expression (Moreno et al., 2014).

In adult males, correlations between the CC isthmus and consistency of right handedness (Witelson, 1989, Denenberg et al., 1991b, Cowell et al., 1993) have been attributed to structure-function relationships emerging from key (plausibly hormonally related) developmental events which shape cortical laterality in temporo-parietal language regions (Habib, 1989). Additional evidence for handedness-based differences in the organisation of language systems was reported by Moffatt et al. (1998), who found a larger CC in left handers with left hemisphere speech lateralisation, compared to left handers with right hemisphere speech, and right handers. Neuroimaging evidence is consistent with a

pivotal role for the CC in establishing left hemisphere specialisation for language. This takes place prior to maturation into the adult pattern of intra-hemispheric connections (Tzourio-Mazoyer, 2016). The impact of atypical CC structure and function is documented in the clinical literature on callosal agenesis (Sauerwein and Lassonde, 1994, Paul et al., 2003), surgical lesions (Pelletier et al., 2011), injury (Benavidez et al., 1999), and degeneration (Reinvang et al., 1994, Varley et al., 2005). Thus, the CC has been studied in relation to its role in mediating behaviour, particularly in lateralised systems, and as an index of developmental and degenerative events which shape cerebral asymmetries in conjunction with changes in callosal connectivity.

A within twin pair handedness discordance model can be pivotal to exploration of structure-function relationships in the callosum relevant to asymmetries in manual fine motor function and language for the following reasons (Gurd et al., 2013). First, it permits simultaneous consideration of the contribution of genes, genes plus environment, and interactions between the two - as a function of experience within a developmental trajectory. Key developmental factors include potential *in utero* foetal positioning of twins, birth effects such as timing and sequence (i.e., potential exposure to anoxic events) (cf. Smith et al. (2007) and Vuoksimaa et al. (2017)), childhood hand usage, and development of laterality preference and practice within the socio-cultural context of dextrally oriented cultures and environments (cf. Gurd et al. (2006)). Second, handedness discordance is a useful model since the environmental bias introduced by living in a right handers' world is readily verifiable and consistent as an effect within twins reared together. In contrast to studies of singletons, the MZHd twin model confers sharper focus on evaluations of structure-function relationships between gross regional CC anatomy and hand preference with its capacity to enhance detection of effects whilst reducing statistical "noise" linked to variance swamping (Rosch et al., 2018).

We describe in-depth profiles of regional CC anatomy in female MZHd twins. We start with a detailed analysis of regional CC size effects to corroborate and expand upon previously published CC results (Gurd et al., 2013). Following this baseline analysis, a fine-grained series of comparisons evaluates patterns of variation in regional CC anatomical size in relation to multifaceted features of handedness, regional neuroanatomical correlations and associations with overall CC size. The intention is to shed light on how the callosum of right and left handers is formed structurally within a life course perspective, and to consider how it functions in relation to the asymmetrical lateralisation of motor and verbal behaviours (Gurd et al., 2013, Gurd and Cowell, 2015, Rosch et al., 2018).

Methods

Participants: Twenty-one MZHd (monozygotic handedness discordant) adult female twin pairs were included (mean age=51.67±1.96; range 37-67 yrs). The right-handed (RH) and left-handed (LH) twins were matched on years of further-plus-higher education (RH (n=21) mean = 3.29, median=3, range 1-11 years; LH (n=19) mean =2.53, median=3, range 1-5 years; $t(18)=1.69$, n.s.) and IQ (RH mean=117.33, range 89-136; LH mean=118.29, range 98-139 years; $t(20)=0.73$, n.s.). All volunteers were purportedly healthy adults, selected randomly from a larger group of 100 similar pairs recruited through the UK Adult Twin Registry (Spector and Williams, 2006) according to the criteria that they be strongly contrastive on laterality and strength of handedness preference for writing, i.e., scoring +/- 2 on item 2 of a 5-point scale of strength of handedness preference for the Handedness Preference Inventory (HPI), be available to travel to Oxford, and suitable to undergo MRI brain scanning. In RH twins, HPI ranged from +70 to +100 (mean=95.71, median 100) and in LH twins, HPI ranged from +20 to -100 (mean=-72.24, median=-90). Zygosity had been established by questionnaire with 91% reliability (Jackson et al., 2001) and/or by zygosity testing through the UK Adult Twin Registry. Moreover, all twins had been raised together

and by their biological parents, had English as a first language, had no indication of atypical or delayed language development (which is less common in females and in female twins (Hay et al., 1987, Thorpe, 2006)), and no indication that any twin had switched handedness in childhood. Testing was carried out between 2003 and 2004, as part of a larger study. The study had local ethics committee approval (COREC), and all participants provided informed consent. All studies were conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Image acquisition: High resolution structural MRIs were acquired at the John Radcliffe Hospital Oxford Centre for Magnetic Resonance, Oxford on a 1.5 T Siemens Magnetom SONATA (Erlangen, FRG) MRI scanner. Anatomical whole brain images were obtained using a T1-weighted, 3D gradient-echo pulse sequence (FLASH, fast low-angle shot) with the following parameters: TR=1200 ms, TE=5.6 ms, TI 19° flip angle, matrix size = 160 x 256 x 208, voxel size = 1mm isotropic, acquisition=coronal, averages=3.

Image analysis: Brain Voyager v1.9 software (www.brainvoyager.com) was used to obtain midline sagittal slices and standardize brain alignment. Images were iso-voxeled to 1 x 1 x 1 mm and spatially transformed to the sagittal plane. Image alignments were then corrected along the anterior commissure-posterior commissure axis such that the sagittal plane was perpendicular to the points at which both commissures crossed the midline. Midline sagittal slices were then extracted and verified through inspection of landmarks in sagittal, coronal and axial views of the three dimensional data set. Criteria for selecting the midline sagittal slice were: (1) In sagittal view, the callosal sulcus, peak of the fourth ventricle and cerebral aqueduct should be clearly visible; (2) in axial view, the slice should pass through the midline of the third ventricle, pineal gland and cerebellar vermis; (3) in coronal view, the slice should pass through the septum pellucidum. Each CC outline was traced by a single rater (PEC) blind to the identity of participants using a computer program (Callosum). Software

developed at the University of Sheffield was based on the principles of the original Denenberg et al. (1991a) software, and has been used in prior anatomical reports (Wignall et al., 2010, Gurd et al., 2013, Rosch et al., 2018). The software automatically bisected the traced outline into dorsal and ventral perimeters. These were divided into percentiles, and the correspondingly numbered points along the dorsal and ventral surface were connected to provide 99 width measurements along the curved anterior-posterior CC axis. The software optimized the placement of the dorsal-ventral division such that the sum of the 99 widths was minimized (Figure 1, panel A).

[Figure 1 here]

Each CC was traced five times to average out variations in manual co-ordination and other forms of rater based error. Coefficients of variation ($CV = SD/mean$) were obtained for the five tracings of area, perimeter, axial length and 99 widths to confirm that CVs for all measures were lower than 10% across the five tracings. If area, perimeter, central length or more than ten individual widths had CVs greater than 10% then a new set of tracings was taken. Measurements for area, perimeter, central length and 99 widths were computed for all cases using the mean of the five tracings. The 99 width measurements were then averaged into clusters following the factor analytic approach of Denenberg et al. (1991b): W3-18, W22-39, W49-62, W65-74, W77-85, W89-94, and W95-99 (Figure 1, panel B). The original factor structure derivation is briefly summarised here. Analysis involved the 99 widths, CC area, length, perimeter and an estimate of total brain area for 104 healthy adults (male and female right and left handers). Data were standardised into z-scores within the four sex by handedness groups and entered into a principal components analysis for purposes of anatomical dimension extraction. The final factor structure was obtained through oblique rotation for a model that retained factors with eigen values greater than 1. Variable loadings of 0.600 and above determined inclusion of which measures were associated with each factor.

Brain area loaded on its own factor, CC widths loaded on seven factors as outlined above, with perimeter and length loading on W95-99.

Ten cases were used for assessment of inter- and intra-rater reliability of area, perimeter, length and the seven regional factor widths. Two raters (PEC, RR) produced measurements with ICCs in the good (W49-62: $r = 0.614$) to excellent range (all other measures: $0.763 < r < 0.946$) (descriptors are from Fleiss (1986) page 7). One rater (PEC) reproduced measurements for the same ten cases within 6 weeks, resulting in ICCs that were all in the excellent range ($0.841 < r < 0.994$). All measurements presented in the current results were from one rater's tracings (PEC).

Statistical analysis. Data analysis was conducted in several stages. First, a detailed profile of the 99 widths was conducted to compare CC form along the anterior-posterior gradient for RH and LH MZ Hd twins. Statistical analyses for global (area, length) and regional (seven factor widths) CC measures were conducted for the full sample 21 twin pairs available in the MRI database. This was done to corroborate results on CC anatomy with the previously published sample of 17 MZ Hd twin pairs from the same database (Gurd et al., 2013).

Next, RH-LH MZ Hd twin pair correlations were examined in detail. The analysis of CC area paralleled the MZ component of a study by Scamvougeras et al. (2003) who examined CC area in MZ vs DZ twin pairs. Additional correlation patterns between RH and LH MZ Hd twins were examined for length and for the seven regional factor widths. Correlations among the global and regional CC measurements were also conducted separately within RH twins and LH twins. Due to the high correlations among CC widths (reported in the Results below), stepwise regression analysis was used to model the relationships between CC regions and area in RH and LH twins as a function of their

background demographics, overall HPI and the seven regional CC measures (Mendenhall and Sincich (2012), page 368).

In two final sets of analysis, CC factor width means for MZHd twins were compared to published findings in singleton women. In the first comparison, the mean values for twins were examined as a function of hand preference direction (right handed = RH; left handed = LH) and hand preference consistency (consistent = c; non-consistent = nc) (Cowell et al, 1993). This comparison of cRH, ncRH, ncLH and cLH women was conducted across all CC regions. In the second comparison, absolute range (in mm) and relative range values (a ratio of the range/median) were computed from the published means of: (a) four handedness groups of cRH, ncRH, ncLH, cLH singleton women mentioned above (Cowell et al, 1993); and (b) four comparably aged groups of singleton women at 31-40, 41-50, 51-60 and 61-79 years (Cowell et al., 1992). This allowed characterisation of variability profiles across the seven CC factor width regions in healthy singleton women. Means for the seven CC regions in LH and RH MZHd twins were considered in relation to these healthy singleton profiles. Comparative analyses involving previously published data are presented in the Discussion.

To support the use of parametric statistical analyses, preliminary tests were conducted for normality and to confirm equal variances. Data distributions for CC area, length and the CC factor width measures for LH and RH twins were normally distributed (as confirmed by non-significant Shapiro-Wilk tests), with the exception of W89-94 in LH ($W(21)=0.88$, $p\leq0.05$). Variances for the paired groups of LH and RH twins were equal (as confirmed by non-significant Pitman's t-tests), with the exception of W77-85 ($t(19)=2.45$, $p\leq0.05$). Variances for the independent groups of consistent and non-consistent right and left handers examined for W3-18 were also equal (as confirmed by non-significant Levene's tests). Therefore, parametric statistical tests have been applied throughout, and have been confirmed

with non-parametric comparisons for regions where normality and equality of variance assumptions were not fully met.

Results.

The ninety-nine percentile widths of the CC for RH and LH twins are plotted in Figure 2. The curves overlapped for the majority of widths except for those spanning CC widths 21 through 43. In this zone, LH twins showed larger widths than RH twins. The span of widths which was larger in the LH twins, overlapped most closely with factor region W22-39.

[Figure 2 here]

Means and standard errors are presented in Table 1 for RH and LH twins for CC area, length and the seven CC factor widths.

[Table 1 here]

Paired t-tests were conducted for area, length and the seven regional factor width measurements. Bonferroni corrections ($p \leq 0.007$) were applied to the seven factor width comparisons. Only one region, W22-39, showed a significant difference with a larger mean width in the LH twins ($t(20)=3.43$, $p \leq 0.01$) comprising a large effect ($ES=0.75$, SD of the difference= 0.738) (Cohen, 1992). This was consistent with the 99 width profile comparison above and with previous results published for a subsample of $n=17$ twin pairs (Gurd et al., 2013). The LH-greater-than-RH difference remained significant when W22-39 was examined as a percent of overall CC area ($t(20)=2.910$, $p \leq 0.01$; correlation between W22-39 and CC area for RH $r(19)=0.764$; LH $r(19)=0.732$, $p's \leq 0.01$). The statistical significance of

within-pair RH/LH contrasts relies in part on the consistency of effect direction. Results on this aspect of the data are reported below.

[Table 2 here]

Pearson's correlations between RH and LH MZHd twins were significant for all measures (Table 2). For the two global measures, the highest correlations were for CC area. For this measure, an equivalent number of points above and below the identity line reflected the lack of significant overall CC size difference between RH and LH twins (Figure 3a). For regional factor width measures, correlations were highest in the anterior, mid and posterior CC body regions of W22-39, W49-62 and W65-74. A pattern of lower correlations was observed in the anterior most (W3-18) and the more posterior regions (W77-85, W89-94 and W95-99) (Table 2). A Pearson's correlation of $r=0.849$ in regional CC size between RH and LH twins was observed for W22-39. This relationship is depicted in Figure 3b. The magnitude of the correlation was similar to that seen in CC area. However, in W22-39, the majority of points fell below the identity line. This reflected the larger regional size of W22-39 in LH twins for 19/21 cases. ICCs for absolute agreement were also significant for all measures, and generally paralleled the patterns observed with Pearson's correlations. In W22-39, the absolute agreement ICC was lower than for the consistency based Pearson's correlation. This pattern is attributable to the consistency of the size difference within MZHd twin pairs. Specifically, the vast majority of LH twins showed larger regional widths for W22-39 than their RH counterparts even when examined as a percent of CC area (Figure 3c).

[Figure 3 here]

Pearson's correlations between adjacent CC regions were significant and ranged from $r(19)=0.602$ to $r(19)=0.787$ in RHs and from $r(19)=0.613$ to $r(19)=0.781$ in LHs (p -values

≤ 0.05). The exception to this was W3-18, which showed a low and non-significant correlation with W22-39 in both RH ($r(19)=0.187$) and LH twins ($r(19)=0.185$). W3-18 was most strongly correlated with the non-adjacent isthmus region W77-85 in both RH ($r(19)=0.494$) and LH ($r(19)=0.525$) twins (p -values ≤ 0.05). A comparably high correlation was observed between W3-18 and W89-94 ($r(19)=0.525$, $p \leq 0.05$) but only in LH twins.

Stepwise regression was used to analyse the dependent measure CC area as a function of the predictor variables Age, IQ, HPI, and the seven regional CC factor widths. Separate regressions were conducted for LH and RH twins. CC area was predicted by the same three variables in both models, which accounted for 95% of the RH twins' variance ($F(3,17) = 117.55$, $p \leq 0.001$), and 92% of the LH twins' variance ($F(3,17) = 66.72$, $p \leq 0.001$). The greatest contributors were CC W49-62 (RH: $b=0.462$, $t(17)=6.19$, $p \leq 0.001$; LH $b=0.384$, $t(17)=3.85$, $p \leq 0.01$), W95-99 (RH: $b=0.397$, $t(17)=7.45$, $p \leq 0.001$; LH $b=0.472$, $t(17)=6.33$, $p \leq 0.001$) and W65-74 (RH: $b=0.421$, $t(17)=5.69$, $p \leq 0.001$; LH $b=0.332$, $t(17)=3.38$, $p \leq 0.01$). This result, together with the correlations described above, indicates a similar RH to LH pattern of inter-regional and region-to-whole CC relationships in these MZ Hd twins.

RH and LH twins were then grouped on the basis of HPI scores. When all responses were right handed or left handed, consistent RH (cRH, $n=16$) and consistent LH (cLH, $n=10$) groupings were assigned. When at least one item was not consistent with writing preference, nonconsistent RH (ncRH, $n=5$) and nonconsistent LH (ncLH, $n=11$) groupings were assigned. All cRHs scored +100 and all cLHs scored -100. For ncRHs, HPI scores ranged from +70 to +90 (mean=82). For ncLHs, HPI scores ranged from -90 to +20 (mean=-47). Means and standard errors of the seven CC regional factor widths for the four groups are plotted in Figure 4. In RH twins, cRHs were larger than ncRHs ($t(19)=2.38$, $p \leq 0.05$) in W3-18 (Table 3). Within LH twins, there was no significant difference in regional CC size between cLH and ncLH groups.

[Figure 4 here]

Discussion

This paper links established findings on twins, handedness and the CC together with new and expanded analyses from our current research programme on MZHd twins to: (a) identify the most enduring trends from the past 25 years in relation to handedness and the CC in females; and (b) use these robust findings as a platform for examining the convergent evidence on CC organisation and the development of functional asymmetries. The approach also built on two important contributions to human CC research: (i) Sandra Witelson's seminal work on regional effects of handedness on the CC (Witelson, 1986); and (ii) Victor Denenberg's stereological measurement and factor analysis method of CC regional quantification in rodents (Denenberg et al., 1989) that was subsequently adapted for use with humans (Denenberg et al., 1991a, Denenberg et al., 1991b). Both employed a continuous reflective method in which biobehavioural paradigms were developed to examine structure-function relationships within a life span development context. This approach underpins a body of work documenting wide ranging regional effects in CC anatomy using stereological and factor analytic approaches to conduct fine-grained analysis of consistent and non-consistent right and left hand preference and to examine sex and age in relation to regional CC form and size (Denenberg et al., 1991b, Cowell et al., 1992, Cowell et al., 1993, Cowell et al., 1994). This furnished the conceptual background, methodology and comparison data for our analyses in our current study.

In-depth anatomical profiles of the CC in MZHd twins used regional divisions based on Denenberg's statistical approach. The method, based on stereological measurements of 99 callosal widths (Denenberg et al., 1991a) and seven CC subregions derived via factor analysis (Denenberg et al., 1989, Denenberg et al., 1991b), has withstood the test of time. Its factor

analysis has been replicated (Cowell et al., 1994, Peters et al., 2002) and is reflected in results of more recent neuroimaging research. Hofer and Frahm (2006) used DTI-based tractography to identify seven distinct fibre tracts which they parcelled into 5 regions. From anterior to posterior, Hofer and Frahm's regions were (I) prefrontal, (II) premotor and SMA, (III) motor, (IV) sensory and (V) comprised of 3 fibre bundles connecting parietal, temporal and occipital cortical regions¹. The above seven fibre tracts identified by Hofer and Frahm (2006) correspond topographically with the seven regions derived through factor analysis of the 99 percentile widths measured along the curved anterior-posterior CC axis, as originally described by Denenberg et al. (1991b). However, not all models partitioning the human CC have reached consensus as to the number and topography of regional subdivisions. As part of a detailed series of analyses including a callosal mapping of cortical regions defined by Brodmann's areas, Chao et al. (2009) compared CC segmentation in 12 adults to DTI based mappings in 8 adults produced by Hofer and Frahm (2006). Both groups isolated three CC regions associated with (pre)frontal, premotor/SMA and primary motor cortices. Yet, the distribution of these regions along the anterior-posterior axis of the CC was not consistently aligned which may be due to the combined differences in methodological approach and human participants sampled (Chao et al., 2009). In relation to our study, it is important to note the difference that handedness can confer on the organisation of interhemispheric fibre tracts of the frontal cortices as a function of right-left hand differences in men and women Hagmann et al. (2006).

The factor structure model was shown to be robust to both handedness and sex (Cowell et al., 1994) and as such remains a stable statistical method for characterizing regional CC structure. This is demonstrated further through a comparison of data from the current study of twins and comparable measures from previous publications in singletons. CC W3-18 means

¹ Overlapping such that they could not be separated geometrically by vertical lines.

for the four handedness groups are presented in Table 3. The pattern of results across the four handedness groups for W3-18 was consistent with the values from the singleton samples (Cowell et al., 1993). In MZHd RH twins, and in singleton right-handed women, cRHs had larger widths than ncRHs. Trends in the opposite direction were observed for consistent left-handers (cLH) versus non-consistent left-handers (ncLH), albeit smaller in magnitude.

[Table 3 here]

Absolute and relative ranges derived from eight groups of singleton women are presented in Table 4 for the seven CC factor widths. These data were from published reports of four groups of singleton women as a function of hand preference direction and consistency (cRH, cLH, ncRH, ncLH) (Cowell et al., 1993) and four groups of singleton women as a function of ages comparable to our twin database (31-40, 41-50, 51-60 and 61-79 years) (Cowell et al., 1992). Data are summarised as a function of samples drawn from the two previous studies of handedness and age and their combined ranges (Table 4). The region with the highest degree of variability across the data from previously published singleton studies was W22-39. For each of the singleton datasets and their combination, W22-39 showed the largest absolute and relative ranges.

[Table 4 here]

In the results and further comparisons above, findings from particular regions were combined to shed light on the complex interplay of variables which contribute to development of neurofunctional relationships in manual and related cognitive asymmetries. Results are considered from the perspective of shared genetic and environmental backgrounds in MZHd twins, in contrast to the differences resulting from a range of environmental and developmental factors that contribute to handedness discordance.

CC area in RH and LH MZ twins (Table 2) showed an intraclass correlation (ICC) of 0.876. This is consistent with Scamvougeras et al.'s (2003) ICC of 0.87 (young adult male and female MZ twins, unstated hand preference). It is also consistent with Haberling et al.'s (2012) ICCs for total CC size of 0.931 (MZ pairs characterized as 'right-shift positive') and 0.932 ('right-shift negative') as related to measures of handedness and cerebral lateralisation for language. These results indicate a stable effect within the literature and provide a solid basis against which to consider regional analysis of the CC in our current sample of MZ twins. They are consistent with a view that the strength of correlation is associated with monozygosity, and appears robust to gender and hand preference differences between the studies and samples.

Against the backdrop of MZ related anatomical covariance, results from the current regional CC dataset confirm that only the anterior body region differed significantly within MZ twins as a function of the direction of hand preference for writing (i.e., RH versus LH twins). This was observed within the profile of the 99 widths, falling between percentile widths 21 through 43, and in the mean width for factor W22-39, the region shown previously to correlate with lateralised covert verbal fluency activation in the inferior frontal cortex (Gurd et al., 2013). This expands on our previous work demonstrating regional differences in CC size between MZ twins (Gurd et al., 2013). Specifically, W22-39 which interconnects the premotor cortex (Hofer and Frahm, 2006) was larger in LH twins for over 90% of the MZ pairs; a finding that holds for the raw measurement and regional width as a percentage of overall CC size.

Given the common genetics and shared early environments within twin pairs, there is a strong argument for the combined role of distinct developmental, environmental and experiential factors in shaping the relationship between left handedness and larger callosal widths in region W22-39. There are several plausible explanations. In terms of directionality

of causation, it is possible that perinatal events in one twin formed the basis for a wider callosum, which in turn provided the neurofunctional basis for left handedness. It is also possible that left hand use *per se* contributed to increases in connectivity between right and left premotor cortex. These combined effects on CC W22-39 may also reflect late development of manual use (Gurd et al., 2006), manual motor learning (Palmis et al., 2017) and changes in interconnectivity between fine motor systems and related language regions used in writing (Varley et al., 2005, Pelletier et al., 2011). Since the above premises are not mutually exclusive, it is possible that all are to some extent involved, either within or between pairs (cf. Gurd et al. (2013)).

Hand preference is evident in early years (age 5 at least), and is well established prior to the maximum life span exposure of twins to shared environmental effects, reached by adolescence and beyond (Brun et al., 2009). In MZHd twins, our view is that unique environmental factors contribute to the differential development of handedness in MZHd twins in early life. This is consistent with reports that genetic effects account for only 24% of sample variance related to hand preference (Medland et al., 2009). Once neurobehavioural differences in the twins' developmental trajectory are in place, they are accentuated by the cumulative long term exposure of living in the context of either a right hander's or a left hander's experiences. Thus, contrasts in interhemispheric measures of premotor cortical white matter in MZHd twins are congruent with left handedness being both a result and a source of unique environmental variance. This sheds new light on the question of what it means to be left handed from a developmental perspective.

Importantly, the wide range of values for CC W22-39, observed in data from female singletons and twins across three studies, is consistent with the interpretation that this region has a high degree of variability across subgroups of the adult female population. What sets the W22-39 region in the MZHd sample apart from samples of singletons is its high rate of

inter-individual variation combined with the high degree of RH-LH twin correlation. Conceptual mapping of the statistical pattern therefore, highlights W22-39 as a CC region with high between twin-pair variability (comparable to the between group variability observed in singletons), and a highly consistent direction of LH greater than RH callosal widths effect. The direction of this effect in MZHd twins ($LH > RH$), mirrors previous findings in healthy adult male singletons (Denenberg et al., 1991b, Cowell et al., 1993) which may be consistent with the presence of perinatal hormone effects (van Hemmen et al., 2017). Moreover, lateralised speech and language systems also appear to be sensitive to hormonally mediated effects in the perinatal development of female twins (Cohen-Bendahan et al., 2004) and singletons (Moreno et al., 2014). Although the source and timing of hormonal exposure cannot be verified in our MZHd twins, perinatal stress may also be involved (Smith et al., 2007).

Evidence from the constellation of characteristics observed for W22-39 in the current and previous reports on our MZHd twin sample (Gurd et al., 2013, Rosch et al., 2018) supports a more general view that handedness-related variation in cortical frontal systems connected via CC W22-39 is likely to be environmentally but also, potentially hormonally mediated. It is important to note that the cross study comparisons to Cowell et al. (1992, 1993) indicate that W22-39 and the interconnected cortical regions of the frontal lobe (Hofer and Frahm, 2006) may be zones of cortical plasticity with particular sensitivity to factors that impact direction of hand preference (Hagmann et al., 2006). Left handed populations have tended to operate in cultural/technological environments better suited to (or biased in favour of), right handers. And yet, left handers still retain a manual superiority for function in right body-centred hemispace (Banissy et al., 2012) and are more likely to have reversal or absence of the left hemisphere population asymmetry for language function (Pujol et al., 1999, Szaflarski et al., 2002, Pelletier et al., 2011), thus increasing the need for interhemispheric

connectivity of premotor regions. This is particularly true in left-handed writers with left hemisphere language lateralisation for tasks involving, e.g., written spelling (Varley et al., 2005, Glickstein and Berlucchi, 2008). Left handers are also more likely to be mixed-handed for other tasks, and/or to use both their left *and* right hands when compared to right-handers (Annett, 2004, Gurd et al., 2006). Thus for left handers, task sequences which involve visual-manual coordination, fine motor and verbal skills may require interhemispheric frontal network connections that are functionally distinct from those in right handers.

In a study of right and left hand fist closures, Pool et al. (2014) observed a contrast between the neural coupling of regions for right and left handers when using their non-preferred hands. In left handers, neural coupling during right hand use mirrored that seen in left hand use (Pool et al., 2014). However, in right handers, the left hand use invoked additional connections, reflecting more negative coupling across motor regions. Thus, additional inhibitory neural coupling (some of which involves motor and premotor regions of the right and left hemispheres), was required for right handers to use their left hand, whereas left handers showed matching patterns of neural coupling (i.e., in mirror image) for left and right hand use. This fundamental distinction highlights a changing role for the CC vis-à-vis handedness: the CC provides inhibitory capacity in right handers, but connective capacity in left handers despite similar behavioural contexts, and particularly when homotopic CC connections of primary motor cortex and heterotopic connections between motor, premotor and supplementary cortical areas are involved. The inference then, is that left handers (and their neural networks) appear well adapted to living in an environment shaped by the human population asymmetry for right hand preference, but use different interhemispheric structure-function mechanisms to adapt when using their non-dominant hand, compared to right handers. In this respect, our current report maintains consistency with our recent findings

pertinent to cerebellar structure and function. This showed distinct patterns of correlations in LH twins between anterior cerebellar asymmetry and CC W49-62 (Rosch et al., 2018).

A multifaceted array of components shapes the developmental trajectories giving rise to different structure-function relationships between the CC and hand preference in adults. These components can in part be disambiguated through comparison of effects observed in CC W3-18, the region anteriorly adjacent to W22-39. The form-function relationships observed for W3-18, the anterior-most callosal region, showed a number of characteristics distinct from those observed for W22-39. With respect to brain structure, W3-18 did *not* show size differences between RH and LH twins. Instead, differences in W3-18 paralleled effects observed in female singletons, specifically, consistent right handers had larger callosal widths than non-consistent right handers (Cowell et al., 1993). There were however, item-based differences in the scales used to measure hand preference indices between the current study and Cowell et al. (1993). HPI scores in the current study of MZHd twins included the full (modified) Oldfield hand preference battery. In contrast, a 5-item subset of the full battery was used with participants studied by Cowell et al. (1993). Nonetheless, cRH and cLH participants from both had completely rightward or completely leftward scores, respectively, on all items. These two groups are the most clearly matched in terms of fine motor asymmetry profiles, especially with respect to writing, for which they strongly preferred either the left or right hand. Indeed, the two groups of cRH women differed by less than two tenths of a millimetre in W3-18.

Callosal W3-18 showed one of the lowest correlations within MZHd twin pairs (i.e., between RH and LH). This indicates a set of developmental influences on regional callosal size for W3-18 (and related prefrontal cortical regions) distinguishable from those of W22-39. This dissociation is also consistent with Caille et al. (2005) who reported localised function mapping of interhemispheric manual motor function by studying people with

different lesions of the anterior callosum. For W3-18 in MZHd twins, values for the hand preference subgroups followed the same pattern as singletons, as indicated by the comparable means for consistent and non-consistent right and left handed women in both samples (Cowell et al., 1993). We interpret these combined effects as indicating that the callosal fibres connecting the prefrontal cortices in MZHd twins may be shaped by a similar set of environmental and neurodevelopmental effects to those of singletons (i.e., they are not artefacts of perinatal twinnedness or twin births). Another distinguishing feature of MZHd twins, is that W3-18 was the only factor width not correlating with its adjacent region. In both RH and LH twins, W3-18 was strongly correlated with W77-85. Moreover, W3-18 exhibited patterns of moderate RH-LH twin correlations similar in magnitude to those observed in the posterior regions (W77-85, W89-94 and W95-99). These four CC factor regions correspond to the four regions shown by Hofer et al. (2006) to have the lowest fractional anisotropy. This supports the view that distinct sets of developmental factors are involved in shaping the relationships between regional CC size in W3-18 versus W22-39 and the outlying cortical systems they interconnect.

A unique feature of W22-39, as revealed here, is its global sensitivity to variation - as expressed in the high variability derived from cross study comparisons, and the significant, directionally consistent, size difference between LH and RH MZHd twins. From a theoretical point of view, it may be concluded that some cortical systems (e.g., W3-18 and prefrontal cortex) are sensitive to factors that differentiate consistent and non-consistent subgroups of right handed female singletons and twins. In contrast, other cortical regions (e.g., W22-39 and premotor cortex), show an enormous range of variability across groups and individuals, yet, within genetically constrained systems such as MZ twin pairs, respond to common environmental influences in a unidirectional manner. Notably, in the regression analysis, a similar structure emerged for both sets of twins, where W22-39 did not contribute

significantly to the variance of total CC area. This result was due in part to the high correlation of W22-39 with other factors selected earlier in the stepwise process. The salience of this finding in the context of the full set of results is that W22-39 behaved in a similar statistical fashion in RH and LH twins in terms of its pattern of association with overall CC area and the surrounding CC regions. Despite this evidence for close organisational parallels in RH and LH twins' CC anatomy, the size of W22-39 differed significantly as a function of their handedness discordance. Thus, future interest will be in unpacking the exact meaning of, and statistical contributors to, these cortically distinct elements of neurodevelopment, and their functional significance to explaining individual and twin neurobehavioural organisation in health and disease.

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List of abbreviations:

Corpus callosum (CC)

Monozygotic twins discordant for handedness (MZHd)

Monozygotic (MZ)

Dizygotic (DZ)

Right-handed (RH)

Left-handed (LH)

Consistent right-handers (cRH) and non-consistent right handers (ncRH)

Consistent left-handers (cLH) and non-consistent left handers (ncLH)

Hand preference inventory (HPI)

Figure captions.

Figure 1. Panel A. Corpus callosum measurement at sagittal midline (anterior left; posterior right) using Callosum software. The perimeter is in blue, the central length in yellow, and the percentile widths in red. See text for details of the software and parameter measures (Figure previously published in Gurd et al (2013)). Panel B. Corpus callosum (anterior left; posterior right) with the seven regional factor width regions depicted. Factor widths were derived using similar measurement algorithms and factor analysis of 99 percentile widths as used by Denenberg et al. (1991b) (Figure previously published in Gurd et al (2013)).

Figure 2. Ninety-nine CC percentile widths presented as the mean values in mm (\pm SE) for right (RH, circles) and left handed (LH, triangles) twins.

Figure 3. CC area (mm^2 ; Panel A), CC W22-39 (mm; Panel B) and CC W22-39 as a percent of CC area (Panel C) for RH and LH MZ Hd twins. Data are plotted in relation to the regression line (solid) and the identity line (dashed) in order to depict the consistency of the direction of effects in W22-39 between LH and RH twins (as a raw measure and as a percent of CC area), in contrast to the CC area overall (for full explanation, see Results).

Figure 4. Mean (\pm SE) regional CC widths (mm) for RH (Panel A) and LH (Panel B) twins plotted as a function of handedness consistency. Data for consistent right (cRH) and left handers (cLH) are plotted in solid lines. Data for nonconsistent right (ncRH) and left handers (ncLH) are plotted in dashed lines.

Table captions.

Table 1. Means (\pm SE) for global and regional CC measures for right (RH) compared to left handed (LH) twins. Only the comparison of W22-39 was significantly different with LH greater than RH twins for this region. (All units are mm except area which is mm²; significance $p \leq 0.01^{**}$).

Table 2. Within twin pair correlations are presented for each CC measure, with Pearson's r values for relative agreement (upper row) and intraclass correlations (ICC) for absolute agreement (lower row). All correlation values were significant (p -values ≤ 0.01).

Table 3. Means (\pm SEs) for W3-18 in consistent and nonconsistent right handed (cRH, ncRH) and left handed (cLH, ncLH) women. Singleton values are from Cowell et al. (1993) and MZHd values are from the present study. cRH MZHd twins had significantly larger W3-18 than ncRH twins ($p \leq 0.05$).

Table 4. Ranges for values (in mm) of each CC region as a function of four age groups in singletons and four hand preference groups in singletons. Relative range ratios [range/median] are in brackets. The singleton datasets were derived from previous reports of the CC as a function of age (Cowell et al., 1992) and handedness (Cowell et al., 1993).

Figure 1.

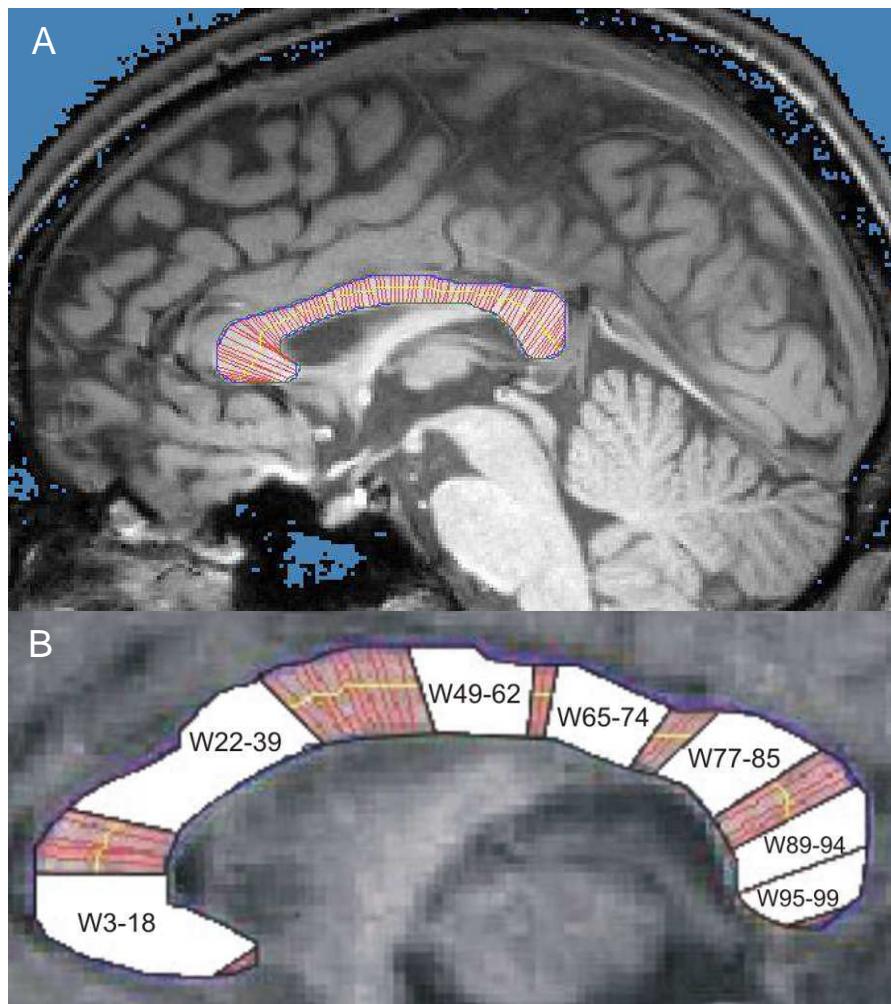


Figure 2.

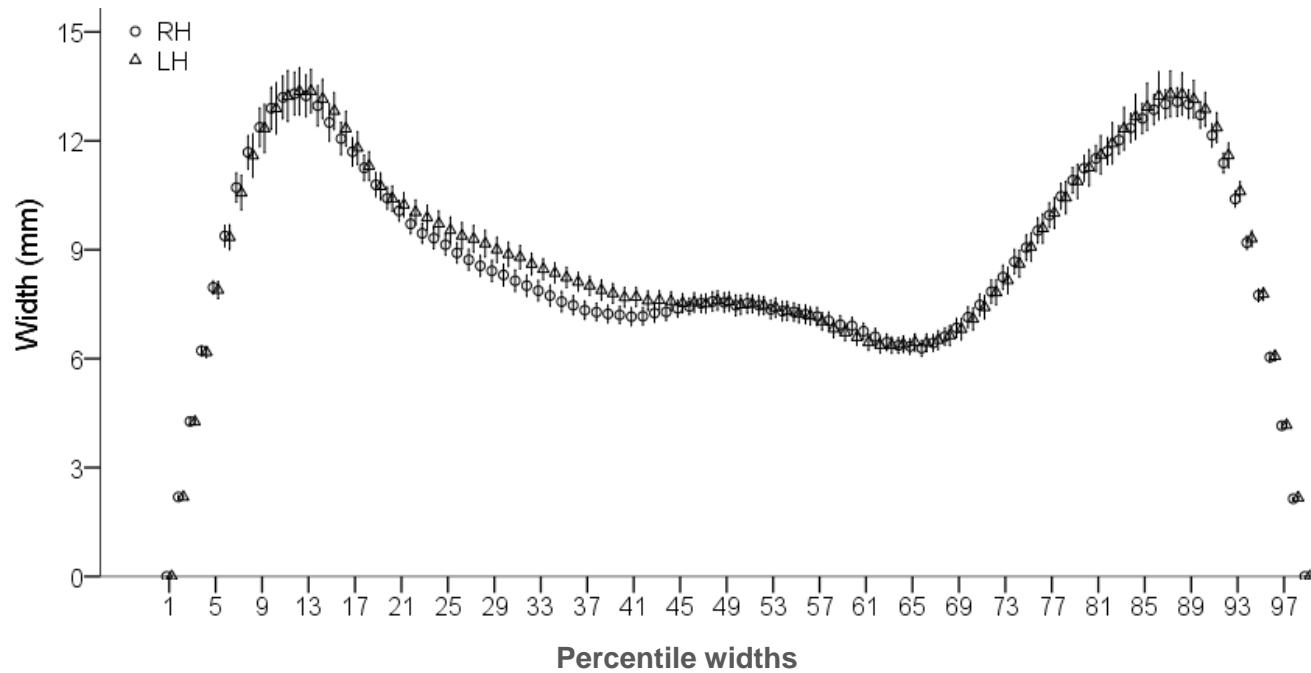


Figure 3.

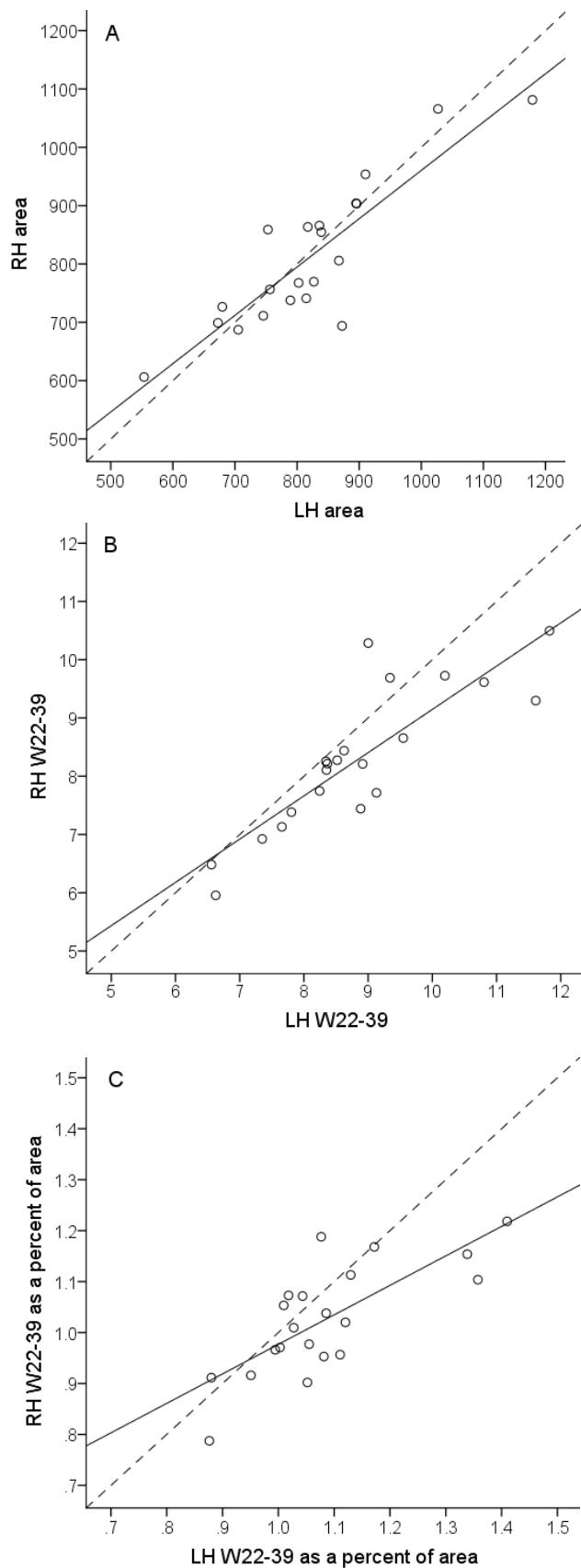


Figure 4.

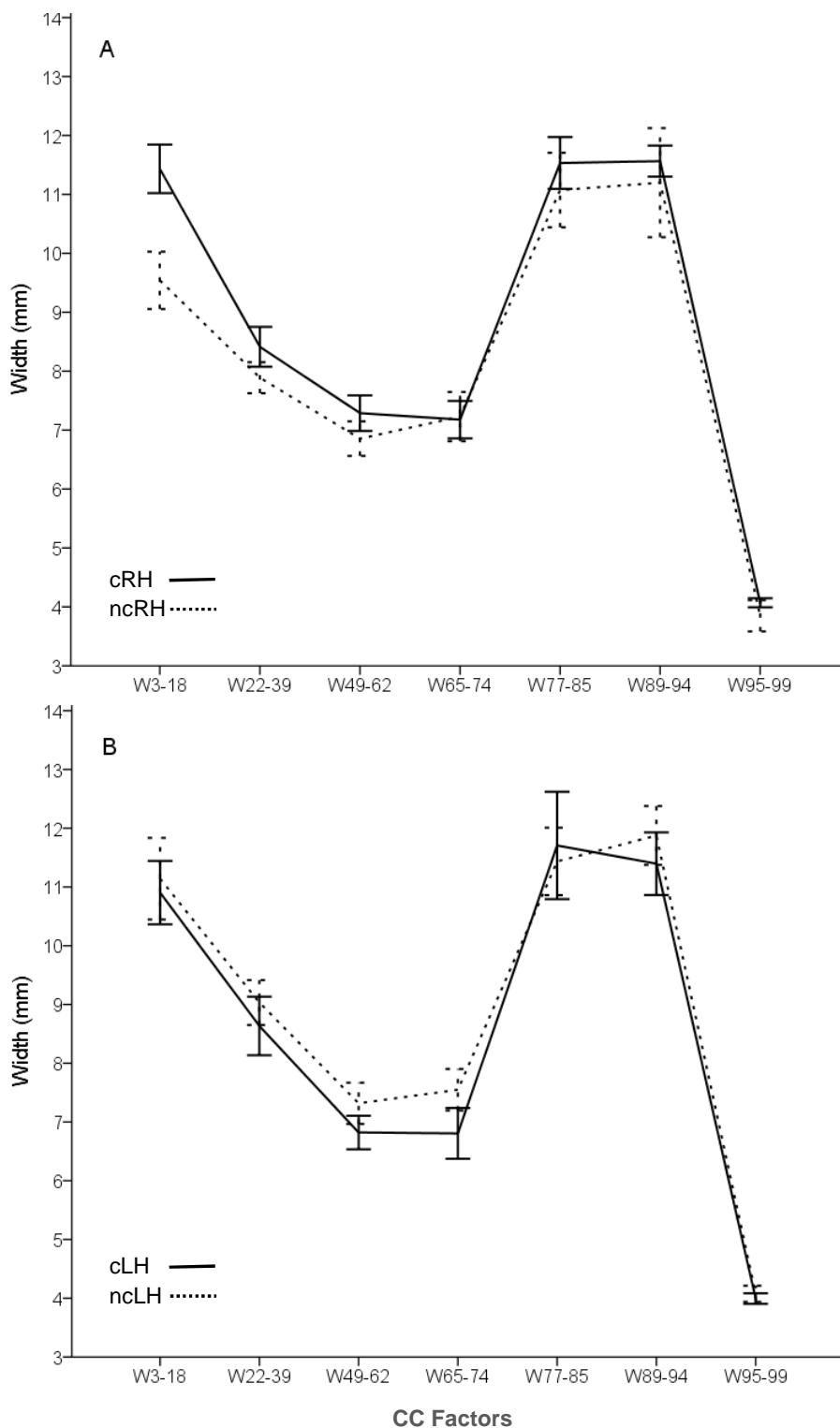


Table 1. Means (\pm SE) for global and regional CC measures for right (RH) compared to left handed (LH) twins. Only the comparison of W22-39 was significantly different with LH greater than RH twins for this region. (All units are mm except area which is mm²; significance p \leq 0.01**).

	Area	Length	W3-18	W22-39**	W49-62	W65-74	W77-85	W89-94	W95-99
RH	812.05 \pm 26.86	99.45 \pm 1.81	10.98 \pm 0.38	8.29 \pm 0.27	7.18 \pm 0.24	7.19 \pm 0.26	11.42 \pm 0.36	11.48 \pm 0.29	4.01 \pm 0.08
LH	820.86 \pm 28.36	99.97 \pm 1.77	11.03 \pm 0.43	8.84 \pm 0.31	7.08 \pm 0.23	7.19 \pm 0.28	11.56 \pm 0.52	11.65 \pm 0.36	4.04 \pm 0.08

Table 2. Within twin pair correlations are presented for each CC measure, with Pearson's r values for relative agreement (upper row) and intraclass correlations (ICC) for absolute agreement (lower row). All correlation values were significant (p-values \leq 0.01).

	Area	Length	W3-18	W22-39	W49-62	W65-74	W77-85	W89-94	W95-99
r	0.874	0.739	0.626	0.849	0.834	0.880	0.765	0.615	0.719
ICC	0.876	0.746	0.631	0.778	0.836	0.882	0.728	0.607	0.727

Table 3. Means (\pm SEs) for W3-18 in consistent and nonconsistent right handed (cRH, ncRH) and left handed (cLH, ncLH) women. Singleton values are from Cowell et al. (1993) and MZHd values are from the present study. cRH MZHd twins had significantly larger W3-18 than ncRH twins ($p \leq 0.05$).

Handedness Groups	Singletons	MZHd twins
cRH	11.57 \pm 0.45 (n=12)	11.43 \pm 0.41 (n=16)
ncRH	10.00 \pm 0.50 (n=15)	9.54 \pm 0.49 (n=5)
cLH	10.49 \pm 1.36 (n=6)	10.90 \pm 0.54 (n=10)
ncLH	10.78 \pm 0.56 (n=20)	11.14 \pm 0.69 (n=11)

Table 4. Ranges for values (in mm) of each CC region as a function of four age groups in singletons and four hand preference groups in singletons. Relative range ratios [range/median] are in brackets. The singleton datasets were derived from previous reports of the CC as a function of age (Cowell et al., 1992) and handedness (Cowell et al., 1993).

	Corpus callosum regional factor widths						
Dataset groups	W3-18	W22-39	W49-62	W65-74	W77-85	W89-94	W95-99
Singletons by Age	2.12 [0.19]	2.17 [0.23]	0.70 [0.11]	0.71 [0.10]	1.47 [0.12]	1.13 [0.10]	0.28 [0.06]
Singletons by Handedness	1.57 [0.15]	1.75 [0.21]	1.00 [0.16]	0.94 [0.14]	1.04 [0.10]	0.48 [0.04]	0.32 [0.06]
Singletons by Age and Handedness	2.22 [0.20]	2.55 [0.29]	1.00 [0.16]	1.11 [0.16]	2.31 [0.21]	1.13 [0.10]	0.99 [0.18]