

# fMRI and corpus callosum relationships in monozygotic twins discordant for handedness

J. M. Gurd · P. E. Cowell · S. Lux ·  
R. Rezai · L. Cherkas · G. C. Ebers

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**Abstract** To further investigate brain structure and function in 26 handedness discordant monozygotic twin pairs (MzHd), MRI and behavioural assessments were carried out. These showed significant correlation between language-specific functional laterality in inferior and middle frontal gyri, and anterior corpus callosum. Previous studies of handedness discordant monozygotic twins failed to resolve the issue concerning handedness and hemispheric laterality for language due to methodological disparities. The results would be relevant to genetic theories as well as to brain structure: function explanations. MzHd twins underwent MRI and fMRI scanning as well as behavioural assessment of motor performance and cognition. There were significant differences on MRI and fMRI laterality measures, as well as a significant correlation

between anterior callosal widths and functional laterality. LH twins showed higher frequencies of atypical functional laterality. There was no significant within-twin pair correlation on fMRI verbal laterality, nor did results show within-twin pair differences on verbal fluency or IQ. Implications for the field of laterality research pertain to frontal hemispheric equipotentiality for verbal processes in healthy individuals. In particular, there can be an apparent lack of cognitive ‘cost’ to atypical laterality. An fMRI verbal laterality index correlated significantly with corpus callosum widths near Broca’s area.

**Keywords** Asymmetry · Broca’s area · Corpus callosum · fMRI · Frontal lobes · Handedness · Laterality · Lateralization · Twins · Verbal fluency

J. M. Gurd (✉) · G. C. Ebers  
Nuffield Department of Clinical Neurosciences  
(Clinical Neurology), University of Oxford,  
The John Radcliffe Hospital, West Wing, Level 6,  
Headley Way, Headington, Oxford OX3 9DU, UK  
e-mail: jennifer.gurd@ndcn.ox.ac.uk

P. E. Cowell  
Department of Human Communication Sciences,  
University of Sheffield, Sheffield, UK

S. Lux  
Institute of Neuroscience and Medicine (INM-1),  
Research Centre Jülich, Jülich, Germany

R. Rezai  
Department of Pediatrics, College of Medicine,  
The University of Tennessee Health Science Center,  
Memphis, TN 38163, USA

L. Cherkas  
Department of Twin Research and Genetic Epidemiology,  
King’s College London, London SE1 7EH, UK

## Introduction

### Background

To further investigate brain structure and function in handedness discordant monozygotic (MzHd) twins, MRI imaging and behavioural assessments were conducted. Is it consequential vis-à-vis cognitive and cerebral function whether an *individual* is left handed? Such enquiries are fundamental to developmental human neurobiology and now addressable at an individual level. With handedness discordant (Hd) twins optimally matched left (LH) and right (RH) handers can be compared by selectively imaging one cortical hemisphere during cognitive paradigms, using region of interest analyses (Pujol et al. 1999). Sommer et al. (2002) first reported fMRI experiments on a series of MzHd twins. Three subsequent papers developed the work (Badzakova-Trajkov et al. 2010; Lux et al. 2008; Rosch

et al. 2010b), although with only 14 single sex pairs as the maximum (Badzakova-Trajkov et al. 2010), and no cross-correlation analyses of fMRI and structural anatomy and behaviour have yet been published. Sommer et al. (2002) described foetal chorionicity, and discordance of handedness and language lateralisation, but the evidence was constrained by subject factors (i.e. small and heterogeneous samples): They reported no within MzHd twin pair correlations among fMRI language laterality indices (cf. Stromswold 2006), their LH laterality indices did not differ significantly from zero, and sample size was limited (i.e.  $N = 13$  MzHd twin pairs, M:F = 5:8).

## Purpose

Following previous single case reports (Lux et al. 2008; Rosch et al. 2010b), Badzakova-Trajkov et al. (2010) asked how many MzHd twins show complete reversal on all fMRI measures, and whether evidence consistent with a single gene model would be found (cf. Annett 2002; McManus 2002; Peters et al. 2006). Their first question was addressed, but their second question remains contentious. Their full set of individual fMRI laterality indices was not provided; therefore it is difficult to compare studies. Badzakova-Trajkov et al. (2010) claimed overall significance of difference from zero on the verbal laterality indices, although cross-task analyses were not provided. They found ‘remarkably little difference in cerebral asymmetries between twins of opposite handedness and those who are both right handed’ (Badzakova-Trajkov et al. 2010, p. 3091–3092), which supported Sommer et al. (2002), with failure to find significant within-pair correlations on verbal fluency (word generation). However, their paradoxical claim was: ‘...consistent with a genetic role ... offering some support for genetic models such as those of Annett (2002) and McManus (2002).’ (p. 3092). 5/21 (24 %) MzHd twins were ‘mirrored’ on a word generation task, while 1/21 (5 %) had switched laterality (i.e. were cross lateralised) on both the verbal and visuo-spatial attention tasks as per Lux et al. (2008) and Rosch et al. (2010b). Differences between studies pertain to subjects, methods, and range of data collected and reported (e.g. subject numbers and homogeneity with respect to twin type, sex, and most importantly, strength of handedness preference, as well as age, chorionicity, and other birth events) (Stromswold 2006); factors with potential to alter statistical power and generalisability of evidence. Outstanding questions remain over within-pair birth weight differences (not reported in Sommer et al. 2002, or in Badzakova-Trajkov et al. 2010), significant due to the potential for twin–twin transfusion syndrome (TTS) in which large birth weight differences may be indicative of neurological risk in the smaller twin (Bhat 2011). fMRI

tasks have differed as have methods of data analysis (such as statistical treatment of asymmetry measures), making it difficult to compare studies. With the exception of Lux et al. (2008), and Rosch et al. (2010a, b), correction for individual differences in fMRI levels of ‘activation’ (bold effect, task versus baseline subtractions) per region of interest (ROI), have been lacking (cf. Nieto-Castanon et al. 2003), thus calling into question the reliability of grouped studies. Some volunteers are better ‘activators’ than others. The importance of highlighting individual values remains outstanding. Our study calculates the left versus right hemisphere activations divided by the sum, but referenced to the entire region’s activation on a percentage basis *per individual* (cf. Lux et al. 2008). Although the published studies share some similarities, there has been a tendency to neglect relationships between function and structure, including cognitive–motor (i.e. verbal) behaviour in well-matched MzHd twins that are required to explain cerebral asymmetry for language and its relationship to left handedness: this is our objective.

The current study builds upon three previous reports from our group (Lux et al. 2008; Gurd et al. 2006; Rosch et al. 2010b), designed to enhance structure–function mapping analyses with series of individual values plotted across four different levels of description: neuroanatomical measures of corpus callosum structure derived from high resolution anatomical MRI, neuro-functional asymmetry measures of the BOLD effect using fMRI, plus cognitive–motor asymmetry preference and performance. All MzHd twin volunteers were strongly RH and LH writers who were ideally matched across several factors, including sex (all female), strength of handedness discordance for writing. We suspected that stronger handedness discordance might be associated with greater lateralisation contrasts (including greater likelihood of atypical functional language asymmetry) (cf. Nettle 2003; Peters et al. 2006). All twins had been reared together and had normal language development. An original hallmark is calculation of fMRI activation levels relative to each individual’s own background level of activation per frontal region of interest (frontal ROI), represented as percentage levels of activation upon which asymmetry values are calculated [(% left hemisphere – % right hemisphere) divided by the sum of the two] (cf. Nieto-Castanon et al. 2003). The richness of the data (MRI and fMRI), and behavioural tests permits the quantification of relationships between different parameters of brain, language and hand asymmetry. These include anatomical measures from high resolution structural MRI scans, functional asymmetry measures from verbal fluency fMRI paradigms, questionnaire measures of limb usage preference, and experimental assessments of cognitive and motor task performance. The value of cross-task correlations and regressions is that they can be adjusted for the

non-Gaussian or heterogeneous nature of the data distributions by employing non-parametric analyses such as rank order statistics. This approach yields sufficiently broad data ranges, which permit detection of significant effects without dropping outliers (cf. Cowell et al. 2011). This non-classical twin paradigm supports case–control analysis and confers sufficient noise reduction to permit the emergence of significant patterns in cross-task relationships.

The broader research objective was to probe the bounds of ‘normality’ within data distributions in terms of central tendency and dispersion, as a function of right versus left handedness. Our study aimed to examine the outer limits of asymmetry in neurobiological substrates underpinning healthy cognition and to elucidate their functional significance in relation to theories of neurodevelopment (cf. Geschwind and Konopka 2009), an approach which contrasts with those of population-based studies in singletons (cf. Pujol et al. 1999). The study posed two broad research questions: (1) What percentage of LH twins show atypical functional cerebral asymmetry in fMRI frontal ROI analyses of inferior and middle frontal gyri (Broca’s area) activation during covert verbal fluency tasks; and (2) Does atypical LH functional laterality impact on cognition (vis-à-vis costs or benefits)? This systematic consideration of individual measures and inter-relationships among brain structure, task-activated brain function, behavioural performance, and behavioural preference examines constellations of characteristics observed in RH and LH twin samples which provide better genetic and environmental controls than comparable studies of unrelated singleton samples.

## Methods

### Overview

Twenty-six pairs of MzHd female twins were scanned using MRI and fMRI language paradigms. Behavioural testing included: laterality preference (Edinburgh Handedness Inventory, Waterloo Footedness Inventory), laterality performance (Tapley and Bryden dot filling, finger tapping, Annett’s peg moving), verbal performance (verbal fluency), and short intelligence scale (WASI).

### Participants

Twenty-six MzHd (monozygotic handedness discordant) adult female twin pairs were recruited (mean age 52.42, SD 9.47, range 33–67 years.). The twins were well matched on approximate mean years of further or higher education (RH = 2.85 SD 1.19, range 1–5 years; LH = 2.54, SD 1.36, range 0–5 years; paired *t* test, *t* = 0.95, *p* = 0.35, ns). All volunteers were purportedly healthy adults, selected

randomly from a larger group of 100 similar pairs according to the criteria that they be strongly contrastive on laterality and strength of handedness preference for writing (i.e. scoring  $\pm 2$  on a 5-point scale of strength of handedness preference for item 2 of the HPI described below), be available to travel to Oxford, as well as suitable (and willing) for MRI and fMRI brain scanning, as recruited by the UK Adult Twin Registry (Spector and Williams 2006). The study had local ethics committee approval (COREC), and all participants provided informed consent. Zygosity had been established by questionnaire with 91 % reliability (Jackson et al. 2001) and or zygosity testing by the UK Adult Twin Registry. Moreover, all twins had been raised together and by their biological parents, there was no indication of abnormal or delayed language development [which is less common in females and in female twins (cf. Hay et al. 1987; Thorpe 2006)], had English as a first language, and no indication that any twin had switched handedness (e.g. from left to right, in childhood). Testing was carried out between 2003 and 2004, as part of a larger study. All studies were conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Details that might disclose the identity of the subjects under study have been omitted. At testing, participants were interviewed for retrospective data on familial sinistrality, birth histories, as well as years of further or higher education (or relevant training), but there was no independent verification available from medical or educational records. In 5/20 pairs there was a sinistral family history (3 mother, 1 father, 1 grandfather). With respect to apparent birth histories: 96 % of pairs (25/26) were born by vaginal delivery. From Table 1 it can be seen that estimated birth order differed between the twins (RH: first born = 19/26, 73 %, LH first born = 7/26 = 27 %,  $\chi^2 = 11.07$ , *p* = 0.001). In those with reported positive familial sinistrality (i.e. parents, grandparents who were LH writers), the LH was born second in 3/5 cases. There was no significant difference in approximate birth weight within pairs (RH: mean = 2.40, SD 0.56, range 0.91–3.41 kg; LH: mean = 2.37, SD 0.63, range 0.91–3.47 kg; paired *t* = 0.41, *p* = 0.68, ns.). In 7/26 (27 %) of pairs, at least one twin had at least one apparent neonatal risk indicator (e.g. low birth weight, prematurity, suggestion of breathing difficulties, and incubation).

### Cognitive motor tasks: laterality preference, performance, verbal fluency, and IQ

#### Overview

All volunteers completed the handedness and footedness preference questionnaires as well as cognitive and fine motor tasks. The modified Edinburgh Hand Preference

**Table 1** Subjects: MzHd female twins ( $N = 26$  pairs), age, birth details, and mean verbal laterality index (xVLI)

Twin	Age	Hand	Birth order	Birth wt. (risks)	xVLI
1	51	RH	1	2.47	0.98
		LH	2	2.47	0.75
2	67	RH	2	2.02	0.74
		LH	1	2.02	0.83
3	48	RH	1	2.95	0.49
		LH	2	3.07	0.76
4	52	RH	1	0.91 (lbw)	0.69
		LH	2	0.91 (lbw)	-1.00
5	46	<i>RH</i>	2	2.47	0.73
		<i>LH</i>	1	2.47	0.76
6	37	RH	1	2.02	0.76
		LH	2	2.02	0.61
7	48	RH	1	3.32	0.50
		LH	2	2.41	-0.08
8	58	RH	1	1.82 (lbw)	0.89
		LH	2	1.56 (lbw)	0.44
9	54	RH	1	2.84	0.91
		LH	2	3.24	0.88
10	56	RH	1	2.27	0.91
		LH	2	2.27	0.35
11	47	<i>RH</i>	1	2.95	-
		<i>LH</i>	2	2.95	-
12	39	RH	2	2.16	0.99
		LH	1	2.73	0.57
13	37	RH	2	1.51 (prem)	0.90
		LH	1	1.11 (prem)	0.93
14	52	RH	1	2.41	1.00
		LH	2	2.19	0.42
15	57	<i>RH</i>	1	2.49	-
		<i>LH</i>	2	3.47	-
16	61	<i>RH</i>	2	2.36	0.86
		<i>LH</i>	1	2.47	-0.96
17	65	RH	1	?	0.26
		LH	2	?	-0.23
18	38	RH	1	3.18 (anoxia)	0.98
		LH	2	2.57	0.97
19	59	RH	1	2.27	0.71
		LH	2	2.27	0.96
20	45	RH	1	2.73	0.89
		LH	2	2.73	-0.23
21	64	<i>RH</i>	1	2.72	-
		<i>LH</i>	2	1.96 (lbw, incubator)	-
22	64	<i>RH</i>	2	2.05	-
		<i>LH</i>	1	2.05	-
23	48	<i>RH</i>	1	3.41	0.07
		<i>LH</i>	2	3.41	0.66

**Table 1** continued

Twin	Age	Hand	Birth order	Birth wt. (risks)	xVLI
24	56	RH	1	2.39	0.55
		LH	2	2.39	0.72
25	55	<i>RH</i>	1	1.91 (lbw)	-
		<i>LH</i>	2	2.18	-
26	33	<i>RH</i>	2	2.38 (prem, c-section, incubator)	-
		<i>LH</i>	1	2.18 (prem, c-section, incubator)	-

Italicized entries indicate subjects dropped from Fig. 4 (twins 5, 11, 15, 16, 21, 22, 23, 25, 26)

Age age at testing, Hand writing hand (strongly preferred), RH right, LH left, Birth wt. approximate birth weight (kg), risks birth risk indicators, lbw low birth weight, prem premature birth, anoxia anoxia at birth, c-section caesarean section, incubator, breath breathing difficulties, order: 1 first born, 2 second born

? Unknown

Inventory (HPI) was administered by post and returned prior to invitation for scanning, for selection and to ensure that no twin had changed hand preference during childhood (e.g. a switched left hander). The remaining tasks administered at testing were the Footedness Preference Inventory (modified Waterloo) (FPI) and fine motor performance tasks which assessed uni-manual asymmetry via finger tapping, dot filling and peg moving, tasks which varied in terms of practise (tap > dot > peg), and skill (dot > peg > tap) (cf. Gurd et al. 2006). Order of administration was counter-balanced with respect to tester, and writing hand of the twin (LH vs. RH, first), across all tasks. The twins were tested on the same day, at the same time (by different testers, in different rooms of the same testing suite), to avoid their discussing the tasks.

#### Handedness Preference Inventory

Prior to testing, the twins completed the handedness questionnaire used by Corey et al. (2001), made up of 16 items from both the Briggs and Nebes (1975) and the Edinburgh Handedness (Oldfield 1971) inventories. For each item on the questionnaire, a self-reported hand preference for the particular motor task is recorded as 'always right' (+2), 'usually right' (+1), 'no preference' (0) 'usually left' (-1) and 'always left' (-2). The questions pertain to: handwriting, drawing, throwing a ball, using scissors for cutting paper, brushing your teeth with a toothbrush, using a knife for eating (later deleted from the analysis), using a spoon for eating, using a hammer, using a sports racket (e.g. for tennis), holding a broom for sweeping (upper hand), holding the top of a shovel, striking

a match, unscrewing the lid of a jar, dealing playing cards (the card being dealt), holding thread to thread a needle, opening the lid of a box. Twins were selected for writing hand strength; RH = +2, LH = -2. The values per item were summed, divided by 30, and multiplied by 100 to yield a handedness preference index (HPI) ranging from -100 (extreme left preference) to +100 (extreme right preference).

Waterloo Footedness Inventory (modified)  
(Elias and Bryden 1998)

The questionnaire contained 10 items of the form “Is your foot preference for \_\_\_\_\_, *always right* (+2), *usually right* (+1), *no preference* (0), *usually left* (-1), *always left* (-2)”. Subjects were asked to circle the response, corresponding to their laterality of foot preference. The ten items we used to fill the blanks are: kicking a ball, hopping on one foot, smoothing sand with one foot, crossing your legs (leg on top), stomping on a bug, picking up a marble with your toes, stepping up onto a chair (foot which goes up first), writing your name in the sand with a big toe, stepping on a shovel (to push down), and standing on one foot. The responses were scored as indicated in brackets. Answers were summed, divided by 20, and multiplied by 100 to yield a footedness preference index (FPI) ranging from -100 to +100. Seven out of ten are items from the Waterloo Footedness Questionnaire—Revised (but reworded into British English): kicking a ball, hopping on one foot, smoothing sand with one foot, stepping up onto a chair (...), stomping on a bug, picking up a marble with your toes, stepping on a shovel (...).

Dot filling (Tapley and Bryden 1985)

The task involves marking as many dots as possible with a pencil, in 20 s (dots per 20 s), and as such, could be considered a task which is both practised and skilled. Subjects were presented with a single sheet of A4 paper, on which open circles (dots) were printed in 4 rows, linked at the top and bottom, to make a ‘W’ shape. There were four such arrays per page. On the upper left-hand array, there was an arrow pointing down at the top left, and the words ‘Left-hand start’. On the upper right array, an arrow pointing down was printed at the far right, and the words ‘Right-hand start’. (The bottom two arrays were identical.) Subjects started with the writing hand (A) and performed 4 trials ABBA. For example, a LH performed the Left-hand start at the top, followed by the Right-hand start at the top, followed by the lower Right-hand start, then the lower Left-hand start. A difference score (right-hand average minus left-hand average), and a laterality score  $[(R - L / R + L) \times 100]$  were calculated (where R and L refer to right and left hand, irrespective of writing hand).

Finger tapping (WPS electronic tapping test, ETT)

Finger tapping performance was measured using an electronic finger tapping test (WPS, ETT, Harcourt Assessment, 2004), (cf. Nalcaci et al. 2001). This unimanual task was non-practised, and non-skilled. The ETT is a compact electronic button box which automatically starts a 10 s timer as soon as the first tap is made. The digital display shows a dash (-) until 10 s are up—at which point it shows the number of taps made during that interval (i.e. number of taps per 10 s). Subjects were required to tap on the button as rapidly as possible with their index finger, for 10 s. Ten trials were run, 5 with each hand. The order of administration was ABABABAB, where A indicates the writing hand. The finger tapping was continued until the five scores for each hand were within a range of five, as per the manufacturer’s instructions. A laterality score was calculated using the equation  $[(R - L / R + L) \times 100]$ .

Peg moving (Annett 2002)

Following the procedures of Annett (2002), subjects were requested to move ten dowel pegs from one row of holes to another row on a purpose-built board which was placed on a desk. This task was not practised, but was skilled. Subjects were timed for their error-free (no dropped pegs) performance of trials for each hand (right versus left). They performed six trials in total, beginning with the writing hand (A), in the form ABABAB (where B refers to the non-writing hand). Results were scored as average time per hand, and the total was ‘left average minus right average’, such that the result is positive if the right-hand is faster (L and R refer to hand used, irrespective of dominance). The method of calculating laterality scores derived from Annett (2002) was  $[(L - R / L + R) \times 100]$ .

Verbal fluency (auditory word association)

The behavioural auditory word association task was spoken out loud. As part of a larger battery, it employed 2 single semantic categories (‘animals’, ‘furniture’). Subjects were requested to say words as quickly and accurately as possible (without repeating any items, or using any proper names such as people or places). This was timed and tape recorded. The score was mean number of correct items produced per 60 s per task type.

Wechsler Abbreviated Scale of Intelligence (WASI)  
(Wechsler 1999)

IQ was assessed using a short form (WASI) comprising Vocabulary, Block Design, Similarities, and Matrix

Reasoning subtests, and providing age-scaled scores (*T* scores), and IQ estimates for Verbal, Performance and Full-Scale IQ.

## Magnetic resonance imaging of the brain

### Image acquisition (MRI)

All images (high resolution structural MRI and functional fMRI) were acquired simultaneously at the John Radcliffe Hospital OCMR Centre (Oxford) 1.5 T Siemens Magnetom SONATA, Erlangen, FRG) MRI scanner. Functional magnetic resonance imaging (fMRI) was carried out using echo planar imaging (EPI) with whole brain coverage, using the standard head coil for radio frequency (RF) transmission and signal reception. Sequences with the following parameters were employed: repetition time (TR) = 3,000 ms, echo time (TE) = 50 ms, voxel size =  $3 \times 3 \times 3 \text{ mm}^3$ . Using a mid-sagittal scout image, 36 axial slices were positioned to cover the whole brain. Anatomical whole brain images were obtained using a T1-weighted, 3D-gradient-echo pulse sequence [Fast Low-Angle Shot (FLASH)] with the following parameters: TR = 1,200 ms, TE = 5.6 ms, TI 19° flip angle, matrix size =  $160 \times 256 \times 208$ , voxel size = 1 mm isotropic, acquisition = coronal, averages = 3).

### Functional imaging paradigm (fMRI)

The functional imaging experiment was performed with eyes open, and back projected printed task instructions. Post processing used a region of interest (frontal ROI) analysis with statistical parametric mapping (SPM2, Wellcome Department of Imaging Neuroscience, London, UK). The region of interest was the inferior and middle frontal gyri comprising Broca's area (e.g. including BA 44, 45 bilaterally). (The temporal lobe activations associated with covert verbal fluency performance do not typically provide reliable signal under these experimental conditions, due in part to artefact susceptibilities from magnetic field inhomogeneities caused by the air in nasal sinuses.)

### Tasks

The first task administered was verbal fluency. In the MRI scanner eight letters (F, A, S, C, T, N, P, L) and eight semantic categories (furniture, animals, fruit, clothing, countries, shops, vegetables, and vehicles) were each presented centrally in yellow Arial font on a dark blue screen. The subjects were requested to covertly list as many words as possible beginning with the given letter (letter-initial, or phonemic fluency), or belonging to the given category

(semantic category fluency) cued on the screen, and to do so as quickly as possible without repeating any words. They were requested to think the words in their heads (rather than speaking them out loud), to avoid movement artifacts during scanning. The individual task instructions were presented as text printed on the screen prior to commencement. The entire experiment was continuous, and lasted for a total of 7.2 min. It began with a baseline condition, in which a plus sign bordered by three asterisks on either side in yellow Arial font was centred on a dark blue screen (\*\*+ \*\*). This baseline lasted for 9 s, and was followed by the stimulus presentation (i.e. a letter), which lasted for 18 s. The 9-s baseline condition was then repeated, followed by the semantic stimulus (i.e. a category name) which lasted 18 s. The paradigm then iterated until each of the letter-initial and semantic fluency cues had been individually presented. Further details of task instructions and imaging procedures are as per Lux et al. (2008). Laterality indices were computed from the mean ROI values (versus baseline) in order to assess the extent of hemispheric functional lateralisation on a subject by subject basis. Particular care was taken in this respect, such that overall cortical activation was represented within the laterality algorithm:  $[(\% \text{ left} - \% \text{ right}) / (\% \text{ left} + \% \text{ right})]$ . Thus, the left–right difference in percent of whole brain activation in the frontal region of interest was divided by the sum of the two, per individual. Positive scores indicate greater left than right hemisphere activation (during covert word retrieval), in the region of Broca's area.

### fMRI image processing

Image analysis included realignment, normalization, and statistical analysis, performed with Statistical Parametrical Mapping (SPM2, Wellcome Department of Imaging Neuroscience, London, UK), in MATLAB (Mathworks Inc, Sherborn, MA, USA). The first two volumes of data acquisition, during which the MR signal reaches a steady state, were not reconstructed. The image time series was realigned to the first image of the remaining time series to correct for head movement between scans. The 3D anatomical data sets were then co-registered to the EPI-image sets and spatially normalized to the stereotactic space of the EPI-MNI (Montreal Neurological Institute) brain using templates provided by SPM2. The voxel size after normalization was then  $2 \times 2 \times 2 \text{ mm}$ . Data were subsequently smoothed with an isotropic Gaussian kernel of 6 mm for single subject analysis at full-width-half-maximum.

### fMRI statistical analysis

Statistical analysis was performed following stereotactic normalization and smoothing. Data were analysed by modelling the different conditions as reference waveforms

(i.e. box car functions convolved with the haemodynamic response function in the context of the general linear model in SPM2). Subject-specific low frequency drifts in signal were removed with a high pass filter of 128 s. Thus, a design matrix was defined for each subject, to model the alternating periods of experimental conditions (using a delayed boxcar reference vector to account for the delayed cerebral haemodynamic response function following stimulus presentation). Six parameters obtained from the realignment procedure were also included as additional regressors in the design matrix. After estimation of all the model parameters, specific effects were tested by applying appropriate linear contrasts to the parameter estimates for each condition, yielding a  $t$  statistic for each and every voxel. The  $t$  statistics (transformed to  $Z$  statistics) constitute subject-specific statistical parametric maps ( $SPM_{\{z\}}$ ). The  $SPM_{\{z\}}$  maps were interpreted with reference to the probabilistic behaviour of Gaussian random fields. For whole brain analyses, areas of activation were identified as significant only if they exceeded a threshold of  $p < 0.05$  (corrected for multiple comparisons at the cluster level) (Friston et al. 1995), with an underlying voxel level of  $p = 0.001$  (uncorrected), and counting of clusters at a criteria of  $>4$  voxels in size within the region of interest (ROI). The anatomic localization of local maxima was assessed using standard neuroanatomy reference tools (Duvernoy, 1999; Talairach and Tournoux 1988), and superposition of the respective  $SPM_{\{z\}}$  maps onto the subjects' anatomical (i.e. structural MRI) images.

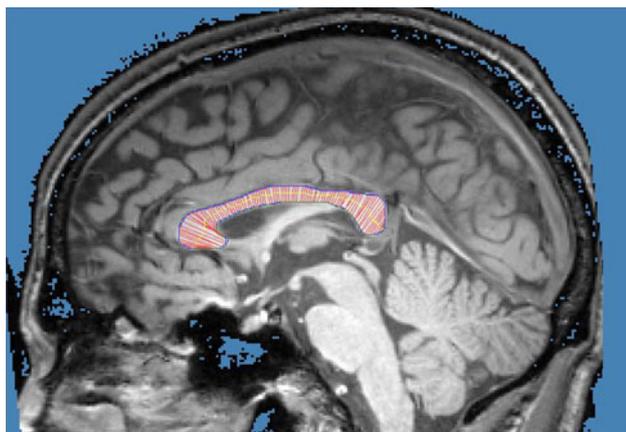
Laterality measures were calculated for the verbal tasks as: percentage of activated voxels per number of voxels in the region of interest (dominant – non-dominant/dominant + non-dominant) where the left hemisphere is considered dominant. The verbal laterality index was obtained from all conditions versus baseline (i.e. mean of letter-initial, and semantic). The laterality indices varied between  $-1$  and  $+1$ . Positive values for laterality indices indicated the expected or typical laterality (i.e. verbal activation greater in the left hemisphere) and negative values indicated the unexpected or atypical laterality (i.e. verbal activation greater in the right hemisphere). Region of interest (frontal ROI) analyses: For verbal tasks, the individual and the summed contrast on both sub-tasks (letter-initial + semantic  $>$  baseline) was employed, to represent the regions activated. Voxels surviving the threshold of  $p = 0.01$  (corrected for peak height) were counted towards the mean letter-initial and semantic fluency (all conditions versus baseline). A frontal ROI comprising the inferior and middle frontal gyri was then defined for each subject, for each hemisphere, using the Talairach Daemon (Lancaster et al. 1997) implemented in (mri3dX <http://www.aston.ac.uk/lhs/staff/singhkd/mri3dX>). (For further details see Lux et al. 2008). Separate laterality values were obtained

for the individual conditions (letter-initial, semantic), and their mean. Use of the mean enhanced the fullness of data sets for within-twin pair comparisons.

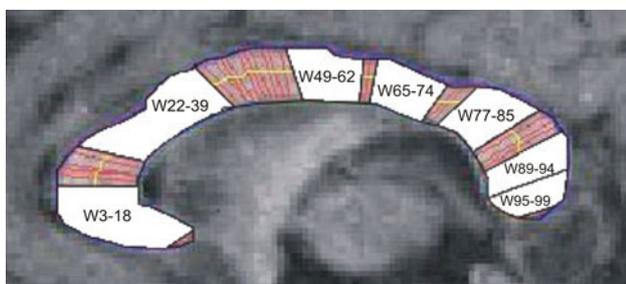
#### MRI high resolution structural imaging of the corpus callosum

Brain Voyager v1.9 software (<http://www.brainvoyager.com>) was used to obtain midline sagittal slices and standardize brain alignment. Images were iso-voxeled to  $1 \times 1 \times 1$  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 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; however, in a minority of participants, the septum pellucidum was deviated from the midline so this latter criterion could not be consistently applied. Each corpus callosum (CC) outline was traced by a single rater (PEC) blind to the identity of subjects using a computer program (Callosum). Software was developed at the University of Sheffield and was based on the principles of the original Denenberg et al. (1991a) software, and has been used in one additional anatomical report (Wignall et al. 2010). The software automatically divided 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 (Fig. 1).

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,



**Fig. 1** 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



**Fig. 2** Corpus callosum (anterior left; posterior right) with the seven regional factor width regions depicted. Factor widths are those derived using similar measurement algorithms and factor analysis of 99 percentile widths (Denenberg et al. 1991b)

W49–62, W65–74, W77–85, W89–94, and W95–99 (Fig. 2). Stability of this factor structure has been replicated within (Cowell et al. 1994) and between laboratories (Peters et al. 2002). 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:  $0.763 < r < 0.946$ ) (Fleiss, 1986, p. 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).

## Results

### Overview

All tasks were analysed separately before within-twin pair effects and cross-task correlations were investigated. For

the anatomical high resolution MRI, only the corpus callosum was measured. For the fMRI language paradigm (frontal ROI), only a summed laterality index was employed. Significant effects were analysed and reported in further detail. The results are presented under the headings of cognitive motor measures, brain imaging measures, and cross-task relationships. *Every effort was made to maximize the data set sizes.* (The exception is calculations which theoretically or practically dictate that data set size be reduced with respect to subject numbers.) Results from 20/26 twin pairs provided fully paired data sets with significant fMRI activations, but this was not the case across all measures (e.g. corpus callosum measurements). *Given the research questions, subject and task effects were analysed using values from subjects for whom fMRI data were available only.* (Within-twin pair analyses necessitated dropping pairs for whom there was only partial data.) Data from 6 twin pairs were also dropped due to excessive neonatal or adult risk indicators, or to scanning-related constraints. fMRI data from 2 pairs were not subject to further analyses, because a brain tumour was detected in one member of a twin pair, and another pair had one member with an atypical looking MRI. Further fMRI data was lost to full pair analysis when 1 member of another twin pair withdrew from the scanner mid-session (that fMRI data set was not further analysed). One member of another twin pair had poor sight and slow reactions which might have affected fMRI task performance. fMRI data from a final pair were not analysed due to multiple risk factors at birth) (see Table 1). fMRI data from 24 pairs (mean age 52 years, SD 8.85, range 37–67) were analysed. However, a further 2 pairs were dropped due to incomplete pair-wise data (there was significant fMRI activation in one twin but failure to exceed threshold for significant activation in the other). Hence a total of 20 pairs ( $20/24 = 83\%$ ) (i.e. ‘full pairs’ with complete fMRI results for both twins) were entered into the following analyses. For the 20 pairs, mean age was 50 (SD 8.80, range 37–65). There was good matching on birth weights (RH: mean = 2.39 kg, SD 0.62, range 0.91–3.41 kg; LH: mean = 2.33, SD 0.64, range 0.91–3.41 kg, paired  $t$  test,  $df = 19$ ,  $T = 0.84$ ,  $p = 0.409$ , ns). In terms of birth order, the RH twin had a greater tendency to be first (birth order, RH: 15/20 first; 5/20 s; LH: 5/20 first, 15/20 s); difference,  $df = 19$ ,  $t = 10$ ,  $p = 0.002$ ). The twins differed slightly on years of further or higher education (RH: mean = 2.9, SD 1.29, median 3, range 1–5; LH: mean = 2.55, SD 1.28, median 3, range 1–5, paired  $t$  test,  $df = 19$ ,  $T = 2.33$ ,  $p = 0.03$ ). The number of pairs with at least one twin having at least one adverse birth indicator was 4/20. Since some results from one pair have already been published (highlighting their distinctiveness), the pair was omitted from the

anatomical MRI callosal analyses here (cf. Lux et al. 2008; Rosch et al. 2010b).

## Cognitive motor measures

### *Hand and foot preference*

Mean laterality preference (XPref) was calculated as the average of HPI and FPI. There was a significant difference between handedness groups ( $N = 40$ , paired  $t$ ,  $df = 39$ ,  $T = 14.26$ ,  $p < 0.0001$ ). There were no significant within-twin pair correlations on raw ( $r = -0.26$ ,  $p = 0.27$ , ns) or on absolute values ( $r = 0.31$ ,  $p = 0.19$ , ns). The RH mean was 84.38 (SD 10.94, median 85, range 60–100). The LH mean was  $-56.35$  (SD 39.99, median  $-63.75$ , range  $-100$  to  $+35$ ).

### *Fine motor performance*

Mean laterality performance (XPerf) was calculated as the average of the three tasks: dot filling, finger tapping, and peg moving. There was a significant difference between handedness groups ( $N = 40$ , paired  $t$ ,  $df = 39$ ,  $T = 11.82$ ,  $p < 0.0001$ ). There were no significant within-twin pair correlations on raw ( $r = -0.13$ ,  $p = 0.57$ , ns), or on absolute values ( $r = 0.15$ ,  $p = 0.53$ , ns). The RH mean was 11.17 (SD 3.71, median 11.63, range 3.11–17.13). The LH mean was  $-7.28$  (SD 5.44, median  $-6.86$ , range  $-19.87$  to  $+3.52$ ).

### *Verbal fluency*

Mean performance (Xflus) was calculated as the mean of single semantic category words per minute (sub-tasks: ‘Animals’ and ‘Furniture’) which were spoken aloud. There was a significant within-twin pair correlation ( $N = 240$ ,  $r = 0.81$ ,  $p < 0.001$ ). Overall, the effect of years of higher or further education was not significant ( $N = 40$ ,  $r = 0.25$ ,  $p = 0.12$ , ns). Overall, the effect of birth order was not significant (Kruskal–Wallis, first born, median = 16.13, second born, median = 17.13,  $H = 0.37$ ,  $df = 1$ ,  $p = 0.54$ , ns). There was no significant difference between handedness groups ( $N = 40$ ,  $df = 39$ , paired  $t$ ,  $T = -1.01$ ,  $p = 0.33$ , ns). The RH mean was 17.20 (SD 4.99, median 16.13, range 10.25–26.25). The LH mean was 17.88 (SD 4.57, median 17.13, range 10.50–26.50).

### *IQ*

The WASI-estimated full scale (IQ) did not differ between handedness groups ( $N = 40$ ,  $df = 39$ , paired  $t$  test,  $t = 0.33$ ,  $p = 0.75$ , ns). There was a significant within-twin pair correlation ( $r = 0.89$ ,  $p < 0.001$ ). The RH mean was 119.40

(SD 11.92, median 121, range 89–136). The LH mean was 119 (SD 11.30, median 120.50, range 98–139).

## Magnetic resonance imaging of the brain

### *Corpus callosum*

Of the 20 twin pairs with complete fMRI laterality data (xVLI) (as described above), 17 pairs had complete structural MRI data which are presented in this section (see Table 2). Statistical power was estimated at 70 % for this reduced sample (where 22 pairs would be needed to attain 80 % power at  $\alpha = 0.05$  for two-tailed tests) (Power and Precision, Biostat, Englewood, NJ, USA). Paired comparisons were used given the significant correlations between RH and LH twins on all measures. There were no significant differences between the RH and LH twins for area, length or any callosal region except for the region W22–39, which showed significantly larger CC widths in the LH twins ( $df = 16$ ,  $t = -2.71$ ,  $p < 0.02$ ; 95 % CI for RH-LH difference:  $-0.76$ ; upper  $-0.09$ ). As shown in Fig. 3, in 15 of the 17 pairs, the LH twin had a larger CC for W22–39. There was no correlation between CC width 22–39 and years of higher or further education ( $N = 34$ ,  $r = -0.21$ ,  $p = 0.23$ , ns). Width at CC 22–39 did not vary significantly as a function of birth order (first born median = 8.27, second born median = 8.62, Kruskal–Wallis,  $df = 1$ ,  $H = 1.02$ ,  $p = 0.31$ , ns). Given the significant difference between handedness groups (i.e. the anatomical salience of W22–39) and the likely anatomical connections through Broca’s area and its right-hemisphere homologue (De Lacoste et al. 1985; Hofer and Frahm 2006; Witelson 1989), CC region W22–39 was selected for further investigation in relation to brain function during verbal fluency. On the CC width measures at widths 22–39, the LH as a group tended to have larger values than the RH, indicating significant width increases in the LH as a group of 17 matched pairs. With respect to the individuals, the number of clear LH outliers was comparatively smaller (i.e. compared to the number of outliers observed for verbal laterality frontal ROI, as described in the section on xVLI below). Again, there were at least twice as many atypical LH as RH (according to either definition), with no atypical RH. This can be defined conservatively as 2 standard deviations from the RH mean ( $N = 17$ , mean = 8.34 mm, SD 1.31). None of the RH, and only 1/17 LH (3 %) fell within this band. Hence there were no matched atypical pairs. (The same result applies if the total summed RH and LH mean and SD are employed.) A less conservative criterion of greater than or less than the RH maximum (10.50 mm) or minimum (5.96 mm), shows that 2/17 LH (12 %), had values falling outside the RH range and all of these were *greater*.

**Table 2** Mean  $\pm$  SE for corpus callosum measures as a function of twin handedness for 17 pairs

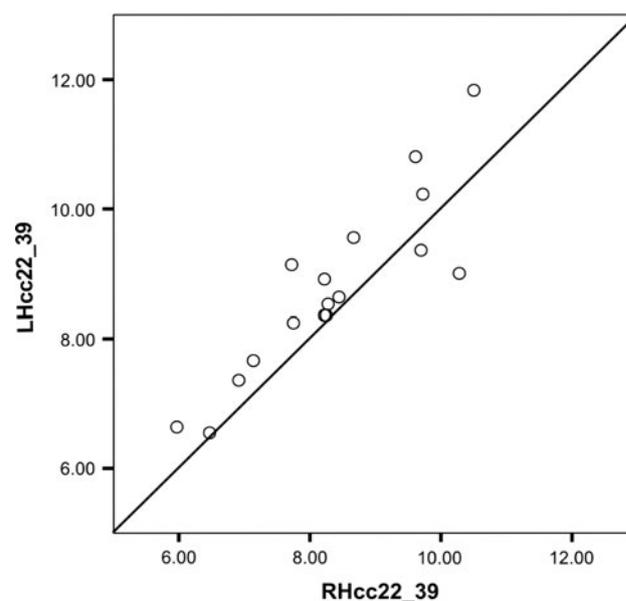
<i>N</i> = 17 pairs	Area	Length	W3–18	W22–39	W65–74	W77–85	W89–94	W95–99
LH (mean $\pm$ SE)	830.45 $\pm$ 30.92	100.89 $\pm$ 1.99	11.20 $\pm$ 0.49	8.77 $\pm$ 0.33	7.21 $\pm$ 0.34	11.53 $\pm$ 0.58	11.73 $\pm$ 0.35	4.10 $\pm$ 0.08
RH (mean $\pm$ SE)	833.98 $\pm$ 29.50	100.75 $\pm$ 1.98	11.22 $\pm$ 0.39	8.34 $\pm$ 0.32	7.20 $\pm$ 0.32	11.48 $\pm$ 0.41	11.65 $\pm$ 0.30	4.10 $\pm$ 0.09
Correlation (RH/LH)	0.92**	0.69**	0.57*	0.89**	0.91**	0.72**	0.52*	0.72**

All measures are in mm except for area which is in mm<sup>2</sup>. The bottom row displays correlations (*r*) for each measure between LH and RH twin pairs

LH left handed, RH right handed, W width

\*  $p < 0.05$

\*\*  $p < 0.01$



**Fig. 3** Mean corpus callosum W22–39 (mm) in right- (RH) and left-handed (LH) twin pairs. The identity line is shown in order to depict that in fifteen out of seventeen MZ pairs, the LH twin had the larger CC width in this region

#### *fMRI bold effect*

Mean frontal ROI verbal laterality index (xVLI) was calculated as the average of semantic category and phonemic (letter-initial) fluency tasks performed covertly within the scanner (shown in Table 1). The total mean verbal laterality index (xVLI) was +0.57 ( $N = 40$ , SD 0.57, median 0.74, range =  $-1$  to  $+1$ , Q3 = +0.89, Q1 = +0.45), which differed significantly from zero ( $N = 40$ , sign test,  $N = 35$  above median,  $N = 5$  below median,  $p < 0.001$ , 1-sample  $T$  test,  $df = 39$ ,  $T = 7.47$ ,  $p < 0.001$ ). Thirty-five percent (14/40) of the values fell above the 95 % confidence interval on the median, but this was not significant (Wilcoxon 95 % CI on the median = +0.52, +0.77, Runs test,  $p = 0.26$ , ns). Thirty percent (12/40) of the xVLI values fell below the lower limit of the 95 % CI, but this was not significant (Runs test,  $p = 0.65$ , ns). Neither the effect of years of higher or further education ( $N = 40$ ,  $df = 39$ ,  $r = 0.06$ ,  $p = 0.70$ , ns), nor the effect of birth order were significant (Kruskal–Wallis, first born, median = 0.76, second born, median = 0.73,  $H = 1.48$ ,  $df = 1$ ,  $p = 0.22$ , ns). For the 20 fully matched twin pairs there was a significant xVLI difference between handedness groups (paired  $t$  test,  $df = 39$ ,  $t = 2.51$ ,  $p = 0.021$ ). However, there was no significant handedness difference on xVLI when absolute values (i.e. sign-free difference from zero) were tested (paired  $t$ ,  $df = 19$ ,  $t = 1.27$ ,  $p = 0.22$ , ns). There were no significant within-twin pair correlations on either the raw ( $r = 0.11$ ,  $p = 0.63$ , ns) or the absolute values ( $r = 0.20$ ,  $p = 0.40$ , ns). The RH mean

was +0.74 (SD 0.25, median +0.81, range +0.07 to +1.00; 95 % CI on the median = 0.63, 0.88), with a significant difference from zero (one-sample  $t$  test,  $df = 19$ ,  $t = 13.03$ ,  $p < 0.001$ ). The LH mean was +0.40 (SD 0.59, median +0.64, range -1.00 to +0.97; 95 % CI on median = 0.17, 0.74), again with a significant, but less pronounced difference from zero (one-sample  $t$  test,  $df = 19$ ,  $t = 3.00$ ,  $p < 0.01$ ). Categorization of individual LH values based on atypical xVLI produced striking effects ranging from 25 to 50 % of individuals, depending on the criteria employed to delineate atypicality (see Table 3):

1. Half of the LH xVLI values fell below the RH lower bound of the 95 % CI on the median (below +0.63);
2. A quarter of the LH xVLI values fell below the RH minimum (below +0.07); and
3. A quarter of LH xVLI values fell below zero (i.e. were negative values).

### Cross-task relationships

For the 17 pairs with complete structural and functional MRI data (Table 1), there was a significant correlation between frontal ROI xVLI, and the width of corpus callosum at W22–39 ( $N = 34$ , Pearson's  $r = -0.34$ , Spearman's  $\rho = -0.36$ ,  $p < 0.05$ ), but not for any of the other CC width values. Though not significant, the magnitude and direction of correlations within handedness groups were comparable to values seen for the whole sample (RH: Pearson's  $r = -0.39$ , Spearman's  $\rho = -0.36$ ; LH: Pearson's  $r = -0.31$ , Spearman's  $\rho = -0.24$ ). However, there was no significant correlation between the spoken behavioural verbal fluency rate (performed in the lab) and xVLI ( $N = 40$ ,  $r = -0.13$ ,  $R = -0.12$ , ns), or between spoken verbal fluency and CC W22–39 ( $N = 34$ ,  $r = 0.16$ ,  $R = 0.10$ , ns). In Fig. 4, the correlation between CCW22–39 and functional frontal ROI asymmetry is

**Table 3** Atypical xVLI and CC 22–39 widths

Group	xVLI <sup>a</sup>	CC 22–39 <sup>b</sup>	dif
RH	4/20 (20 %)		
LH	9/20 (45 %)	1/17 (6 %)	
Total	13/40 (32.5 %)	1/34 (3 %)	
Dif	5/20 (25 %)	1/17 (6 %)	1/17 (6 %)

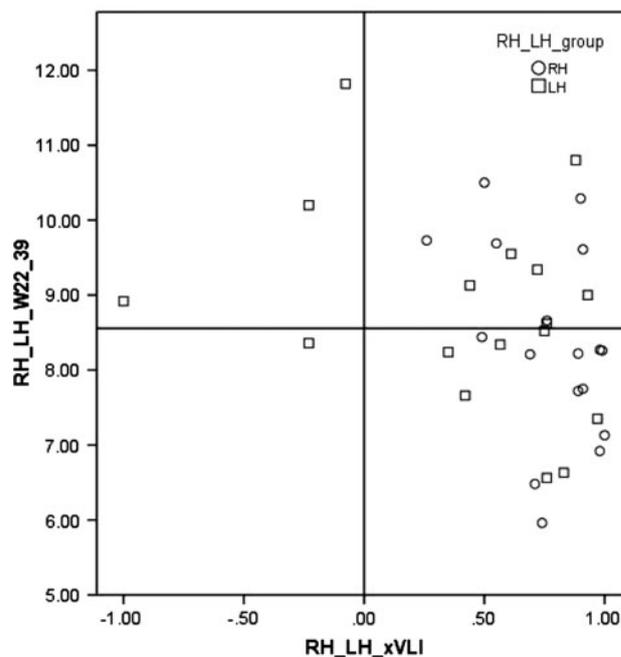
*dif* difference, *xVLI* mean verbal laterality index (fMRI), *CC 22–39* corpus callosum width at slices 22–39 (mm)

<sup>a</sup> Criteria = summed atypical xVLI (-0.52 to +0.52; outside 95 % CI for difference from zero,  $N = 40$ , Wilcoxon), and all negative values (-0.52 to -1)

<sup>b</sup> Criteria = both atypical: xVLI and CC W22–39 ( $\geq 2$  SD from RH mean,  $N = 17$ )

shown as a function of twin handedness. The y axis is split for the mean corpus callosum width for the whole sample (mean = 8.56) and the x axis is split at zero. Data points for LH twins exclusively (squares) were present in the quadrants of Fig. 4 to the left of the zero-line (i.e. symmetry). Notably, three of these LH twins were located in the top left quadrant of the plot. RH twins by contrast (circles) were most prevalent in the lower right quadrant which shows 10/17 RH twins. There was some overlap in the distribution for both dependent measures. However, LH twins were more likely to have a larger corpus callosum with either less leftward or more rightward functional asymmetry. In contrast, RH twins were more likely to have smaller corpus callosum and greater leftward functional asymmetry.

Statistical modelling according to Crawley's (2007) principles and procedures was employed to derive a *minimal adequate model* for the explanatory variables. The variables are described as 'statistical' to avoid unintended implications of causation, or directionality thereof. The statistical response variable xVLI was chosen a priori, and



**Fig. 4** Corpus callosum width of region W22–39 (mm) plotted against frontal ROI functional asymmetry during verbal fluency (xVLI) in right- (RH: circles) and left handed (LH: squares) twin pairs. The x axis is split at zero and the y axis is split at the mean CC width. Data are for 17 pairs with complete structural and functional MRI data. Functional asymmetry values for the typical left-hemisphere-greater-than-right activation pattern are represented as positive xVLI. The atypical right-greater-than-left pattern is depicted as negative xVLI

the statistical explanatory variable mean width for CC region W22–39, was chosen a posteriori.

The best fit regression equation was:  $xVLI = 1.66 - (0.125)W22-39$

Consistent with the patterns of correlations in the previous section, the model can be interpreted as predicting larger regional widths at W22–39 when xVLI is more rightwardly lateralised. There was no evidence of lack of fit for the model, and it was not unduly influenced by unusual observations. The model was arrived at by background regressions, such that this choice of explanatory variable was based on a best subsets regression (MINITAB) on xVLI, for continuous variables which correlated significantly with xVLI. These variables included anatomical corpus callosum region W22–39, fine motor dot filling, and finger tapping performance laterality, all of which regressed significantly with xVLI on their own. Corpus callosum W22–39 was the highest single significant predictor of xVLI. Dot filling was the second highest single predictor. Since dot filling and finger tapping were highly inter-correlated, adding finger tapping to the model did not contribute significantly towards enhancing its correlational power. Once added to the CC W22–39 regression model, dot filling was no longer a significant predictor. Moreover, spoken verbal fluency rate did not appear as a significant factor in the regression model.

#### *Qualitative fractionation by behavioural task performance*

Fractionations by *subject* (i.e. within-twin pair contrasts) were employed to probe the cross-task relationships. Pair-wise fractionation by subjects could be performed in several ways depending upon different criteria for atypicality vis-à-vis differences in atypical versus typical pairs. The objective was to elucidate sources and consequences of atypicality particular to the LH twins, so a modified case–control method was employed (RH = ‘control’; LH = ‘case’). When similarities and differences were investigated conservatively (applying parametric, Gaussian calculations), 3/20 (15 %) were atypical as the LH had an atypical (i.e. negative) xVLI, and/or an atypical CC W22–39 width (i.e. large  $\geq 2$  SD from the RH control mean). The RH values were within the normal range on both (i.e.  $xVLI \geq 0$ ; CC W22–39 within normal limits). Differences fell into three patterns, 2/3 with negative (atypical) LH: xVLI values, and 2/3 with wide (atypical) LH: CC 22–39 values. In one pair, the RH:  $xVLI = +0.69$ ; LH:  $xVLI = -1$ ; the RH group-referenced Z score for CC W22–39 for the RH =  $-0.06$ ; LH =  $+0.49$ . In this pair, xVLI for the LH was atypical, but CC W22–39 was in the normal range. For another pair, the RH:  $xVLI = +0.50$ ; LH:  $xVLI = -0.08$ , the RH group-referenced Z score for

CC W22–39 for the RH =  $+1.71$ ; LH =  $+2.74$ . In this pair, xVLI for the LH was atypical, and CC W22–39 was wider than the norm. For a third pair, the RH:  $xVLI = +0.91$ ; LH:  $xVLI = +0.88$ ; the RH group-referenced Z score for CC W22–39 for the RH =  $+1.02$ ; LH =  $+1.95$ . In this case, both RH and LH had typical xVLI, and the LH had a CC W22–39 wider than the norm. In one of the three pairs, the LH had atypical xVLI and CC W22–39. (See “Appendix” for details.)

#### *The big picture*

There was a significant correlation between brain structure and function, such that atypically right lateralised frontal ROI xVLI was associated with wider CC W22–39 width. The relationship was more representative of LH than RH. The selective patterns of atypical LH results differed between cerebral function (xVLI) and structure (CC W22–39). As a task measure, xVLI produced more heterogeneous results, which tended to cluster in the typical direction of left-greater-than-right asymmetry (i.e. positive values), and the values were algorithmically derived. There were significant group effects, but the individual patterns of atypical results were more meaningful. By contrast, as a measure, CC W22–39 yielded more homogeneous values which were more evenly spread across a narrower distribution range, and constituted by raw values. There were significant group effects which were moderate in size (Estimated ES = 0.64), but yet pervasive in the direction of greater size within-pairs in the LH twin. On xVLI the ten individuals with clearly atypical values lacking asymmetry (i.e. values not differing significantly from zero) were composed of four RH and six LH twins, with only 2/20 coming from within an MZ pair. The five individuals who had asymmetrical values going in the opposite direction (i.e. negative values indicating right hemisphere lateralisation) comprised entirely LH twins. Overall if the two atypicality types are combined, there is a striking difference such that twice as many LH as RH show atypical xVLI, and the LH twins are one and a half times (6:4) more frequently represented in the non-lateralised set.

#### *Summary of results*

Table 4 shows that: on laterality preference (Edinburgh Handedness Inventory, Waterloo Footedness Inventory), there were significant differences between right and left handers. On laterality performance (Tapley and Bryden dot filling, finger tapping, Annett’s peg moving), there were significant differences between handedness groups. On verbal performance (spoken verbal fluency), there was no difference between handedness groups. On the IQ-estimate, there was no significant difference between groups. The

**Table 4** Summary of main results

Similarities for RH and LH twins	Differences between RH and LH twins
IQ	
Verbal Fluency	xVLI laterality
Corpus callosum area, length and six of the 7 factor widths, within-twin correlations for all measures	Corpus callosum W22–39
<b>Overlapping on same continuum but with greater representation on opposite ends</b>	
The correlation with frontal ROI fMRI and corpus callosum W22–39	

groups differed with respect to magnetic resonance imaging of the brain, with significant difference on corpus callosum width at frontal widths 22–39, and significantly more LH with atypical fMRI verbal laterality indices. There was a significant cross-task correlation only between the CC widths at 22–39, and the frontal ROI fMRI laterality indices.

## Discussion

### Overview

Based on MRI and fMRI, there was a significant correlation between language-specific functional laterality indices and anterior corpus callosum width in the contiguous region. The LH showed a greater tendency towards atypicality of brain structure and function. Twenty percent of RH and 45 % of LH, a total of 25 % of MzHd twins had atypical xVLI lateralities. Although the mean callosal width at W22–39 was significantly larger in LH, the distributions for RH and LH twins overlapped considerably. Significant difference arose due to the strong consistency in direction of the effect, with larger callosal width in left-than-right-handers for 88 % of pairs (rather than because of a large mean difference per se).

### Critical discussion

Methodological considerations include study design, subject sample size, and distribution shapes. Interpretations are best limited to within-study effects, given that population-based inferences require considerably larger sample sizes than reported to date (Badzakova-Trajkov et al. 2010; Lux et al. 2008; Rosch et al. 2010b; Sommer et al. 2002). Emphasis here has been on within sample analyses using within-twin differences, as well as cross-task correlations. The percentage of atypical LH xVLI results (approx.

25–50 %), is broadly consistent with those reported elsewhere for LH singletons (Hund-Georgiadis et al. 2002; Pujol et al. 1999; Szaflarski et al. 2002), in support of earlier studies (lacking perfect match on: age, sex and other demographics). As per Hund-Georgiadis et al. (2002), the twins were grouped for cross-task comparisons in order to increase the subject numbers (and hence power). (It is not appropriate to analyse hand and foot preference, which by definition yield bimodal distributions.) Our results fit well within the literature on verbal fluency fMRI paradigms in singleton RH; activation of frontal, parietal, and occipito-temporal cortices, as well as anterior cingulate, is ‘limited largely to the left hemisphere’ (Birn et al. 2010, p. 1101). Two potential sources of age-related bias might arise from the UK Adult Twin Registry, originally established to investigate osteoporosis, whilst younger individuals are perhaps less available for study participation (i.e. travel mid-week to Oxford for scanning). Age as a factor in neurological fluctuations over the menstrual cycle is reduced and matched in our sample (cf. Stromswold 2006). That homotopic regions in both cerebral hemispheres are linked by interhemispheric connections of the corpus callosum (cf. Putnam et al. 2008), which may perform a ‘modulatory’ role under some conditions, is an assumption based on studies of healthy singleton brains (Stephan et al. 2005); but twins may be different (cf. Jahanshad et al. 2010). Evidence from split brain cases (cf. Gazzaniga 2000) has shown that: (a) Phonological aspects of lexical processing are more left lateralised versus semantic aspects which are more bilateral. (b) Split brain cases with atypical lexical functions in both hemispheres have been limited (i.e.  $N = 2$ , one each; cf. Lutsep et al. 1995). (c) Evidence of duplicate lexicons is infrequent. (d) Evidence from split brain commissurotomy cases derives from damaged brains, and overlooks the potential role of subcortical connections between the hemispheres (cf. Holtzman 1984). Therefore, we now provide evidence from individuals with healthy brains, development, and cognition.

*Research question 1: what percentage of the LH twins show atypical functional cerebral asymmetry in frontal ROI fMRI analyses during verbal fluency?*

Responses to this question depend upon the operational definition of ‘atypical’: (1) Values falling below the lower bounds of the RH 95 % confidence interval; (2) below the RH minimum; (3) negative values (right > left hemisphere activation); or (4) other (e.g. differing from literature reports of RH singletons, albeit confounded by differing methods affecting variance within and between individuals). Overall, there is 25–50 % LH atypicality. A quarter of LH values (5/20) fell below zero (i.e. were negative), a quarter of the LH values (5/20) fell below the RH minimum (i.e. below +0.07), and half (10/20) fell below the RH lower bound of the 95 % CI on the median (below +0.63). Hund-Georgiadis et al. (2002) compared 17 RH with 17 non-RH using fMRI of the inferior frontal gyrus and reported 8/17 (47 %) non-RH had either right lateralised or bilateral activations on a lexical encoding task. Pujol et al. (1999) showed significant differences between 50 RH versus 50 LH singletons on silent verbal fluency frontal lobe fMRI lateralisation, such that RH had 96 % left lateralisation (4 % bilateral), whereas LH had 76 % left lateralisation, with 14 % bilateral (i.e. non-lateralised), and 10 % right lateralised. Szafarski et al. (2002) used fMRI to scan 50 non-RH, calculated laterality indices for entire hemispheres using the formula  $(L - R)/(L + R)$ , and reported that 22 % had right lateralised or bilateral language activation. Child studies differ, with greater right hemisphere activation, which: ‘... may reflect developmental plasticity for the ongoing organization of neural networks, which underlie language capacity’ (Gaillard et al. 2000, p. 180). For twins, in utero environment may have twice the effect on left hemisphere development (versus right), ‘consistent with less genetic control over the left hemisphere’ (Geschwind et al. 2002, p. 3176).

*Research question 2: what is the mechanistic significance of atypical frontal ROI fMRI asymmetry in relation to other neurological structures, and to behavioural functions?*

With respect to the structure and function of the corpus callosum, uncertainty remains concerning anatomical width [and area (cf. Welcome et al. 2009, 2010)], as well as fractional anisotropy measures, in terms of factors such as neurite density, myelin content, axon diameter, axon numbers, size, myelin thickness, membrane integrity, and fibre width (cf. Johansen-Berg 2010). ‘Variation in any of these features could have consequences for the physiology of the axon by modulating conduction velocity, refractory time, the probability of transmission along an axon, and even the wide-scale synchronization of signals across a distributed neuronal

network’ (Johansen-Berg 2010, p. 353). Witelson (1985) showed larger CC in LH, replicated by O’Kusky et al. (1988) and Welcome et al. (2010), but not by Moffat et al. (1998). In our sample, this difference occurs only at widths 22–39, and it is more marked with right hemisphere language lateralization; a finding not contradicted by genetic studies (Chiang et al. 2009; de Zubicaray et al. 2008). Welcome et al. (2009) showed that the direction of correlation between regional callosal area and functional asymmetry depend on both sex and handedness, although this remains controversial; diverse demographic and biobehavioural factors may contribute to individual differences in callosum–asymmetry relationships. It would be possible to account for our findings with a speculative 2-level model of adverse perinatal events, or with overlay of epigenetic effects of gene expression in a 3-level model (cf. Beaton 1997, 2004; Dubois et al. 2008; Yoon et al. 2010).

#### Level 1

Specific adverse effects related to birth order in twin births might lead to *thickening* of the corpus callosum at W22–39 (cf. Lerman-Sagie et al. 2009), due to combined effects of faulty repair and/or lack of pruning in a second born LH twin at greater risk of anoxia (cf. Miu 2005; Ohno et al. 2009). In a mouse model (Bilali et al. 2008; Ohno et al. 2009), hypermyelination can occur due to a neuron-specific over expression of a zinc peptidase (NRDc) binding partner of a growth factor (HB-EGF) which (under normal healthy circumstances) regulates the shedding of a wide range of membrane proteins (i.e. pruning). NRDc appears to regulate myelination specifically. Its expression is confined to neurons, and affects axon signalling between neurons and oligodendrocytes, and may be implicated in delayed remyelination after injury (Ohno et al. 2009). A neonatal model of hypoxia *down regulates* the expression levels of genes important for growth and development of oligodendrocyte progenitor cells (OP) in developing brain (Bilali et al. 2008), such that normal pruning fails to take place.

#### Level 2

Environmental effects of motor practise during development could exert an additional effect (cf. Gogtay et al. 2004), due to left hand crossing the midline (which draws upon callosal mechanisms, and is more frequent in LH). The right hemisphere controls the left hand, and the left hemisphere the right (and similarly movement from left to right space). The left hand of LH prefers to be in right space (cf. Banissy et al. 2012). If the effects of twin birth and LH manual practise interact cumulatively, then LH could have thicker CCs at W22–39 without adverse impact on cognitive function. Being a twin could enhance

atypicality overall (Lerman-Sagie et al. 2009), although thinning rather than thickening of the CC would be more predictable given the association with neurodevelopmental abnormalities leading to cognitive deficits (but cf. Fujii et al. 1994; Lerman-Sagie et al. 2009; Stromswold 2006). In summary, the LH prefers to be in right space, so if dominant, crosses the midline more frequently, leading to potential CC thickening at W22–39. Neural aspects of the motor function may develop at the expense of optimal requirements for lexical retrieval networks. Fink et al. (2006) have demonstrated how Broca's region changes function: '...consequent upon the simultaneous activation of other regions that are effectively connected to a given region' (p. 264); its *functional fingerprint* is dependent upon a *connectional fingerprint*.

### Concluding remarks

'Left handed' (or sinistral) as a term, indicates difference from the population norm (dextral, or right handedness), more aptly called 'non-right handed' (cf. Beaton, 2004). Considering how the brain works is often predicated on an assumption of normality; perhaps obscuring the potential redundancy 'built in' to the developing nervous system (cf. Provins 1997). Trade-offs and priorities in the developing nervous system (cf. Gogtay et al. 2004) can be explored using methods presented here. The outcome may have more to do with *effects* than with *causes*: Why the right rather than the left hand became dominant may be dictated by laterality differences in visuo-spatial mechanisms of neural attention which confer an enhanced spatial attention to the right side of body-centred space (cf. Banissy et al. 2012; Driver et al. 2001; Fink et al. 2001; Rosch et al. 2010a; Toni et al. 2001). With the maximally streamlined experimental design framing the background in terms of matched demographics, verbal fluency rate, and WASI-estimated IQ, but contrastive writing hand preference and fine motor performance laterality, the important relationships between frontal functional laterality and corpus callosum width at Broca's area (inferior and middle frontal gyri) are revealed: The observed relationships occur in a cognitive context of significant within-twin pair correlations on verbal fluency and IQ only. The major finding (Fig. 4) of significant correlation between frontal brain activation and the corpus callosal widths in a comparable region (W22–39) shows atypical right hemisphere blood flow increases in Broca's area analogue during covert verbal fluency performance associated significantly with thicker corpus callosum measures anatomically. This supports evidence from O'Kusky et al. (1988), and focuses on the anterior corpus callosum W22–39. Surrounding controversies are reviewed in Beaton (1997). Luders et al.

(2010) suggested that weaker handedness lateralization on HPI was associated with larger callosal dimensions; however, our twins match well for strength of handedness preference for writing in both RH and LH. (In our data set, the overall HPI measure across both hand groups is not suitable for correlational analyses because it is bimodal, with RH values clustering closely within a narrow range of the distribution.) The relationship demonstrated between functional MRI (bold effect) and high resolution structural MRI measures is difficult to interpret because it rests on assumptions concerning aspects of an unusual and comparatively small population. This addresses Deason et al. (2005, p. 596): '*Studies with humans have failed to produce evidence that any direct causal relation exists between the asymmetry of one function in an individual and the asymmetry of a different function in that individual.*' Due to the region of interest methodology adopted for fMRI analysis, only the inferior and middle frontal activations were investigated statistically (using SPM). Some factors may have helped reveal the correlation pattern in Fig. 4, such as reduced noise (i.e. statistical variance) in the data combined with extremes of a normal continuum of development (cf. Geschwind and Konopka 2009; Stromswold 2006), revealed by strong handedness discordance and twinnedness. However, there is no simple account currently supported by further independent evidence. A hypothesis based on human brain development shows the potential interaction of two independent processes expressing differently in different individuals: (1) iterative feed-forward effects between callosal thickness and left-hand use (cf. Banissy et al. 2012; Sainburg and Eckhardt 2005); and (2) neonatal effects on neural structure. In some people, left handedness could be symptomatic of atypical neural development (cf. Mitwood 2008). Such effects may be pointers to epigenetic/environmental/developmental, rather than purely genetic effects (cf. Medland et al. 2006). The developmental trajectory may be multi-layered (cf. McBeath and Sugar 2005), spanning several stages ranging from neonatal to adult. What is remarkable is that it can and does yield healthy development underpinning normal adult language and intelligence. The evidence presented here is compatible with unshared environmental variance effects (or combined genetic, epigenetic, environment, *unshared* environment) (cf. Hustler and Galuske 2003). Further research is needed (cf. O'Kusky et al. 1988). Implications for the field of laterality research pertain to frontal hemispheric equipotentiality for verbal processes in healthy individuals.

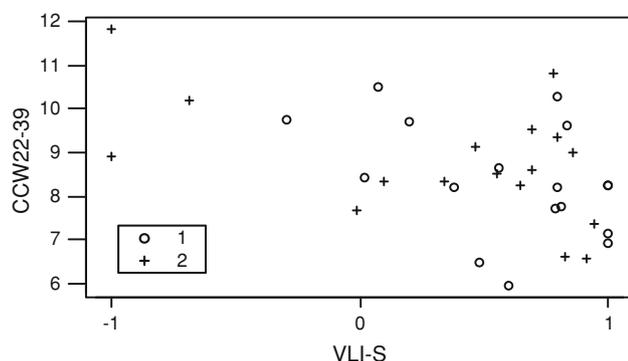
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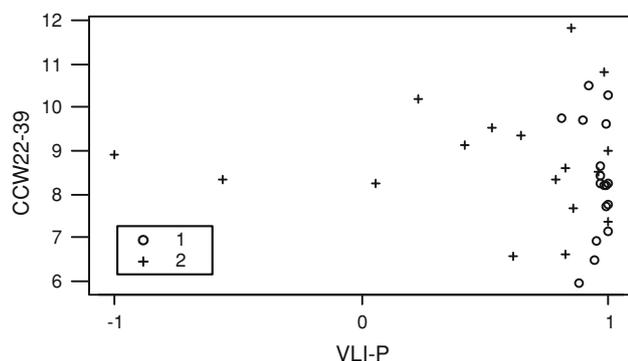
## Appendix

### Cross-task relationships

Post hoc fractionation on the fMRI sub-tasks (VLI-P, phonemic or letter-initial; and VLI-S, semantic or category fluency) of mean  $xVLI$  checked that a small number of data points did not confer undue weight on the significance of the  $XVLI$  by CC 22–39 correlation. (Task administration was inter-leaved throughout the scan session, and it was not originally designed to be fractionated.) The response curve distribution for VLI-P and VLI-S differ in shape (Figs. 5, 6). VLI-P is asymmetric and indicative of lateralised effects, whereas VLI-S is comparatively symmetric (i.e. bilateral). Corpus callosum W22–39 represents a raw width measure (i.e. not a derived value) and could arguably be considered as possessing inherent ‘symmetry’, given its



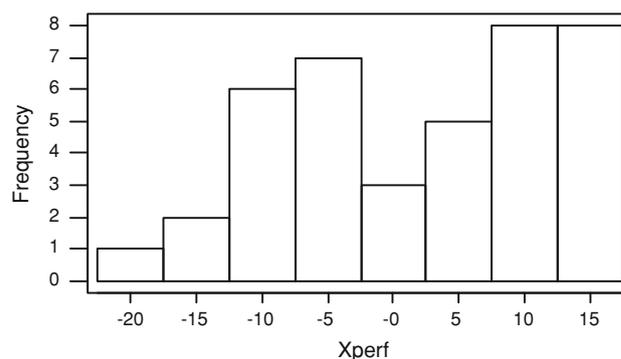
**Fig. 5** VLI-S by CC 22–39 width plot ( $N = 34$ ; 17 matched pairs) (RH = 1; LH = 2)



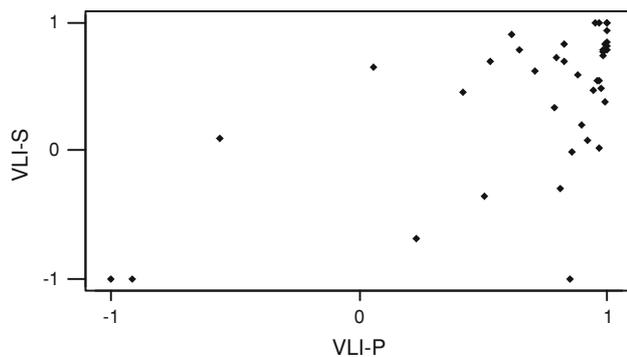
**Fig. 6** VLI-P  $\times$  CC 22–39 plot ( $N = 34$ ; 17 matched pairs) (RH = 1; LH = 2)

identity as a measure of the diameter of an anatomical fibre bundle. Given such concurrences between distribution shape and identity, there is a greater likelihood of significant cross-task relationships in values with greater symmetry; thus CC W22–39 lends itself more readily to significant correlation with VLI-S (versus VLI-P). The results fit this prediction; for the 20 matched pairs combined ( $N = 40$ ; RH = 20, LH = 20), the correlation between CC 22–39 and VLI-S is significant ( $R = -0.40$ ,  $p = 0.02$ ), but not between CC 22–39 and VLI-P ( $R = -0.16$ ,  $p = 0.37$ , ns).

Three broad dimensions to the large overlap between the RH and LH twins’ values are: First, VLI-S is right lateralised with large CC 22–39 widths (LH = 3, RH = 1); second, VLI-S is bilateral with medium CC 22–39 widths (8.5–10.5 mm; LH:  $N = 2$ , RH:  $N = 3$ ); and third there are values representing balanced overlap between the RH ( $N = 16$ ) and LH ( $N = 15$ ). There is no evidence of artefacts such as IQ effects. There is no significant relationship between WASI-IQ and VLI-S ( $IQ \times VLI-S$ ,  $R = -0.03$ ,  $p = 0.83$ , ns), or VLI-P ( $IQ \times VLI-P$ ,  $R = -0.08$ ,  $p = 0.16$ , ns). The relationship between the fMRI sub-tasks and *performance* asymmetry (mean: dot filling, finger tapping and peg moving; XPerf) were also tested (Fig. 7). It was expected that the more lateralised of the fMRI sub-tasks (i.e. VLI-P) would bear the stronger relationship to performance asymmetry (based on the distribution shapes explained above). The results provided a good fit; for the 20 matched pairs ( $N = 40$ ; RH = 20, LH = 20), there is a significant correlation between XPerf and VLI-P ( $R = +0.54$ ,  $p < 0.001$ ), but not between XPerf and VLI-S ( $R = +0.13$ ,  $p = 0.42$ , ns). It is particularly noteworthy that *none of the subjects with right hand performance advantage showed right cerebral hemispheric dominance on VLI-P*. Checks for bimodality effects within the XPerf distribution to ascertain whether the result was backed-up by significant correlations between XPerf and VLI-P for the sub-group with exclusively positive XPerf values (RH:  $N = 20$ , LH:  $N = 1$ ) showed support from significant

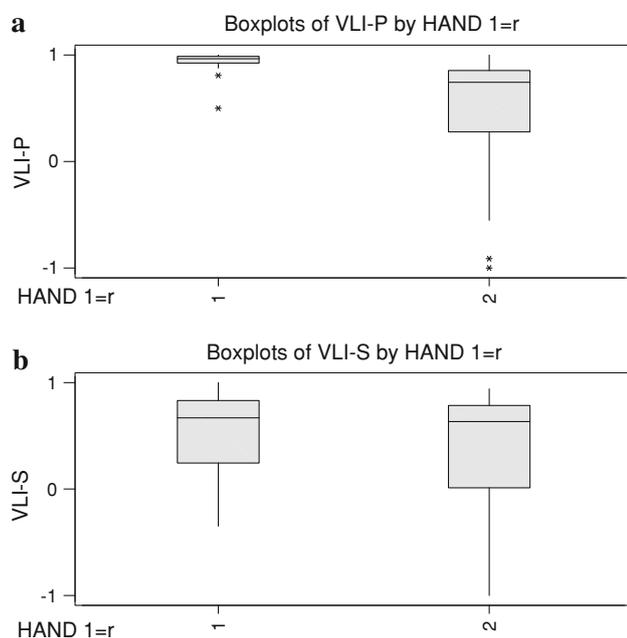


**Fig. 7** Mean performance (Xperf) histogram ( $N = 40$ )



**Fig. 8** Relationship between the fMRI subtask VLI-P (phonemic, letter-initial) and VLI-S (semantic) fractionations ( $N = 40$ )

overall correlation (i.e. for  $N = 40$ ), such that the correlation with VLI-P for the sub-group with positive XPerf values ( $N = 21$ ) remains significant ( $r = +0.43$ ,  $p = 0.05$ ), while that with negative XPerf values ( $N = 19$ ) does not ( $r = 0.08$ ,  $p = 0.75$ , ns). The bimodal aspect of the XPerf distribution does *not* weaken the contrasts demonstrated; it provides a satisfactory fit to the descriptive interpretation. A small number of outliers are *not* biasing the significance of the correlational curve relationships between fMRI (i.e. xVLI) and CC widths (i.e. 22–39). This can be expanded upon by examination of the within task VLI-P by VLI-S relationships (Fig. 8). In Fig. 8 (20 matched pairs,  $N = 40$ ) the pattern is: *If VLI-P is negative, then VLI-S will also be negative* (although the reverse is not necessarily true). VLI-P appears to ‘swing’



**Fig. 9** Boxplots of VLI-P (a) and VLI-S (b) by hand ( $N = 40$ ), (RH = 1; LH = 2)

the asymmetric component of the XVLI distribution, because VLI-P is inherently more sensitive to laterality effects. VLI-P is more sensitive to handedness *preference* differences, as borne out by the resultant differences per group (RH vs. LH) which are greater for VLI-P (Mann–Whitney test,  $W = 541$ ,  $p = 0.0004$ ), than for VLI-S (Mann–Whitney test,  $W = 448$ ,  $p = 0.310$ , ns) (RH:  $N = 20$ , VLI-P median =  $+0.97$ , VLI-S =  $+0.67$ ) (LH:  $N = 20$ , VLI-P =  $+0.75$ , VLI-S =  $+0.63$ ). There was a significant group (RH vs. LH) difference on VLI-P (20 matched pairs,  $N = 40$ , RH vs. LH, Mann–Whitney test,  $W = 541$ ,  $p = 0.004$ ), but *not* on VLI-S ( $N = 40$ , Mann–Whitney test,  $W = 448$ ,  $p = 0.31$ , ns) (see Fig. 9a, b).

### Behavioural task fractionations

Spoken verbal fluency rate was not a significant regressor. The qualitative relationship between ‘atypical xVLI and larger CC W22–39’ and spoken fluency could be further probed in the doubly atypical pairs. Criteria for LH atypicality was based on values falling outside the 95 % CI on the RH mean difference between semantic category and letter-initial fluency rates (RH:  $N = 20$ , one-sample  $t$  test,  $df = 19$ ,  $t = 4.23$ ,  $p < 0.001$ , 95 % CI = 1.59, 4.71). Seventy-five percent of LH twins belonging to the atypical pairs had atypical values (mean differences =  $+1$ ,  $-0.50$ , and  $+7.00$ , respectively). When a within-twin pair difference analysis of the semantic minus letter-initial values is employed (paired  $t$  test, 95 % CI  $-2.55$ ,  $2.55$ ), again 75 % of pairs are atypical (but with LH-RH pair-wise differences = 3.5, 9.0,  $-5.5$ , respectively). However, all of the RH twins were also atypical (half above and half below the outer bounds for the 95 % CI\*) (semantic – letter = RH:  $0^*$ , LH:  $1.00^*$ ; RH:  $+6.5^*$ , LH:  $3.00$ ; RH:  $+9.5^*$ , LH:  $-0.50^*$ ; RH:  $+1.5^*$ , LH:  $+7.00^*$ ). A contrast emerges in three of the four pairs; the direction of the difference is opposite in RH versus LH, so if one has the semantic > letter, the other has (i.e. letter > semantic). This atypicality on a pair-wise level is more marked in the LH, making birth histories relevant: In all 4 pairs, the RH twin was first born, and all twins had IQ values within the average or above average range.

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