Brain structural and functional asymmetry in human situs inversus totalis

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Brain Structural and Functional Asymmetry in Human Situs Inversus Totalis

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Abstract:	Magnetic resonance imaging was used to investigate brain structural and functional asymmetries in 15 participants with complete visceral reversal (situs inversus totalis, SIT). Language related brain structural and functional lateralization of SIT participants, including peri-Sylvian grey and white matter asymmetries and hemispheric language dominance, was similar to those of 15 control participants individually matched for sex, age, education, and handedness. In contrast, the SIT cohort showed reversal of the brain (Yakovlevian) torque (occipital petalia and occipital bending) compared to the control group. Secondary findings suggested different asymmetry patterns between SIT participants with (n=6) or without (n=9) primary ciliary dyskinesia (PCD, also known as Kartagener syndrome) although the small sample sizes warrant cautious interpretation. In particular, reversed brain torque was mainly due to the subgroup with PCD-unrelated SIT and this group also included 55% left handers, a ratio close to a random allocation of handedness. We conclude that complete visceral reversal has no effect on the lateralization of brain structural and functional asymmetries associated with language, but seems to reverse the typical direction of the brain torque in particular in participants that have SIT unrelated to PCD. The observed differences in asymmetry patterns of SIT groups with and without PCD seem to suggest that symmetry breaking of visceral laterality, brain torque, and language dominance rely on different mechanisms.

REPLY TO THE REVIEWERS

Reviewer #1: Excellent paper showing a dissociation between brain "brain torque" (petalia) on the one hand and other structural (perisylvian) and functional markers of cerebral L-R-asymmetry in patients with situs inversus totalis (n=15). The results further support the notion that the typical direction of brain laterality is not affected by this visceral condition.

Of course, an even larger sample would always be desirable in studies of this kind, but the authors' one is already the largest reported so far.

Reply: We thank the reviewer for a positive appreciation of our manuscript. We fully agree that an even larger sample would have better, in particular with regard to the handedness data and the fact that it appears relevant to discriminate between (at least) two types of SIT. But SIT it is a rare condition and we were happy that most of the SIT individuals we found were willing to participate in MRI-scanning.

Reviewer #2: This is a very interesting study investigating the relationship between structural and functional brain asymmetries, handedness and visceral organ situs.

The most valuable points of this work are (1) imaging on a very large sample (n=15) of individuals with situs inversus totalis even including two subgroups of different aetiology, i.e. Kartagener Syndrome and SIT unrelated to primary ciliary dyskenisia and (2) collection of a broad range of information about laterality and cognitive data. The results of this study will help other researches in the field of laterality and embryology research to generate new hypotheses, which has the potential to push research in these areas. The figures included are very illustrative.

Reply: Thank you for a most thorough reading of our manuscript. Your comments and references have been of great value and have contributed to a considerably improved version of the manuscript.

The major drawback of the manuscript as it is, is that many of the citations the authors referring to are very old and the theories they build their research ideas on are outdated. Most strikingly they did not include the latest model on different embryogenetical origins of situs inversus by Vandenberg and Levin (2013). Although the theory of ciliary movement as originator is commonly accepted and seems not to be particularly wrong, evidence suggests that laterality is established much earlier in development which renders ciliary movement not always the primary cause of SIT.

Furthermore, the following points should be addressed during revision:

Acknowledgments, Page 1, Line 3: I'm sure you mean "control participants" instead of "controls participants".

Reply: We have corrected this typo.

Introduction, Page 3, Line 1-2: Please refer at least to the considerations of Vandenberg and Levin (2013) and also mention mechanical aspects of visceral asymmetry establishment.

Reply: Thank you for the most interesting references on recent advances in the biology of embryonic laterality. We have adapted and expanded the first few lines of our introduction accordingly.

Page 3, Lines 1-12. Visceral asymmetry in humans emerges in embryogenesis according to complex genetic mechanisms that remain to be elucidated. The predominant model posits that the origin of left right asymmetry is due to the movement of motile cilia and the resultant directed fluid flow during late gastrulation/early neurulation that gives rise to an asymmetric signaling cascade. Recent findings suggest a much earlier origin of symmetry breaking, perhaps as early as fertilization, and in which cilia merely operate as a downstream amplification/correction mechanism (Vandenberg and Levin 2013). Whichever the primary cause or the role of the cilia in the establishment of developmental chirality, consistent left-right asymmetry is a highly conserved feature in many animal species all of which orient their hearts and viscera with similar biases in placement and morphology. Although the mechanical aspects of visceral asymmetry establishment fall outside the scope of this paper (but see (Grimes and Burdine 2017)), its development results in is characterized by a typical organization of thoracic and abdominal organs including heart on left and liver on right, a condition named situs solitus (solitus (Lat.): customary, habitual).

Line 10-13: This sentence needs specification. E.g. due to genetical abnormality motor protein Dynein is synthesized imperfectly → hypomotility in primary cilia. There is also more recent literature on this e.g. Leigh et al. (2009).

Reply: We added some more information on the background of ciliary hypomotility.

These comorbidities may be the result of associated left-right axis malformations (in particular of the cardiac circulation), or may originate from a putative common underlying etiology such as primary ciliary dyskinesia (PCD), a rare genetic disorder characterized by imperfect synthetization of the motor protein Dynein and resulting in hypomotility of the primary cilia (Kosaki and Casey 1998; Leigh et al. 2009).

Line 13-15: This sentence requires a reference.

Reply: Reference was added.

Bush et al. (1998). Primary ciliary dyskinesia: diagnosis and standards of care. Eur. Respir. J.

Line 16-18: Kartagener-Trias is marked by: situs inversus, chronic sinusitis and bronchiectasis. Please correct this sentence.

Reply: The sentence is corrected.

Combination of situs inversus, chronic sinusitis, and bronchiectasis is known as Kartagener syndrome.

Line 18-19: This sentence should also refer to the more recent model on SIT by Vandenberg and Levin (2013).

Reply: The reference is added.

However, only 20 to 25% of individuals with SI have PCD which indicates that causal mechanisms of SI other than chronic PCD must exist (Rott 1979), an observation which is in agreement with alternative suggestions on the origin of symmetry breaking (Vandenberg and Levin 2013).

Page 4

Line 1-2: Here I would also suggest adding more recent literature e.g. Goto et al. (2010); (Long et al., 2003).

Reply: Thank you for referring to these additional citations on the relation between situs inversus and brain asymmetries in animals. They were added.

All report either reversed vascular or gross lobar brain asymmetry, suggesting a possible relation between visceral and neural asymmetries that was also noted in animals (Wehrmaker 1969; Bisgrove et al. 2000; Goto et al. 2010; Long et al. 2003).

Line 14-17: This sentence needs some connecting passage between retained handedness and brain asymmetries. Although there is a huge corpus of evidence suggesting a relationship between both functions, this is not to be assumed trivial, as recent research even challenges this view by suggesting that handedness and language lateralization are completely dissociable in certain cases (Schmitz et al., 2017).

Reply: We agree that our wording may have been somewhat confusing and disregarded the fact that the relation between handedness and language dominance cannot be taken for granted. We rephrased both sentences and added the suggested reference.

In the first study all three right handed SIT participants showed reversed petalia and no dominant occipital bending, while typical left hemisphere dominance for language and leftward planum temporale asymmetry were retained (Kennedy et al. 1999). Although the association between language dominance and handedness is by no means absolute (Schmitz et al. 2017) the retained brain structural and functional language asymmetries seem in agreement with multiple reports of normal handedness patterns in SIT individuals (McManus et al. 2004; Afzelius and Stenram 2006).

Line 19-21: You say: "In contrast with the first report, … " you only mention that two of the subjects in Ihara et al. (2010) were weakly left/right handed in the discussion. Since the question of handedness was a big issue in your study data and you even replaced control participants after realizing that handedness is a factor subjects should be matched for, this finding of Ihara et al. (2010) should be addressed in one sentence in the introduction. It is also mentionable that in contrast to Kennedy et al. (1999) they included one neurological patient.

Reply: We agree with the comment of the reviewer and now mention the handedness of all individuals from previous imaging studies in the introduction.

In the first study all three right handed SIT participants showed reversed petalia and no dominant occipital bending, while typical left hemisphere dominance for language and leftward planum temporale asymmetry were retained (Kennedy et al. 1999). Although the association between language dominance and handedness is by no means absolute (Schmitz et al. 2017) the retained brain structural and functional language asymmetries seem in agreement with multiple reports of normal handedness patterns in SIT individuals (McManus et al. 2004; Afzelius and Stenram 2006). The second study also observed reversed petalia in three other SIT participants, but reported atypical (right hemisphere) language dominance in two of the three SIT individuals despite normal leftward planum temporale asymmetry (Ihara et al. 2010). In contrast with the first report, the second study suggests an increased probability of atypical language dominance in SIT and a possible link with reversal of the cerebral torque, but here two participants showed weak left/right handedness and the other suffered from left temporal lobe epilepsy (Ihara et al. 2010). A recent case-study, described typical left-lateralized neural language organization in a right handed boy with SIT that showed reversed frontal and occipital petalia (Schuler et al. 2017).

Line 21-22: Since you are addressing language related structural asymmetries revealed by Kennedy et al. (1999) you should also mention that Schuler et al. (2017) found a typical structural asymmetry pattern in the fetal superior temporal sulcus in SIT.

Reply: We have added this finding.

A recent case-study, described typical left-lateralized neural language organization in a right handed boy with SIT that showed reversed frontal and occipital petalia and typical structural asymmetry in the superior temporal sulcus on an antenatal scan (Schuler et al. 2017).

The authors should also mention the study by Tanaka et al. (1999) that found laterality of ear advantage in a dichotic listening paradigm to be typical in a sample of nine SI subjects. Although, they do not report structural features of the brain, they have to date (I mean until you came) the largest sample evidence for typical functional language lateralization. This evidence additionally relativizes the results of Ihara et al. (2010).

Reply: We have added the evidence reported by Tanaka et al.

Although no brain structural data were reported, further functional evidence of typical language lateralization in nine right handed participants with SIT was provided using a dichotic listening paradigm (Tanaka et al. 1999). Eight of the nine SIT-participants showed right-ear-advantage indicative of left hemisphere language dominance, a ratio similar to that of a control cohort.

Line 22-p5 Line 4: The research question the authors ask does not emerge from the study evidence they described before. In fact, in the previous section they clearly summarize that reversed brain torque is in the right-handed healthy population of SIT cases not associated with atypical language. I am missing a clear explanation on the relationship between torque asymmetry and language lateralization on healthy and patient samples.

Reply: We agree that the research question was formulated oddly given the evidence listed before. We have rephrased this sentence. The reviewer also mentions a missing explanation on the relation between brain torque asymmetry and language lateralization on healthy and patient samples. The problem is that this relation is speculative at best and hardly investigated in healthy people, let alone in clinical cohorts. Measurement of the (different qualities of the) torque in itself is methodologically demanding, and we used a new and state-of-the-art approach to quantify them. We mention the different points of view on the relevance of the torque for functional laterality in the discussion (we did not want to overload the already long introduction with yet another complex concept), but have to conclude that at least with regard to language lateralization, there does not seems to be a relationship.

Together these results suggest that SIT, which is apparently associated with reversed brain torque, in general presents with typical brain structural and functional lateralization for language (Geschwind and Levitsky 1968; Geschwind and Galaburda 1987; McManus and Bryden 1991). This conclusion is however based on neuroimaging data of only seven SIT participants using three different language paradigms and qualitative rather than quantitative measures of a limited set of brain structural language indices. By recruiting a larger cohort of SIT participants to a brain imaging study than ever before we aim to confirm that human SIT is associated with typical brain structural and functional asymmetry. We present new brain imaging data for 15 SIT participants in comparison with an age, sex, handedness, and education matched control cohort concerning quantitative asymmetries of petalia and bending, and putative language associated areas like the trajectory of the Sylvian fissure, cortical surface area of planum temporale, anterior insula, Heschl's gyrus, and pars opercularis and triangularis of the inferior frontal gyrus, and number of white matter tracts in the arcuate fasciculus (Galaburda et al. 1978; Toga and Thompson 2003; Catani et al. 2005; Catani and Mesulam 2008; Chiarello et al. 2013) as measured using a 3-D high-resolution T1-weighted anatomical images of the whole brain and Diffusion Tensor Imaging (DTI) respectively. In addition, we describe the BOLD-activation pattern during a word generation paradigm to assess hemispheric language dominance (Wagner et al. 2014), while taking possible brain structural group differences into account (Goebel et al. 2006). In addition, we aim to explore possible differences with regard to these measures in SIT participants with or without PCD.

Page 5

Line 10: Please delete the blank before the comma.

Reply: Done

Materials and Methods

Page 7

Line 12: Did you mean "was" instead of "were"?

Reply: We corrected this grammatical error.

Page 8/12/26

You mention that you could not assess in scanner fluency performance, but you did additional fluency assessment outside the scanner. This is quite creditable of you. I have, however, two concerns about your approach. First, you assess semantic-categorical fluency outside and phonemic fluency inside the scanner. Although both tasks are associated with memory retrieval from temporal lobes, phonemic fluency requires stronger involvement of the prefrontal cortex (Baldo et al., 2006; Chapados and Petrides, 2013; Kopp et al., 2013; Papagno et al., 2010; Robinson et al., 2012). So you should at least mention this at some point in the paper. Second, you report that there is no group difference between SIT and control group in out of scanner fluency performance. The interesting question in this case would be: how does outside scanner performance relate to BOLD signal. Therefore, I would like to see the correlation between outside-scanner performance and percent signal change during in scanner phonemic fluency.

Reply: Assessment of in scanner word fluency performance is difficult to achieve without risk of movement artefacts. To counter arguments that eventual group differences are merely the result of performance differences researchers sometimes use an outside scanner fluency task to estimate performance levels. We fully agree with the reviewer's remark that it would have been more ideal to have used an identical phonemic fluency task to measure inside and outside scanner performance. We now acknowledge in the text that both tasks rely on similar but not identical brain activation patterns and that a comparable performance level merely suggests, but by no means assures that both groups performed similarly.

In order not to over interpret the implications of eventual performance differences we think it is not warranted to correlate outside-scanner semantic fluency performance and percent signal change during in scanner phonemic fluency. First, it is highly unlikely that these variables will correlate and second, a negative finding will be impossible to interpret as both tasks are different.

Changes on page 9

To avoid image acquisition disturbances due to speech the phonemic fluency task was (as usual) performed covertly. As a result, no in-scan performance data are available. Instead, we asked the participants to perform an out-of-scan word generation exercise that required them to name as many fruits/vegetables in one minute. Despite very similar brain activation during the execution of either phonemic or semantic verbal fluency tasks, phonemic fluency reveals stronger response of the opercular cortex (Wagner et al. 2014). This dissimilarity should be taken into account when interpreting performance results.

Changes on p 28

On the out-of-scan word fluency task SIT participants named on average 21 (SD=3.0) items and controls 23 (SD=3.6). This difference is not significant, but should be interpreted with caution given the dissimilarity of the inside and outside word fluency tasks (Table A2). Put differently, the comparable between-group performance level merely suggests, but by no means assures that both groups performed similarly.

Page 10: Caption of Figure 1: Maybe you meant "coordinate system" instead of "coordinate".

Reply: Caption has been adapted.

Page 12: On your part on Sylvian fissure trajectory: which measure did you use for quantifying fissural length and how exactly did you quantify sulcal depth?

Reply: The length and depth measures are calculated based on a BrainVISA's "Morphometry Statistics" toolbox measure explained more fully in Cykowski et al. (2008) and used for example in Leroy et al (2015). It is based on a model-driven parameterization which is used to define a coordinate system on the sulci. The length is then calculated as the voxel length of the external sulcal line that joins the fold segmentation to the brain hull. The average depth of the sulci is calculated based on the distance between the most and least superficial location at each length coordinate line of the sulcal fold on the brain hull to the bottom of the sulcus, and is calculated by the geodesic distance map that follows the curve of the sulcus itself (and not just an Euclidean distance). This has been summarized and incorporated into the text, along with the relevant reference, on Pages 13-14.

1. Cykowski MD, Coulon O, Kochunov PV, Amunts K, Lancaster JL, Laird AR, Glahn DC, Fox PT, "The central sulcus: an observer-independent characterization of sulcal landmarks and depth asymmetry", Cerebral Cortex, 18(9):1999-2009, 2008.

2. Leroy, F., Cai, Q., Bogart, S. L., Dubois, J., Coulon, O., Monzalvo, K., et al. (2015). New human-specific brain landmark: The depth asymmetry of superior temporal sulcus. *Proceedings of the National Academy of Sciences*, *112*(4), 1208–1213. http://doi.org/10.1073/pnas.1412389112

Changes on page 13-14

To analyze Sylvian Fissure (SF) asymmetry the 3D MR images were processed using BrainVISA software (http://brainvisa.info/web/index.html, version 4.5.0) blind to group. The SF were analyzed carefully in 3D space to identify the presence of the bifurcation point, and when present, split into the anterior-horizontal-SF (AH-SF) and vertical-SF (V-SF) segments. The length and depth of each segment was then automatically measured in each of the scans based on the length and the distance depth between the most and least superficial location respectively within a parameterized coordinate system using the Morphometry Statistics toolbox (see Cykowski et al., 2008). Asymmetry indices (AI = ((R-L)/(R+L))*0.5) were computed for each of the segment length measurements. In four individuals (two with SIT and two controls) V-SF lengths and/or depth measurements in one hemisphere could not be computed, leading to a comparison of 13 individuals for each group.

Page 14

Line 12: As far as I understand you only had one dependent variable, namely asymmetry index of AF tracts, in this analysis. So how were you able to perform multivariate analysis of variance?

Reply: Thank you for noticing this typo. We performed a two-way anova, but named it a multivariate by mistake. We have corrected the error.

Page15. A two-way anova was used to evaluate the effects of Group and Side.

Results

Page 18

Line 2: You write "Table S2" instead of "A2".

Reply: Corrected

Page 20

You should consider performing an additional comparison including the handedness as factor, as handedness and brain torque might relate to each other irrespective of organ situs.

Reply: The aim of this study is to investigate the patterns of brain torque in the two groups with or without situs inversus. It is worth noting that the handedness has been matched between the two groups. We thank the Reviewer for the suggestion to additionally including the handedness as a factor in exploring brain torque and will consider the relationship between handedness and brain torque in a subsequent publication with larger sample size.

Page 21

Line 4: According to your reported degrees of freedom (16) I assume you performed an unpaired t-test here. However, in this case paired t-test is indicated since you are comparing the study group to a matched control group.

Reply: Yes, we chose to perform an unpaired t-test to compare between the study group and the control group, because the two cannot be perfectly paired considering the larger individual variations, even though they have been carefully matched for general conditions, such as sex, handedness and age.

Line 6-10: I think it is sufficient, if you report only the results including the forced right-handers. The subjects' anlagen and the brain development before the onset of forced right-handedness should be more influential on brain torque than forced right-handedness.

Reply: We agree with the reviewer that forced right handedness is unlikely to have an impact on brain torque, although we used this argument to discard the forced right handers from the correlation. Unfortunately, we used the wrong argument here. The correct argument is that forced right handedness influences handedness inventory score (both cases scored weakly right handed according to the EHI) and hence the EHI is not a proper reflection of the participant's original handedness. In fact we don't know what the participant's original handedness score would have been. For this reason it is more appropriate to leave the two cases of forced right handedness out of the equation when correlating EHI with petalia. We rephrased our argument on page 22.

It is also worth noting that for the total cohort (excluding the forced right handers SI09 and SI13) both the frontal and occipital petalia were significantly correlated with the handedness inventory score ($r=0.48$, $p=0.010$ for the frontal petalia and $r=0.43$, $p=0.021$ for the occipital petalia). Including the forced right handers gave similar results, but since forced right handedness influences handedness scores, we chose to exclude them.

Page 23: Figure 3: According to an older post-mortem study by Witelson and Kigar (1992) the anterior segment showed no asymmetry, the horizontal segment shows leftward asymmetry and the vertical segment shows rightward asymmetry. Since your asymmetry patterns are differing from this standard cohort, I would love this observation to be discussed in the end of your report.

Reply: For the current study, we decided to compound the anterior and horizontal segments identified and measured by Witelson & Kigar (1992) together as the identification of Heschl's sulcus as a separating landmark is challenging based on MR images alone. As expected based on the observation of a symmetric anterior segment and leftward horizontal segment, the controls did have an overall leftwardly asymmetric anterior-horizontal segment. It was surprising that the controls did not have a rightward vertical SF (though the SIT cohort did) in this case and we can't offer any specific explanation of this.

In terms of the spread of data points in the control cohort, 8 of them did have a rightward asymmetry of some magnitude, 4 had leftward asymmetry and 1 was considered symmetric. One measure of each direction was considered an outlier and not counted in the statistics, but even though nearly twice as many of the control cohort's V-SF had rightward asymmetry, the magnitude was smaller, bringing the average to be leftward, though any difference was not significant between the SI cohort. This will be discussed at the conclusion of the paper, and we will recommend that further study in a bigger cohort will be necessary to definitively consider whether the SF asymmetries differ in Situs Inversus.

Suggested Text Addition page 32

The findings of the present study confirm the typical leftward planum temporale asymmetry in SIT participants and extend this finding to other language-related peri-Sylvian asymmetries. This includes the SF length asymmetries, though our control cohort did not have the expected rightward asymmetries in the vertical aspect (Witelson & Kigar, 1992) unlike the SIT participants, and so further replication is needed.

Page 27

Line 14-19: This part belongs to the discussion section. And you might also relativize your finding on posterior insula and somatosensory regions, since your clusters are not controlled for multiple comparisons. Anyway, your finding on decreased (not "increased") somatosensory activation in the SIT group seems quite interesting and I would love to have it discussed in one or two additional sentences.

Reply: We downplayed the importance of the difference findings by pointing at the uncorrected statistics on page 28. We moved the interpretation part with regard to the insular region to the discussion (page 33). We have looked carefully at the literature on interoception and visceroception, but found no reports on lateralized activity in the posterior insula. We feel that any discussion of this finding from our part would be highly speculative and we prefer to mention the finding just as we did, but to refrain from any interpretation as it is not central to the aim of the study and indeed not statistically corrected, as you rightly pointed out.

Page 28

Direct comparison of word generation activation maps (Figure 6B) revealed that the SIT participants showed increased activation of right posterior insula compared to controls. In addition, they showed reduced BOLD-response in left medial frontal and left middle frontal gyrus. The relevance of these differences is tempered by their being achieved by statistics uncorrected for multiple comparisons and by the observation that the frontal differences lie outside the classic peri-Sylvian region associated with language.

Page 33

Functional MRI revealed only minor between-group differences during word generation. Further exploration of potentially relevant insular differences revealed that word generation-related (within-group) insular activation was located in anterior insula of both hemispheres (though stronger on the left), whereas the between-group related difference was located in right posterior insula. Asymmetric anterior insular involvement of the former contrast is in agreement with its putative role in communication and language (Craig 2002; Chiarello et al. 2013). Posterior insula has been associated with interoceptive representation, including responses to visceral sensations (Craig 2002). Apparently, the insular between-group difference found does not pertain to a region underlying language or speech. The increased and lateralized activation of this visceroceptive region in SIT participants compared to the control cohort warrants further attention.

Page 28-29: Caption of Figure 6: Please recheck punctuation. Also you should add colour bars to your fMRI cluster images.

Reply: Color bars are added to all figures with fMRI data.

Page 29/30

Line 12-p30 3: This section is already part of a discussion.

Reply: We removed the sentence ‘These atypical hand preference/language dominance combinations are known to occur in small numbers in the general population (Mazoyer et al. 2014).’ from this section. It is already mentioned in the discussion on page 33.

In addition, we separated observation from interpretation with regard to participant SI07 and moved the latter to the discussion section on page 33.

Discussion

Page 30

Line 12-15: Here I would also be glad, if you had some more recent citations, e.g. Kasprian et al. (2010), Habas et al. (2012).

Reply: Thank you for these relevant references. They were added to the statement.

Page 31: Does SI07 have left-handers in their family?

Reply: No, her parents, grandparents, and her brother were all right handed.

We added … and no familial sinistrality… to the short description of this case on page 33.

Page 32: Figure 7 is very informative, thank you for that one.

Reply: Glad you liked the figure.

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Brain Structural and Functional Asymmetry in Human Situs Inversus Totalis

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Abstract

Magnetic resonance imaging was used to investigate brain structural and functional asymmetries in 15 participants with complete visceral reversal (situs inversus totalis, SIT). Language related brain structural and functional lateralization of SIT participants, including peri-Sylvian grey and white matter asymmetries and hemispheric language dominance, was similar to those of 15 control participants individually matched for sex, age, education, and handedness. In contrast, the SIT cohort showed reversal of the brain (Yakovlevian) torque (occipital petalia and occipital bending) compared to the control group. Secondary findings suggested different asymmetry patterns between SIT participants with (n=6) or without (n=9) primary ciliary dyskinesia (PCD, also known as Kartagener syndrome) although the small sample sizes warrant cautious interpretation. In particular, reversed brain torque was mainly due to the subgroup with PCD-unrelated SIT and this group also included 55% left handers, a ratio close to a random allocation of handedness. We conclude that complete visceral reversal has no effect on the lateralization of brain structural and functional asymmetries associated with language, but seems to reverse the typical direction of the brain torque in particular in participants that have SIT unrelated to PCD. The observed differences in asymmetry patterns of SIT groups with and without PCD seem to suggest that symmetry breaking of visceral laterality, brain torque, and language dominance rely on different mechanisms.

Key words: brain asymmetry; situs inversus; primary ciliary dyskinesia; language dominance; handedness.

Introduction

Visceral asymmetry in humans emerges in embryogenesis according to complex genetic mechanisms that remain to be elucidated. The predominant model posits that the origin of left right asymmetry is due to the movement of motile cilia and the resultant directed fluid flow during late gastrulation/early neurulation that gives rise to an asymmetric signaling cascade. Recent findings suggest a much earlier origin of symmetry breaking, perhaps as early as fertilization, and in which cilia merely operate as a downstream amplification/correction mechanism (Vandenberg and Levin 2013). Whichever the primary cause or the role of the cilia in the establishment of developmental chirality, consistent left-right asymmetry is a highly conserved feature in many animal species all of which orient their hearts and viscera with similar biases in placement and morphology. Although the mechanical aspects of visceral asymmetry establishment fall outside the scope of this paper (but see (Grimes and Burdine 2017)), ~~I~~its development results in ~~is characterized by~~ a typical organization of thoracic and abdominal organs including heart on left and liver on right, a condition named situs solitus (solitus (Lat.): customary, habitual). Individuals with situs inversus (SI), exhibit either a complete reversal of thoracic and abdominal organs (situs inversus totalis) or a partial reversal of some internal organs (situs ambiguus). The prevalence of SI in adults is quite rare and estimated to be 1 in 10,000 (Torgersen 1950; Rott 1979). As visceral reversal does not necessarily hinder normal organ functioning, SI may go unnoticed in some individuals, whereas in others an increased incidence of circulatory, digestive, and respiratory disease is observed. These comorbidities may be the result of associated left-right axis malformations (in particular of the cardiac circulation), or may originate from a putative common underlying etiology such as primary ciliary dyskinesia (PCD), a rare genetic disorder characterized by imperfect synthetization of the motor protein Dynein and resulting in hypomotility of the primary cilia~~a general defect of ciliary motion~~ (Kosaki and Casey 1998; Leigh et al. 2009). Since about half of patients with PCD present with SI, disturbed movement of nodal cilia during embryogenesis is believed to result in a random allocation of visceral laterality (Bush et al. 1998). After birth, defective ciliary motion leads to recurrent infections of the upper and lower respiratory tracts and subfertility in both sexes. Combination of situs inversus, chronic sinusitis, and bronchiectasis ~~When PCD occurs in combination of chronic respiratory infections and subfertility this~~ is known as Kartagener syndrome. However, only 20 to 25% of individuals with SI have PCD which indicates that ~~other~~ causal mechanisms of SI other than chronic PCD must exist (Rott 1979), an observation which is in agreement with alternative suggestions on the origin of symmetry breaking (Vandenberg and Levin 2013).

Published studies of brain asymmetries in individuals with SIT are relatively few and include an autopsy report of one subject (Tubbs et al. 2003) and three neuroimaging studies (Kennedy et al. 1999; Ihara et al. 2010; Schuler et al. 2017). All report either reversed vascular or gross lobar brain asymmetry, suggesting a possible relation between visceral and neural asymmetries that was also noted in animals (Wehrmaker 1969; Bisgrove et al. 2000; Goto et al. 2010; Long et al. 2003). In particular, all available brain images obtained for people with SIT report atypical ‘Yakovlevian’ or brain torque. The brain torque refers to a counter-clockwise twist of the brain about the vertical axis of the body which is observed in the majority of humans and gives rise to a more anteriorly protruding frontal lobe on the right and even more posteriorly protruding occipital lobe on the left that are referred to as ‘petalia’ (Figure 1a-c), (Toga and Thompson 2003). The torque’s twisting effect is also credited for a more anterior position and more vertical trajectory of the right Sylvian fissure relative to the left and a so-called ‘bending’ of the left occipital lobe across the midline resulting in a rightward turning of the posterior interhemispheric fissure. The three neuroimaging studies also investigated whether the atypical brain torque of the

SIT individuals was associated with reversed hemispheric language dominance. In the first study all three right handed SIT participants showed reversed petalia and no dominant occipital bending, while typical ~~together with usual~~ left hemisphere dominance for language and leftward planum temporale asymmetry were retained (Kennedy et al. 1999). Although the association between language dominance and handedness is by no means absolute (Schmitz et al. 2017) ~~T~~the~~se~~ retained brain structural and functional language asymmetries seem in agreement with multiple reports of normal handedness patterns in SIT individuals (Mcmanus et al. 2004; Afzelius and Stenram 2006). The second study also observed reversed petalia in three other SIT participants, but reported atypical (right hemisphere) language dominance in two of the three SIT individuals despite normal leftward planum temporale asymmetry (Ihara et al. 2010). In contrast with the first report, the second study suggests an increased probability of atypical language dominance in SIT and a possible link with reversal of the cerebral torque, but here two participants showed weak left/right handedness and the other suffered from left temporal lobe epilepsy (Ihara et al. 2010). A recent case-study, described typical left-lateralized neural language organization in a right handed boy with SIT that showed reversed frontal and occipital petalia and typical structural asymmetry in the superior temporal sulcus on an antenatal scan (Schuler et al. 2017). Although no brain structural data were reported, further functional evidence of typical language lateralization was provided in nine right handed participants with SIT using a dichotic listening paradigm (Tanaka et al. 1999). Eight of the nine SIT-participants showed right-ear-advantage indicative of left hemisphere language dominance, a ratio similar to that of a control cohort. Together these results suggest that ~~leave open the interesting question of whether~~ SIT, which is apparently associated with reversed brain torque, in general presents with typical brain structural and functional ~~may be related to reversed functional~~ lateralization ~~of the brain~~ for language ~~and whether or not this is independent of more local brain asymmetries in structures~~

~~such as the planum temporale thought to be associated with language lateralization~~ (Geschwind and Levitsky 1968; Geschwind and Galaburda 1987; McManus and Bryden 1991). This conclusion is however based on neuroimaging data of only seven SIT participants using three different language paradigms and qualitative rather than quantitative measures of a limited set of brain structural language indices. By recruiting a larger cohort of SIT participants to a brain imaging study than ever before we ~~additionally~~ aim to confirm ~~explore whether~~that human ~~visceral reversal in~~ SIT is associated with ~~or without PCD would show different effects on~~typical brain structural and functional asymmetry. We present new brain imaging data for 15 SIT participants in comparison with an age, sex, handedness, and education matched control cohort concerning quantitative asymmetries of petalia and bending, and putative language associated areas like the trajectory of the Sylvian fissure, cortical surface area of planum temporale~~-~~, anterior insula, Heschl's gyrus, and pars opercularis and triangularis of the inferior frontal gyrus, and number of white matter tracts in the arcuate fasciculus (Galaburda et al. 1978; Toga and Thompson 2003; Catani et al. 2005; Catani and Mesulam 2008; Chiarello et al. 2013) as measured using a 3-D high-resolution T1-weighted anatomical images of the whole brain and Diffusion Tensor Imaging (DTI) respectively. In addition, we describe the BOLD-activation pattern during a word generation paradigm to assess hemispheric language dominance (Wagner et al. 2014), while taking possible brain structural group differences into account (Goebel et al. 2006). In addition, we aim to explore possible differences with regard to these measures in SIT participants with or without PCD.

Materials and Methods

Participants

The study was approved by the Research Ethics Committee and following institutional approval the database of Ghent University Hospital was searched for the term ‘situs inversus’ in radiological protocols of patients aged 18-70 years. By using the contact information available, these individuals were sent information describing the rationale for the study together with the procedures to be followed and invited to participate. Written informed consent was obtained according to the Declaration of Helsinki. A similar procedure was applied at Middelheim Hospital, Antwerp. Seventeen participants suspected to have SI (SI01 through SI17) agreed to take part and, after informed consent was obtained, underwent a neuroimaging protocol described below. The participants provided written consent to access the actual radiological files that were consulted to determine the type of situs inversus and possible comorbidities. Radiological information (RX or CT) of thorax and complete abdomen was available in 9 participants, and of thorax and upper abdomen in 8 participants. The medical reports confirmed that all participants presented with radiologically documented situs inversus totalis, except SI01 who had situs ambiguus (levocardio) and SI10 whose protocol mentioned the term ‘situs inversus’ in a context unrelated to the visceral condition of the patient. The latter two participants were excluded from the study. The patient sample thus consisted of 15 individuals with situs inversus totalis, 7 women and 8 men that were between 18 and 50 years old (Table A1).

In five participants with SIT a formal diagnosis of primary ciliary dyskinesia or Kartagener syndrome was found in their medical records. All were referred for radiological investigation on account of respiratory problems. A sixth SIT-participant was identified on account of a radiological consultation regarding infertility. The participant also complained about chronic sinusitis and mild chronic bronchitis, symptoms that were confirmed by his general practitioner and lung specialist. Although no formal diagnosis of PCD was obtained in this case, the presence of chronic upper and lower respiratory infection and infertility in an individual with SIT warrants suspicion of Kartagener syndrome. Consequently, we ranked the participant with the PCD group. The PCD prevalence of 40% in our SIT-sample is higher than generally estimated (20-25%, (Rott 1979)) and can be explained by the fact that individuals with PCD are likely to seek more medical attention because of their chronic symptoms and thus become more easily detectable in a search based on hospital records. Three PCD-unrelated SIT participants had been previously diagnosed with congenital heart disease that required surgical treatment and their radiological files all referred to their cardiac condition. Congenital heart disease is a frequent comorbidity of SI as the cardiac circulation appears particularly sensitive to perturbation in normal left-right positional information (Kosaki and Casey 1998). The other six PCD-unrelated SIT participants reported no medical complications. They underwent radiological examinations for various reasons including gastric complaints (n=1), general fatigue (n=1), accidents (traffic related (n=1) or sport related (n=1)). In two cases the reason for referral could not be determined from the radiologist's report.

A group of healthy control participants was~~ere~~ also recruited, individually matched with the SIT participants with respect to age, sex, handedness and years of formal education. Recruitment was performed via the social networks of the researchers and word of mouth and all participants underwent an identical research protocol as the SIT participants. Although no radiological verification was obtained, it is reasonable to assume that all conform to a situs solitus configuration. Demographics and relevant medical data of all participants are listed in Table A1. Initially, the control group was not matched for handedness but given recent evidence of this trait's influence on cognitive performance and neuroanatomy (Herve et al. 2013; Mellet et al. 2014; Marie et al. 2015) several control participants were replaced with participants to ensure this matching. This explains the extended notation in some of the control participant ID-codes in Table A1. The control group thus also consisted of 15 individuals, 7 women and 8 men, aged between 19 and 51 years (Table A1). Mean age of the SIT-group is 33.0 years (SD = 10.1) with 12.9 (SD = 2.3) years of formal education. Average age of the control group is 33.0 years (SD = 10.0) with 12.9 (SD = 1.6) years of formal education. These measures were not significantly different from the SIT participants.

Behavioral assessment

Before MRI investigations were performed participants completed a Dutch version of the National Adult Reading Test (NART (Nelson and Willison 1991); DART (Schmand et al. 1992)) to estimate intelligence. In two non-Dutch speakers (SI06 and CO17) the Standard Raven Progressive Matrices were used for the same purpose (Raven 1976) (Table A2). Participants also completed the Edinburgh Handedness Inventory (EHI) (Oldfield 1971). The EHI requires participants to indicate the side of the preferred limb for the execution of 12 tasks (i.e. writing, throwing, brushing teeth). The total number of left and right preference marks on the 10 hand-items only was used to calculate a lateralization index (R-L)/(R+L) that reflects the individual's general handedness. This LI ranges from +1 (consistent right hander) to -1 (consistent left hander) (Table A1). The absolute value of the LI makes abstraction of handedness direction, and is used as an indication of handedness strength.

To avoid image acquisition disturbances due to speech the phonemic fluency~~fMRI~~ task was (as usual) performed covertly. As a result, no in-scan performance data are available. Instead, we asked the participants to perform an out-of-scan word generation exercise that required them to name as many fruits/vegetables in one minute. Despite very similar brain activation during the execution of either phonemic or semantic verbal fluency tasks, phonemic fluency reveals stronger response of the opercular cortex (Wagner et al. 2014). This dissimilarity should be taken into account when interpreting performance results.

MR acquisition details

MRI data were acquired using a 3.0 tesla TIM Trio (release VB17) and standard 32-channel head coil (Siemens Healthineers, Erlangen, Germany). First, a high-resolution anatomical image of the whole brain was acquired using an MPRAGE sequence with 1.0x1.0x1.0mm³ resolution and 176 sagittal slices (TR/TE/TI= 2250/4.18/900ms, flip angle 9°).

Functional T2* weighted echo planar images (EPI) with blood oxygenation level-dependent (BOLD) contrast were acquired with voxel size 3.0x3.0x2.5mm³, FOV = 192mm, 33 ascending axial slices, TR/TE=2500/27ms, flip angle=62° and PAT=2. A total of 245 volume scans were acquired over 10 minutes.

Finally, Diffusion Tensor Imaging (DTI) was performed using a twice refocused single-shot echo-planar diffusion pulse sequence with an isotropic image resolution of 2.5mm³, 64 different non-collinear directions, b-values of 0 and 1200 s/mm², 60 contiguous slices, TR/TE=10800/83ms, FOV=240mm, matrix size 96x96, bandwidth 1736 Hz/Px, epi factor=96, PAT=3 and acquisition time= 12:36min.

Processing of the structural MRI scans

Brain torque: Petalia and bending

All 3D MR images were pre-processed in FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) including skull strip, bias field correction, and brain normalization using 7 degree of freedom transformations (i.e., 3 translations, 3 rotations and 1 uniform scaling). The uniform scaling factor by which the acquired brain dataset was scaled so as to be co-registered to the standard MNI152 template (http://www.bic.mni.mcgill.ca/ServicesAtlases/ICBM152NLin2009) was recorded. The processed brain images were then analyzed in the standard FreeSurfer processing stream (https://surfer.nmr.mgh.harvard.edu/), in which the surface-based module enables high quality cerebral surface reconstruction from the brain volume data by following the T1 intensity gradient between grey matter and CSF with subvoxel accuracy (Dale et al. 1999).

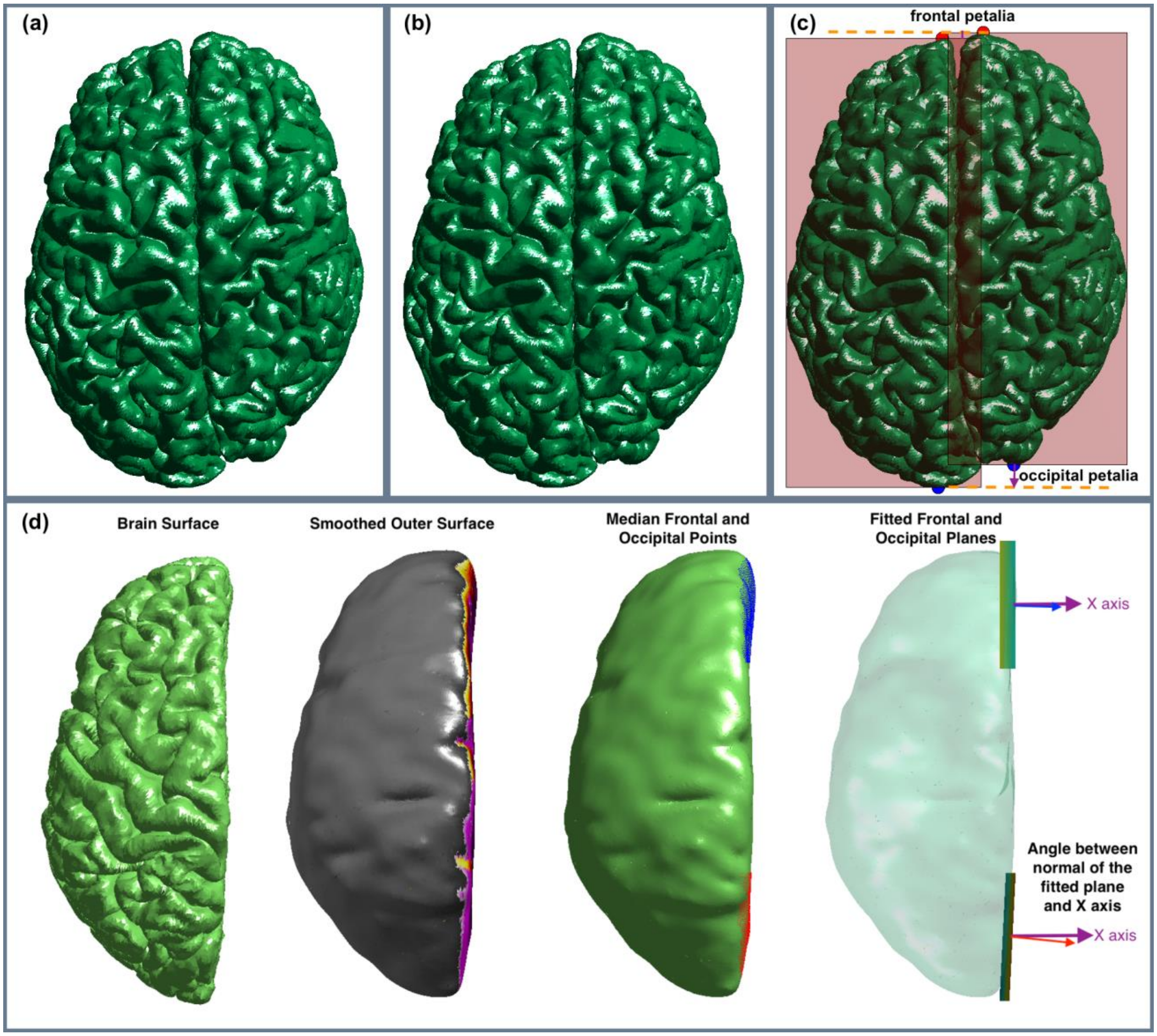

Figure 1. *Petalia and frontal/occipital bending computation. The original cerebral surface in MNI coordinate* system *is demonstrated in (a) and the re-oriented surface whose mid-sagittal plane was aligned parallel to x=0 is shown in (b). The measurement of petalia at the frontal and occipital poles is depicted in (c). The computation of the frontal and occipital bendings in the left and right direction is illustrated in (d), in which the bending angles are estimated as the angle between x-axis and the normal of the least squares plane that best fits the vertices relating to the medial surface of the brain at corresponding regions..*

To measure the frontal and occipital petalia and bendings, it is important to put brains in the standard orientation with the mid-sagittal plane parallel to x=0 plane in the MNI coordinate system. After pre-processing in FSL, the brain has already been normalized to the MNI coordinate space. However, due to the asymmetric shape of brains, the low-dimensional linear registration often fails to align the mid-sagittal plane to x=0. The following steps were therefore applied: i) the mid-sagittal plane (MSP) was computed as the least squares plane that best fits the 3D vertices on the medial surface of the brain lying within 5 mm of plane x=0 (the MSP is therefore not influenced by the fact that the interhemispheric fissure is not entirely planar or by asymmetries of the lateral surface of the brain), ii) following computation of brain-MSP, the angle between the brain-MSP and x=0 was estimated as the 3D angle α between the surface normals of the brain-MSP and x=0 and iii) the whole brain surface reconstructed in FreeSurfer (see Figure 1a) was rotated through angle $-\alpha$ to align the brain-MSP parallel to plane x=0 (see Figure 1b). The frontal and occipital poles were determined automatically as the most extensive points in the anterior-posterior direction for each 3D cerebral hemisphere surface respectively. The petalia were computed as the relative displacement between the homologous points of the left and right cerebral hemispheres in the anterior-posterior direction, see Figure 1c. The computation of the frontal and occipital bendings in the left-right direction is demonstrated in Figure 1d. The procedure can be broken into the following subtasks: i) generate a smoothed outer surface for each cerebral hemisphere by the tessellation on top of the cerebral hemisphere volume that was sulcus-filled using the morphologic closing operation, ii) at each surface location compute the local surface normal and angle between the estimated normal and x-axis, iii) locate the vertices lying within the mid-sagittal plane by thresholding the angle computed in step 2) at 40° (vertices on the lateral surface or the edge of the brain normally are associated with larger angles) and from which further identify the vertices belonging to the frontal and occipital regions by restricting to the first and last quarter of the anterior-posterior direction coordinate (Y coordinate), iv) compute the least squares planes that best fit the points obtained in step 3) for the frontal and occipital regions respectively, and v) compute the frontal and occipital bendings as the angles between the normal of the plane and x-axis at associated regions. Asymmetries of the above measurements were statistically analyzed for the control and SIT-participants independently at the respective frontal and occipital regions using one-tailed one-sample t-tests. The group difference was explored by one-tailed two-sample t-tests.

Sylvian fissure trajectory

To analyze Sylvian Fissure (SF) asymmetry the 3D MR images were processed using BrainVISA software (http://brainvisa.info/web/index.html, version 4.5.0) blind to group. The SF were analyzed carefully in 3D space to identify the presence of the bifurcation point, and when present, split into the anterior-horizontal-SF (AH-SF) and vertical-SF (V-SF) segments. The length and depth of each segment was then automatically measured in each of the scans based on the length and the distance depth between the most and least superficial location respectively within a parameterized coordinate system using the Morphometry Statistics toolbox (Cykowski et al. 2008). Asymmetry indices (AI = ((R-L)/(R+L))*0.5) were computed for each of the segment length measurements. In four individuals (two with SIT and two controls) V-SF lengths and/or depth measurements in one hemisphere could not be computed, leading to a comparison of 13 individuals for each group.

Between-Group differences of the four factors (AH-SF and V-SF length and depth AI) were analyzed using a multivariate analysis of variance (MANOVA). Differences between individual matched pairs were also analyzed separately using a paired sample t-test.

Peri-Sylvian surface areas

FreeSurfer was used to automatically label brain surface into sulco-gyral regions by comparing geometric information (e.g., curvature) derived from 3D cortical surface to a pre-trained atlas that embeds the neuroanatomical convention (Fischl et al. 2004). By virtue of this labelling technique, based on a so-called Destrieux surface label atlas (Destrieux et al. 2010), individual brains are parcellated into 148 regions (two hemispheres x 74 maps). In this study, the primary focus lies at peri-Sylvian language related regions, therefore, the average values of the cortical surface area were extracted for planum temporale, anterior insula, Heschl’s gyrus, pars opercularis and pars triangularis. We applied multivariate analysis of variance to evaluate the effects of Group and Side.

Arcuate fasciculus

Diffusion weighted MRI brain scans were corrected for eddy current distortion and head motion using eddy (Andersson and Sotiropoulos 2016) from FDT (FMRIB diffusion toolbox, part of FSL) and using a 12 parameter affine registration to a reference volume (volume without diffusion-weighting), whereby the gradient directions were rotated accordingly (Leemans and Jones 2009). Deterministic tractography was performed using Euler integration (Basser et al. 2000). Fiber pathways were reconstructed until fiber tracts entered a voxel with fractional anisotropy <0.20 or when the angle between two consecutive tractography steps was >35°, the step size was 1 mm. Manual fiber tracking was performed with the TrackVis software (Wang et al. 2007) using a two-ROI approach to delineate the direct segment of the arcuate fasciculus (AF) in each hemisphere as previously described (Lebel and Beaulieu 2009; Catani et al. 2005). After delineation in each subject, the total numbers of tracts in the AF were computed. To assess the robustness of the manual tractography, two independent raters performed tractography and the reliability was calculated based on the number of tracts of both AF's of all subjects. The agreement between the two raters was very high (Cronbach's alpha = 0.97). In subsequent analyses, the numbers of tracts were averaged over the two raters. A repeated measures analysis of variance was performed with Side (left versus right hemisphere) as within-subject variable and Group (SIT versus controls) as between-subject variable on the tract count of the arcuate fasciculus. A lateralization index (LI) was determined by calculating the difference in number of tracts between right- and left-hemisphere AF and dividing it by the sum of the tracts of the AF of both hemispheres (i.e. (R-L)/(R + L)). A two-way anova~~multivariate analysis of variance~~ was used to evaluate the effects of Group and Side.

fMRI Word generation paradigm

Stimuli. This task was an adapted Dutch version of a paradigm used to ascertain language dominance in volunteers with typical and atypical language lateralization (Cai et al. 2010). Ten letters served as stimuli (b, d, k, l, m, n, p, r, s and t) and were displayed in white on a black background.

Task. The task consisted of 10 cycles. Each cycle comprised four blocks, namely a word generation task (duration 15s), followed by a rest period (15s), followed by a control task (15s), and finally a rest period (15s). A cycle started with a generation task during which a letter was displayed at the center of the screen and participants were requested to covertly generate as many words as possible that started with that letter. The generation task was followed by a rest period. In the subsequent control task, the letter sequence “BABA” was displayed on the screen and participants were instructed to covertly repeat baba, which is pronounceable but meaningless in Flemish-Dutch. The four-block cycle was repeated 10 times, once for each of the 10 letters chosen in random order. The task took 10 min to complete and the investigated contrast was word generation > baba.

Procedure

Each participant was screened for MRI safety before entering the scanner. Prior to scanning, participants completed the behavioral tests. Next, participants received instruction in the task they would have to perform in the scanner. In particular, participants were presented with several examples of the stimuli until they correctly understood all instructions.

Participants were positioned head first and supine in the MR system and with the head gently held in place by means of foam padding. Arms were positioned comfortably alongside the body on the scanner table. Stimulus presentation was controlled by a commercially available software (Presentation, Neurobehavioral Systems Inc., Albany CA, USA) synchronized with the MRI-scanner. The display was projected on a screen at the back of the magnet bore and viewed via a mirror attached to the head coil.

fMRI data processing

Analysis of the functional data was performed using Brain Voyager QX (http://support.brainvoyager.com/) for preprocessing and functional inference. A standard sequence of preprocessing steps (slice scan time correction, 3-D motion correction, and temporal filtering) was used. Functional data were co-registered with the anatomical scan in Talairach space. A volume time course was created and spatially smoothed using a Gaussian filter (FWHM = 8mm). For each participant, a protocol file representing the onset and duration of each block for the different conditions was derived. Factorial design matrices were constructed for each protocol. The BOLD response in each condition was modeled by convolving the defined conditions with a canonical hemodynamic response function (gamma) to form the main predictors in the GLM. Finally, a cortex-based mask that was individually created from the merged segmented hemispheres of the anatomical scan was applied (see below). After the GLM had been fitted, individual t-maps were generated to evaluate the effects of relevant contrast; word generation > baba. For each individual a threshold of $p < .05$ corrected for multiple comparisons using False Discovery Rate (FDR) was applied. The individual t-maps were used to calculate the individual laterality indices (see below).

Group comparison of word generation activation using cortex based aligned time courses

To improve spatial correspondence over and above normalization to standard space, the cortex based alignment procedure provided by Brain Voyager QX was applied. First, the anatomical data of each participant were corrected for signal non-uniformity and transformed to Talairach space. Next, the brain was skull-stripped and segmented into gray and white matter. The results of the automatic segmentation results were inspected, manually corrected and the segmented algorithm was rerun. The borders of the segmented sub-volumes produced a surface reconstruction (mesh) of the left and right hemispheres. By means of an automatic 3D morphing algorithm, the resulting meshes were transformed into inflated cortex representations (spheres) that allow between-subject non-rigid alignment. The curvature information drives inter-cortex alignment in an iterative fashion by minimizing the mean squared differences between the curvature of a source and a target sphere. A moving target approach was used, which means that no actual target was selected, but instead the goal function is specified as a moving target computed repeatedly during the alignment process as the average curvature across all hemispheres (Goebel et al. 2006). This procedure was performed separately for the left and right hemisphere resulting in a group-aligned left and right hemisphere mesh.

Mesh time courses were then derived from the volume time courses for each participant and each hemisphere. A multi-subject random-effects GLM-analysis was performed across the cortically aligned time courses. This method maps each 'source' participant to the group-aligned 'target' to align the mesh time courses. Finally, functional brain asymmetry of the SIT and control groups was compared using an ANOVA random effects analysis with one within-subjects (condition) and one between-subjects factor. Statistical maps were generated to evaluate the main effects of condition and group. In these group-analyses a threshold of $p < .001$ uncorrected for multiple comparisons was used.

Determination of individual laterality indices (LI) during word generation

LI's were calculated for Regions of Interest (ROI) reported to coincide with brain regions in which lesions can cause functional disruption of verbal fluency in patient studies: Brodmann areas 44 and 45 (Baldo et al. 2006; Costafreda et al. 2006; Price 2012). To define the Brodmann areas in our participants, the segmented image of each individual was used to create a left and right hemisphere mesh along the grey-white matter border and the result was visually inspected and manually corrected where necessary. This mesh was also used to create the grey matter mask for use in the GLM-analysis of the functional data. Next, the cortical-based alignment procedure was applied to copy the Brodmann areas provided as patches-of-interest by the BrainVoyager template to the aligned individual hemisphere meshes of our participants. This procedure calculates the differences in cortical folding of the surface mesh of the template and that of the participant. These transformations are then applied to the template Brodmann patches to produce ROIs tailored to individual participants. Left and right hemispheric ROIs were composed for each individual as defined above. The significant voxels in each ROI were used to calculate a lateralization index (LI) based on the magnitude of signal change defined by the t-values by selection of voxels above a particular threshold (mean t-value of 5% most active voxels/2) over the left and right ROI taken together, and then calculate a LI on the summed t-values between the selected voxels of the left and right ROIs (Fernandez et al. 2001; Jansen et al. 2006). A correction was applied to adjust for the unequal size of individually determined left and right ROIs. Bilateral language representation was defined as an LI < |30|. This cut-off is based on the distribution of the language LI’s of a large sample of left and right handed participants (Mazoyer et al. 2014).

Results

Behavioral data

Mean estimated IQs of both groups were in the average range (SIT: 104, SD=20; Controls: 108 (SD=15) and the 4 IQ points difference between the groups was not significant (Table A~~S~~2). The mean handedness LI of the SIT-group measured +0.35 (SD = 0.80) and mean handedness lateralization strength (i.e. mean LI regardless of the sign indicating direction of handedness) was

0.83 (SD = 0.15). For the control group these values were +0.49 (SD = 0.82) and 0.92 (SD = 0.17) respectively. Neither direction nor strength of handedness differed significantly between the two matched groups (Table A1).

The handedness inventory revealed that four SIT participants were left handed. Two of the 11 right handed SIT participants reported being forced to write with the right hand in primary school which brings the number of natural left handed individuals to 6 in the group of 15 SIT participants (40%). The odds of such a result following a random selection in the general population with a prevalence of 10-15% left handedness is less than one percent (0.57%). The increased probability of sinistrality in the present cohort at first appears to contradict previous reports on hand preferences in relatively large groups of SI that found that left handedness is no more common in this condition than it is in the general population (Mcmanus et al. 2004; Afzelius and Stenram 2006). It is important to keep in mind, however, that the present cohort consists of a mix of PCD-related and PCD-unrelated SIT whereas the previous studies on handedness recruited exclusively PCD-related SI. Interestingly, and in agreement with the previous reports, all six SIT individuals of the PCD-related sub-group appeared to be right handed with only one participant claiming to be forced to write with the right hand. Therefore, five of the nine participants in the sub-group with SIT unrelated to PCD (55%) were born naturally left handed, a ratio that is close to a random allocation of handedness. To obtain a more qualitative report of the difference in hand preference between the SIT-subgroups, we plotted the difference in handedness LI between the SIT sub-groups (Figure A1.) PCD-related SIT showed clear (high mean) and less variable (low SD) right hand preference (mean LI=0.77, SD = 0.20) compared to an almost absent directional hand preference (mean LI close to 0) despite maintained individual hand preference (reflected in high SD) in the sub-group with PCD-unrelated SIT (mean LI=0.08, SD = 0.93).

Due to the very small sample sizes, basic assumptions for Chi-square type tests are violated and statistical comparison of the hand preference data between the SIT-subgroups is unjustified. The odds of there being five left handed participants in a random sample of nine individuals from the general population is 0.23%.

Brain structural results

Petalia and bending

A pattern of right frontal and left occipital petalia was observed in the majority of control participants (see blue dots in Figure 2, left panel). The rightward frontal extension was found in 9 out of 15 control participants with the average frontal petalia being -0.09±1.38mm; while the leftward occipital protrusion was observed in 11 out of 15 control participants with the average posterior petalia being -0.79±1.31mm. One-sample one-tailed t-tests revealed that only the leftward occipital petalia was significant in controls (t(14)=-2.334, p=.018). With regard to SIT participants, a leftward frontal protrusion was observed in 10 out of 15 participants with the average frontal petalia being 0.40±1.50mm; while the right occipital protrusion was found in 8 out of 15 participants with the average posterior petalia being 0.30±2.00mm. Though neither was found to be significant, a two-sample t-test between the control and SIT participants revealed a significant Group difference in the latter that suggests a reversal of the occipital petalia in the SIT cohort compared to the controls ($t(28)=1.765$, $p=0.044$). The frontal and occipital bendings were also examined (see Figure 2, right panel). A pattern of rightward occipital (13/15) and rightward frontal bending (10/15) was observed in the majority of the control cohort and proved to be significant by a one-tailed one-sample t-test ($t(14)=2.812$, $p=0.007$ for the right occipital bending and $t(14)=2.022$, $p=0.031$ for the right frontal bending). There is no significant directional asymmetry in the SIT cohort although a trend of leftward occipital bending was seen in 9 out of 15 participants. Based on a one-tailed two-sample t-test the occipital bending was significantly reversed in the SIT cohort compared to controls ($t(28)=-2.910$, $p=0.004$), whereas the frontal bending was not. So, despite marked variability in both groups, on average the SIT cohort showed a significant reversal of the occipital petalia as well as the occipital bending. The difference of petalia and bendings between the SIT sub-groups and their corresponding control cohorts were also explored. Interestingly, a significant reversal of petalia and bending was observed only in SIT participants unrelated to PCD compared to the matched control subjects (i.e., occipital petalia $t(16)=2.694$, $p= 0.008$; occipital bending $t(16)=3.269$, $p=0.002$), but not in PCD-related SIT whose occipital petalia and bending were not significantly different from controls. It is also worth noting that for the total cohort (excluding the forced right handers SI09 and SI13) both the frontal and occipital petalia were significantly correlated with the handedness inventory score ($r=0.48$, $p=0.010$ for the frontal petalia and $r=0.43$, $p=0.021$ for the occipital petalia). Including the forced right handers gave similar results, but since ~~the effect of~~ forced right handedness influences handedness scores~~on brain torque is unknown~~, we chose to exclude them. The combination of right frontal and left occipital petalia was more common in right handed participants while left frontal and right occipital petalia were more common in left handed participants.

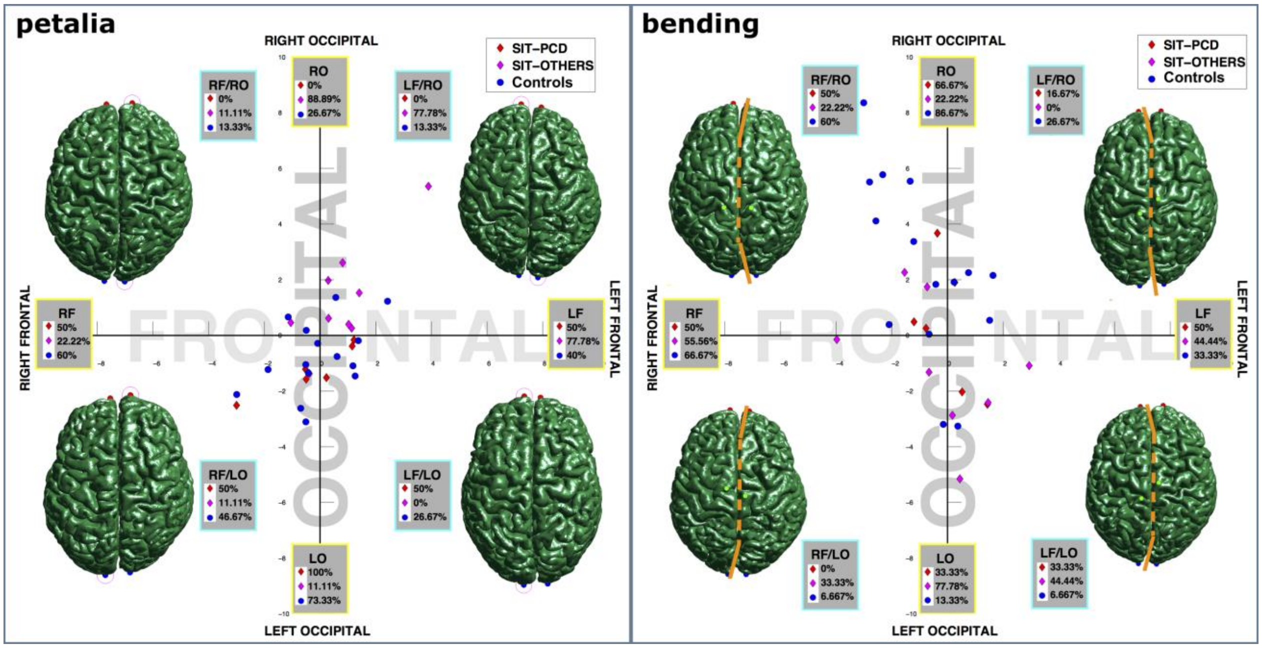

Figure 2. Distribution of petalia and bending in participants with SIT and in matched controls. Left panel: The typical petalia pattern is reflected by the lower left quadrant where the majority of control (blue dots) and PCD-related SIT participants (red dots) fall in. Most PCD-unrelated SIT participants (magenta dots) have a reversed petalia pattern shown in the upper right quadrant. Right panel: Rightward occipital bending is most frequently observed in controls (blue dots) and PCD-related SIT (red dots) participants (upper quadrants). Most PCD-unrelated SIT participants (magenta dots) show leftward occipital bending (lower quadrants).

Sylvian fissure

The Sylvian fissure (SF) typically has a longer horizontal course on the left and reaches a higher end-position on the right. This pattern was not investigated in the second and third MRI study (Ihara et al. 2010; Schuler et al. 2017) but was reported typical in two of the three SI cases in the first MRI-study (Kennedy et al. 1999) and reported to be reversed in the cadaver study (Tubbs et al. 2003). In the present cohort no significant differences were found for length nor depth of the anterior-horizontal (AH-SF) and vertical (V-SF) segments of the SF between SIT and controls (Figure 3 and Table A3; AH-SF (*F* (1, 24) = 0.002, *p* = 0.96) and V-SF (*F* (1, 24) = 0.26, *p* = 0.15) length or AH-SF (*F* (1, 24) = 1.13, *p* = 0.26) and V-SF (*F* (1, 24) = 0.91, *p* = 0.77) depth). Given the missing data of two individuals in each cohort, the MANOVA was not applied on closely matched groups. Therefore, multiple t-tests were also run that maximized the number of paired SIT and control participants for each measure to see if there were any significant differences. Reinforcing the group findings, there were no significant differences for any of the SF measurements.

SI cohorts with (n=6) and without PCD (n=9) were not statistically different from each other based on AH-SF and V-SF lengths and depths (F (4, 5) = 0.44, *p*= 0.78). Each SIT sub-group was also compared to only their matched control cohort to investigate potential Group differences though neither PCD-unrelated SIT (*F* (4, 7) = 1.14, *p* = 0.41) or PCD-related SIT (*F* (4, 4) = 0.98, *p* = 0.51) were significantly different to controls based on two separate four-factor (AH-SF and V-SF) MANOVA analyses. In short, no difference in SF trajectory was found between SIT sub-groups with and without PCD.

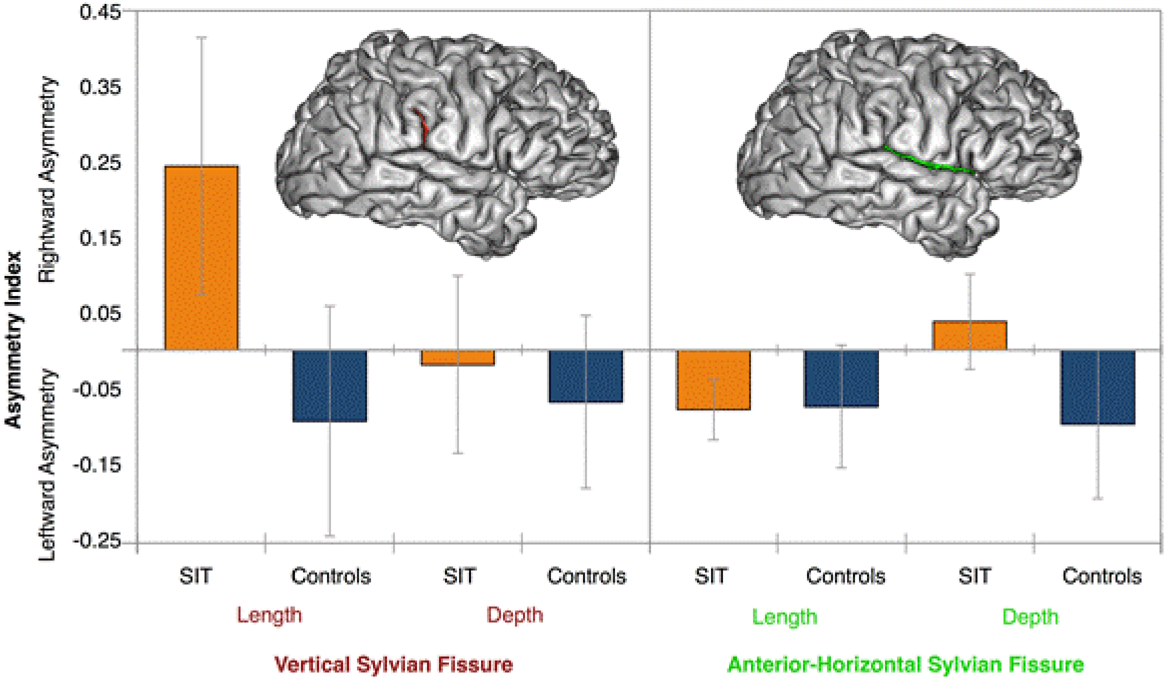

Figure 3: Vertical (red) and Anterior-Horizontal (green) SF depth and length asymmetry indices for SIT (n=13) and control (n=13) participants with standard error bars. Positive values over +0.025 are rightward asymmetric, negative values under -0.025 are leftward asymmetric. The two sulcal aspects are demonstrated on a right hemisphere mesh.

Peri-Sylvian surface areas

Whereas asymmetry of the planum temporale was assessed in all available SIT-brain scans thus far, other common leftward peri-Sylvian asymmetries like Heschl's gyrus (a region associated with auditory perception), the anterior insula (an integrative region associated with social communication and language), and the pars opercularis and pars triangularis of the inferior frontal gyrus (that form Broca's area and are relevant for speech production) were largely ignored. A repeated measures analysis of variance was performed with Side (left versus right hemisphere) as within-subjects variable and Group (SIT versus controls) as between-subjects variable on the surface areas of the planum temporale, anterior insula, Heschl's gyrus, and the pars opercularis and pars triangularis of IFG computed from the FreeSurfer segmentations. A significant main effect of Side was obtained ($F(5,24)=20.61$, $p<.001$) with generally larger regions on the left side of the brain. Univariate analyses showed this to be the case for all areas except the pars triangularis (Figure 4). No main effect for Group nor a Side by Group interaction effect was found which indicates that the surface areas of language-associated regions of SIT participants showed the same leftward asymmetries as the control group. Similar results were obtained when comparing the surface asymmetries of the SIT sub-groups with and without PCD versus their respective control participants.

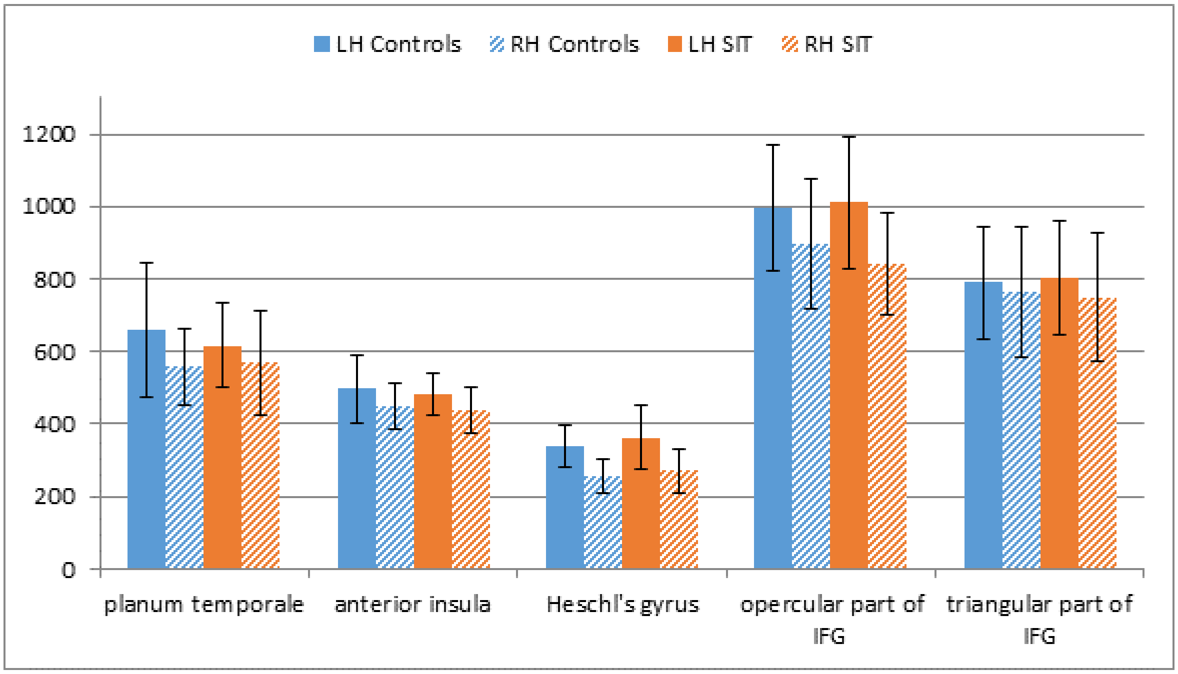

Figure 4. *Left hemisphere (LH) and right hemisphere (RH) surface areas (in* mm^2*) of language-related peri-Sylvian regions in SIT and matched control participants. Error bars reflect 95% confidence intervals. LH regions are significantly larger than RH regions (except for the pars triangularis). No significant Group difference is found.*

Arcuate fasciculus

We also investigated whether a significant asymmetry existed in the number of white matter tracts in left and right arcuate fasciculus, a peri-Sylvian white matter bundle that connects Broca's and Wernicke's areas and is associated with language (Catani et al. 2007). A significant main effect of Side (*F*(1,28)=34,97, *p*<.001) was obtained with a higher number of tracts on the left side of the brain (Figure 5). No main effect of Group nor a Side by Group interaction was found. Similar results were obtained when comparing tract asymmetries of the SIT sub-groups with and without PCD versus their respective control participants, with the exception that PCD-unrelated SIT participants on average showed a significantly lower total number of tracts in the arcuate fasciculus in the left and right cerebral hemispheres than their control counterparts (*F*(1,16)=5.04, *p*=.039). Figure A2 shows examples of the tractography results of two participants, one with a symmetrical (SI13) and one with a left lateralized tract count (CO02).

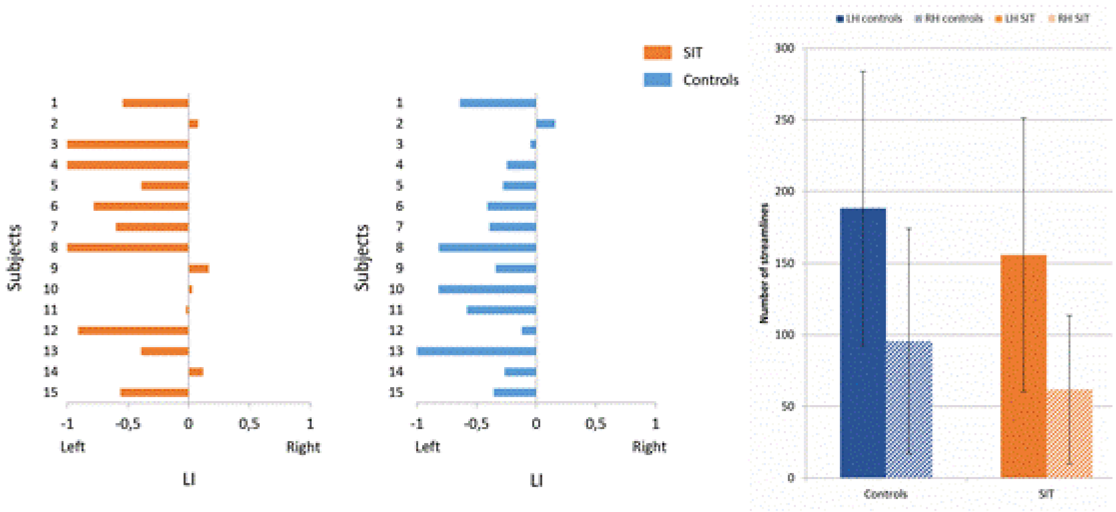

Figure 5: Manual tractography results of the arcuate fasciculus. Graphs on the left show the LI's of each participant based on the number of tracts between the two hemispheres. The graph on the

right illustrates the average number of tracts for each hemisphere with standard error bars. The asymmetry patterns of SIT and controls reveal no significant difference.

Brain functional results

On the out-of-scan word fluency task SIT participants named on average 21 (SD=3.0) items and controls 23 (SD=3.6). This difference is not significant, but should be interpreted with caution given the dissimilarity of the inside and outside word fluency tasks (Table A2). Put differently, the comparable between-group performance level merely suggests, but by no means assures that both groups performed similarly. In SIT and control participants the word generation task elicited the typical response principally involving the left cerebral hemisphere and especially in inferior frontal regions (extending superiorly to the dorsolateral prefrontal and premotor regions) and the supplementary motor area. Both groups showed additional activation in posterior parietal and inferior temporal regions of the left hemisphere, and in caudate nucleus and cerebellum. As a group, the SIT participants displayed the usual leftward lateralization of language with only minor between-group differences outside the left peri-Sylvian region (Figure 6, more details in Figure A3 and Table A4; Individual lateralization indices are listed in Table A2). Even at the voxel level, peak coordinates of both groups were very similar (Table A4). Direct comparison of word generation activation maps (Figure 6~~5~~B) revealed that the SIT participants showed increased activation of right posterior insula compared to controls. In addition, they showed reduced BOLD-response in left medial frontal and left middle frontal gyrus. The relevance of these differences is tempered by their being achieved by statistics uncorrected for multiple comparisons and by the observation that ~~T~~the frontal differences lie outside the classic peri-Sylvian region associated with language. ~~, but~~ Activation of the insular region is potentially relevant though. Figure A4 explores the insular activation found in the within-group (word generation > baba) and between-group (word generation$_{\text{SIT}}$ > word generation$_{\text{Controls}}$) contrasts. It appeared that word generation-related (within-group) insular activation was located in anterior insula of both hemispheres (though stronger on the left), whereas the between-group related difference was located in right posterior insula. ~~Asymmetric anterior insular involvement of the former contrast is in agreement with its putative role in communication and language (Craig 2002; Chiarello et al. 2013). Posterior insula has been associated with interoceptive representation, including responses to visceral sensations (Craig 2002). Apparently, the insular between-group difference found does not pertain to a region underlying language or speech. The increased and lateralized activation of this somatosensory visceral region in SIT participants compared to the control cohort warrants further attention.~~

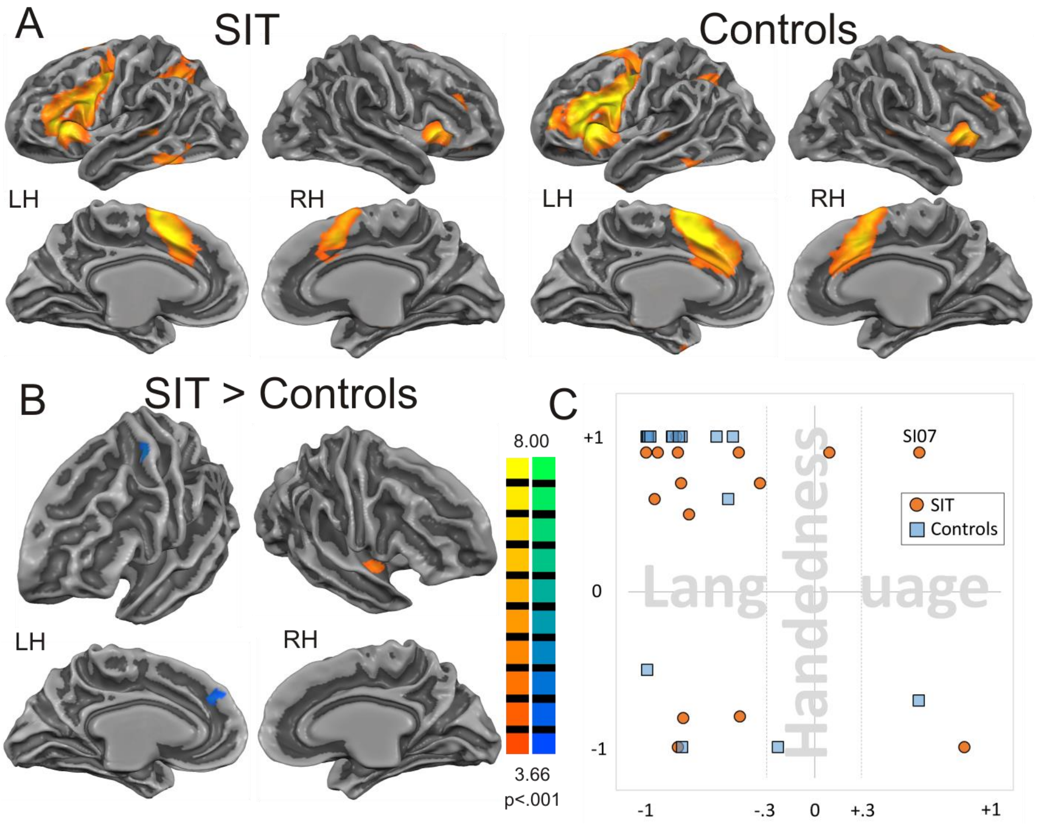

Figure 6. A. Left and right hemisphere activation during word generation in participants with SIT (left, n=15) and controls (right, n=15). -Both groups reveal clear leftward inferior frontal and supplementary motor area activation. Additional posterior parietal and inferior temporal BOLD-response in both groups is seen exclusively in the left hemisphere. B. Regions where significant differences between SIT and control participants are found, with hot colors indicating SIT>control and cool colors indicating the opposite. All analyses at p<.001, uncorrected for multiple comparisons. C. Scatterplot showing the relationship between hemispheric language lateralization and hand preference (from -1 (leftward) to +1 (rightward)). Participants located between vertical dashed lines are considered to have bilateral language representation. Most participants are situated in the upper left quadrant representing the most typical combination in humans. Most left handed participants also show left language dominance (lower left quadrant) but some show right hemisphere language dominance (lower right quadrant). Atypical left handedness/right language dominance combination (lower right quadrant) is observed in SIT and control participants. Right handed individuals with clear right hemisphere language dominance are probably extremely rare, and this very atypical combination is only seen in one of the SIT participants (SI07, upper right quadrant).

No significant Group differences in LI-strength (using absolute values of LIs) were found between SIT and controls, nor between SIT participants with or without PCD. However, individual BOLD lateralization indices (LI) calculated over cortically aligned Brodmann areas 44 and 45 (Broca's area) revealed that three SIT participants had atypical language lateralization (two were right hemisphere language dominant and one had bilateral language representation (the latter defined as an LI < |.30|), and two participants in the control group showed atypical language dominance (one was right hemisphere dominant, the other had bilateral representation). Panel C in Figure 6 shows the relation between language lateralization and hand preference in the two groups. The two participants with right hemisphere language dominance (one SIT and one control) were left handed and had first degree relatives that were left handed. Of the two participants with bilateral language representation one with SIT was left handed and the other who was a control participant was right handed. ~~These atypical hand preference/language dominance combinations are known to occur in small numbers in the general population (Mazoyer et al. 2014).~~ A final observation is~~comment should be~~ reserved for the very atypical individual SI07, who showed visceral reversal, right hand preference, and atypical right language dominance despite typical lobar asymmetry and leftward grey and white matter peri-Sylvian organization (Figure 7d). ~~This 35-year-old woman with PCD-unrelated SIT may exemplify a complete randomization of all anatomical and functional features measured here (Figure 7d). Although language lateralization appears most resistant to atypicality of all asymmetries assessed, it seems to be able to lateralize independent of putative 'pressure' from right hand preference and leftward peri-Sylvian brain structural organization.~~

Discussion

Despite a generally bilateral symmetric body plan, vertebrates show consistently asymmetric placement of visceral organs such as heart and liver, and asymmetric development of paired organs such as the lungs and brain. We investigated the relation between visceral, brain structural and brain functional asymmetry in a large cohort of individuals with SIT in comparison with a matched control group.

Detailed analysis of petalia and bending of SIT individuals confirms previous reports of situs-associated reversal of the usual counter-clockwise brain torque. Although reversal is not present in every SIT-participant and may even occur in control participants, the posterior lobar asymmetry is on average significantly reversed in participants with SIT. The functional significance and neurodevelopmental origin of the brain torque remain unknown, but its features have been used for developing theories that explain cognitive lateralization by relating them to differences in maturation rates between the cerebral hemispheres. Geschwind and Galaburda, for example, noted the lobar asymmetries and speculated that they were the result of an intrauterine maturation effect involving selective areas, rather than slower development of only one (the left) hemisphere (Geschwind and Galaburda 1987; McManus and Bryden 1991; Kasprian et al. 2011; Habas et al. 2012). A more explicit causal relation was suggested by Best who proposed dynamic directional gradients along the main axes of neuroembryological development allegedly illustrated by the petalia and torque, reflecting a morphological growth vector that defines both language lateralization and handedness (Best 1988), but see also (Previc 1991). However, the present data show that despite reversed posterior lobar asymmetry typical language dominance is maintained.

The findings of the present study confirm the typical leftward planum temporale asymmetry in SIT participants and extend this finding to other language-related peri-Sylvian asymmetries. This includes the SF length asymmetries, though our control cohort did not have the expected rightward asymmetries in the vertical aspect (Witelson and Kigar 1992) unlike the SIT participants, and so further replication is needed. Functional MRI data add to the picture of generally retained language lateralization by showing predominantly left hemisphere language dominance in SIT in general that is not different from a matched control group, including the occasional bilateral or right hemispheric language dominance, the latter being associated with left hand preference. As suggested by the distribution patterns of language lateralization in a large data set of left and right handed individuals, reversed language lateralization occurs only in a small number of left handed individuals, whereas in right handed and most left handed people hand preference and hemispheric language dominance appear unrelated (Mazoyer et al. 2014). In other words, atypical language lateralization is expected to occur in some left handed individuals, regardless of their visceral organization. It remains to be noted however, that one SIT-participant (SI07) showed an unexpected right hand preference combined with clear right hemisphere language dominance.~~, a rare merger in the human population~~. This 35-year-old woman with PCD-unrelated SIT and no familial sinistrality may exemplify a complete randomization of all anatomical and functional features measured here. Although language lateralization appears most resistant to atypicality of all asymmetries assessed, it seems to be able to lateralize independent of putative 'pressure' from right hand preference and leftward peri-Sylvian brain structural organization.

The present study ~~thus~~ helps to resolve the seemingly contradictory findings concerning language dominance reported in two previous SIT brain imaging studies, one using fMRI (Kennedy et al. 1999) and the other MEG (Ihara et al. 2010) and which did not mention PCD-related symptoms in their participants. In particular, the MEG study included more SIT participants with atypical handedness than those recruited to the fMRI study and this perhaps resulted in an increased probability of atypical language dominance.

Functional MRI revealed only minor between-group differences during word generation. Further exploration of potentially relevant insular differences revealed that word generation-related (within-group) insular activation was located in anterior insula of both hemispheres (though stronger on the left), whereas the between-group related difference was located in right posterior insula. Asymmetric anterior insular involvement of the former contrast is in agreement with its putative role in communication and language (Craig 2002; Chiarello et al. 2013). Posterior insula has been associated with interoceptive representation, including responses to visceral sensations

(Craig 2002). Apparently, the insular between-group difference found does not pertain to a region underlying language or speech. The increased and lateralized activation of this visceroceptive region in SIT participants compared to the control cohort warrants further attention.

Secondary findings, which are more speculative because of the even smaller sample size, hint at the interesting possibility of different lateralization patterns for different types of SIT. Because half of the people with PCD syndrome show SIT, it is taken that dysfunction of nodal cilia during embryogenesis results in a random allocation of visceral lateralization. At the same time individuals with PCD show a distribution of handedness that is not different from that of the general population, which led McManus et al. to construct a model of symmetry breaking in which visceral and cerebral asymmetry are caused by different mechanisms (Mcmanus et al. 2004). The authors proposed the model for PCD depicted in Figure 7a. Results of the present study show that in PCD-related SIT language lateralization and posterior lobar asymmetry also adhere to the default directional bias of lateralization (Figure 7b). In fact, PCD only seems to cause visceral inversion but does not appear to impact normal brain structural and functional lateralization, brain torque included.

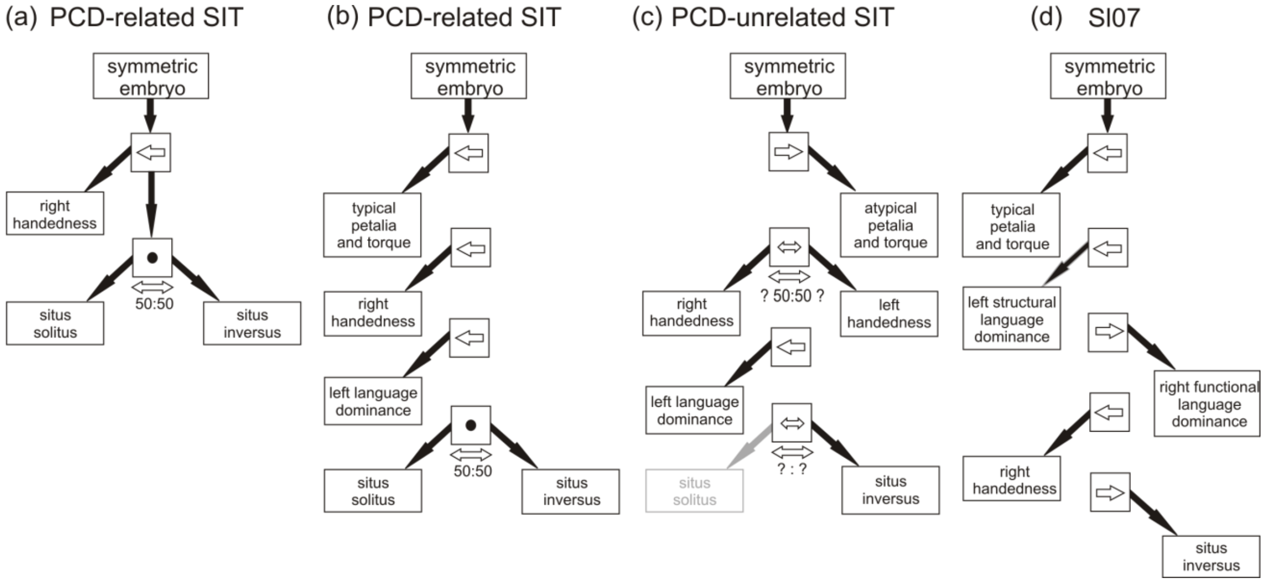

Figure 7. *Models of the relationship between visceral and cerebral situs. (a) This shows the model compatible with the PCD-related SIT findings on handedness presented by McManus et al. (2004). Disruption of ciliary flow, as in PCD, will give rise to SI in 50% of the individuals, but both visceral conditions will show the same distribution of (predominantly right) hand preference as the rest of the population. According to McManus et al. hand preference is determined upstream by a mechanism that is not dependent on ciliary rotation. (b) Data of the present study expand the McManus et al. model by adding language dominance and lobar asymmetry to the typically distributed asymmetries. We refrain from using vertical arrows in the model to avoid the idea of a causal order in symmetry breaking. (c) Hypothetical pattern of asymmetry in PCD-unrelated SIT showing generally reversed petalia and torque and increased incidence of left handedness (possibly random allocation of handedness) but preserved typical language dominance. In model 7c the resulting distribution of situs cannot be predicted, as ciliary motion is preserved in this SIT sub-group and visceral laterality must have another origin that is presently unknown. (d) Participant SI07, a woman with PCD-unrelated SIT, shows unexpected right language dominance despite right hand preference, typical lobar asymmetry and leftward*

peri-Sylvian brain structural organization. (adapted and expanded from McManus et al. 2004, with kind permission from (will be completed on acceptance of the manuscript)).

The data further suggest that the sub-group of PCD-unrelated SIT shows a more complex picture which hypothetical pattern is depicted in Figure 7c. First, they appear to have the typical leftward language dominance as controls and PCD-related SIT. Second, based on the unexpected observation that five out of nine participants with PCD-unrelated SIT are left handed, it can be hypothesized that this subgroup may present with random individual hand preference, or at least increased frequency of left handedness. Third, it appeared that the petalia and occipital bending are only significantly reversed in SIT unrelated to PCD, whereas in PCD-related SIT there is no significant difference from controls. This suggests that participants with PCD-unrelated SIT show at least more frequent reversal of petalia and occipital bending. Interestingly, the data also point to a possible relation between petalia and handedness, an observation that has been reported before (Lemay and Kido 1978), but not language dominance.

We conclude that brain structural and functional asymmetries in SIT participants with or without PCD show little evidence that ciliary movement plays a role in the genesis of human brain laterality. In addition, the data suggest that symmetry breaking of visceral laterality, brain torque, and language dominance rely on different mechanisms, as each of these asymmetries can be atypical irrespective of the laterality of the others.

Appendix

Supplementary Tables and Figures (in order of reference in the main text)

Table A1. Demographic, relevant medical, and hand preference data of the situs inversus totalis (SI) and Control (CO) participants.

SI ID	SI comorbidities	Gender	Age	Educ*	Handedness**	CO ID	Gender	Age	Educ*	Handedness**
SI02	No complications	Male	50	8	0.9	CO02	Male	51	10	1.0
SI03	No complications	Female	26	12	-0.8	CO03bis	Female	26	15	-1.0
SI04	Sacral agenesis, congenital heart disease	Male	23	13	-1.0	CO04	Male	22	13	-0.5
SI05	No complications	Male	27	12	0.9	CO05	Male	27	12	1.0
SI06	Primary ciliary dyskinesia	Male	46	12	1.0	CO06bis	Male	43	12	1.0
SI07	Congenital heart disease	Female	35	12	0.9	CO07	Female	33	12	1.0
SI08	Primary ciliary dyskinesia	Female	23	15	0.9	CO08	Female	22	14	1.0
SI09	No complications	Female	36	15	0.7***	CO09bis	Female	38	15	0.6
SI11	Primary ciliary dyskinesia	Female	32	11	0.9	CO11	Female	34	12	1.0
SI12	No complications	Female	40	12	0.9	CO12	Female	38	12	1.0

SI13	Suspect primary ciliary dyskinesia	Male	48	14	0.6***	CO13bis	Male	46	12	1.0
SI14	Congenital heart disease	Male	18	12	-0.8	CO14	Male	19	12	-1.0
SI15	Primary ciliary dyskinesia	Female	31	18	0.7	CO15	Female	35	16	1.0
SI16	No complications	Male	21	14	-1.0	CO16bis	Male	20	14	-0.7
SI17	Primary ciliary dyskinesia	Male	39	14	0.5	CO17	Male	41	12	1.0

*education is expressed in years of full time formal education; ** Based on Edinburgh Handedness Inventory score: Lateralization index calculated on the 10 hand items and ranges from +1 (consistent right handedness) to -1 (consistent left handedness); *** Forced to right hand writing at primary school.

Table A2. Behavioral data and language laterality index (LI) of the situs inversus totalis (SI) and Control (CO) participants.

SI ID	**Estimated IQ**	**WGEN performance***	**WGEN LI****	**CO ID**	**Estimated IQ**	**WGEN performance***	**WGEN LI****
SI02	65	15	-0.93	CO02	89	25	-0.99
SI03	80	20	-0.78	CO03bis	107	22	-0.22
SI04	123	18	-0.81	CO04	107	23	-0.99
SI05	118	23	-0.81	CO05	80	17	-0.48
SI06	111	22	-0.82	CO06bis	122	25	-0.84
SI07	91	16	0.61	CO07	111	26	-0.79
SI08	118	21	-0.99	CO08	122	22	-0.84
SI09	120	25	-0.33	CO09bis	128	27	-0.51
SI11	94	20	-0.45	CO11	101	29	-0.80
SI12	76	22	0.08	CO12	105	20	-0.99
SI13	90	22	-0.95	CO13bis	89	21	-0.99
SI14	131	25	-0.44	CO14	110	19	-0.78
SI15	123	21	-0.79	CO15	119	26	-0.58

SI16	121	24	0.88	CO16bis	129	18	0.61
SI17	97	18	-0.75	CO17	100	27	-0.97

* Out-of-scan word generation performance; **Laterality index of fMRI-based brain activation during word generation, LI ranges from +1 (strong right hemisphere language dominance) to -1 (strong left hemisphere language dominance).

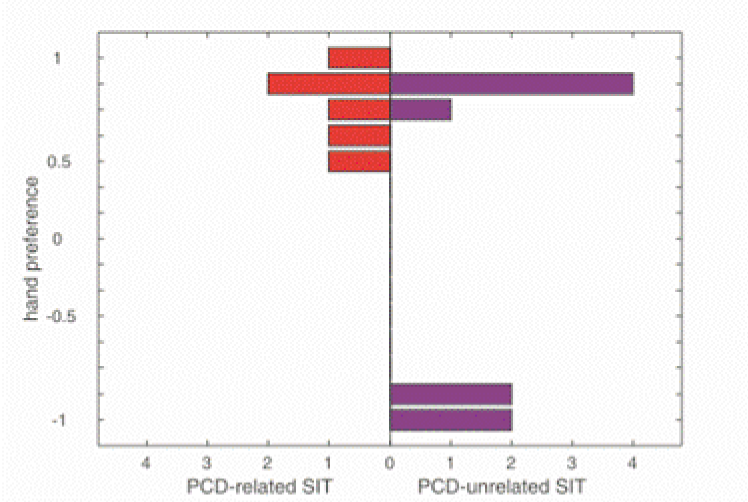

Figure A1. Histogram of EHI hand preference LI's for SIT participants with or without PCD. Whereas PCD-related SIT (red bars) shows the usual directional right handedness of the human population, PCD-unrelated SIT (purple bars) shows retained individual hand preference, but the expected directional bias appears lost as half of the cohort shows left hand preference and half prefers the right hand.

Table A3. Overview of the Sylvian fissure length and depth asymmetries in SIT and control participants.

		AH-SF *	**S.D**	***n******	**V-SF ****	**S.D**	***n******
Length (Mean)	**Situs Inversus**	-0.079	0.144	13	+0.243	0.615	13
	Controls	-0.075	0.282	13	-0.095	0.527	13
Depth (Mean)	**Situs Inversus**	+0.372	0.217	13	-0.020	0.406	13
	Controls	-0.985	0.368	13	-0.069	0.428	13

*AH = Anterior-Horizontal; ** V= Vertical; *** absence of clear bifurcation point resulted in incomplete data

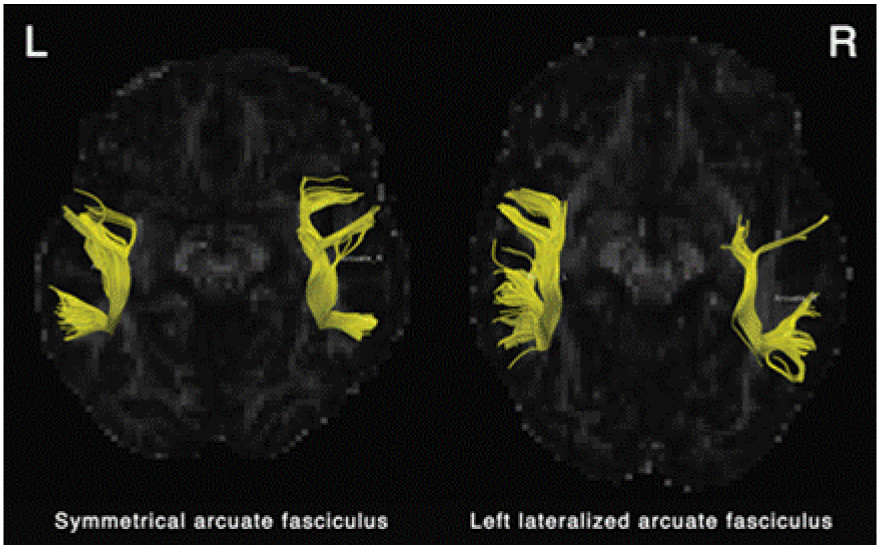

Figure A2. Illustration of the long direct segment of the arcuate fasciculus in a participant with symmetrical (SI13) and leftward (CO02) arcuate fasciculus.

Table A4. Averaged voxel coordinates (Talairach space) and peak voxel t-values of significant activation clusters of the Word generation > baba contrast for SIT and Control cohort, and of the difference in word generation BOLD-response between SIT and Control.

Word generation > baba for Control and SIT cohort										
			Controls				SIT			
Region	BA	Side	X	Y	Z	t_{max}	X	Y	Z	t_{max}
Frontal regions										
Medial frontal gyrus	6	L	-2	11	49	10.59	-3	13	48	9.73
Inferior frontal gyrus	44	L	-42	5	30	10.13	-46	4	32	9.52
Middle frontal gyrus	9	L	-40	18	25	9.58	-44	16	29	6.11
Inferior frontal gyrus	45	L	-45	30	14	9.50	-40	25	9	7.66
Middle frontal gyrus	6/8	L	-26	-4	56	8.12	-30	-3	43	6.37
Anterior insula		R	32	19	3	7.47	31	15	6	7.03
Middle frontal gyrus	46	R	40	37	26	4.47	33	32	26	6.05
Parietal regions										
Inferior parietal lobule	40	L	-27	-53	38	4.96	-27	-59	36	4.95
Inferior parietal lobule	40	L	-44	-36	43	4.75	-35	-41	37	5.52
Temporal regions										
Inferior temporal gyrus	37	L	-52	-46	-12	4.34	-49	-48	-14	6.58
Middle temporal gyrus	22	L	-60	-27	4	3.78	-53	-33	4	4.44
Subcortical regions										
Caudate nucleus		R	18	5	15	11.30	17	7	10	6.24

Caudate nucleus		L	-17	-1	13	10.06	-15	5	9	7.12
Cerebellum			0	-51	-11	9.59	0	-50	-13	4.63
Caudate nucleus		L	-33	23	6	8.61	-37	22	6	7.60
Cerebellum		R	28	-59	-21	7.97	23	-62	-24	6.89
Cerebellum		L	-45	-51	-21	5.54	-44	-51	-19	6.58
Cerebellum		L	-51	-48	-14	4.40	-49	-48	-14	6.58
Word generation SIT > Word generation Controls										
Region	BA	Side	X	Y	Z	t_{max}				
Right posterior insula	13	R	40	-7	-5	5.62	Increased in SIT			
Left middle frontal gyrus	6	L	-24	-8	54	-4.14	Decreased in SIT			
Left medial frontal gyrus	9	L	-10	42	33	-3.91	Decreased in SIT			

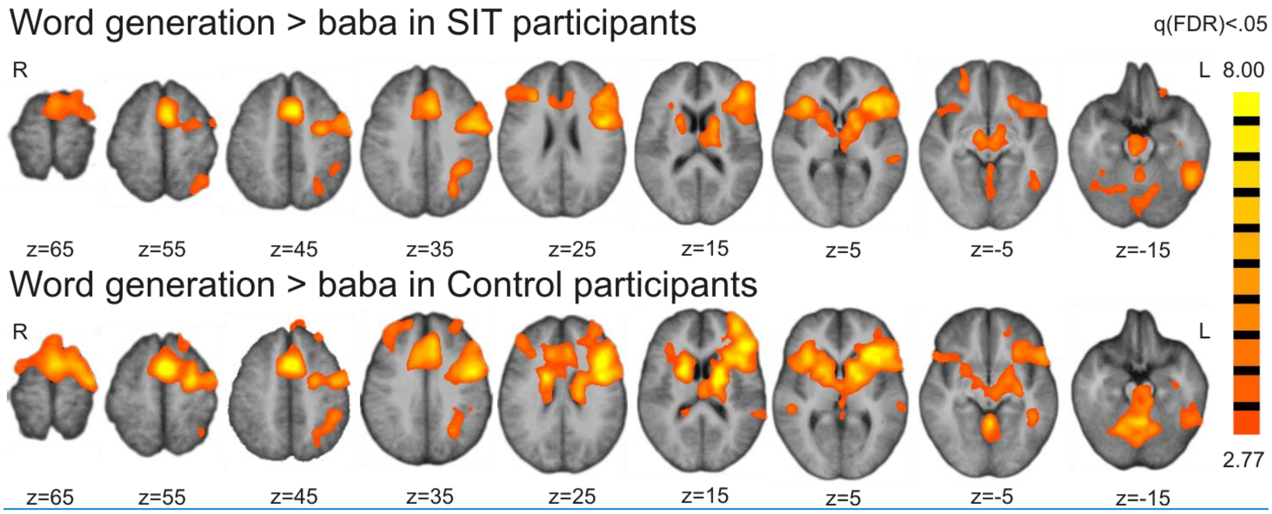

Figure A3. Primary contrasts of the word generation > baba differences in participants with SIT (upper row) and controls (lower row). Depicted activation at alpha < .05 FDR corrected over group averaged horizontal brain slices.

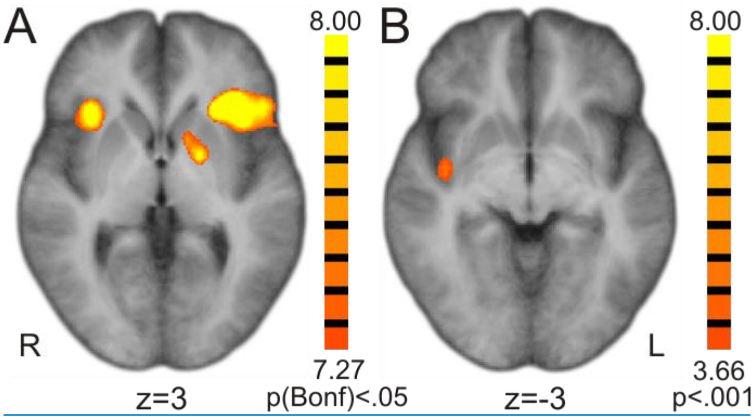

Figure A4. (A) BOLD-response of the within-subjects word generation > baba contrast for the whole group (SIT + Controls). We used a high threshold to demonstrate that the peak activity is located in the anterior part of the insular cortex in both hemispheres. (B) BOLD-response of the between-subjects (SIT>Controls) contrast of word generation at p<.001, uncorrected. The increased activation of SIT participants is located in the right posterior insula.

Compliance with Ethical Standards

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figure 1

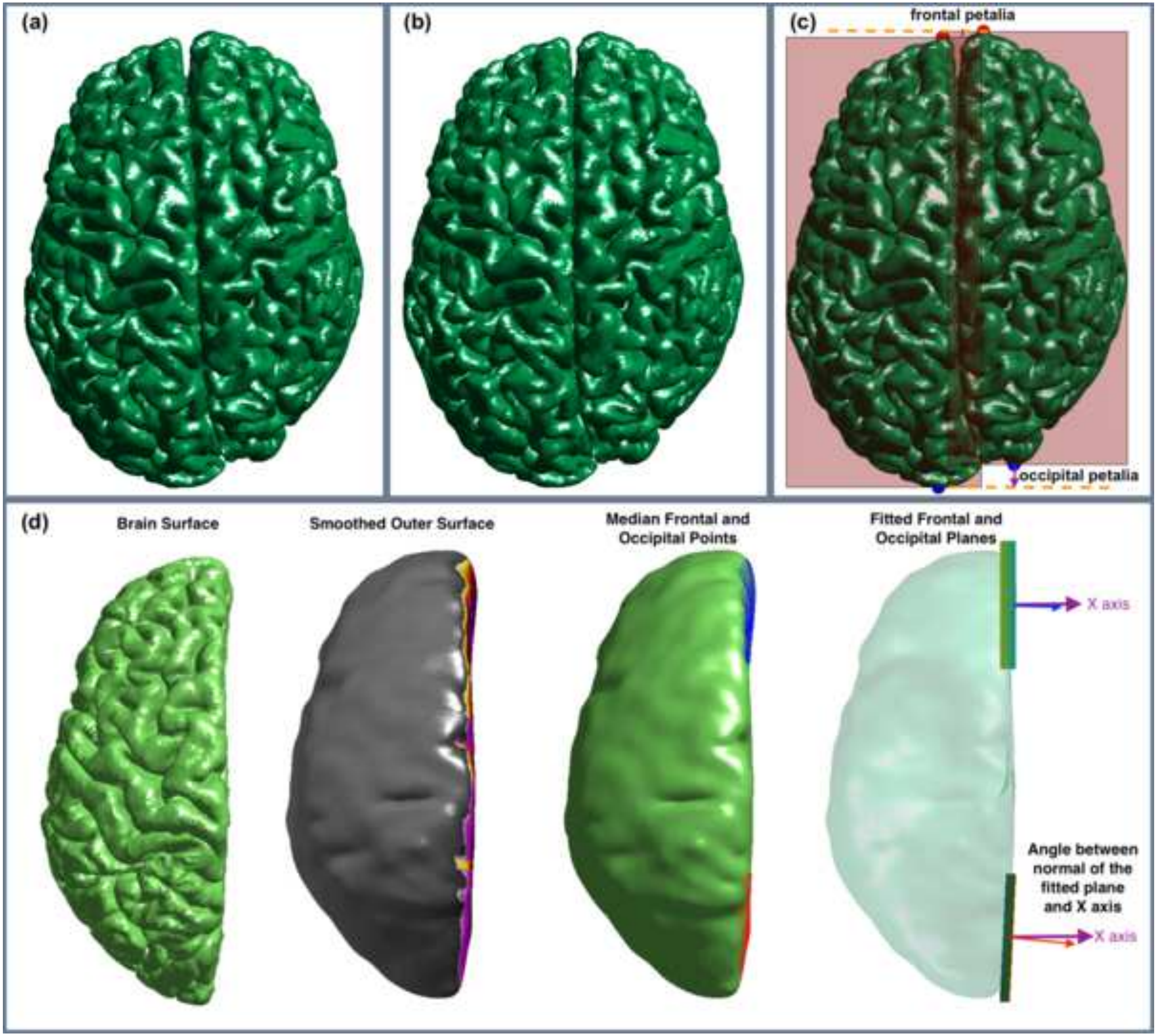
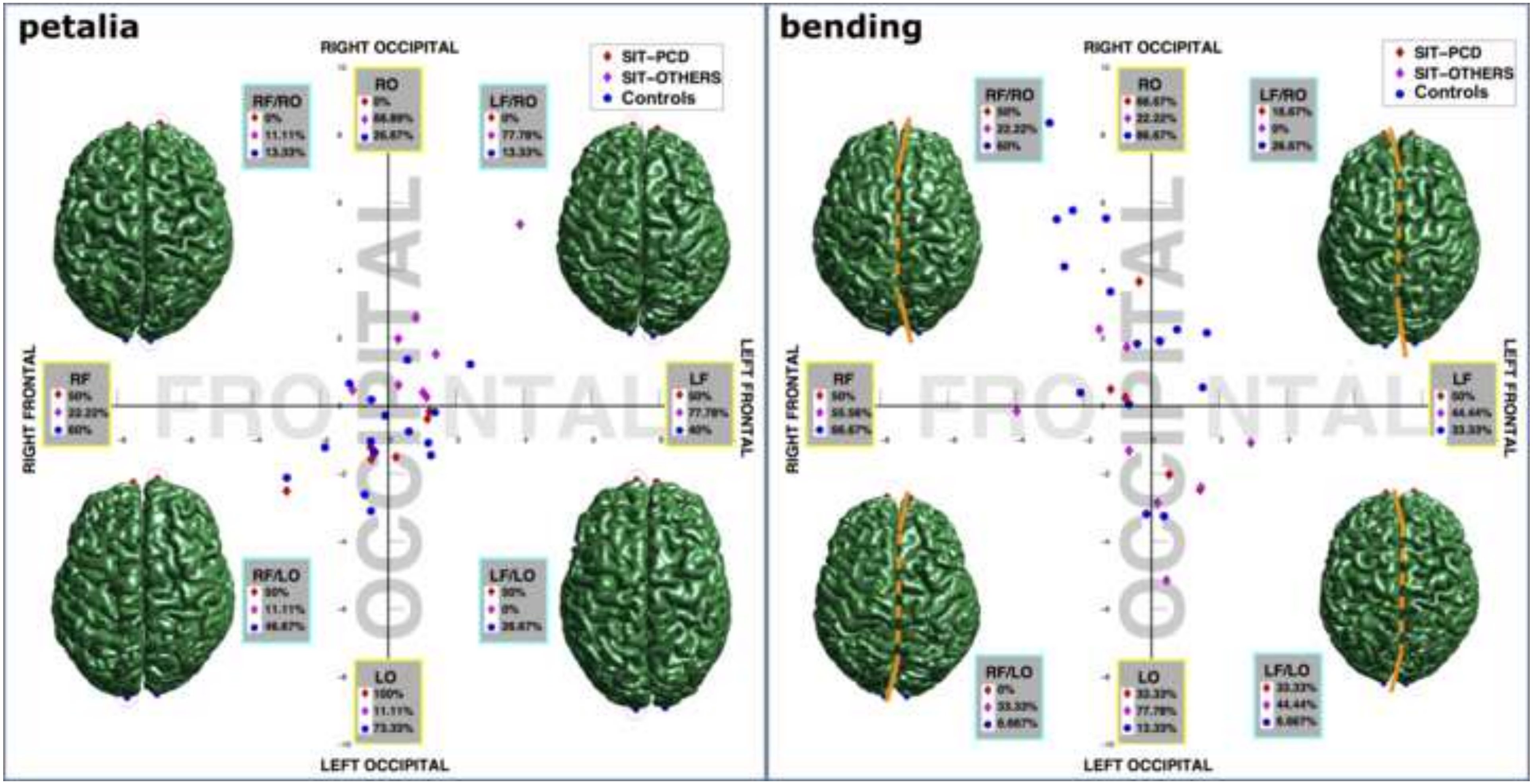

(a)
(b)
(c)
frontal petalia
occipital petalia
(d)
Brain Surface
Smoothed Outer Surface
Median Frontal and Occipital Points
Fitted Frontal and Occipital Planes
X axis
Angle between normal of the fitted plane and X axis
X axis

Figure 2


petalia
RIGHT OCCIPITAL
LEFT OCCIPITAL
RIGHT FRONTAL
LEFT FRONTAL
FRONTAL
OCCIPITAL
SIT-PCD
SIT-OTHERS
Controls
RF/RO 0% 11.11% 13.33%
RO 0% 88.89% 26.67%
LF/RO 0% 77.78% 13.33%
RF 50% 22.22% 60%
LF 50% 77.78% 40%
RF/LO 50% 11.11% 86.67%
LO 100% 11.11% 73.33%
LF/LO 50% 0% 26.67%
bending
RIGHT OCCIPITAL
LEFT OCCIPITAL
RIGHT FRONTAL
LEFT FRONTAL
FRONTAL
OCCIPITAL
SIT-PCD
SIT-OTHERS
Controls
RF/RO 50% 22.22% 60%
RO 66.67% 22.22% 86.67%
LF/RO 16.67% 0% 26.67%
RF 50% 55.56% 66.67%
LF 50% 44.44% 33.33%
RF/LO 0% 33.33% 6.667%
LO 33.33% 77.78% 13.33%
LF/LO 33.33% 44.44% 6.667%

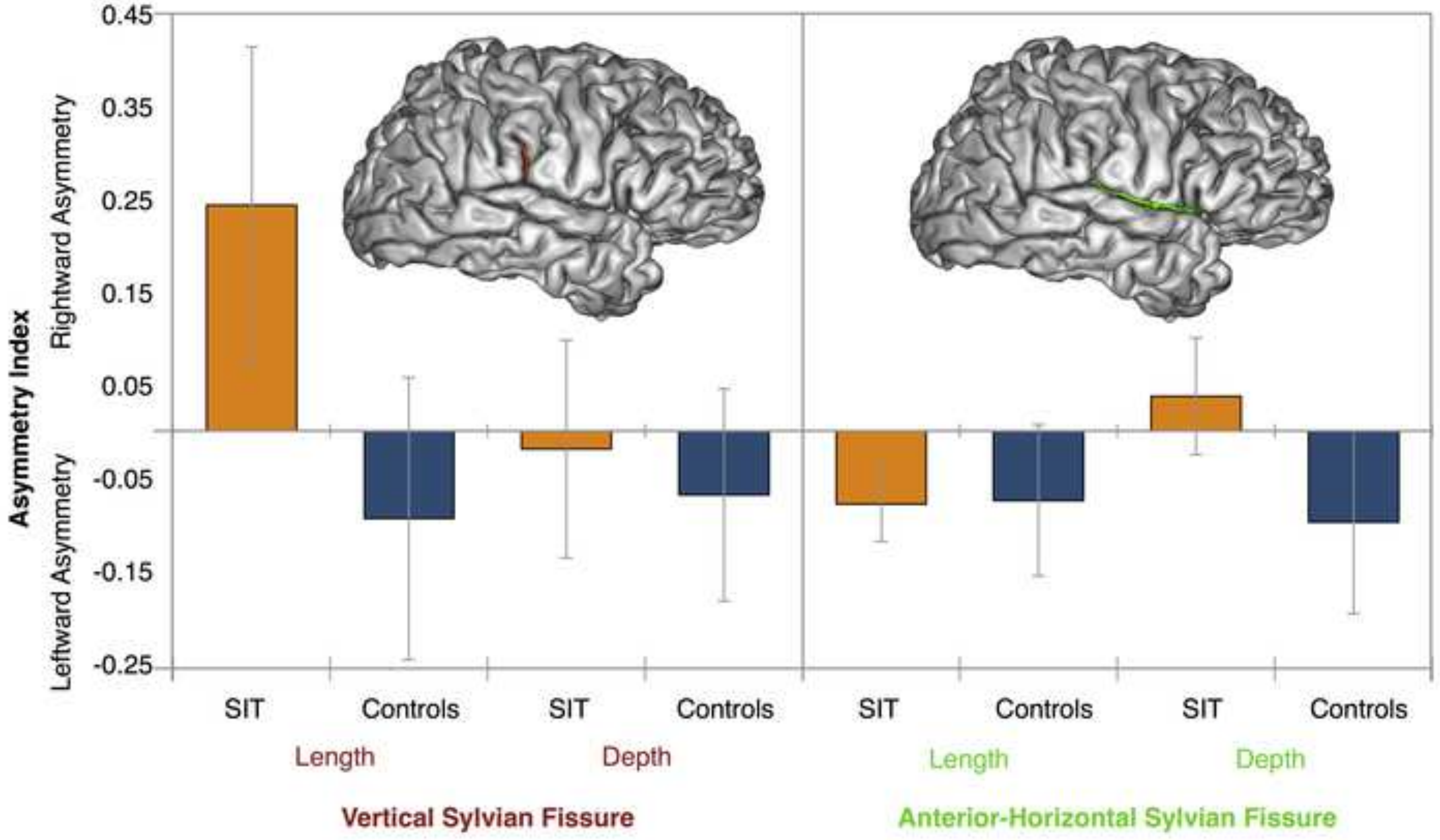

Asymmetry Index
Rightward Asymmetry
Leftward Asymmetry
0.45
0.35
0.25
0.15
0.05
-0.05
-0.15
-0.25
SIT
Controls
SIT
Controls
SIT
Controls
SIT
Controls
Length
Depth
Length
Depth
Vertical Sylvian Fissure
Anterior-Horizontal Sylvian Fissure

Figure 4

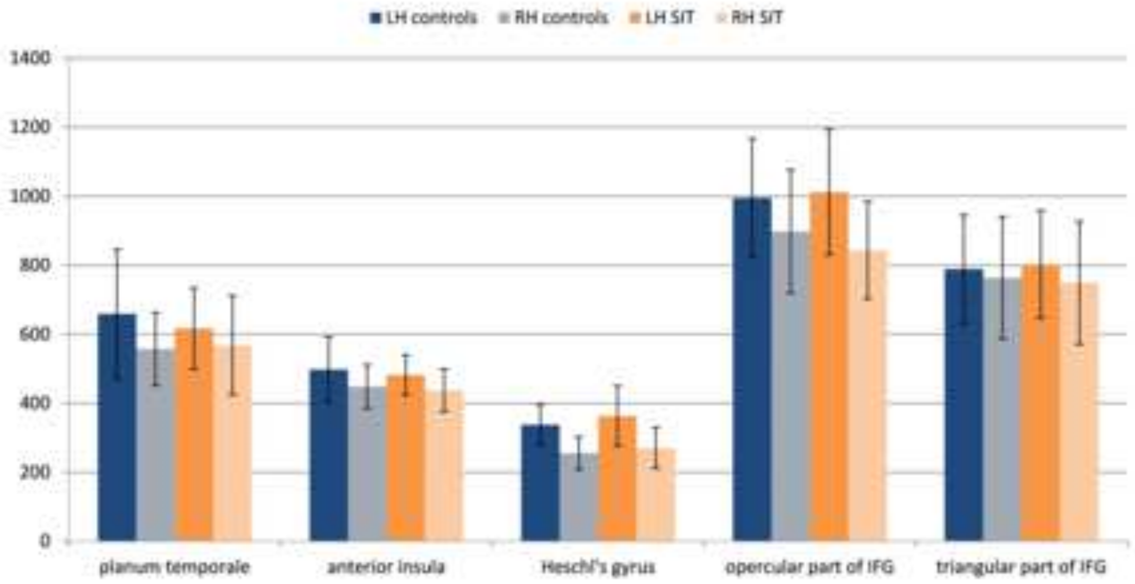

LH controls
RH controls
LH SIT
RH SIT
1400
1200
1000
800
600
400
200
0
planum temporale
anterior insula
Heschl's gyrus
opercular part of IFG
triangular part of IFG

Figure 5

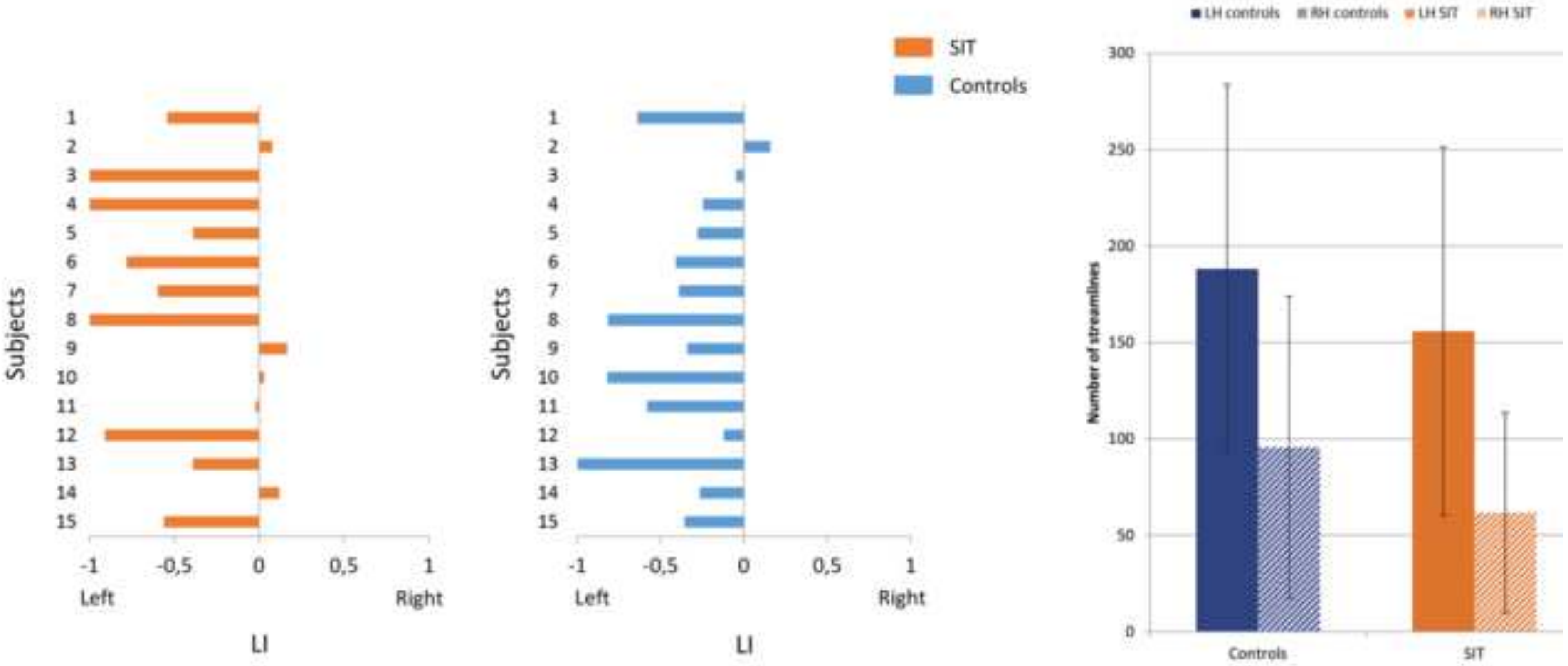

SIT
Controls
Subjects
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
-1
-0,5
0
0,5
1
Left
Right
LI
Subjects
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
-1
-0,5
0
0,5
1
Left
Right
LI
LH controls
RH controls
LH SIT
RH SIT
Number of streamlines
0
50
100
150
200
250
300
Controls
SIT

Figure 6

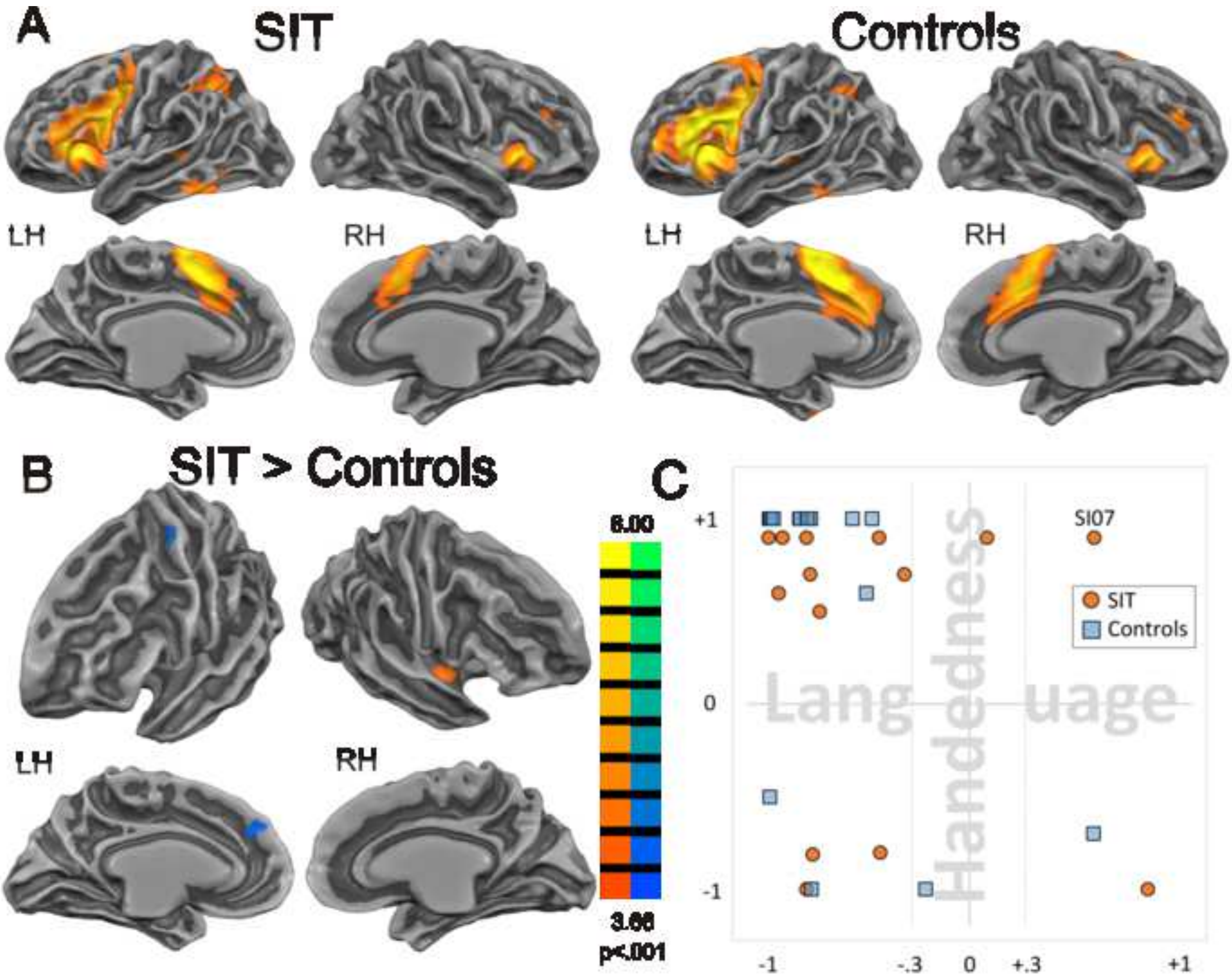

A
SIT
Controls
LH
RH
LH
RH
B
SIT > Controls
LH
RH
6.00
3.66
p<.001
C
+1
0
-1
-1
-.3
0
+.3
+1
SI07
SIT
Controls
Language
Handedness

Figure 7

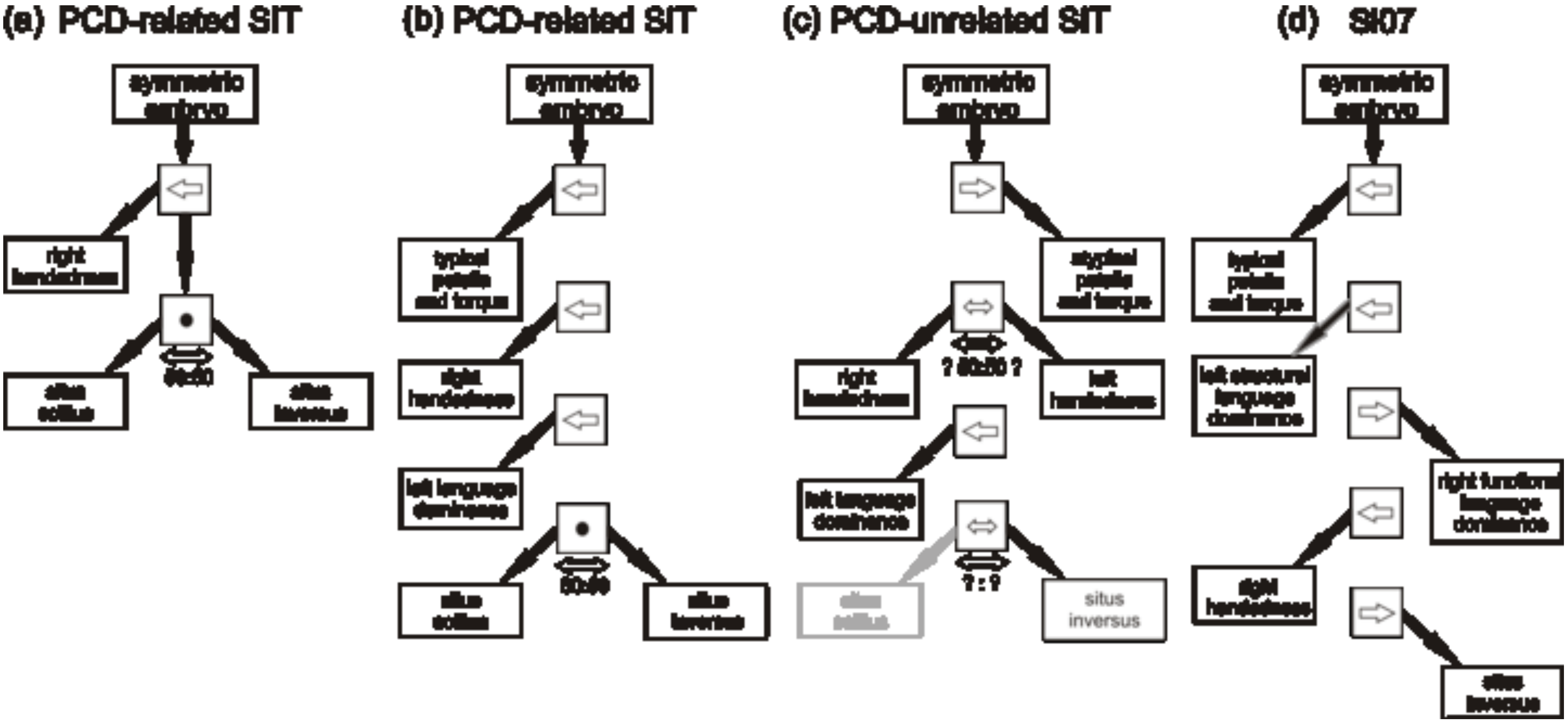

(a) PCD-related SIT
symmetric embryo
right handedness
50:50
situs solitus
situs inversus
(b) PCD-related SIT
symmetric embryo
typical petalia and torque
right handedness
left language dominance
50:50
situs solitus
situs inversus
(c) PCD-unrelated SIT
symmetric embryo
atypical petalia and torque
? 50:50 ?
right handedness
left handedness
left language dominance
? : ?
situs solitus
situs inversus
(d) SI07
symmetric embryo
typical petalia and torque
left structural language dominance
right functional language dominance
right handedness
situs inversus

Table A1. Demographic, relevant medical, and hand preference data of the situs inversus totalis (SI) and Control (CO) participants.

SI ID	SI comorbidities	Gender	Age	Educ*	Handedness**	CO ID	Gender	Age	Educ*	Handedness**
SI02	No complications	Male	50	8	0.9	CO02	Male	51	10	1.0
SI03	No complications	Female	26	12	-0.8	CO03bis	Female	26	15	-1.0
SI04	Sacral agenesis, congenital heart disease	Male	23	13	-1.0	CO04	Male	22	13	-0.5
SI05	No complications	Male	27	12	0.9	CO05	Male	27	12	1.0
SI06	Primary ciliary dyskinesia	Male	46	12	1.0	CO06bis	Male	43	12	1.0
SI07	Congenital heart disease	Female	35	12	0.9	CO07	Female	33	12	1.0
SI08	Primary ciliary dyskinesia	Female	23	15	0.9	CO08	Female	22	14	1.0
SI09	No complications	Female	36	15	0.7***	CO09bis	Female	38	15	0.6
SI11	Primary ciliary dyskinesia	Female	32	11	0.9	CO11	Female	34	12	1.0
SI12	No complications	Female	40	12	0.9	CO12	Female	38	12	1.0
SI13	Suspect primary ciliary dyskinesia	Male	48	14	0.6***	CO13bis	Male	46	12	1.0
SI14	Congenital heart disease	Male	18	12	-0.8	CO14	Male	19	12	-1.0
SI15	Primary ciliary dyskinesia	Female	31	18	0.7	CO15	Female	35	16	1.0
SI16	No complications	Male	21	14	-1.0	CO16bis	Male	20	14	-0.7

SI17	Primary ciliary dyskinesia	Male	39	14	0.5	CO17	Male	41	12	1.0

*education is expressed in years of full time formal education; ** Based on Edinburgh Handedness Inventory score: Lateralization index calculated on the 10 hand items and ranges from +1 (consistent right handedness) to -1 (consistent left handedness); *** Forced to right hand writing at primary school.

Table A2. Behavioral data and language laterality index (LI) of the situs inversus totalis (SI) and Control (CO) participants.

SI ID	**Estimated IQ**	**WGEN performance***	**WGEN LI****	**CO ID**	**Estimated IQ**	**WGEN performance***	**WGEN LI****
SI02	65	15	-0.93	CO02	89	25	-0.99
SI03	80	20	-0.78	CO03bis	107	22	-0.22
SI04	123	18	-0.81	CO04	107	23	-0.99
SI05	118	23	-0.81	CO05	80	17	-0.48
SI06	111	22	-0.82	CO06bis	122	25	-0.84
SI07	91	16	0.61	CO07	111	26	-0.79
SI08	118	21	-0.99	CO08	122	22	-0.84
SI09	120	25	-0.33	CO09bis	128	27	-0.51
SI11	94	20	-0.45	CO11	101	29	-0.80
SI12	76	22	0.08	CO12	105	20	-0.99
SI13	90	22	-0.95	CO13bis	89	21	-0.99
SI14	131	25	-0.44	CO14	110	19	-0.78

SI15	123	21	-0.79	CO15	119	26	-0.58
SI16	121	24	0.88	CO16bis	129	18	0.61
SI17	97	18	-0.75	CO17	100	27	-0.97

* Out-of-scan word generation performance; **Laterality index of fMRI-based brain activation during word generation, LI ranges from +1 (strong right hemisphere language dominance) to -1 (strong left hemisphere language dominance).

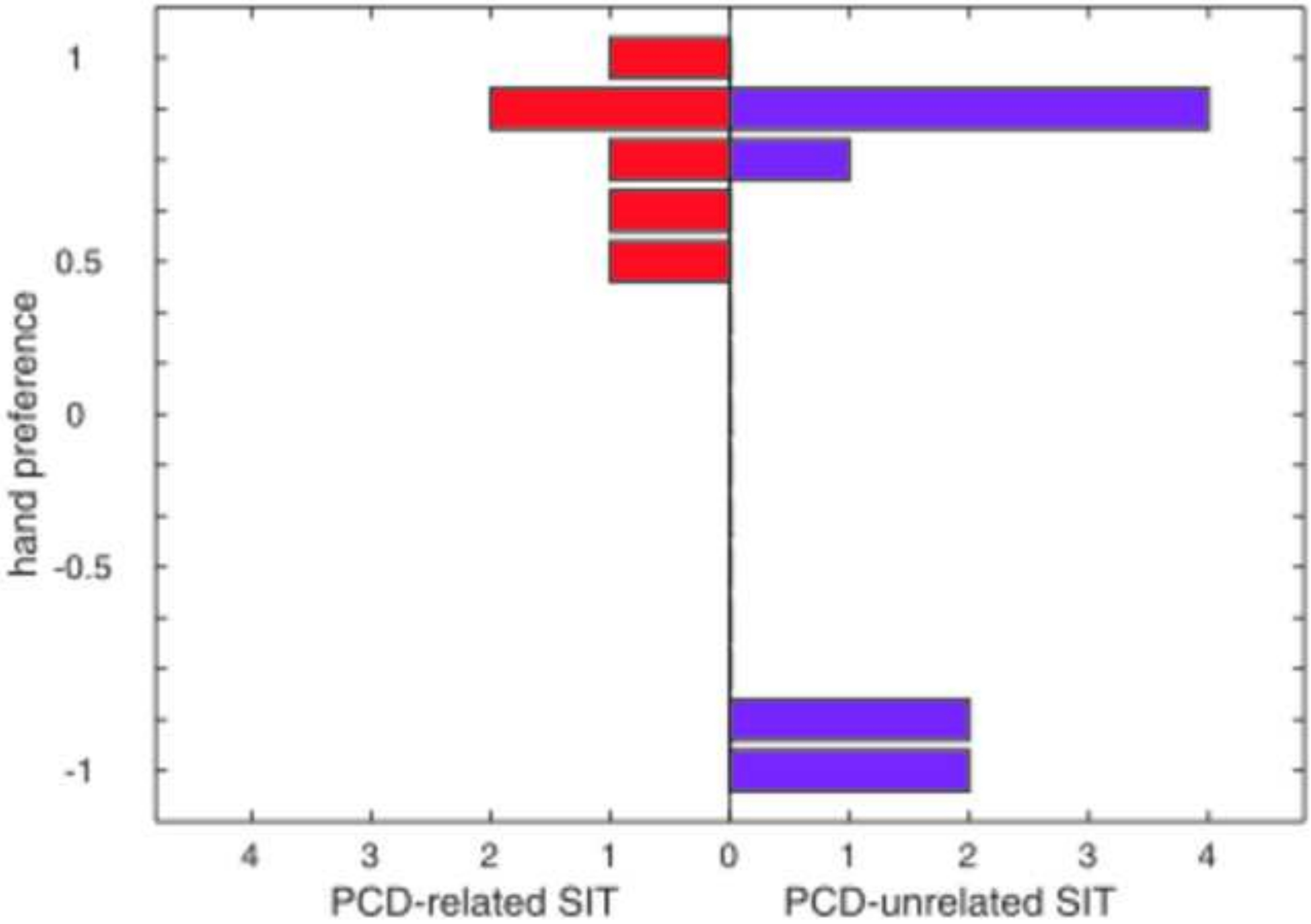

1
0.5
0
-0.5
-1
hand preference
4
3
2
1
0
1
2
3
4
PCD-related SIT
PCD-unrelated SIT

Table A3. Overview of the Sylvian fissure length and depth asymmetries in SIT and control participants.

		AH-SF *	**S.D**	***n******	**V-SF ****	**S.D**	***n******
Length (Mean)	**Situs Inversus**	-0.079	0.144	13	+0.243	0.615	13
	Controls	-0.075	0.282	13	-0.095	0.527	13
Depth (Mean)	**Situs Inversus**	+0.372	0.217	13	-0.020	0.406	13
	Controls	-0.985	0.368	13	-0.069	0.428	13

*AH = Anterior-Horizontal; ** V= Vertical; *** absence of clear bifurcation point resulted in incomplete data

Figure A2

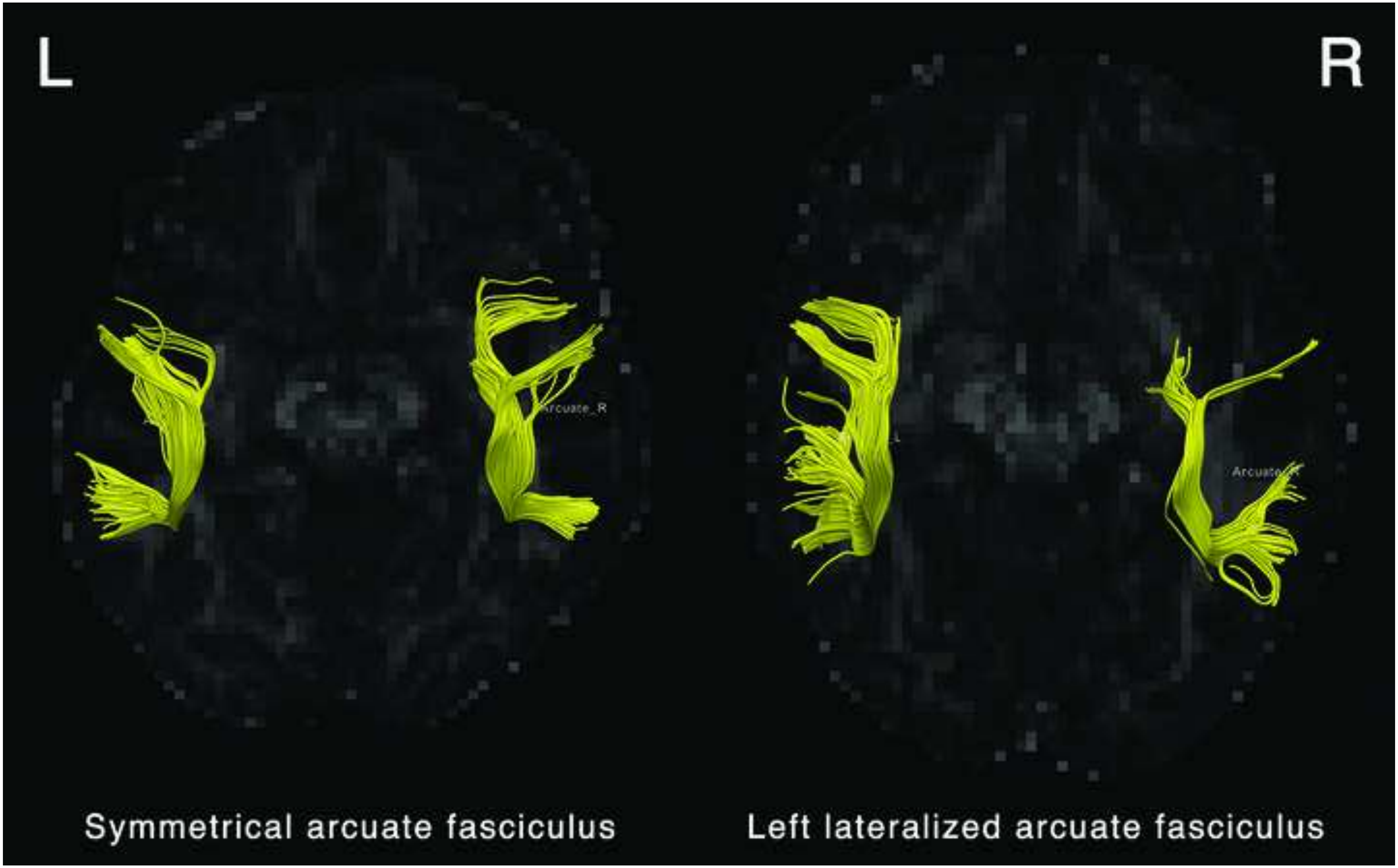
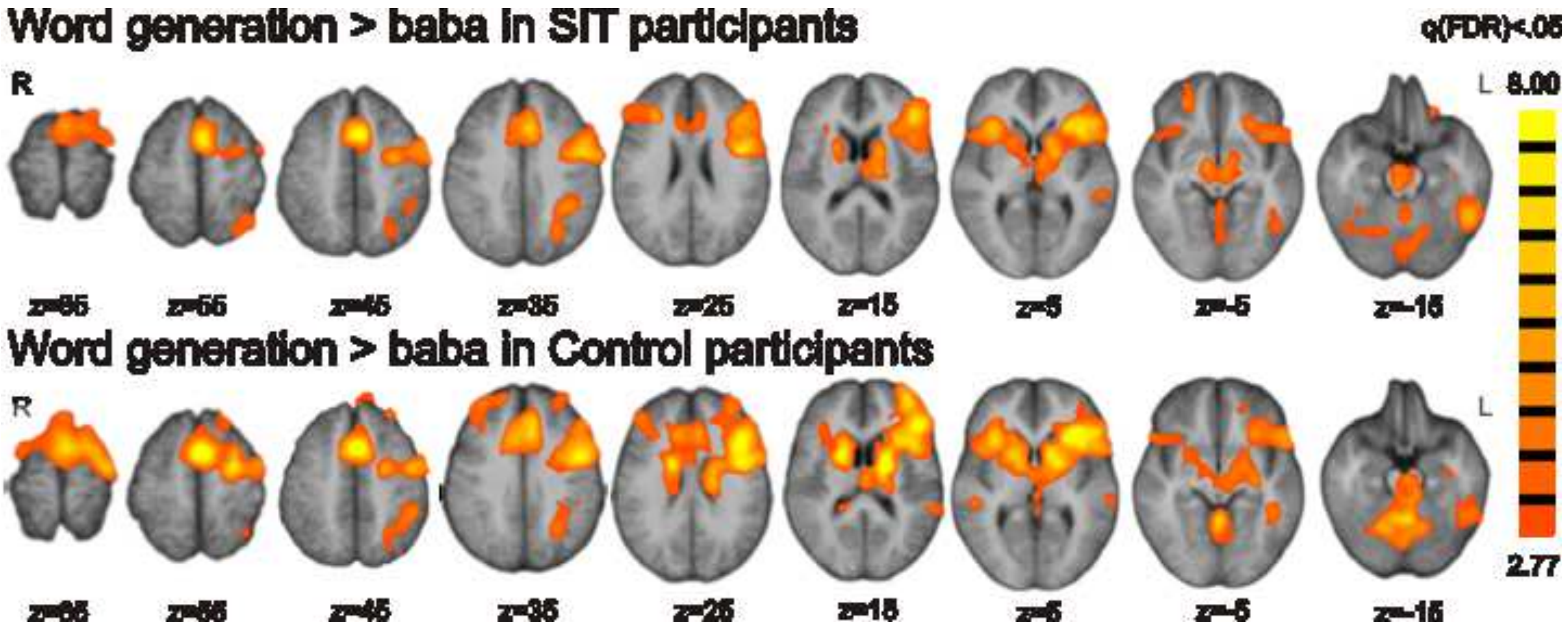

L
R
Arcuate_R
Arcuate_R
Symmetrical arcuate fasciculus
Left lateralized arcuate fasciculus

Table A4. Averaged voxel coordinates (Talairach space) and peak voxel t-values of significant activation clusters of the Word generation > baba contrast for SIT and Control cohort, and of the difference in word generation BOLD-response between SIT and Control.

Word generation > baba for Control and SIT cohort										
			Controls				SIT			
Region	BA	Side	X	Y	Z	t_{max}	X	Y	Z	t_{max}
Frontal regions										
Medial frontal gyrus	6	L	-2	11	49	10.59	-3	13	48	9.73
Inferior frontal gyrus	44	L	-42	5	30	10.13	-46	4	32	9.52
Middle frontal gyrus	9	L	-40	18	25	9.58	-44	16	29	6.11
Inferior frontal gyrus	45	L	-45	30	14	9.50	-40	25	9	7.66
Middle frontal gyrus	6/8	L	-26	-4	56	8.12	-30	-3	43	6.37
Anterior insula		R	32	19	3	7.47	31	15	6	7.03
Middle frontal gyrus	46	R	40	37	26	4.47	33	32	26	6.05
Parietal regions										
Inferior parietal lobule	40	L	-27	-53	38	4.96	-27	-59	36	4.95
Inferior parietal lobule	40	L	-44	-36	43	4.75	-35	-41	37	5.52
Temporal regions										
Inferior temporal gyrus	37	L	-52	-46	-12	4.34	-49	-48	-14	6.58
Middle temporal gyrus	22	L	-60	-27	4	3.78	-53	-33	4	4.44
Subcortical regions										

Caudate nucleus		R	18	5	15	11.30	17	7	10	6.24
Caudate nucleus		L	-17	-1	13	10.06	-15	5	9	7.12
Cerebellum			0	-51	-11	9.59	0	-50	-13	4.63
Caudate nucleus		L	-33	23	6	8.61	-37	22	6	7.60
Cerebellum		R	28	-59	-21	7.97	23	-62	-24	6.89
Cerebellum		L	-45	-51	-21	5.54	-44	-51	-19	6.58
Cerebellum		L	-51	-48	-14	4.40	-49	-48	-14	6.58
Word generation SIT > Word generation Controls										
Region	BA	Side	X	Y	Z	t_{max}				
Right posterior insula	13	R	40	-7	-5	5.62	Increased in SIT			
Left middle frontal gyrus	6	L	-24	-8	54	-4.14	Decreased in SIT			
Left medial frontal gyrus	9	L	-10	42	33	-3.91	Decreased in SIT			

Figure A3



Word generation > baba in SIT participants

Word generation > baba in Control participants

Figure A4