Structural Connectivity in Ventral Language Pathways Characterizes Nonverbal Autism

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Abstract

Autism spectrum disorder (ASD) involves a large variety of language capacities, from normal scores on standardized language tests to absence of functional language in a substantial minority of 30% of individuals with ASD. Due to practical difficulties of scanning at this severe end of the autism spectrum, insights from MRI are scarce. Here we used manual deterministic tractography to investigate, for the first time, the integrity of the core white matter tracts defining the language connectivity network in nonverbal ASD (nvASD): the arcuate (AF), inferior fronto-occipital (IFOF), inferior longitudinal (ILF) and uncinate (UF) fasciculi, and the frontal aslant tract (FAT). A multiple case series of nine individuals with nvASD were compared to normative benchmarks from matched individuals with verbal ASD (vASD) and typical development (TD). Bonferroni-corrected repeated measure ANOVAs were performed separately for each tract – *Hemisphere (2:Left/Right) x Group (3:TD/vASD/nvASD)*. Results revealed (i) a main effect of group consisting in a reduction in fractional anisotropy (FA) in the IFOF in nvASD relative to TD; (ii) a significant interaction of hemisphere and group in the UF, which showed reduced volume in the left hemisphere when compared to the right, in the vASD group only; and (iii) main effects of hemisphere in both the AF (left-lateralized in volume) and the ILF (left-lateralized in FA). These results do not replicate deficits of the dorsal language route previously observed in nvASD, and instead point to a disruption of the ventral language pathway, which is in line with semantic deficits observed behaviourally in this group.

1. Introduction

Non- or minimally verbal individuals with autism (nvASD) belong to the low-functioning section of autism spectrum disorder (ASD). They are defined by a severe expressive language deficit, which limits their spoken language acquisition to a handful of single words, with no compensation on the part of sign or written language (Tager-Flusberg and Kasari 2013). Current insights from magnetic resonance imaging are minimal and largely limited to two studies using diffusion tensor imaging to assess white matter (WM) structural connectivity, mainly focused on the exploration of WM tracts linked to mapping auditory information to articulatory motor representations. In particular, a reversal of a neurotypical left-right asymmetry of the arcuate fasciculus (AF) has been documented in four out of five nonverbal children with ASD (Wan et al. 2012). Similarly, when assessing treatment-based change in speech production of 10 minimally verbal children with ASD, an improvement during therapy was related to the integrity of both the left AF and right frontal aslant tract (FAT) (Chenausky et al. 2017). Further in line with this evidence, at least a subset of nvASD children have been reported to show childhood apraxia of speech (Chenausky et al. 2018), a developmental motor speech impairment (ASLHA 2021).

Language deviance in children and adults with nvASD, however, is not confined to expressive language. Language comprehension also falls far below the one expected from their chronological age (CA), and some evidence suggests that expressive and receptive language levels correlate in nvASD (Hartley et al. 2019; Pickles et al. 2014; Chenausky et al. 2019; Slusna et al. 2021). By definition, furthermore, nvASD are not characterized merely by a speech production deficit, but more broadly by an expressive language

deficit, which as such reaches beyond the vocal-auditory modality. In the present study, therefore, we aimed to provide the first characterization in nvASD of the fronto-temporal language network as a whole.

This language network distributes information along both dorsal and ventral processing streams (Price 2012; Friederici 2011; Skeide and Friederici 2016). Broadly, the dorsal pathway is argued to support sound-to-motor mapping, that is, the mapping of auditory speech sounds to articulatory representations, while the ventral pathway subserves sound-to-meaning mapping, i.e., extracting meaning from auditory speech sounds (Hickok and Poeppel 2004). Structurally, the dorsal stream incorporates the superior longitudinal fasciculus (SLF)–AF complex, often referred to as SLF/AF, which can be segregated into one direct and two indirect segments (Catani et al. 2005). The SLF/AF underpins sensorimotor processes during speech production and perception (Rauschecker and Scott 2009; Hickok and Poeppel 2007) and is also argued to support higher-level syntactic processes (Friederici 2015). In addition, the FAT contributes to the dorsal stream with a function argued to be specific to speech production (Catani et al. 2013) or speech-specific cognitive control processes (Dick et al. 2014). Within the ventral processing stream, the inferior fronto-occipital fasciculus (IFOF) is regarded as a crucial pathway subserving semantic processes (Saura et al. 2008; Duffau et al. 2005). Running laterally to the IFOF, the inferior longitudinal fasciculus (ILF) has also been hypothesized to aid semantic processing, namely lexical retrieval (Herbert et al. 2019; Shin et al. 2019). Finally, the uncinate fasciculus (UF) potentially hosts local phrase structure building (Friederici et al. 2006) and might be recruited as an indirect pathway for semantics-related processes (Harvey et al. 2013; Duffau et al. 2009).

In the present study, we used manual deterministic tractography to reconstruct the entire aforementioned structural language connectome, comprising the IFOF, UF, FAT, ILF, and the three segments of the AF (long segment, anterior segment, posterior segment), in a case series of 9 nvASD children and adolescents. While this approach is highly labor-intensive and difficult to pursue in large sample sizes, smaller samples provide an opportunity to allow for an individualized approach to the neuroanatomy of each participant (López-Barroso et al. 2013) and the combination of dissection proposals from different authors for the selected tracts (Catani and Thiebaut de Schotten 2008; Fekonja et al. 2019). After reconstructing these pathways, we estimated their WM micro- and macrostructural characteristics by extracting their corresponding fractional anisotropy (FA) and volume measures bilaterally. To obtain benchmarks of the tracts' volume and FA, data from 9 typically developing (TD) and 9 verbal children with ASD (vASD), obtained from an online ASD neuroimaging database (ABIDE II), pair-matched on sex, age and handedness, were also explored. We hypothesized structural alterations in both ASD groups, showing deviance in the neural organization of language within both the dorsal and ventral streams. This was based on widespread structural anomalies along both of these routes previously documented in vASD cohorts (Travers et al. 2012; Li et al. 2017). In particular, there have been reports of a loss of hemispheric lateralization of the AF (Fletcher et al. 2010; Liu et al. 2019; Joseph et al. 2014), and of aberrant WM integrity in the UF associated with socio-affective deficits (Samson et al. 2016; Li et al. 2019), while some studies have also pointed to structural alterations in the IFOF and ILF (Jou et al. 2011; Aoki et al. 2013). By comparing nvASD to both a neurotypical and a vASD group, we hoped that a differential pattern specific to nvASD would transpire.

2. Methods And Materials

2.1 Ethics approval

This study was approved by the corresponding institutional review board (CEIC Fundació Sant Joan de Déu; PIC-99-17). Written informed consent was obtained from legal guardians of all participants.

2.2 Participants

Nine non- or minimally verbal school-aged children and adolescents diagnosed with ASD (nvASD, 3 females, mean age = 12.5 ± 3.23) were recruited from special schools in Barcelona, Spain. Recruitment criteria included: (a) a parent / center-reported ASD diagnosis confirmed during recruitment via the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 2012) and the Autism Diagnostic Interview-Revised (ADI-R) (Rutter et al. 2003) tests; (b) an absence of phrase-level functional speech.

To compare the Barcelona-recruited sample of nvASD individuals with standards across typical and ASD development, a database collected at the San Diego State University (SDSU) was used. Specifically, we included two control groups consisting of (i) nine typically developing (TD) children, and (ii) nine verbal ASD (vASD) children matched on age, sex and handedness. Recruitment criteria for vASD consisted of a clinical diagnosis of ASD confirmed by the ADIR-R, ADOS, and a DSM-5-based clinical judgment, while TD participants required a parent-reported absence of personal / family history of ASD or other neurological or psychiatric conditions. See Table 1 for demographic and neuropsychological data from all three groups.

Table 1
– Demographic and neuropsychological participant profile

	TD Mean ± SD (n = 9)	vASD Mean ± SD (n = 9)	nvASD Mean ± SD (n = 9)	*p* value
Demographic information				
Sex (Male/Female)	6/3	6/3	6/3	1.000
Handedness (Right/Left)	8/1	8/1	8/1	1.000
Age at MRI acquisition (Years;Months)	12;6 ± 3.49	12;8 ± 3.07	12;6 ± 3.23	0.992
Neuropsychological profile				
Verbal Mental Age (VMA)/IQ	110.33 ± 10.33	93.56 ± 13.34	24.25 ± 15.74	–
Non-verbal IQ	108.44 ± 8.69	104.56 ± 16.38	63.75 ± 16.91	–
Diagnostic score (ADOS)	–	15.11 ± 2.76	17.88 ± 3.64	–

Table 1 Demographic information – Data are means ± SD unless otherwise stated. Between-group differences were explored using X^2-tests for sex and handedness and one-way ANOVA for age at MRI acquisition. Statistical tests confirmed the lack of significant different across the three groups. Neuropsychological profile – The neuropsychological tests applied were different for the three groups due to their intrinsic characteristics. Tests administered were: Verbal Mental Age (VMA)/IQ – Peabody Picture Vocabulary Test-III (PPVT-III) in nvASD, Wechsler Abbreviated Scale of Intelligence in vASD and TD; Non-Verbal IQ – Leiter International Performance Test-Revised (Leiter-R) in nvASD, Wechsler Abbreviated Scale of Intelligence in vASD and TD; ADOS – Autism Diagnostic Observation Schedule-2/-Adapted (ADOS-2/ADOS-A) in nvASD and vASD. Abbreviations: TD = Typically development; vASD = Verbal Autism Spectrum Disorder; nvASD = Non-verbal Autism Spectrum Disorder; IQ = intelligence quotient; MA = mental age; ADOS = Autism Diagnostic Observation Schedule.

2.3 MRI acquisition

Non-verbal ASD participants were scanned under anaesthesia, as approved by the corresponding institutional review board (CEIC Fundació Sant Joan de Déu; PIC-99-17), on a Philips Ingenia 3T scanner using a 64-channel head coil at the Sant Joan de Déu Hospital, Barcelona. Diffusion-weighted images (DWI) were acquired with a spin-echo echo-planar imaging (EPI) sequence (TR = 10100 ms, TE = 102 ms, 64 axial slices, 36 directions, 90° flip angle, slice thickness = 2.1 mm, FOV = 23 cm, acquisition matrix = 112 x 112, voxel size = 2.05 mm3) with three non-diffusion (b = 0s/mm2) and 36 diffusion weighted volumes (b = 1250 s/mm2). Data from TD and vASD subjects were collected on a GE 3T Discovery MR750 scanner using an 8-channel head coil (UCSD–CFMRI). DWI were acquired with an EPI sequence (TR = 8500 ms, minimum TE by scanner protocol, 68 axial slices, 61 directions, slice thickness = 2.0 mm, FOV = 24 cm, acquisition matrix = 128 x 128, voxel size = 2.05 mm3) with one non-diffusion (b = 0 s/mm2) and 61 diffusion weighted volumes (b = 1250 s/mm2).

2.4 MRI preprocessing

A visual inspection was performed by an expert for all data prior to the preprocessing to ensure the absence of any major artifact (due to acquisition errors, movement or others) that could not be corrected during the subsequent processing steps. All images were pre-processed using FMRIB Software Library (FSL www.fmrib.ox.ac.uk/fsl/fdt) and Diffusion Toolkit software (DTK) (Wang and Weeden 2015). DWI were processed as follows: (i) eddy-current correction using FMRIB's Diffusion Toolbox (FDT), part of FMRIB Software Library (FSL www.fmrib.ox.ac.uk/fsl/fdt); (ii) brain extraction using FSL's Brain Extractor Tool (Smith 2002; Smith et al. 2004; Woolrich et al. 2009) with 0.3 as threshold value; (iii) rotation of the b-vectors; (iv) reconstruction of the diffusion tensors using DTK (Wang and Weeden 2015); and (v) whole-brain deterministic tractography using DTK with 35 degrees as maximum curvature and a minimum FA threshold of 0.2.

2.5 Tract dissections

Manual deterministic tractography was performed focusing on the five main language-related tracts: arcuate (AF), inferior fronto-occipital (IFOF), inferior longitudinal (ILF), uncinate (UF) fasciculi, and frontal

aslant tract (FAT). Tracts were dissected for each participant in native space, in both hemispheres, using Trackvis software (v.0.6.0.1, http://trackvis.org/) by manually placing Regions of Interest (ROI) as identified in previous reports (Catani and Thiebaut de Schotten 2008; Fekonja et al. 2019).

AF. The three segments of the AF were dissected using three ROIs drawn in a single slice as described in previous studies (Catani et al. 2005; Lopez-Barroso 2013): a first ROI was delineated in the coronal view encompassing the fibers going to the inferior frontal gyrus (IFG) (including BA44 and 45); a second ROI was drawn in the axial plane covering the WM fibers traveling to the superior temporal gyrus; finally, a third ROI was depicted on the sagittal view, covering supramarginal and angular gyri. These ROIs were combined to reconstruct the three subdivisions of the AF: the long (fronto-temporal), the anterior (fronto-parietal), and the posterior (temporo-parietal) segments. However, in order to reduce the number of statistical comparisons, only measures for the whole AF were included in the analysis: the volume of the three segments was summed, and the FA across the three branches was averaged in order to obtain a single value for the whole tract.

FAT. To dissect the frontal aslant tract, two ROIs were delineated: the first was a spherical ROI of radius 8 mm located in the IFG and the second one was a single slice ROI placed in the WM of the superior frontal gyrus, encompassing fibers traveling to the Supplementary Motor Area (SMA) and pre-SMA (Catani et al. 2013).

ILF, UF & IFOF. For the delineation of the WM pathways supporting the ventral stream for language processing (i.e., ILF, IFOF and UF) (Rauschecker and Scott 2009; Hickok and Poeppel 2007), we used the combination of four ROIs according to previous publications (Catani and Thiebaut de Schotten 2008; Fekonja et al. 2019). The first ROI was placed axially at the level of the anterior temporal lobe (temporal ROI) spreading throughout an average of 5 slices; the second one on the anterior floor of the external/extreme capsule covering an average of 3 slices (frontal ROI); a third one on the region located between the occipital and temporal lobe (occipital ROI); and a fourth spherical ROI of radius 6.5 mm was placed in the middle temporal region, anterior to the radiation of the corpus callosum (temporoccipital ROI). To define each of the tracts of interest, we applied a two-ROI approach: ILF was comprised by fibers going through the temporal and occipital ROIs; streamlines going through both anterior and frontal ROIs were considered as part of the UF; finally, the fibers crossing the frontal and temporoccipital ROIs formed the IFOF (following Fekonja's method) (Fekonja et al. 2019).

Fekonja's method of dissection was selected here for reconstructing the IFOF because we found it to be more permissive in the inclusion of fibers than other methods, generating a more plausible outcome for our type of data, processed following a diffusion tensor kind of analysis (as opposed to higher resolution data that could be processed using spherical deconvolution methods, for instance). Nonetheless, and as stated in the main text, we additionally followed the Catani and Thiebaut de Schotten's (2008) approach, which defines the IFOF as the fibers travelling through the frontal and occipital ROIs as described above. As expected, both dissection approaches generated similar results in our analyses. See the Online

Resource 1 for details and comparison of the ANOVA test performed with the data extracted using each type of IFOF reconstruction.

Finally, artefactual fibers, if present in any of the tracts / hemispheres, were removed using exclusion ROIs, as is standard practice in manual reconstructions (Elmer et al. 2019; Vaquero et al. 2021). Tract volume and Fractional Anisotropy (FA) values were extracted in every participant for each tract and hemisphere. The dissections for all participants of the nvASD group are given in Figure 1 and dissections of the vASD and TD participants can be found in the Online Resource 4. For visualization purposes, rendering of the streamlines was performed using the “tube” render option of TrackVis with a radius of 0.15 mm. Examples of ROI placement are depicted in Figure 2.

2.6 Statistical analysis

Statistical analyses were performed using IBM SPSS software (v25.0). Hemisphere (2: Left/Right) x Group (3: TD / vASD / nvASD) repeated measures ANOVAs were performed separately for each tract (i.e., AF, FAT, IFOF, ILF, UF) and WM measure (volume, FA), resulting in 10 ANOVAs (5 tracts per 2 measures). Bonferroni correction for multiple comparisons at $p < 0.005$ was applied and only results with a *p*-value below this threshold will be presented below; for uncorrected trends see supplementary tables A2 and A3).

3. Results

ANOVA results are detailed in the Online Resources 2 and 3, and data distributions for AF, ILF, IFOF and UF are visualized in Figure 3.

Tract volume. A main effect of hemisphere was observed for AF ($F(1,24) = 47.323$, $p < 0.001$), which showed larger volumes in the left compared to the right AF across all groups. Importantly, an interaction of hemisphere and group was observed in the UF ($F(2,24) = 9.974$, $p = 0.001$), showing a reduced volume in the left compared to the right UF, in the vASD group only, with Bonferroni corrected *post-hoc* tests confirming this effect (volume differences between left and right UF in vASD: $F(1,24) = 13.225$, $p = 0.001$; in TD: $F(1,24) = 3.382$, $p = 0.078$; in nvASD: $F(1,24) = 3.341$, $p = 0.080$).

Fractional Anisotropy. A main effect of hemisphere was found in the ILF ($F(1,23) = 63.097$, $p < 0.001$), where larger FA values were found in the left compared to the right hemisphere across the three groups. Moreover, a main effect of group was encountered in the IFOF ($F(2,24) = 8.062$, $p = 0.001$), showing a gradual tendency to decrease in FA in both ASD groups compared to TD individuals (TD > vASD > nvASD). *Post hoc* comparisons (Bonferroni corrected) showed that this effect was driven by differences between TD and nvASD groups ($F(2,24) = 8.062$, $p = 0.002$), whereas the comparisons between TD and vASD ($F(2,24) = 8.062$, $p = 0.061$) or between vASD and nvASD ($F(2,24) = 8.062$, $p = 0.448$) groups did not reach significance.

4. Discussion

This study aimed to investigate language-related WM structural connectivity alterations in nvASD individuals compared to matched verbal ASD (vASD) and typical development (TD) individuals. Manual DWI deterministic tractography was used for reconstruction of the main WM fiber tracks associated to language processing and measured by computing fractional anisotropy (FA) and volume measures. The two main findings are, firstly, a main effect of group consisting in a reduction in FA in the IFOF in nvASD relative to the TD group, and secondly, a significant interaction of hemisphere and group in the UF, which showed reduced volume in the left hemisphere when compared to the right only in the vASD group.

The reduction of FA in the IFOF in nvASD compared to TD individuals is a new finding. Although the exact involvement of the IFOF in language functions is still unclear, previous reports have demonstrated its role in reading, writing and attention (Catani and Thiebaut de Schotten 2008; Dorrichi et al. 2008), but it has been first and foremost considered as a crucial pathway subserving semantic processing (Fekonja et al. 2019; Catani and Thiebaut de Schotten 2008; Dick et al. 2014). In line with this, several lesion and tumor studies using electric stimulation have shown the relationship between IFOF integrity and proficiency in a semantic matching task (Sierpwoska et al. 2019), a verbal fluency task (Almairac et al. 2015), and the number of semantic paraphasias (Duffau et al. 2005; Sierpwoska et al. 2019), but not for semantic learning (Ripolles et al. 2017). In that sense, the anatomical course and terminations of the IFOF can also be of great value to understand its contribution in language processing.

Recently, both DTI and anatomical dissection studies have described the main course of the IFOF at the level of the insula and the temporal lobe (Martino et al. 2010; Catani and Thiebaut de Schotten, 2008), but more debate has been generated with respect to its anterior and posterior terminations. Sarubo and colleagues (2013) attempted to describe the frontal terminations of the IFOF by combining anatomical dissections and DWI. The authors proposed a division of the tract in two major components: a superficial one, terminating in the inferior frontal gyrus (IFG) and a deeper one, connecting with the middle frontal gyrus (MFG), dorso-lateral prefrontal cortex (DLPFC), the orbitofrontal cortex and the frontal pole. Similarly, Wu and colleagues (2016) used high resolution diffusion tensor tractography to identify five subcomponents of the IFOF based on its frontal terminations (which overlapped greatly with those described by Sarubo and colleagues, 2013). These results would support the idea of the IFOF as a “multi-function” tract, with a clear involvement in language processing due to its role in conveying information to crucial language-related regions and nearby ones (IFG, MFG, DLPFC and orbitofrontal cortex). In most cases, these are associated to semantic processing functions (Binder et al. 2009; Plaza et al. 2008). Similarly, Martino and colleagues (2010) used post-mortem anatomical dissections to investigate and describe the posterior terminations of this tract. In this case, the authors also suggested the division of the IFOF into a superficial and a deeper component based on the posterior terminations. The former would project to the superior parietal lobe and posterior parts of the superior and middle occipital gyrus, whereas the latter would be associated with terminations in the inferior occipital gyrus and the posterior temporo-basal area. Again, the terminations of the IFOF in the associative extra-striate cortex and

posterior temporo-basal area would further support the involvement of this tract in semantic functions (Martino et al. 2010, Price 2000, Vilha et al. 2004).

Despite this evidence, no study until now has attempted to elucidate the role of this pathway in a disorder with a clear semantic impairment such as individuals with nvASD. In standardized settings, language comprehension measures in this group have yielded scores far below those expected by individuals' CA (Chenausky et al. 2019; DiStefano et al. 2016; Garrido et al. 2015; Slusna et al. 2021), and caregiver reports consistently document a lack of understanding or following of complex linguistic constructions (e.g., three-step instructions) in individuals with nvASD (Skwerer et al. 2016). Although children with nvASD show variation in how many single words they produce, there is evidence that those words are not semantically understood as carrying referential meaning (Preissler 2008), unlike what is seen already even in very young neurotypical infants (Marno et al. 2015). In line with this, experimental assessments using EEG have uncovered anomalous patterns of lexico-semantic neural processing in a mixed group of nonverbal and preverbal children with ASD (Cantiani et al. 2016), effectively pointing to an aberrant rather than delayed language processing in line with the neural patterns observed here. Although lexical semantic anomalies are seen throughout ASD (Tek et al. 2008; Arunachalam and Luyster 2016), these certainly do not reach the level of the essential absence of neurotypical word use in nvASD, suggesting that ventral structural alterations of the IFOF may indeed be unique to nvASD.

Although it was not the original focus of this investigation, anomalies in the ventral language route were also found here for the vASD group. Specifically, higher volume of the UF on the right compared to the left hemisphere was observed in this group, a result that converges with previous findings in both children and adults with vASD (Samson et al. 2016; Li et al. 2019; Catani et al. 2016). Some of this previous work proposed that the maldevelopment of the UF, a tract connecting the lateral orbitofrontal cortex and Brodmann area 10 with the anterior temporal lobe (Von der Heide et al. 2013), is a potential neural substrate for the socio-affective deficits observed in this group (Samson et al. 2016; Li et al. 2019). Our vASD and nvASD individuals, however, shared a diagnosis and were selected so as to differ in language, not in socio-affective deficits. Further work is therefore required to corroborate what functions the UF supports. Given anomalies relating to the ventral route of language processing found in both ASD groups in our study, our results are consistent with a more localized ventral impact in vASD, as reflected by macrostructural alterations in a short and restricted associative bundle such as the UF, while nvASD shows a more global effect underpinned by a microstructural anomaly in the IFOF, a massive tract crossing the entire brain ventrally. Furthermore, as neural profiles between nvASD and vASD diverge, it is possible that nvASD should not be viewed as continuous with vASD, but as a relatively separate group within the autism spectrum, with distinct structural correlates.

In this study we capitalized on manual dissection, despite it being labor-intensive and making larger samples difficult. This method was selected as it allowed a more suitable neuroanatomic approach for the research question of this study. First, manual dissections make the tract reconstruction adaptable to individual differences, which in the present case of developing brains (children and adolescents) is crucial, since most automatic dissection tools are based on adult anatomical landmarks / atlases.

Second, we wanted to combine different authors' proposals for dissecting the IFOF, a complex tract for which both anterior and posterior terminations are highly controversial. Despite the multiple possible frontal terminations discussed for this tract, all the streamlines are compacted when passing through the external/extreme capsule, so a first region of interest placed in this bottleneck should include all of the tract's fibers, as suggested by Catani and Thiebaut de Schotten (2008). However, the posterior ROI proposed by these authors is a lot more restrictive as it does not encompass some of the parietal and superior occipital terminations observed postmortem by other authors, such as Martino and colleagues (2010). Hence, we opted for a more inclusive ROI in the middle temporal gyrus, anterior to the radiation of the corpus callosum (Fekonja et al. 2019), comprising all the fibers coming from the temporal isthmus before they spread into their final cortical destination. The aim of this approach was to be as comprehensive as possible when selecting fibers, to ensure a complete and anatomically reliable characterization of the structural connectivity of this tract, which seems to be crucial for the understanding of this disorder. Nonetheless, very similar results were obtained when using the two ROIs proposed by Catani and Thiebaut de Schotten (2008) for the dissection of the IFOF as compared to the more comprehensive approach (see Online Resource 1).

Unlike in the case of our predictions for the ventral language pathway, our findings did not confirm our predictions based on previous literature in nvASD for structural alterations of the dorsal language pathway (Wan et al. 2012). These predictions were based on the study by Wan and colleagues (2012), who compared volume lateralization of the Arcuate Fasciculus between five completely non-verbal ASD and five TD children. Their results showed a rightward laterality (instead of the typical leftward asymmetry) in nvASD, which the authors argue could be critical for the language deficits observed in this group. Several factors could explain the divergence between theirs and our results: a difference in the selection of the tractography method (probabilistic vs. deterministic) or sample size (five vs nine participants per group), or even the inclusion criteria applied (completely vs minimally verbal ASD children). While not ruling out dorsal route involvement, our results do not support that the severe language problems in nvASD can be due only to problems of sensory-motor integration related to the AF and the dorsal processing route. Instead, they point to a deficit involving anomalous comprehension and semantic language processing.

Limitations of the current study include different scanning sites and protocols, although generalized scanner artifacts seem unlikely given the specificity of the patterns observed. Also, the fact that dissections were performed in native space for every participant, extracting individual values from selected tracts, implies less methodological issues than voxel-based techniques performed at a group-level. Another limitation may be the reduced sample size, which prevents us from extracting definitive conclusions from our results. Although limited, the sample used in this study is similar to the ones recruited in previous studies on nvASD, which makes evident the difficulty of scanning and working in the lab with this population, therefore supporting the value of the present results. Finally, as previously discussed, manual dissection was used for this study, but future work should try to expand the sample size and complement the analyses with other tractography methods like TRACULA (a global probabilistic approach - Yendiki et al. 2011), AFQ (an automated deterministic method - Yeatman et al. 2012), or tract-

based spatial statistics (TBSS, to compare at group and voxel-based-like levels - Smith et al. 2006). This would help to better understand the neurobiological basis of this extreme side of the ASD spectrum, from which we know so little in terms of structural neural underpinnings despite its prevalence.

5. Conclusions

Our investigation revealed a more complex pattern of WM structural differences in nvASD than the one expected from previous findings. Unlike the previously reported disruption of the dorsal language processing route, the key finding of the present study is a reduction of FA in the IFOF in nvASD compared to TD. These results suggest the disruption of the ventral language pathway as contributing to the severe language problems exhibited at this end of the autism spectrum, in line with behavioural findings of semantic deficits in this group. Our results suggest that further investigations should not merely be centered on the articulatory-motor or dorsal route (only comprising tracts such as the AF and FAT), but that a more comprehensive investigation of the language network is needed. We also observed an increased volume in the right compared to the left UF in vASD, possibly indicating a more localized ventral processing problem in this group, which, interestingly, did not generalize to nvASD.

Declarations

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Authors' contributions - **CRediT author statement: Guillem Olivé**: Methodology, Software, Formal Analysis, Writing- Original draft preparation, Visualization. **Dominika Slušná**: Investigation, Data curation, Writing- Original draft. **Lucía Vaquero**: Validation, Formal analysis, Writing- Review & Editing, Visualization. **Jordi Muchart**: Investigation, Resources. **Antoni Rodríguez-Fornells**: Conceptualization, Writing- Review & Editing, Supervision. **Wolfram Hinzen**: Conceptualization, Resources, Writing- Review & Editing, Supervision, Project administration, Funding Acquisition.

Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the corresponding institutional review board (CEIC Fundació Sant Joan de Déu;

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Consent to participate: Written informed consent was obtained from legal guardians of all participants.

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Figures

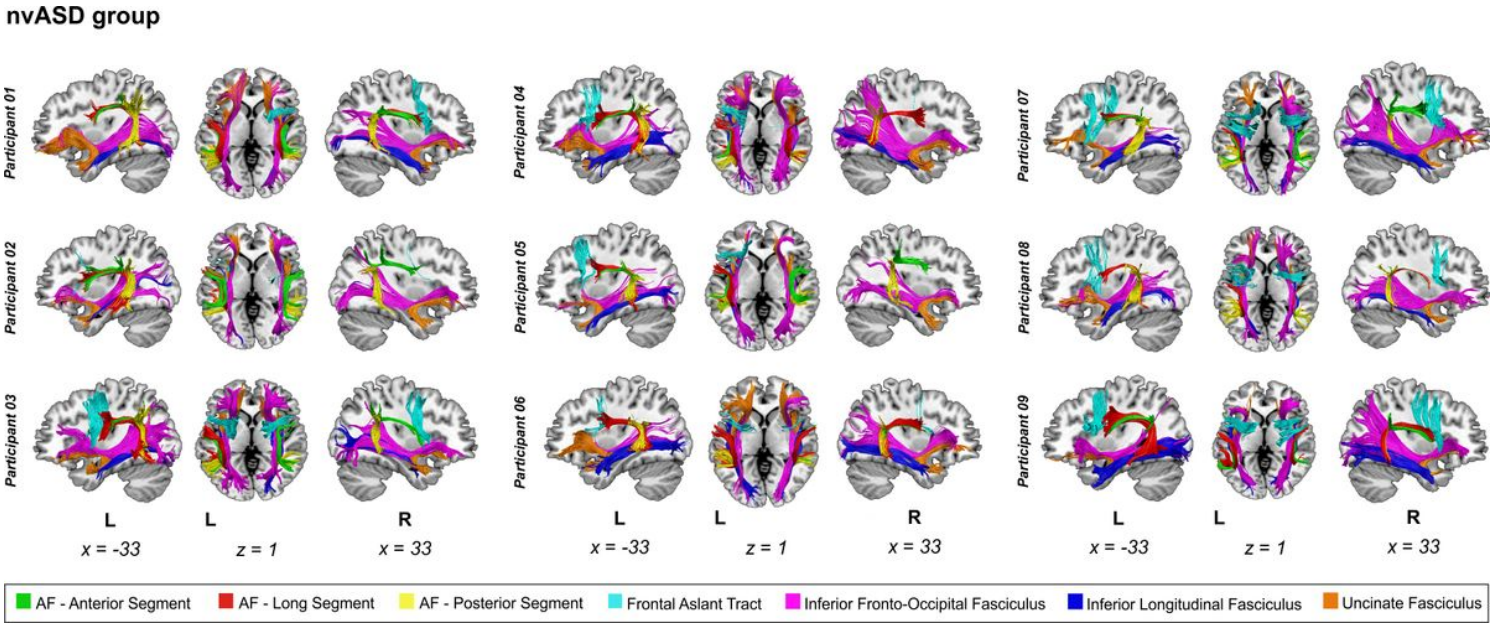

Figure 1

Dissections of nvASD participants Manual deterministic tractography reconstructions from all participants of the nvASD group. Tracts reconstructed were the three segments of the arcuate fasciculus (AF) [Green = anterior, red = long, yellow = posterior segments], Frontal Aslant tract (FAT) [Cyan], Inferior Frontal Occipital Fasciculus (IFOF) [Purple], Inferior Longitudinal Fasciculus (ILF) [Dark blue] and Uncinate Fasciculus (UF) [Orange]. Abbreviations: L, left. Montreal Neurological Institute space coordinates of the structural template slices are specified at the bottom of the image.

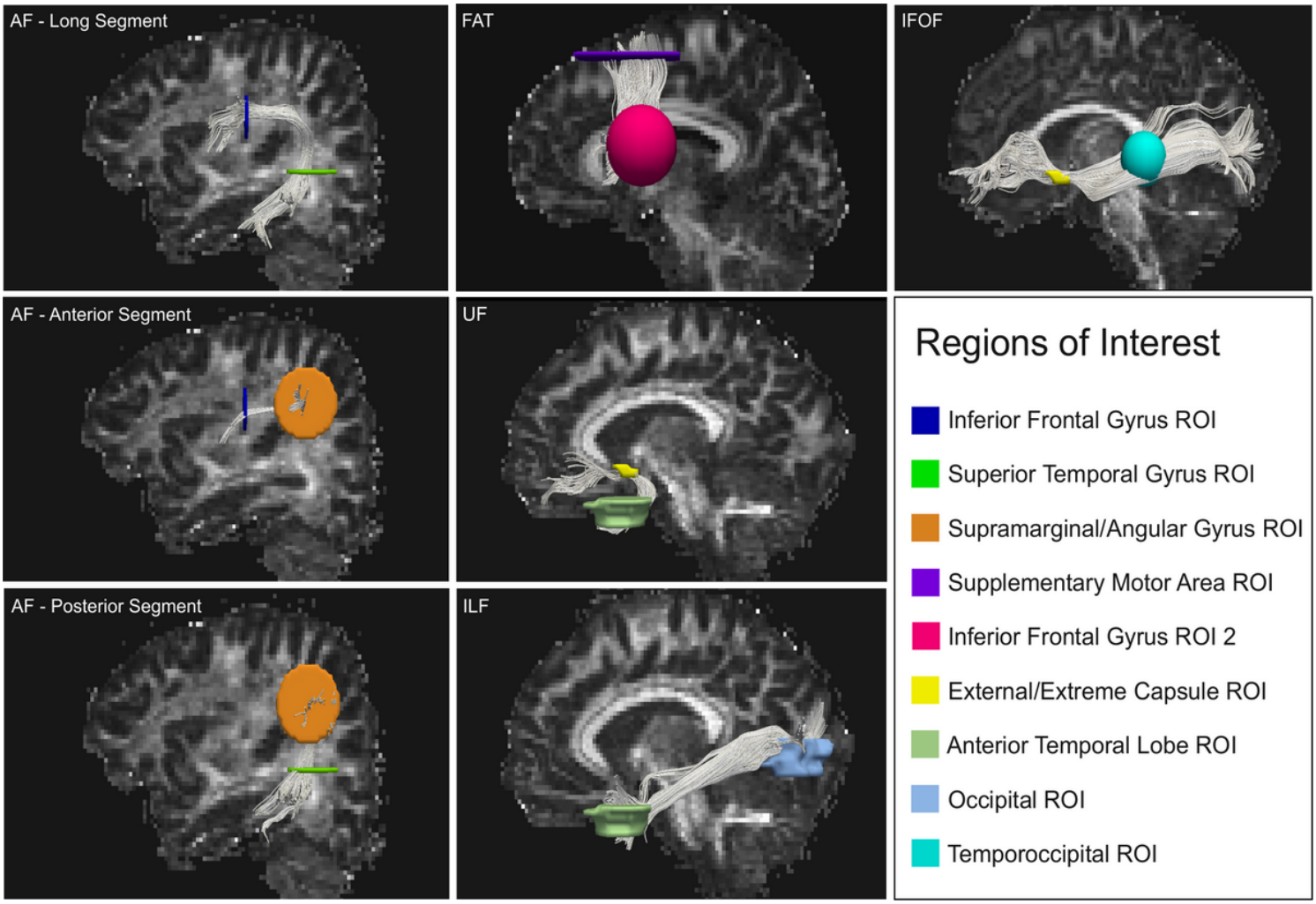

Figure 2

Regions of Interest placement examples Regions of Interest (ROI) placements for manual deterministic tractography reconstructions of the selected tracts. Tracts reconstructed were the three segments of the arcuate fasciculus (AF), Frontal Aslant tract (FAT), Uncinate Fasciculus (UF), Inferior Longitudinal Fasciculus (ILF) and Inferior Frontal Occipital Fasciculus (IFOF).

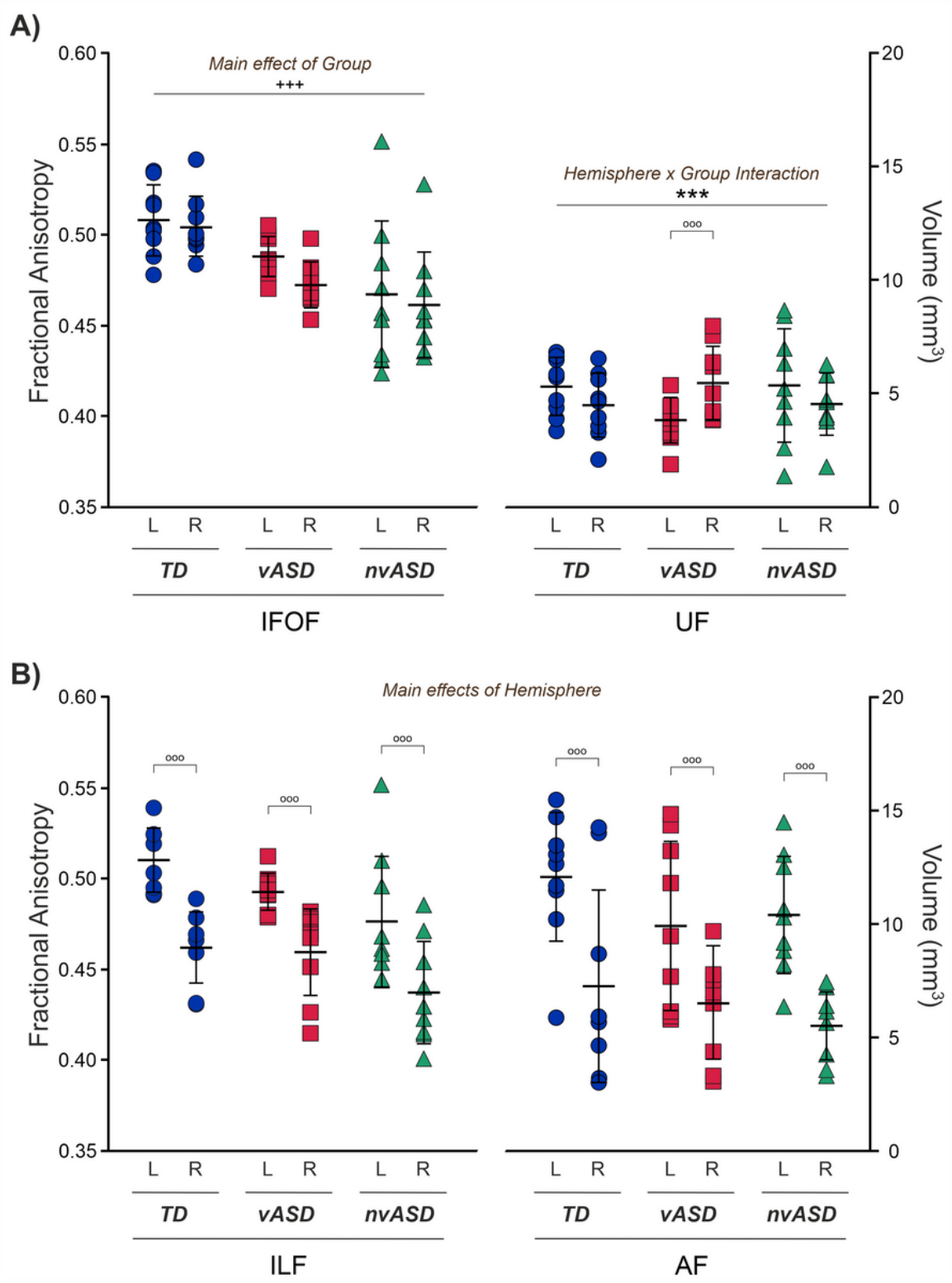

Figure 3

Structural connectivity results: volume and fractional anisotropy. Significant results of the repeated-measures ANOVA performed for the structural connectivity measures (volume and FA) extracted from each tract, with values for both hemispheres depicted in each group (blue circles correspond to TD participants, dark pink squares show vASD participants, and teal triangles represent nvASD participants). A) Left side of the graph shows the distribution of FA values in the IFOF, with the Main effect of Group

specified; right side of the graph illustrates the distribution of volume values in the UF, marking the significant Group x Hemisphere interaction. B) Main effects of Hemisphere for both FA values of the ILF (left side of the graph) and volume values of the AF (right side of the graph). All results were Bonferroni corrected ($p < .005$). Abbreviations: IFOF = Inferior Frontal Occipital Fasciculus; UF = Uncinate Fasciculus; ILF = Inferior Longitudinal Fasciculus; AF = Arcuate Fasciculus; L = Left; R = Right.

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