# The structural and functional organization of the language network in non- or minimally verbal autism spectrum disorders

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# Table of contents

## Abstract

Language impairment forms a broader spectrum in humans than current diagnostic labels such as specific language impairment suggest. This thesis is about language capacities at the neglected bottom end of severity across this spectrum, with a focus on non- or minimally verbal children and adults with autism (nvASD). Despite fundamental challenges that this population poses in terms of investigating affected individuals, language impairment at this level also provides opportunities for illuminating aspects of the neural basis of language through their absence. Currently, insights from fMRI are unavailable. In this context, this thesis sets out firstly to reconceptualize the landscape of language impairment with a view to integrating language impairment of this kind. It then provides the first neuropsychological profiling of this population from a lifetime perspective. The remainder of the thesis is devoted to a first exploration using fMRI of the intrinsic functional connectivity (FC) of whole brain and language networks in nvASD, and of responses to auditory verbal stimuli, mapped against resting state auditory components, in order to probe deeper into the functionality of language regions. Structural connectivity and microstructure of language tracts was also assessed. The final chapter expands the view to a comparative case study of language capacities at a similarly severe end, yet in the absence of ASD. Overall, results of the neuropsychological profiling revealed an apparent glass ceiling of language capacities in this population at any age, with generally correlated levels of productive and receptive language, despite some variation in nonverbal IQ. In FC, despite surprising maintenance of intrinsic coupling of language regions as reflective of very early language endowment in humans generally, a crucial difference emerged within the hierarchy of auditory language processing between the superior and middle temporal gyri. In stimulus-driven auditory processing, I found a generally manifest left-lateralized language network at a group level, overlapping to different degrees with an auditory network as revealed by independent component analysis. The structural connectivity analysis revealed anomalous right-lateralization of the arcuate fasciculus, expanding previous volumetric findings at a microstructural level. Unlike in nvASD, the non-ASD comparative cases were revealing by showing more profound across both anatomical and functional organization of the brain, which were meaningful in relation to behavioural measures. Overall, these new results expand our vision of language impairment in nvASD and beyond, revealing that, to a large extent, and despite the uniform absence of phrase speech, it is not the existence of the neural infrastructure of language itself, but its functional tuning, which is aberrant in this

population. This raises essential questions about this neural infrastructure, as well as informing the need to investigate conditions of minimal language at other levels.

## Resumen

Los trastornos del lenguaje forman un espectro más amplio en los seres humanos de lo que sugieren las categorías de diagnóstico actuales como la del trastorno específico del lenguaje. Esta tesis explora la capacidad del lenguaje en el extremo inferior de la gravedad en este espectro, frecuentemente marginado en la investigación, con un enfoque particular en niños y adultos con autismo no o mínimamente verbal (nvASD). A pesar de los desafíos fundamentales que plantea esta población en términos de investigación de los individuos gravemente afectados, el trastorno del lenguaje de este grado también brinda oportunidades para iluminar aspectos de la base neural del lenguaje, concretamente, a través de su ausencia. Actualmente, existe un abismo en la investigación respecto a la exploración por resonancia magnética funcional (fMRI) de esta población. Enmarcada en este contexto, esta tesis se propone como objetivo, en primer lugar, re-conceptualizar el panorama de los trastornos del lenguaje con el fin de integrar el trastorno del lenguaje de este tipo. A continuación, se esboza el perfil neuropsicológico de esta población por primera vez desde una perspectiva que recorre tanto la edad infantil como la adulta. El resto de la tesis está dedicado a la exploración por fMRI de nvASD y, en concreto, 1) de la conectividad funcional intrínseca (FC) de todo el cerebro y de las redes del lenguaje, como también 2) de la activación a los estímulos verbales auditivos, mapeados contra la red auditiva en el estado de reposo; la segunda con el fin de explorar más a fondo la funcionalidad de las regiones lingüísticas. También se examinó la conectividad estructural y la microestructura de las fibras nerviosas relacionadas con el procesamiento del lenguaje. Por último, el capítulo final amplía la perspectiva sobre las bases neurales de trastornos graves del lenguaje incluyendo una serie de casos clínicos a modo de comparación, particularmente, de niños con alteraciones lingüísticas graves sin autismo. A modo general, la exploración neuropsicológica reveló una atenuación significativa del desarrollo del lenguaje en esta población a cualquier edad, con niveles del lenguaje receptivo significativamente estimables por los niveles del lenguaje productivo, sin distinción por la variación en el coeficiente intelectual no verbal. En el análisis de conectividad funcional, a pesar de una general preservación de la interacción intrínseca entre las regiones del lenguaje tal como se encuentra en el desarrollo neural normotípico temprano, se ha podido observar una alteración crucial a lo largo de la jerarquía del procesamiento auditivo del lenguaje, particularmente entre

el giro temporal superior y medio. La exploración de procesamiento auditivo por activación a estímulo verbal ha revelado lateralización de la señal neural hacia el hemisferio izquierdo a nivel de grupo, con diferentes grados de extensión de la actividad a nivel individual. A su vez, el análisis de la conectividad estructural ha indicado un patrón de lateralización atípico del fascículo arqueado hacia el hemisferio derecho; expandiendo los hallazgos volumétricos de estudios previos al nivel de la integridad de las fibras. A diferencia de lo que se ha observado en nvASD, los casos comparativos sin autismo han brindado una perspectiva esclarecedora al mostrar una organización neural atípica más profunda tanto a nivel anatómico como funcional; con sus características relacionadas de manera significativa con el perfil neuropsicológico de cada caso. En general, los resultados de esta tesis brindan una visión más amplia respecto a la alteración de lenguaje en nvASD; más allá revelando que, en gran medida, y a pesar de la ausencia uniforme del habla espontánea en esta población, las bases neurales de este trastorno no prescinden de las características globales de la infraestructura cerebral en sí, sino que estas bases, en vez de ausentes, más bien se muestran alteradas. A partir de estos resultados, surgen unas incógnitas esenciales sobre la infraestructura neuronal en cuestión, que urgen a investigar las bases de trastornos graves de lenguaje a niveles de análisis más profundos.

## Resum

Els trastorns del llenguatge formen un espectre més ampli en els éssers humans del que suggereixen les categories de diagnòstic actuals com ara la del trastorn específic del llenguatge. Aquesta tesi explora la capacitat del llenguatge a l'extrem inferior de la gravetat en aquest espectre, sovint marginat en la investigació, amb un enfocament particular en nens i adults amb autisme no o mínimament verbal (nvASD). Tot i els reptes fonamentals que planteja aquesta població en termes de recerca dels individus greument afectats, el trastorn del llenguatge d'aquest grau també ofereix oportunitats per il·luminar aspectes de la base neural del llenguatge, concretament, a través de la seva absència. Actualment, hi ha un abisme a la investigació respecte a l'exploració per ressonància magnètica funcional (fMRI) d'aquesta població. Emmarcada en aquest context, aquesta tesi es proposa com a objectiu, en primer lloc, reconceptualitzar el panorama dels trastorns del llenguatge per tal d'integrar el trastorn del llenguatge d'aquest tipus. Tot seguit, s'esbossa el perfil neuropsicològic d'aquesta població per primera vegada des d'una perspectiva que recorre tant l'edat infantil com l'adult. La resta de la tesi està dedicada a l'exploració per fMRI de nvASD i, en concret, 1) de la connectivitat funcional intrínseca (FC) de tot el cervell i de les xarxes del llenguatge, com també 2) de

l'activació als estímuls verbals auditius, mapejats contra la xarxa auditiva a l'estat de repòs; la segona per explorar més a fons la funcionalitat de les regions lingüístiques. També es va examinar la connectivitat estructural i la microestructura de les fibres nervioses relacionades amb el processament del llenguatge. Finalment, el capítol final amplia la perspectiva sobre les bases neurals de trastorns greus del llenguatge incloent-hi una sèrie de casos clínics a manera de comparació, particularment, de nens amb alteracions lingüístiques greus sense autisme. A manera general, l'exploració neuropsicològica va revelar una atenuació significativa del desenvolupament del llenguatge en aquesta població a qualsevol edat, amb nivells del llenguatge receptiu significativament estimables pels nivells del llenguatge productiu, sense distinció per la variació del coeficient intel·lectual no verbal. En l'anàlisi de connectivitat funcional, malgrat una preservació general de la interacció intrínseca entre les regions del llenguatge tal com es troba en el desenvolupament neural normotípic primerenc, s'ha pogut observar una alteració crucial al llarg de la jerarquia del processament auditiu del llenguatge, particularment entre el gir temporal superior i mig. L'exploració de processament auditiu per activació a estímul verbal ha revelat la lateralització del senyal neural cap a l'hemisferi esquerre a nivell de grup, amb diferents graus d'extensió de l'activitat a nivell individual. Alhora, l'anàlisi de la connectivitat estructural ha indicat un patró de lateralització atípic del fascicle arquejat cap a l'hemisferi dret; expandint les troballes volumètriques d'estudis previs al nivell de la integritat de les fibres. A diferència del que s'ha observat a nvASD, els casos comparatius sense autisme han brindat una perspectiva aclaridora en mostrar una organització neural atípica més profunda tant a nivell anatòmic com funcional; amb les característiques relacionades de manera significativa amb el perfil neuropsicològic de cada cas. En general, els resultats d'aquesta tesi ofereixen una visió més àmplia respecte a l'alteració de llenguatge a nvASD; més enllà revelant que, en gran mesura, malgrat l'absència uniforme de la parla espontània en aquesta població, les bases neurals d'aquest trastorn no prescindeixen de les característiques globals de la infraestructura cerebral en si, sinó que aquestes bases, en comptes d'absents , més aviat es mostren alterades. A partir d'aquests resultats, sorgeixen unes incògnites essencials sobre la infraestructura neuronal en qüestió, que urgeixen a investigar les bases de trastorns greus de llenguatge a nivells més profunds d'anàlisi.

## 0. Preface

At birth, human infants have already embarked on what normally is a seemingly effortless route through the developmental language cascade. Roughly 7% of children, however, stumble on their way to language, despite of no other apparent concomitant developmental or physical difficulties, showing impairments in vocabulary, sentence structure, discourse, and speech (Tomblin et al., 1997). Decades of research on language have been centred on these 'specific' language disorders, aiming to study language in its own terms, unspoiled of 'noise' from other cognitive domains. Although important progress has been achieved, this spotlight paradoxically dims our understanding of the human language ability in its global cognitive dimension and breakdown. Thus, the most severe cases of language impairment can be found outside of this spotlight, and, at the same time, inside a broader context of collapsing human cognition. This PhD thesis revolves around such a population, where arguably even the basics of language acquisition milestones have not been met, namely, children and adults with autism spectrum disorders (ASD) that have no or only minimal expressive language, limited to single words ('nonverbal ASD', nvASD). Such a severe language profile paves the way to two fundamental questions about the language faculty: Firstly, what can go wrong in the language acquisition and how deep can an impairment in language run? And secondly, under what cognitive conditions does a severe language impairment occur? These two overarching questions guided my research itinerary during the last four years and inspired the subsequent delineation of workable research questions.

As I was pursuing this thesis, my efforts concentrated on determining the (neuro-)linguistic circumstances of the failure to develop phrase speech, and on probing whether human-specific conceptual knowledge can arise in and despite an absence of language. To address these questions practically, I zoomed in on five central points that became fully developed research studies: (1) An appropriate place to look for conceptual knowledge amid minimal language are gestures. I thus explored the conservation of the form (e.g., iconicity) and function (e.g., declarative communication) of gestures in absence of phrase speech. (2) Secondly I analysed vocal production in nvASD in an effort to determine whether these vocalizations abide by the basic acoustic-phonetic features observable in neurotypical infant's transition from early vocalizations through babbling to first words. (3) I designed an eye-tracker experiment to assess the capacity of nvASD

individuals to form categorical representations and consolidate them in short-term memory, starting from basic-level perceptual categories. (4) I further explored whether a discrepancy exists between language production and comprehension abilities in nvASD with a particular focus on a lifetime perspective. Finally, (5) I investigated the neural circumstances of severe language dysfunction examined through multimodal magnetic resonance imaging (MRI).

External factors, especially the ongoing sanitary crisis due to Covid-19 pandemic, halted major parts of my research plan, literally on the verge of being implemented. This led me to re-focus my thesis on the last two studies – the pervasiveness of the language deficit in nvASD and its neural basis. The analysis of the neural underpinning then expanded to encompass structural and functional aspects of brain organization and function. To provide depth to this thesis, I also put forward a comprehensive and critical view of the current research landscape in developmental language disorders, to contextualize severe language dysfunction within its proper clinical, cognitive, and conceptual space. The anchor of this thesis was placed on the nvASD population, although I also obtained the possibility to explore a single case series of diverse pervasive language disorders linked to rare biomedical conditions beyond ASD (see Chapter 7).

The narrative of this thesis goes as follows. In Chapter 1, I situate severe language dysfunction in a broader landscape of language development and breakdown as seen from a conceptual and evidence-based point of view. The second chapter proceeds to expose the current state of knowledge and research perspective regarding the nvASD population and transitions into a lifespan-focused assessment of the neuropsychological profile of nvASD in Chapter 3. This is the first existing study to offer an insight, albeit cross-sectionally, into this population's long-term outcomes, which directly bear on the nature of their language impairment. This initial triad of chapters constitutes a conceptual and evidence-based backbone which we will see specifically motivates the examination of the kind of neural underpinnings targeted in this thesis. The fourth chapter opens up to this aim, pioneering a functional magnetic resonance imaging (fMRI) exploration of this population. The focus of this chapter is placed on gross functional organization of the language network as assessed via a functional connectivity (FC) analysis. Chapter 5 further elaborates on language functioning in nvASD by examining their low-level auditory processing in response to language stimuli. Chapter 6 is dedicated to assessing the integrity of the structural connectome underlying the core language regions. The final research study, in Chapter 7, ventures away from ASD into a broader realm of rare (genetic) diseases with severe manifestations of

language difficulties. This last study reproduces the assessment of neural circuity as done in the previous chapters for nvASD, this time in a series of cases of severely affected but non-autistic children. The final discussion goes through what has been learned in this thesis and proposes principal paths for the field to embark on.

Most of the research in this thesis has been sustained by work in collaboration. While the theoretical chapters are a result of my sole contribution, each empirical chapter counted with the guidance of several researchers. I was nevertheless at the forefront of these studies, leading the conceptualization, analysis and writing of these works, yet slightly less so with respect to the analysis in chapter six that explores the microstructure of the structural language connectome. Of all chapters, Chapter 3 has been published as Slušná et al. (2021), and Chapter 7 is a continuation of another published paper of which I am a second author (Linke et al., 2021).

# 1. The landscape of neurodevelopmental language disorders

The drive to acquire language runs deep. Yet, it is also fragile. It is language that is pervasively affected in neurological disorders, even beyond those that are said to be 'specific' to language (Hinzen, 2017). This chapter offers a research landscape that accommodates current conceptualizations of language disorders in order to contextualize severe the language-impaired phenotypes studied in this thesis. I begin by calling into question the idea of an emergence of language viewed as detached from early conceptual development. Sections 1.2 and 1.3 further pursue this line of thought by inciting the field to open to new models of language dysfunction that breach a priori archetypes of the language faculty. I finish by introducing the scientific value of severe-language impaired phenotypes, thereby motivating this thesis as a whole.

## 1.1 Rethinking the 'pre-linguistic' stage

The end of the first year of a human life marks an incontrovertible sign of an acquired language capacity: infants begin to produce their first words. It is through their first words that we can catch a glimpse of the construction of meaningful semantic and referential representations of the outside world, a cornerstone of language. Decades of research, however, have also tried to reach an understanding of the infant mind *before* it is reflected back onto the world in this way. For many, this reflection, as and when it happens came to signify the arrival of language itself, while the period that leads to this point was termed 'pre-linguistic', or in other words, representing the mind 'before language'.

The neural template enabling the processing of language, however, is far from absent in the early postnatal months. The emergence of white matter pathways including all dorsal and ventral language tracts, which constitute the skeleton of the language network, starts early in fetal development (Horgos et al., 2020; Mitter et al., 2015), guided by genetically specified molecular clues. The structural fine-tuning of these connections and their functional consolidation is then preferentially led by firing of its neurons, firstly generated endogenously and later via sensory-driven activity during the last prenatal weeks (Pan & Monje, 2020; Penn & Shartz, 1999; Yamamoto & Lo, 2012). By the time of the first postnatal weeks, the adult-like asymmetry of the arcuate fasciculus can already be appreciated (Dubois et al., 2016). The early neural activity in the

fetal brain parcels itself into correlated assemblies (Jakab et al., 2014; Thomason et al., 2014), which by the time of birth topologically resemble canonical resting-state networks in adults (Cao, Huang, et al., 2017; Gao et al., 2015). The configuration of the primary auditory network completes its maturation process by the time of birth, but higher-order networks continue to develop postnatally in response to predominantly sensory-driven activity (Cao et al., 2017; Smyser et al., 2010). Despite this, starting from the 31st gestational week, the intrinsic language network already encompasses the future Broca's and Wernicke's areas in a highly functionally integrated module, which exhibits leftward lateralization (Thomason et al., 2014). However, these early network assemblies are not mature functional circuits. They rather represent an underlying structural and functional connectivity template that primes the formation of circuitry-led transfer of information. By three months after birth, the connectivity pattern of the language network is similar to the adult one in strength and hierarchical structure (Cusack et al., 2018).

Clearly, then, much of the infrastructure of the neural substrate of language at birth comes into being without prolonged speech processing or rich linguistic experience, but rather as a result of a interaction of genetic and activity-dependent processes starting early during prenatal development (Blankenship & Feller, 2010). The established neural template sets the ground to accommodate the linguistic information from the external world. Speech-specific information is integrated into the neural template circuitry from the first days of life (May et al., 2018; Peña et al., 2003; Sato et al., 2012; Vannasing et al., 2016). Speech elicits a differential activation pattern to other complex sounds: it is predominantly processed in the left hemisphere (May et al., 2018; Peña et al., 2003; Sato et al., 2012; Vannasing et al., 2016) along an adult-like fronto-temporal hierarchical cascade, beginning at 3 months (Dehaene-Lambertz et al., 2006a). The right hemisphere, in turn, shows specialization for prosodic features of speech (Homae et al., 2006). Clearly, the generative mechanisms that give rise to a functioning language circuitry change dramatically during development, a process that spans several years and is further reflected onto the neural substrate (Edde et al., 2021; Li et al., 2014; Su et al., 2018). At the same time, this substrate appears strikingly adapted for early processing of linguistic information.

How is linguistic information processed in this circuity? Traditionally (see Kuhl, 2004, for review), the formation of speech representations has been considered as a sensory-grounded process at first, leading to full-fledged language-like, abstract representations from 12 months on. This perspective has its roots in in the early 20th century, when the scientific community began to

conceptualize the first year of infant life as the pre-linguistic period as mentioned above, based on early observations of infant vocalizations regarded as lacking any intonation, modulation or timbre, i.e., linguistic quality (O'Shea, 1907). Meanwhile, it has become clear that neonates are not only sensitive to intonation patterns of their ambient language, but reproduce them in their own non-reflexive vocalizations (Mampe et al., 2009; Wermke et al., 2016). The notion of a pre-linguistic period has, nevertheless, lingered behind and fuelled sustained research efforts to disentangle such a sensory, 'bottom-up' route to speech representations.

By now the endeavour of tracing this route has accumulated robust evidence of sophisticated perceptual abilities in neonates, aided alongside by computational and pattern-detection skills. These abilities are assumed to guide the infants' discovery of native language phonology, which in turn scaffolds their speech representations. Such early skills appear specifically tuned to language: newborns prefer speech to non-speech sounds, and their native-language speech pattern to non-native language, and they can differentiate phonetic contrasts. Throughout this period, speech segmentation skills continue to develop exponentially while gradually tuning exclusively into infant's native language (see Dehaene-Lambertz & Spelke, 2015; Kuhl, 2004, for review). Grounding in these pre-trained models of phonology-driven perception and articulation, infants become skilled in segmenting the speech input into the phonological word forms and subsequently link these word forms to semantic representations – the founding stone of language, mirrored in their first words (Skeide & Friederici, 2016).

While this 'bottom-up route' approach has fossilized the term 'pre-linguistic' in the scientific literature until today, the earlier foundational arguments of the 1900s for a so-called pre-linguistic period no longer hold. An emerging line of research has begun to challenge the established developmental cascade and shaken our understanding of the emergence of speech representations and language-like processing. Thus, contrary to what would be expected on the bottom-up account of language acquisition, infants learn word forms simultaneously to individual speech sounds (Feldman et al., 2013). This finding curves the posited straight bottom-up route to word learning into a looped system where it is the initial proto-lexicon of word forms that constrains and guides the infant's attainment of individual phonetic categories in a top-down fashion (Bortfeld et al., 2005; Obrig et al., 2017; Swingley, 2021; Swingley & Alarcon, 2018). Crucially, moreover, this proto-lexicon appears not to be solely a catalogue of word forms void of meaning. Evidence shows that infants are specifically sensitive to words as guiding their formation

of object categories from three months (Friedrich & Friederici, 2017; for review, see Perszyk & Waxman, 2017). The symbolic nature of these early speech representations has been classically questioned in favour of an account based on associative learning. Yet, recent evidence suggests that 5 month-old infants readily use words to form abstract symbolic representations of a complex percept (Kabdebon & Dehaene-Lambertz, 2019). Midway through their supposed pre-linguistic period, 6-month old infants already know meanings of several common words (Bergelson & Swingley, 2012; Tincoff & Jusczyk, 1999), organize their lexicon via semantic relations (Bergelson & Aslin, 2017), consolidate novel words in lexical-semantic long-term memory (Friedrich & Friederici, 2017), and grasp the referential-communicative function of unfamiliar speech (Vouloumanos et al., 2014).

Taken together, neither the neural substrate nor early linguistic processing appear dissimilar in nature to their adult counterpart, but rather parallel. Importantly, these findings do not preclude the role of nurture in linguistic attainment, nor further development and maturation, which spans several decades after birth. They merely point to the sophistication of infant processing as arguably amply linguistic, which questions the assumption of a pre-linguistic stage. Once such a perspective on language development is taken, profound ramifications follow for the conceptualization of early cognitive development and their mutual interplay. This includes the ontogeny of concepts and words, where instead of characterizing words as 'labels' to be attached to preceding concepts (e.g., Fodor, 1975; Perszyk & Waxman, 2017), words may be seen as inherently identifying human concepts. Taking early linguistic endowment into consideration also becomes practically important for understanding possible aetiological factors leading a child not to develop language, and for designing interventions rooted in putative precursors to language – such as gestures – when primary language milestones have not been met. Rather than as evolutionary and developmental *precursors* to language, gestures could be explored as an aspect of language development itself. Further still, questioning the temporal detachment of early cognition from language leaves us pondering the very existence of 'pure' impairments in either 'language' (e.g., specific language impairment) or early 'cognition' (e.g., autism spectrum disorders). In the following two subsections I will explore the evidence for such 'pure' and 'secondary' language impairments, which speaks to the status of language within cognition in the current conceptual space.

## 1.2 Language impairment arising with preserved cognition

Language acquisition demands a time-restricted alignment between the maturation of the neural substrate and the environment that the human mind is embedded in, even from before birth (Gervain, 2018). After the general structural features of the brain are established and the sensory canals are open, at the end of the second gestational trimester (Borsani et al., 2019), environmental factors hijack the late prenatal and early postnatal brain maturation mechanisms. These progress at different rates across the cortex (Vasung et al., 2019) opening differently timed vulnerability windows (Martín, 2016). Physical defects that either sever the neurological structures or deprive infants of early speech input and output impose a clear threat to this carefully orchestrated process. Thus, congenital or acquired brain injuries are associated with a range of language deficits that hamper grammatical, speech or lexical-semantic functioning (Dennis, 2010). Congenital hearing loss or orofacial clefts represent a barrier between the environment and the mind that introduces delays in acquisition of language milestones, which, nevertheless, gradually subside with age to the extent they are dealt with (Caselli et al., 2021; Lancaster et al., 2020; Petitto & Marentette, 1991).

However, the prevalence of language delay in the general population does not consist only in 0.14 to 0.29 % of live births suffering from these primarily physiological deficits (Tanaka et al., 2012; Tang et al., 2006). More often – between 2.3% and 29% of the general population – failure to meet language milestones on time arises in the absence of any sensory or frank neurological insult (Mondal et al., 2016; Silva, 1980; Silva et al., 1983). Unlike in the case of manifest aetiologies, the first two years in these cases often pass without alarm, but by the second birthday, a child that has acquired less than 50 words and no two-word combinations receives a referral for an early language delay. From that point on, the prognosis of early language delay is highly variable. Roughly half of the children grow out of these difficulties and catch up with their peers by school age, yet the remaining half continues on a protracted developmental path at and beyond 5 years of age (Matte-Landry et al., 2020; Petinou & Spanoudis, 2014). Clinically, such a protracted path is operationalized with a cut-off that generally corresponds to language skills below the 15$^{th}$ percentile for the child's chronological age (Matte-Landry et al., 2020). Beyond standardized tests, however, even recovered children frequently show subclinical difficulties with complex language skills (Manhardt & Rescorla, 2002; Rice et al., 2004) and related cognitive abilities (Aro et al., 2014).

By elementary school, those children that continue to fall behind on their way to language receive a diagnosis of developmental language disorder (DLD; Bishop et al., 2017; Capone Singleton, 2018). Two considerations motivate this terminology. Firstly, the language difficulties observed in DLD are regarded as of unknown origin, often repercussing in a discrepancy with non-verbal ability (ICD-11; World Health Organisation, 2019). A reference to 'unknown origin' is deeply rooted in the medical history since the 1800s, when physicians had to examine children with language difficulties but no apparent severe condition – such as intellectual disability, hearing impairment, or neural damage – which might give rise to the observed difficulties (Leonard, 2020). In need of clearer diagnostic boundaries, the language disorder was operationalized in the early 1980s in contrastive terms, with nonverbal ability set to surpass a threshold of nonverbal IQ (NVIQ) > 85 (Stark & Tallal, 1981). The term 'specific language impairment' (SLI) started to be applied in the literature and caught the attention of linguists in search of selective mechanisms to inform linguistic theories. One of the most prominent proposals on the nature of language was put forward by Pinker (1999) arguing for the autonomy of the language system rooted in a double dissociations seen in SLI and Williams syndrome.

However, decades of research have shown that conceptualizing language difficulties of unknown origin behind the closed doors of preserved cognition is hardly tenable, indicating a more complex picture in the interplay of language and cognition. A study by Plante & Vance (1994) showed early on that many children with unexplained language difficulties do not surpass below-average NVIQ level. The NVIQ level was also found to be unstable over time with children fluctuating between specific to non-specific language impairment (Tomblin et al., 2003). Even in children with 'specific' language impairment, research beyond NVIQ tests revealed deficits in motor skills (Hill, 2001; Sanjeevan & Mainela-Arnold, 2019), mental imaginary (Kamhi, 1981), executive functioning (Kapa et al., 2015; Leonard et al., 2007) or processing speed (Leonard et al., 2007). In line with these findings, Tomblin et al. (2004) did not find substantial language differences between children on either side of the NVIQ cut-off. Gradually, strict conceptualizations of pure language learning disability have come to be discarded in favour of a somewhat broader developmental perspective, which allows for difficulties in nonverbal cognition, insofar as they do not warrant a diagnostic label; hence the term 'developmental language disorder' in use today (Bishop et al., 2017; Leonard, 2020). The definition of DLD,

however, continues to be an exclusionary one, with language as a primary domain of concern in an idiopathic sense (Schwartz, 2017).

Moving beyond the specificity of the language difficulties in DLD, there is a second consideration regarding terminology, which concerns the term ‘disorder’. It reflects a significant impact that the language difficulties impose on everyday social interactions and academic progress. The language deficit is clinically operationalized via standardized language scores falling 1 to 2 standard deviations below the population average, but this leaves room for quite heterogenous manifestations and levels of severity. Attempts to delimit subgroups of difficulties in DLD did not produce consistent results (for review see Leonard, 2014), yet a recent multinational and multidisciplinary consensus of problems in language development (CATALISE) has proposed to pinpoint principal areas for clinical intervention and research recruitment within several language domains– from phonology through word learning, syntax, to discourse (Bishop et al., 2017). In particular, children with DLD may struggle to distinguish and substitute phonemes (Aguilar-Mediavilla et al., 2002), exhibit an elevated frequency of errors compared to typically developing children in inflectional morphology (Marchman et al., 1999), show word-finding difficulties and shallow semantic knowledge (McGregor, 2009; Messer & Dockrell, 2006), have difficulty disambiguating prosodic cues (Marshall et al., 2009), or fail to grasp the overall meaning of a narrative (Karasinski & Weismer, 2010). Salient obstacles in DLD arise especially in the morphosyntactic domain, and while some children receive particularly low scores equivalent to half their chronological age (Lely, 2005; van der Lely et al., 1998), they do not appear to represent a qualitatively different DLD subtype (Bishop et al., 2000). Isolated production deficits tend to resolve overtime, but concomitant receptive language difficulties (Beitchman et al., 1996; A. Clark et al., 2007) and low NVIQ (Rice & Hoffman, 2015) set a child on a poor overall prognosis. When receptive language is vastly affected, productive language follows its lead at a comparable level (Clark et al., 2007).

In short, with the coming of a new century, attempts to model the language faculty in its own terms, with ‘cognition’ as a separate and confounding domain, have proven to be complicated, both from the perspective of prelinguistic development or selective impairments to language. With the rising understanding of the brain as an integrated system of functional networks (Sporns, 2013), both DLD and infant development were readily acknowledged as non-specific and complex. Yet, the reminiscence of this methodological approach lingers in research to this day, as the clear-cut

dichotomy of impaired/preserved or in-place/not-yet-in-place is attenuated via the modifier ‘not entirely’ (e.g., Frausel et al., 2020, p.2). Despite this, the abandonment of clear-cut boundaries opens the field to new models of language dysfunction in the non-specific sense (Bishop et al., 2017), which raises the question of whether DLD provides the full picture of the depth and nature of a language breakdown.

## 2.3 Broadening the spectrum of language disorders: language impairments of ‘explained’ origin

Language dysfunction forms part of numerous language-non-specific neurodevelopmental conditions, where it often takes severe manifestations. Dyslexia, speech sound disorders, coordination disorder or attention-deficit-hyperactivity disorder (ADHD) frequently appear alongside language dysfunction. Substantial effort has been devoted to elucidating the causal relation of these conditions to the language difficulties in question, but the evidence remains unclear (Leonard, 2014). This has led to their conceptualization as ‘co-occurring’ with a yet again ‘unexplained’ DLD (Bishop et al., 2017), but the mere fact of frequent co-occurrence has undermined the biological construct validity of these behaviourally-based disorder categories (Reiss, 2009).

When language problems arise as a part of such a more complex pattern of impairments and have the potential to be qualitatively different from DLD, they are said to be linked to a certain ‘differentiating condition’ (Bishop et al., 2016; Bishop et al., 2017). The complexity of the overall pattern of language problems may in this case stem from a concrete genetic aetiology (e.g., the trisomy of the chromosome 21 in Down syndrome), and may influence the prognosis and intervention approach, as further cognitive difficulties must be considered. Recently (Bishop et al., 2017), these forms of language dysfunction have evolved into being considered as ‘language disorders’ as well; a paradigm shift that has started to reverberate in research in this domain. Thus, a 2017 review of the language genome by Deriziotis & Fisher (2017) incorporates intellectual disability (ID) and ASD among the reviewed disorders, which an earlier review by Graham & Fisher (2013) still lacks. However, this broadening of the spectrum of language disorders has not been fully adopted yet, specifically in the field of linguistics, and a systematic overview of language disorders in their full scope is lacking. This stems from the fact that language dysfunction in this context is oftentimes still viewed as a secondary consequence of a broader cognitive

dysfunction that ‘explains’ it, an association interpreted as causal (Diagnostic and Statistical Manual of Mental Disorder, DSM-5; International Classification of Diseases, ICD-11).

This dichotomy – with neurodevelopmental deficits defined according to a causal chain of apparently clear-cut ‘primarily’ or ‘secondarily’ arising deficits – is a behavioural and not biological construct. As such it is vulnerable to how are cognitive domains a priori conceptualized and where the dividing lines are drawn. An explicit dichotomy is specifically maintained between the domain of language as opposed to that of cognition, as language is generally a priori assumed to be either a mere expression device (Fodor, 1975) or else a cognitive ‘booster’ (Perszyk & Waxman, 2017): two current widespread perspectives on the language faculty, which maintain it at the periphery of cognition. This is coherent with the fact that DLD is considered less severe and pervasive than, for example, ID or ASD, and that language difficulties associated to a biomedical condition are seen as deriving from an overarching difficulty in a broader cognitive domain that bears on the language ability as its means of expression. However, in recent years, research has started to examine language as central to neurocognitive disorders (Hinzen, 2017), specifically when looking across genetic, neurological, neurocognitive, behavioural or environmental levels of description. Arguably, together these levels of description question the contribution of language to the neurodevelopmental difficulties as peripheral or secondary.

Orofacial clefts, hearing loss or brain injuries are examples of primary physical deficit triggering secondary language difficulties. Yet, even with a ‘clear’ physical cause, the exact cascade of primary and secondary deficits remains difficult to untangle since both cognitive development and brain functioning themselves involve highly hierarchical and parallel maturation and information processing (e.g., Mioni et al., 2014). Similarly, Down syndrome (DS) is the major differentiating condition linked to ID, which in turn is generally conceptualized as a primary factor leading to the observed language difficulties. Yet, beyond the behavioural definition of ID, at the biological neural level the first noticeable early brain abnormalities in DS transpire in delayed postnatal myelination particularly of language-related fronto-temporal association tracts, in brains otherwise indistinguishable from normal development (Nadel, 2003). Children with DS also show particular deficits in expressive vocabulary, grammar and verbal short-term memory, relative to normal development at equivalent nonverbal mental ages (Næss et al., 2011), although few insights into more fine-grained qualitative aspects of these deficits exist. In the case of ASD, with the incorporation of the Asperger syndrome on the autism spectrum as an instantiation of a ‘pure’

language-non-impaired form of autism, the definition of the disorder left out language impairment from its diagnostic criteria in favour of broader social 'communication deficits' (DSM-V). And yet, just like there appears to be a non-language-impaired part of the spectrum (yet see (Boucher, 2012), there are also profoundly language-impaired individuals on the spectrum, constituting no less than almost a third of ASD (Norrelgen et al., 2015). The above clinical definition of ASD, thus, opened a breach along the autism spectrum: can an overreaching difficulty in social communication really lead to such a severe language profile as seen in non- or minimally verbal autism?

Severely language-impaired phenotypes will in what follows be understood as *pervasive* language impairments – in the sense that they hardly allow for localization of principal or single area of difficulties in the traditional linguistic domains, i.e., phonology, syntax, semantics, pragmatics, and discourse. Such phenotypes are in dissonance with the conceptualization of penetrant language difficulties as resulting from a single overreaching differentiating condition (as is the case of social cognition deficits in nvASD common to the entire autism spectrum), since they point to fundamental biological principles gone awry in this domain of language itself (Lee et al., 2020). It is specifically the profoundly impaired cases that should guide the research on where the dividing lines should be drawn when conceptualizing diagnostic and cognitive domains. Paradoxically, so far it was the model of DLD, with milder linguistic deficits, that has been taken to exemplify the separation of the domain of language, in its entirety, from cognition.

Clearly, severe phenotypes pose greater difficulties in conducting standardized research that assumes certain levels of functioning. Valuable insights into the underpinnings of language at the neural, genetic, and cognitive levels can be obtained, even if a complex amalgam of deficits is involved. For example, recent papers by Brignell et al. (2021) and Tabet et al. (2017) related the extension of gene deletions to the language profile of children with Phelan-McDermid syndrome (PHMDS; Online Mendelian Inheritance in Man, OMIM 606230), a genetic syndrome characterized by a gene deletion on the 22q13 chromosome resulting in a complex cognitive profile of ID, autism and language disorder. The researchers managed to link a specific locus on the 22q13 region and the deletion extension particularly to absent phrase speech, a phenotypic feature of a subset of the PHMDS spectrum. In a similar vein, Paldino et al. (2016) assessed 37 patients with diverse malformations of cortical development with varying degrees of language impairment. Absent speech was specifically linked to bilateral absence of the arcuate fasciculus, also reported

in children with Angelman syndrome (Wilson et al., 2011a), a rare genetic syndrome involving ID, motor problems and no speech (OMIM 601623).While contrasting views exist on the clinical research value of a rare event leading to a disease, rare diseases with severe phenotypes specifically have been recognized to offer invaluable insights into core biological mechanisms. Thus, mechanisms that fail to come in place are a reference point leading to a system that would otherwise be too complex to untangle (Lee et al., 2020).

In summary, non-specific sorts of language dysfunction are gradually more recognized and incorporated into language models, although they are inwrought with conceptual difficulties that relate to viewing them as secondary deficits embodying a broader cognitive impairment. As we open up the range of language disorders, venturing further into this non-specific territory, we often encounter severe manifestations of language difficulties, specifically in rare genetic diseases. Such severe phenotypes represent unique entry points to fundamental biological and cognitive principles that would otherwise be difficult to arrive at. Yet the field lacks a comprehensive phenotypic nomenclature, which leads to further disregard of these phenotypes in research. In the following section I will discuss grammar as one fundamental building block of language, which comes to the for when considering language acquisition at the single-word level and the stagnation at this level across many rare genetic diseases.

## 1.4 Severely language-impaired phenotypes as models of foundational principles of language

To elucidate mechanistic factors central to a particular disorder, detailed delineation of its phenotypic outcome is needed. This may be particularly difficult in pervasive language impairment, as standardized cognitive measures assume presence of certain skills such as picture understanding, fine-motor-skills, or understanding of verbal instructions, and alternative methods such as neuroimaging require high levels of patient compliance. Further contributing to the underrepresentation of these populations in research is the low prevalence of rare genetic diseases in particular. Currently, general handbooks that touch on the matter of language and/or communicative disorders (e.g., Damico et al., 2010; Hickok & Small, 2016; Schwartz, 2017) cover major disorder categories and occasionally include some genetic disorders mainly with major prevalence. But largely no mention is made about severe manifestations of language disorders, as in the case of a developmental outcome of no speech attainment, which may go beyond cognitive

profiles typically observed in speech disorders such in dysarthria or aphasia. Thus, in dysarthria resulting from cerebral palsy, a subset of school-aged children may indeed be referred to as ‘minimally verbal’, if they struggle or are unable to produce *comprehensible* speech (Mei et al., 2020). But as will become clear in the following chapters, nvASD children crucially differ from children with dysarthria along several dimensions. Thus, many people with nvASD produce a handful of comprehensible single words, though they use these words sporadically. They can even be fluently echolalic or else produce linguistic vocalizations non-functionally, even when language in its normal function is a mystery to them. Severe neuroanatomical deficiencies, on the other hand, such as brain injuries resulting in extensive tissue damage or loss, do not need to lead to such severe language profiles, thanks to neural coping mechanisms and plasticity. Thus, inborn and acquired focal lesions of the left hemisphere tigger contralateral reorganization of the cerebro-cerebellar network involved in language production (François et al., 2016; Lidzba et al., 2008). Severe phenotypes, such as nvASD, thus offer the opportunity for an approximation to the fundamentals of language, in a way that these other profiles do not.

The nvASD section of the ASD spectrum is specifically defined in terms of language, since they are specifically identified as ‘non- or minimally verbal’. That is, absence of phrase speech in this population provides a dividing line that sets it apart from the rest of the spectrum (DiStefano & Kasari, 2016). Phrase speech signals emergence of grammar, which, in neurotypical development, is the point in development where a spurt of language acquisition is unleashed (Bloom, 1976). Before that point, communication by means of single words reaches its factual limit of roughly 50 words (Carey, 1978; Nazzi & Bertoncini, 2003), after which meaning is given a fundamental turn. When meaning as arising at a grammatical level is reached, an unconstrained space of meaning is restrained and bound to the external world as words are being coerced into parts of speech, hierarchically interlinked in the surge of sentences, and bound to objects referentially (Hinzen, 2017). A fundamental principle seems to be operative: for some reason, as words reach a certain quantitative threshold, grammar has to come into play. The same we strikingly see in people whose speech only consists of single words – the repertoire of words they possess *also* does never seem to exceed this threshold. Thus, although some variation in vocabulary exists in nvASD – oscillating on a range from several words, through no words, to no linguistic or reflexive sounds – this does not counterbalance the uniform lack of this fundamental principle in all of nvASD: the absence of grammar.

Pervasive language impairments are not confined to the autism spectrum, but as mentioned above, are frequently encountered in rare genetic diseases. We currently fall short of a systematic exploration of such phenotypes, which would allow for comparison of the level of severity, detection of commonalities, exploration of attainment of early developmental milestones (babbling, declarative gestures, onset of first words, etc.), or basic comprehension skills (understanding of simple/2-step/3-step verbal commands outside of routine actions and contexts). Currently, the human phenotypic ontology (HPO) initiative, used to clinically describe impairments in language acquisition (mainly in genetic diseases), does not permit to address these developmental indicators.

Thus, HPO's language impairment category distinguishes between 'delayed speech and language development' and 'spoken word recognition deficit'. The former ontological category further branches out into more specific 'expressive language delay', 'receptive language delay', and 'absent speech'. Although the this ontology was developed based on databases of (rare) genetic diseases such as OMIM, the catalogue of reference for genetic diseases, or Orphanet, this terminology falls short of marking disease severity in language, apart from the descriptor 'absent speech'. Even this descriptor, however, allows for important variability in the overall language ability, which is rarely reported or formally assessed in pertinent research studies. A search for severe language-impaired phenotypes in OMIM based on the term 'absent speech' already returns a total of 333 entries out of a total of ~6 000 diseases. This list of genetic diseases involving absent speech is quite certainly not exhaustive, as OMIM does not make use of HPO's standardized ontology.

Because of space limitations, Table A1 (accessible online) lists 66 rare genetic diseases involving 'absent speech' retrieved from GARD (Genetic and Rare Information Center), a database of a limited number of rare genetic diseases yet described with HPO criteria. Once again, this is not an exhaustive list of 'absent speech' phenotype, but it offers a literature-based corroboration of this phenotypic profile in individuals beyond 6 years of age, with a particular focus on receptive-expressive language discrepancies. A special regard for this chronological age threshold was taken as varied trajectories of phenotypic progression have been reported before this developmental point (Pickles et al., 2014a). After it, the phenotypes start being more constant. Even in DLD, language problems present at 5 years of age are likely to persist (Stothard et al., 1998) and nvASD is specifically defined with respect to school-age. Reports of all 66 diseases

involved late childhood or adult cases, hence allowing to discard the possibility of speech delay instead of speech absence.

To further elucidate the fundamental contribution of grammar to what language is, I have paid special attention to the description of speech in single words whenever reported in the reviewed literature. It turns out that, comparably to the speech profile in nvASD, single words – if present – were limited to only a handful, among case reports of all the 66 investigated diseases, except one. This study is Nabais Sá et al. (2019), which reported 17 cases with a DEAF1-related mutation, out of which one case with limited speech is said to have had 300 to 400 words, albeit used in simple sentences. It is of theoretical importance why this should be so. If we conceptualize grammar simply as a system of rules to combine units of meaning – with the rules simply being an additional cognitive system extrinsic to the constitution and use of meaning – we should not expect any particular boundary for children that do not acquire this system to communicate via a myriad of content words, even when their phonological repertoire is restricted. Instead, a concrete boundary appears to exist, albeit for now reviewed only non-systematically.

Taken together, language and speech disorders covered in the scientific literature fail to encompass the full constellation of the often severe speech and language difficulties observed in nvASD as well as in many genetic diseases resulting in absent speech. These constitute new and as yet underexplored models of language and speech breakdown, which allow us to tap into fundamental aspects of the language faculty such as grammar, and of cognition more broadly, while raising novel questions about what the relations between these, and ultimately what it means to have language.

## 1.5 Summary

The view of language as a bootstrapping or expressive system at the periphery of cognition is deeply rooted in the science of language. This view has fuelled a research program aimed at dissecting language out of cognition, creating in this way a construct that came to represent – via a circular reasoning – what language is and how it breaks down. In the context of this research panorama, I have tried to bring to the foreground models of language dysfunction at the most severe end of the spectrum of such dysfunction, where the dividing line between language and cognition fades away. I have argued that such severe language deficits open an inroad into the core principles of language and human cognition, which frequently entertained models of speech and

language deficits cannot reach. The foundational principles of language can be uncovered precisely and paradoxically through their absence – that is, by scrutinizing neurodevelopmental disorders where language dysfunction takes severe manifestations.

## 1.6 General aims of this thesis

The thesis to follow engages in a detailed exploration of one particular severe language dysfunction, namely that of non- or minimally verbal autism. The following two Chapters (2 and 3) aim to characterise – via a critical overview of nvASD and a neuropsychological study using a lifetime perspective – this phenotype by exploring the severely constrained linguistic space that lies behind single-world speech. This characterisation will fuel the neural exploration of the language connectome in its structural and functional aspects in Chapters 4 and 5. The theoretical framework of this exploration is the coarse wiring and further activity-dependent refinement of the brain circuity as discussed above. The principal aim of the exploration is to characterize brain development when language fails to arise – through a population whose key unifying feature is the lack of a grammatical organization of a lexicalized space of meaning – with the view of disentangling possible neural contributors to absent language development. Lastly, in Chapter 6, to open a window to more variation at the severe end of language dysfunction, I venture into the broader landscape of severe language disorders beyond ASD, by conducting a case series of principally rare genetic diseases. The specific aims relevant to each of the above studies in chapter three to seven will be specified in those chapters.

# 2. The nature of language deficits in non- or minimally verbal individuals on the autism spectrum

Before diving into specific questions about the neural substrate of nvASD addressed in the main parts of this thesis, the present chapter surveys current knowledge about the linguistic and neuropsychological profile of people with nvASD. This will highlight areas that need more research attention and motivate the studies reported in subsequent chapters. As noted, the definition of non- or minimally verbal individuals on the autism spectrum refers specifically to language. Thus, speech production in nvASD is, by definition, limited to single words (e.g., *more, no, water*), at most simple fixed phrases (e.g., *Want more*), or even no or only non-speech-like vocalizations. However, no or minimal language is a common developmental trajectory in many children with ASD in the *preschool* years. Current estimations suggest that around 70% of these preschoolers go on to develop phrase speech *during* their preschool years (Wodka et al., 2013), hence can be said to be 'pre-verbal', while the remaining 30% show deeper expressive language deficits, remaining non- or minimally verbal at school entry and beyond. So far, we do not know what is driving these diverse outcomes, given that both groups of children have ASD and both have similar exposure to relevant linguistic stimuli, which propel language acquisition in one of these groups but not the other.

Pre-verbal children not only find their way to language, but also continue to develop early cognitive and communication skills such as gestures and joint attention (DiStefano & Kasari, 2016), which are indicative of experience-dependent neural development (Kraus & White-Schwoch, 2015). NvASD children, however, stagnate in these skills. Apart from driving learning, experience-dependent mechanisms also secure the plasticity of the developing brain well into adulthood (Kraus & White-Schwoch, 2015; Marín, 2016; Tierney & Nelson, 2009). Beyond 5 years of age, however, speech acquisition in nvASD continues to be minimal. Thus, a literature review by Pickett et al. (2009) on late acquisition of first words or phrases during school years in nvASD estimates gains only in a minority of 0,1% of nvASD children. The nature of these deeper expressive language deficits seen in nvASD (and low-functioning ASD more broadly) remains underexplored, with calls to action made only recently (Jack & Pelphrey, 2017; Tager-Flusberg & Kasari, 2013).

Directly related to the lack of understanding of expressive language deficits in nvASD is the prevailing unclarity about how exactly language is affected in nvASD. Inferring levels of language capacity in this population has proven challenging, leading to questions about the accuracy of receptive language measures (Plesa Skwerer et al., 2016). The difficulty mainly lies in the severely limited use of alternative and augmentative means of communication (AAC) in nvASD, such as gesture, sign, or writing, and the severe difficulty of affected people to follow verbal, gestural, written or signed instructions. The difficulty to engage in such alternate communication is, however, telling per se. In conditions where absence of speech (along with gesture and writing) results due to a severe fine-motor skill impairment, clear evidence for language capacities can often arise. For example, Gernsbacher (2014) reports a case where a keyboard-adapted communicative system finally revealed normal language comprehension and production through typing, in a child with severe motor disabilities. By contrast to such cases, when pictures are used in nvASD for communication, e.g. in the Picture Exchange Communication System (PECS), care is needed when equating such means to vocabulary or language in general. Although of an enormous practical relevance, PECS as a form of AAC is based on behavioral principles of learning by stimulus and response. Current evidence does not support such principles to play a central role in neurotypical language acquisition (Guasti, 2002), and success in PECS does not seem to translate into gain in language (Flippin et al., 2010).

Illuminating language and cognitive abilities in nvASD is limited due to the difficulty or impossibility of administering many standardized tests in this case. Such tests, however low-level they are, require certain skills (e.g., the understanding of pictures in the case of the Peabody Picture Vocabulary test, PPVT). Nonetheless, Plesa Skwerer et al. (2016) corroborated score consistency in between the PPVT and other non-standardized vocabulary measures such as parent reports, eye-tracker, and touch screen measures, in nvASD. Furthermore, the PPVT vocabulary scores in this study showed similar levels as scores reported in several other studies on children, adolescents and young adults with nvASD (Bal et al., 2020; Chenausky et al., 2019; DiStefano et al., 2016; Garrido et al., 2015; La Valle et al., 2020; Maljaars et al., 2011; Plesa Skwerer et al., 2019). Overall, the standard deviations reported in the studies reviewed above, seen against the background of the chronological ages (CAs) of the samples, show a degree of variance in vocabulary comprehension, but severely restricted at a low level far below participants' CA. Moreover, the variability in receptive or productive vocabulary, however, does not extend to the absence of grammar in this

population, which is uniform. NvASD children matched on receptive vocabulary to typically developing children also show significant deficits in comprehension of grammatical structures (Comprensión de estructuras gramaticales, CEG, *M* = 2,15) and verbal instructions (Token test for children, TTCF-2, *M* = 10,31) (Garrido et al., 2015). In Plesa Skwerer et al. (2016), too, parental responses on the Vineland receptive communication subdomain revealed that 82% of the sample could not follow three-step instructions, although variation was observed in their understanding of one-step instructions and if-then statements. The exact structures that the remaining 18% comprehended were not reported. Overall, these findings, albeit initial, suggest that language comprehension in nvASD is a mirror image of their language production insofar as the former does not appear to exceed the latter to a significant extent.

The functions and underlying meaning of speech production in nvASD offer further clues about the nature of the underlying language deficit, although once again, formal studies have been scarce, particularly due to the minimal repertoires of single words involved and their sporadic rate of production. Word production in nvASD primarily serves an imperative communicative function, i.e. regulating behavior via requesting or refusing, which contrasts sharply to declarative communication predominant in fluent phrase speech users with ASD (La Valle et al., 2020). Declarative reference, a hallmark of language (Tomasello & Call, 2018), signals the use of lexicalized concepts that can be retrieved outside of the here-and-now for purposes of reference and predication (Hinzen & Sheehan, 2015), and is found reflected in the earliest production of words and gestures in infants (Colonnesi et al., 2010). The first words in nvASD do not necessarily appear in the pre-school years, but can appear beyond 5 years of age, after the developmental trajectory of no or minimal language is sealed (Pickett et al., 2009). With such a deflected trajectory of word acquisition, the nature of these late-acquired words might be fundamentally different. In line with this possibility, a study by Preissler (2008) suggests that children with autism and ID tend to learn words in relation to pictures and objects by association, rather than as carrying referential meaning (see also Hartley & Allen, 2013). A recent study of word-object matching using EEG has similarly suggested that there are anomalies in how the meanings of words are processed in nvASD (Cantiani et al., 2016).

Contrasting with this evidence, one of the currently prominent hypotheses suggests that not semantic factors but difficulties in praxis underpin the lack of phrase speech in nvASD (Chenausky et al., 2018). At least a subset of minimally verbal ASD children show speech

characteristics compatible with childhood apraxia of speech (CAS) or else a more general motor speech impairment. These characteristics predict the number of different words produced by a child (Chenausky et al., 2019), as would be expected. Chenausky et al. (2019) additionally reported receptive vocabulary as a significant predictor of number of words in a mixed group of non-verbal and low-verbal children without motor speech deficits. This was not the case for the non-verbal only group with speech motor deficits, although their receptive vocabulary and number of different words were at floor level – a consistency between the two measures that in fact may not allow to test for a predictive relationship due to minimal variability. Further research is needed on this matter: the presence of CAS is not unexpected in children that have severely minimal experience with speech production, but conceptualizing CAS as the origin of the lack of phrase speech in nvASD falls short of explaining the cognitive characteristics that transpire through word production in nvASD, the lack of compensation through different language modalities, or their cognitive dissimilarity to minimally verbal children that acquire phrase speech during their school years.

The nature of language deficits in nvASD, therefore, runs deep, and so do levels of ID in this group. Severely low NVIQ is a significant early predictor of pre-school children that eventually will remain non-verbal (Weismer & Kover, 2015). Traditionally, ID was indeed conceptualized as a driving factor of language difficulties in ASD and beyond. However, the very few studies on nvASD and low-verbal ASD that exist (Maljaars et al., 2011; Maljaars et al., 2012a), suggest that such children communicate differently from children with ID but without ASD matched on nonverbal mental age, and have different language profiles. The overall picture further gains in complexity once NVIQ is assessed in nvASD at school-age: thus, a small subset of children in fact shows NVIQ above the cutoff (NVIQ < 70) (Bal et al., 2016). Interestingly, not even in these cases does a higher or normal NVIQ predict gains in verbal mental age (VMA) in nvASD, while slightly more words in production do (Bal et al., 2016; Maljaars et al., 2012). This contrasts with both typical development (TD) and ID, where NVIQ is a strong predictor of concomitant language abilities, thus suggesting a different developmental route in nvASD towards levels of nonverbal cognition, which are nevertheless similar, in terms of their absolute punctuations, in standardized tests.

Indeed, in a subsequent study by Maljaars at al. (2012b), a nvASD and low-verbal ASD population matched on NVIQ to both TD and ID groups, had significantly lower levels of ‘sense-

making', an index of nonverbal cognition measured by the ComFor test; see Noens et al., 2006 and section 3.2.2.5 for details). In this test, sense-making is assessed via perceptual categorization of objects by criteria of progressive difficulty, namely first by perceptible features (e.g., colour, shape), then by primary and functional meaning (e.g., different-looking same kind of objects). Such lower levels of sense-making in nvASD despite commensurate non-verbal IQ could indeed point to a divergent cognitive style, which additionally matches with more severe diagnostic symptomatology in social interaction, communication and imagination domains, as also assessed in Maljaars et al. (2012b).

Taken together, nvASD represents a severe constellation of deficits in language and cognition in general, which can be relatively varied in its manifestation, but only up to the point where it reaches an apparent glass ceiling of absent grammatical understanding, arguably in both productive and receptive language domains. Linking back to the previous chapter, this population thus offers an opportunity to explore this core principle of linguistic endowment via its putative absence from the human neuro-cognitive substrate, which prevails despite optimal exposure of pertinent stimuli during early development. In neurotypical development, sensitivity to sensory experience gives rise to the opening of developmental windows of plasticity, critical for maintenance and further fine-tuning of neural circuits, which assures optimal responses to the external world (Toyoizumi et al., 2013). Experience-dependent shaping of the brain circuits operates on a large time scale from late prenatal development well into adulthood, scaffolding the learning that drives language development (Ghio et al., 2021; Kraus & White-Schwoch, 2015; Marín, 2016; Tierney & Nelson, 2009; Ismail et al., 2017). In nvASD, such learning fails to arise during early childhood and arguably beyond, throughout lifetime, suggesting a disruption or a failure in the onset of a critical window of plasticity (Bragg-Gonzalo et al., 2021).

The failure for grammar to arise in nvASD rises important questions about the extent of variability in the language capacity that can be nevertheless achieved once language development is deprived of its fundamental steppingstone. The following chapter opens up to this question, by exploring variability in language and cognitive abilities of individuals with nvASD. It is the first study to capture variability in nvASD from a lifespan perspective, allowing this variability to potentiate. The remaining chapters of the thesis will build on the considerations reviewed above and direct the research spotlight to early emerging aspects of cortical wiring, upon which experience-dependent mechanisms build when leaving behind their functional fingerprint. In

particular, they will target the structural and functional organization of auditory language circuitry along with its sensitivity to the external speech stimuli.

# 3. Profiling expressive language, receptive language, nonverbal cognition, and their interrelations in nvASD across the lifespan

## 3.1 Introduction

The severity of the nvASD cognitive-behavioural phenotype outlined in the previous chapter has paradoxically kept this population marginalized from ASD-targeted empirical studies, and consequently the conceptualization of ASD as a disorder. Very recently, this neglect has come into the spotlight (Jack & Pelphrey, 2017; Tager-Flusberg & Kasari, 2013). An especially alarming aspect that made this population emerge from the backstage has been the apparently limited malleability of such a profile in response to extensive interventions and learning opportunities during early development (Tager-Flusberg & Kasari, 2013). Thus, Pickett et al. (2009) set out to review evidence of expressive language growth in minimally verbal ASD children at age 5 and beyond. They encountered 167 reports of gains in speech, which, according to their estimates of nvASD prevalence, amounts to roughly 0.1% of the nvASD population. The definitional characteristics of nvASD have come to represent this lack of gains in speech and centred on the persistence of minimal speech beyond five years (Bal et al., 2016). Incipient research efforts, therefore, have also concentrated on elucidating the two core features brought forward by the definition: a) lack of phrase speech attainment, in b) children beyond five years.

The present study aimed at contributing to this by exploring the interrelation of general cognitive and receptive language abilities in nvASD alongside expressive language limited to single words. The key component of this study is a prospective sampling of a child and adult population with nvASD. This not only offers a view of long-term outcomes of the population but in part also challenges the current conceptual space regarding who individuals with nvASD are. In particular, while they are clinically defined by their expressive language deficits, language comprehension in nvASD – though also impaired as noted in the previous chapter – is left out of the definition, based on the reasonable consideration that it could in principle be more variable and independent of production (Kasari et al., 2013). In line with this possibility, it has been asserted that language comprehension varies greatly in nvASD (DiStefano et al., 2016; Plesa Skwerer et al., 2016; Helen Tager-Flusberg & Kasari, 2013), just as it does more generally in autism with

intellectual disability (ASD+ID). At the core of this claim are anecdotal reports that many children with nvASD understand spoken language to a substantial extent (Kasari et al., 2013). But this empirical observation disregards the possible facilitatory effects of context and daily routines for the communication informing such claims. Thus, parental reports compiled in Plesa Skwerer et al. (2016) showed that 82% of the study cohort could not follow three-step instructions, while variation was rather observed in their understanding of one-step instructions and if-then statements.

The general difficulty of assessing this population through standardized tests, as mentioned in the previous chapter, has further contributed to views of receptive language as possibly wide open to variation, despite formal assessments yielding overall very low scores. Few standardized measures have been regarded as suitable for such assessment (Kasari et al., 2013). Following these guidelines, the majority of studies has opted for the Peabody Picture Vocabulary test (PPVT) to assess receptive language in nvASD. While the PPVT directly measures vocabulary comprehension, it computes an estimate of verbal mental age (VMA), which nevertheless practically corresponds to the number of words understood. In Maljaars et al. (2011) and DiStefano et al. (2016), mean VMA as measured in this way corresponded to a mean of 1;6 years (*SD* = 0;7) and 2;7 years (*SD* = 0;8), in groups with CA = 3-11 and CA = 5-8, respectively. Studies reporting PPVT raw scores (Chenausky et al., 2019; Garrido et al., 2015; Plesa Skwerer et al., 2019), attest average scores falling between 5.15 and 49.69 in a mixed group of children and adolescents. Plesa Skwerer et al. (2016), applying both standartized and non-standartized measures, reported VMA falling 1.5 to 4 *SD* below neurotypical population average. Contrasting with the foregoing reports is a study by Rapin et al. (2009) that reported low-average to average comprehension and NVIQ in a subset of its sample (N=4), but they classified them as having severe articulation deficits and did not state directly whether these children were nonverbal.

Despite the above relatively convergent findings, a precise interpretation of the exact spread of receptive language abilities in this population remains currently hampered by several confounds in previous studies, including: (a) different recruitment criteria determining non- or minimally verbal status; (b) sampling children below 6 years of age, thus possibly including some ‘pre-verbal’ children (as characterized in DiStefano & Kasari, 2016); (c) sampling from a broader low-functioning ASD population without distinguishing expressive language levels (present / absent phrase speech), (d) reporting only central tendency statistics (*M* and *SD*) for CA and VMA,

even in cases of non-normal distributions, thus not allowing for the interpretation of their relative spread. I address these limitations here.

Accounts that have aimed to elucidate reasons behind limited speech development in nvASD have typically located the space of possible explanations outside of the language domain (as noted in Saul & Norbury, 2020). Thus, Paul et al. (2013) put forward the theory of speech attunement behind poor expressive language development in ASD. On this account, core ASD features would explain the expressive deficits across the spectrum: ASD individuals fail to 'tune in' to others' communications, while their limited social motivation represents a barrier to practice their own speech output. However, as Pecukonis et al. (2019) have observed, predictors of expressive language vary along the autism spectrum, and expressive language difficulties in nvASD specifically have not been predicted based on social aspects such as joint attention. Other recent accounts have targeted motor skills as an underlying cause of nvASD speech deficits. Pecukonis et al. (2019) found that among several social-communication measures, imitation skills in the manual modality were the only significant predictor of concurrent expressive language in nvASD. Similarly, Chenausky et al. (2019) set out to investigate whether speech-motor deficits comorbid to an ASD diagnosis might result in lack of phrase speech in nvASD. The conclusions drawn by these authors are specifically formulated around motor speech impairment as a predictor of expressive language in minimally verbal ASD, but not low-verbal ASD. In turn, receptive language predicted speech capacity in low-verbal but *not* in minimally verbal ASD. Such a result, indeed, advocates for taking language out of the equation of expressive language deficits in nvASD.

However, in that last study, the low-verbal ASD group in which receptive vocabulary was a predictor of expressive language, was, in fact, a mixture of minimally verbal and low-verbal children without motor speech deficits. Further still, in the minimally-verbal-only group and motor-speech problems, both receptive vocabulary and expressive vocabulary were at floor level, as noted in the previous chapter – a telling consistency between these two measures. This relation might not have turned into a significant statistical prediction due to the minimal variability of those measures, and thus made the authors conclude that no predictive relationship exists. Related evidence from the lower end of ASD further suggests that nvASD's receptive and expressive language capacities might be intertwined. In Hartley et al. (2017), in particular, language production in low and minimally verbal children correlated with their receptive language. More

generally, in a 17-years follow-up of 2-year-olds with ASD (Pickles et al., 2014), expressive and receptive language developed in tandem throughout the entire period even in development groups with very low and markedly delayed language. Assessing the degree of variation in comprehension alongside language production in this population, therefore, remains important to clarify, and was therefore a first primary aim here.

A second key aspect to arrive at the core of expressive and receptive language co-variation in nvASD, are long-term outcomes in this population. According to the above-mentioned study by Pickles and colleagues, the rate of language development beyond 6 years of age in ASD in general enters a steady stage, exhibiting only small variation in increase rate across years and different language outcome groups. In this study, even children with very low language levels at 2 years, progressed in their receptive and expressive language development over the 17-year period, but they did so very slowly, reaching between 2-year-old and 5-year-old language equivalents at 19 years of age. These two age equivalents, however, both correspond to phrase speech in neurotypical development, thus contrasting with the existence of an adult population with nvASD, as well as with the limited estimated growth in speech reported by Pickett et al. (2009).

Lifetime development in nvASD, however, remains an important research gap. Thus, studies of the adult nvASD population are virtually absent and the adult-adapted version of the Autism Diagnostic Observation Schedule (ADOS) has been made available for research only recently (Bal et al., 2020; Bal et al., 2011). Among rare exceptions is a study by Bal et al. (2020), which, nevertheless, assessed a mixed group of adolescents and young adults at a speech level of no or only some words, and reported receptive language (VMA: PPVT) to average to 20.10 months (*SD* = 14.3) and 44.83 months (*SD* = 14.6), respectively. To my knowledge, no study so far has recruited an adult-only group and assessed their language abilities alongside those of children with nvASD, thus addressing the question of later outcomes, comparability of the receptive language profile at different life stages in nvASD, and possibly divergent development of receptive language relative to the expressive one.

A third important concern regarding the variability of the language profile in nvASD is the interaction of receptive and expressive language with non-verbal cognitive abilities. This particularly includes the issue of whether non-verbal cognition can explain language deficits in nvASD, as has been proposed for ID. As noted in the previous chapter, in pre-school children with minimal language, NVIQ is one of the major predictors of later language gains for those children

that acquire some language before age 5 (Thurm et al., 2007, 2015; Weismer & Kover, 2015; Wodka et al., 2013). In turn, concomitant ID, especially at the severe end of the affectation, predisposes the preschool children with ASD to a higher probability of remaining non-verbal at school age. However, a minority of non- or minimally verbal children at school-age shows nonverbal IQ (16% in Bal et al. (2016)) outside of the disability range. Despite this, once meeting criteria for nvASD, that is at and after school age, higher or normal NVIQ in nvASD does not translate into higher concurrent receptive language. Slightly more words in production, however, do. In nvASD, NVIQ thus seems to be uncoupled from their language profile.

To provide further evidence of this effect across the lifespan, I have thus assessed NVIQ in the present cohort of children and adults with nvASD, while additionally examining their broader cognitive abilities in relation to both language and NVIQ measures. This specifically concerned conceptualization skills as measured with the ComFor test, which is specifically adapted to populations with very low verbal profiles, providing an index of 'sense-making' relative to categorization of objects (Maljaars et al., 2012b). As such, it reflects sensory-guided conceptualization abilities that open a first inroad into how cognition develops under conditions of minimal language. While it is common to distinguish concepts from the words that express or encode them lexically, it is an open question which concepts depend on words and how concepts function when they are not supported by language (de Villiers, 2014). Understanding the spread and depth of other cognitive abilities under minimal language conditions is thus theoretically and conceptually revealing, while also bearing practical significance with regard to designing and administering interventions.

In summary, several key desiderata characterize the current state of the art and conceptualization of nvASD: (a) the relationship between expressive and receptive language in nvASD along the lifespan; (b) the variability of language abilities in nvASD as informed by long-term developmental outcomes, i.e. the comparability of child and adult nvASD populations; (c) the role of NVIQ for the variability in the language profile; and (d), more specifically, how both NVIQ and language variation in nvASD bears onto broader cognition, namely the emergence of conceptualization capacities.

### 3.1.1 Present study

I recruited a large population of 49 individuals of all ages beyond the age of six years, to address the following specific research questions:

1. What is the overall variability with regards to receptive language, NVIQ, and sense-making scores in an ASD population with no phrase speech, seen across the lifespan and through the lens of significant differences between youngster and adult groups?
2. Do receptive language abilities co-vary with and predict expressive language levels regardless of age?
3. What association is there between NVIQ and both expressive and receptive language measures?
4. Which cognitive factors predict sense-making capacities in nvASD (ComFor)?

Answers to these questions would significantly inform current theories and therapies of nvASD and autism at large, as well as expectations for lifelong development. Predictive relations between receptive and expressive language, in particular, would indicate a global language deficit not reducible to a speech-motor problem The variability in NVIQ together with an absence of relations between NVIQ and language would point to its relative independence from linguistic cognition, impairments in which cannot be compensated for by non-linguistic cognition (Hinzen et al., 2019). Based on both previous studies and clinical experience, we predicted both a correlation between receptive and expressive language and a failure of NVIQ to predict language capacities in nvASD.

## 3.2 Methods

### 3.2.1 Participants

The sample consisted of 49 nvASD participants beyond 6 years of age (Table 1), who were recruited from several special schools and daily centers across Spain (Barcelona, Vitoria and Seville) for the purpose of two different studies sharing procedures of cognitive profiling. The recruitment criteria were[1]: (a) a parent or center-reported ASD diagnosis, (b) absence of phrase level-functional speech (according to a formal ASD classification) with a speech profile consisting of no words, single words or fixed phrases as determined by parental, school, or center reports,

[1] 9 participants out of this present sample (N = 49) were specifically recruited, additionally, based on their cognitive performance in the ComFor test (having reached the ‘representation’ level), but were disregarded in the sample composition analysis, but included in correlational analysis with other cognitive measures.

and (c) evidence of absence of speech in other modalities (written, sign) or contexts (home, etc.) (Hinzen et al., 2019). The speech status and diagnosis of all participants were re-evaluated after recruitment over the course of the study assessments with specific reference to Module 1 of The Autism Diagnostic Observation Schedule (ADOS) and, in case of the school-aged subsample, additionally The Autism Diagnostic Interview-Revised (ADI-R).

There was one participant with a comorbid genetic disorder diagnosis (22q11.2 deletion syndrome), which, by not representing a divergent trend in the results, was kept in the analyses. School-aged participants (i.e., youngsters) have participated and/or were currently part of early intervention programs (such as applied behavioural analysis or the Denver method) to boost their communicative abilities. Older adults, however, were not generally part of early intervention programs. However, each day center adults are currently enrolled in, put into place life-long educational programs and activities. Nevertheless, this may have introduced a bias in the present study comparison analyses. 82% of the participants lived in a bilingual environment (i.e., Spanish-Catalan and Spanish-Basque). The standardized tests, insofar as they involved minimal verbal instructions, were administered in the language participants heard at home. This was Spanish for most subjects, although Catalan was also applied. In the case of three participants, the subjects' native languages partially used at home were other than the above, but two of these were excluded for reasons specified below. 10 additional participants were recruited but excluded during assessment based on: uncooperative behavior (N = 1), ADOS or ADI-R did not confirm ASD diagnosis (N = 5), mutism (N = 1), possible use of another language in vocalizations during the ADOS (N = 2), some flexible phrase speech (N = 1). To ensure comparability to previous research on receptive language in children and adolescents, the sample was divided along a cut-off of 19;0 years for the purposes of some analyses (Table 3.1).

**Table 3.1:** Participant demographics (N=49)

| | |
|---|---|
| Chronological age (years) | Mdn = 20;0, IQR = 9;95 - 27;10, Range = 5;11 - 57;1 |
| Subsample - youngsters (N=24) | Range = 5;11 - 16;9 |
| Subsample - adults (N=25) | Range = 20;0 - 57;1 |
| Gender: Male (N, %) | 40 (82%) |
| Ethnicity: Non-white, non-Hispanic (N, %) | 3 (6%) |

| | |
|---|---|
| ASD Diagnosis: Core autism/ASD (N, %) | 45/4 (92%) |
| ADOS-2/A-ADOS score (M, SD) | 19.08 (3.26) |

### 3.3.2 Measures

#### 3.2.2.1 ASD diagnosis

*ADOS*. The Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2; Lord et al., 2012) and The Adapted Autism Diagnostic Observation Schedule (A-ADOS; Bal et al., 2020) is a comprehensive semi-structured ASD diagnostic observation instrument. Participants were administered Module 1 Pre-Verbal/Single Words for individuals with 30+ months (ADOS-2) and 13+ years (A-ADOS).

*ADI-R*. The Autism Diagnostic Interview-Revised (ADI-R; Le Couteur et al., 2003) is a semi-structured diagnostic interview conducted with a primary caregiver. Individuals that do not produce flexible 3-word phrases are considered minimally verbal.

#### 3.2.2.2 Expressive language

Functional speech was determined according to Item A1 (General level of non-echolalic language) on the ADOS insofar as it represents spontaneous speech production with a communicative intent. Speech levels were subclassified as in Bal et al. (2020; 2016), into two groups: (1) 'FNW' ('few-to-no-words'): subjects with no or less than five different words or approximations to words produced during the ADOS session; (2) 'SW' ('some-words'): subjects with more than 5 different words or simple fixed phrases produced during the ADOS session. While the number of different words taps into lexical semantic ability, it has been shown to correlate with more complex syntax and morphology measures in children with phrase speech (Tager-Flusberg et al., 2009).

#### 3.2.2.3 Receptive language

*PPVT-III*. The Peabody Picture Vocabulary Test–III (PPVT-III; Dunn & Dunn, 1997; Dunn et al., 2010) is a standardized receptive vocabulary test that does not require a verbal response and yields a measure of VMA. This test was selected to ensure comparability to previous studies reporting receptive language levels in children. The PPVT is recommended as one of the language measures most suited to this population (Kasari et al., 2013); and it correlates with other vocabulary comprehension measures (parent reports, eye-tracker and touch screen measures), though some

variability is also noted (Plesa Skwerer et al., 2016). In administering the test trials, hand-over-hand guidance was used during test trials if necessary (adapted from Tsatsanis et al., 2003). Subjects were provided with a small cube to place it onto a picture sheet to deal with absence of pointing or touch. All participants started the task at the lowest starting age point corresponding to 2;6-years.

A further language assessment was envisioned via Child Early Language Fundamentals (CELF; Wiig et al., 2004), however, due to the fact that only a few individuals met a criterion of 3 years of VMA on PPVT to be administered the test, we had to exclude it from the study.

#### 3.2.2.4 Nonverbal IQ

*LEITER-R.* The Leiter International Performance Test-Revised (Leiter-R; Roid et al., 2013) is a standardized test used to assess nonverbal IQ with minimal need for instructions and for motor skills to respond. We followed recommendations for administration in low-functioning autism described in Tsatsanis et al. (2003).

#### 3.2.2.5 Sense-making

*COMFOR.* The ComFor (Noens et al., 2006) is a clinical assessment tool primarily targeted for individuals with ASD+ID to obtain an individualized indication of a most suitable means of augmentative communicative interventions at a precise level of sense-making. Sense-making – classified into levels of ‘sensation’, ‘presentation’, and ‘representation’ - is assessed through largely perceptual discrimination and generalization tasks. Along the sense-making levels, the subject’s interaction with the outside world is described as guided by sensory experiences (sensation), the situational context (presentation) or symbolic conceptualization (representation). Since the ComFor is a perceptual sorting-and-matching categorization task of non-transient and frequent daily-use objects and images that represent them, it can only directly determine a selected number of the cognitive aspects of the different sense-making levels. In particular, it does not assess the referential meaning of words or gestures, displacement of referents outside of here-and-now, object categorization by other means than matching, or even categorization of non-daily use objects.

In this test, the level of ‘sensation’ is attributed when sorting of identical non-functional objects is achieved only with support of a box with an object-shaped hole. The ‘presentation’ level consists of the sorting of identical objects (presentation A) or images of them (presentation B) into

open baskets according to concrete and directly perceptible visual features. The level of ‘representation’ proceeds with the sorting of non-identical kinds of objects or pictures, which goes beyond identical perceptible features and includes primary and functional meaning. An intermediate level of ‘developing representation’ involves minimal sorting of non-identical objects.

### 3.2.3 Procedure

The caregiver’s informed consent was received for all subjects. The standardized tests were administered by a trained psychologist (AR) specifically chosen for her extensive expertise in diagnosis and intervention programs targeted at nvASD population, and independent with respect to the formulation of research questions, analyses, and writing. In case of some participants, PPVT-III, ComFor and ADI-R were administered by trained examiners and revised by AR. Participants were individually tested at their respective schools or centres over the course of three videotaped sessions interspaced on different days that lasted between 45 and 75 minutes. Finally, caregivers were administered the diagnostic interview ADI-R.

### 3.2.4 Analyses

First, sample composition on the neuropsychological measures (i.e., VMA, expressive language level, NVIQ, and sense-making) was examined. Group differences between youngsters and adults in receptive language and expressive language were assessed via a bootstrapped independent sample t-test and a chi-square test of homogeneity, respectively. Additionally, CA modulation of VMA was explored in both groups to further explore findings in adolescents as in Pickles et al. (2014). Second, receptive and productive language abilities were assessed in relation to each other and to other cognitive predictors across the lifespan. CA was entered as a covariate in analyses, although it was excluded in analyses concerning NVIQ, as NVIQ is itself estimated according to CA. Specifically, a rank analysis of covariance was conducted to identify differences in VMA between the FNW and the SW expressive language level (less or more than five words in production, respectively) while controlling for CA, followed by a binomial logistic regression to ascertain the predictive effects of VMA on the likelihood of having FNW or SW. Subsequently, the associations between NVIQ and expressive language levels were explored through an independent sample t-test, while VMA and NVIQ failed to show a linear relationship.

Finally, the association between the ComFor and expressive language was explored via a rank-biserial correlation, while two Kendall's Tau b correlations assessed its relationship to both NVIQ and VMA (with CA as a covariate). Kendall's Tau b was selected as it is more robust against tied ranks than Spearman's correlations (Howell, 1997). Lastly, a cumulative odds ordinal regression with proportional odds was run to explore NVIQ and VMA as predictors of the ComFor. Expressive language level had to be excluded from the model as it was shown to be associated to VMA.

In case of a non-normal distribution, which was inspected visually and via a Shapiro-Wilk test (*p* values were <.002), bias-corrected and accelerated (BCa) bootstrapped parametric statistical analysis based on 10 000 samples was reported, since non-parametric analyses yielded identical findings. Correlational analyses were conducted only when the data followed linearity and/or monotonicity. Comparison analyses were checked for homogeneity of variances via Levene's Test for Equality of Variances. Effect sizes are reported in *r*-values.

Analyses were conducted in IBM SPSS Statistics (version 26) and in R (R Core Team, 2014) and figures were produced using the package ggplot2 in R (Wickham, 2009).

## 3.3 Results

### 3.3.1 Neuropsychological measures across the sample

Table 3.2 shows summary statistics of neuropsychological measures of the entire sample (N = 49) and its youngster and adult subgroups, with Bias Corrected and Accelerated 95% Confidence Intervals and Standard Errors. On the VMA measure, the PPVT-III, all participants (N = 49) scored 2 SDs below the population average. Figure 3.1 shows the relative distributions of VMA and CA. Five participants did not reach a basal score (VMA = 0;0). However, they were kept in the subsequent analyses. Different comprehension measures correlate between each other in nvASD as reported in Plesa Skwerer et al. (2016), thus such a score is not likely to be attributable to the test characteristics or administration bias. Although VMA scores ranged between 0;0 and 5;0, the interquartile variability was limited to IQR = 1;1-2;6. Figure 1 shows the distribution of VMA with higher probability values clustered around the Median = 1;7, BCa 95% CI [1;4, 1;7].

As expected, NVIQ was above the intellectual disability limit (NVIQ > 70) in a small proportion of the sample (N = 5, 10,2%), all of whom belonged to the Youngsters group. However, the NVIQ distribution peaked dramatically at the lowest NVIQ score (Mode = 30, N = 14, 28,6%).

Finally, according to the ComFor, while 62.2% of the sample (N = 28) reached sense-making levels that imply correct categorization of pictures, the remaining 37.8% (N = 17) persisted at manipulation of tridimensional objects, among which were also those participants that did not reach a basal score in PPVT-III (VMA = 0;0). Out of the participants able to categorize pictures, 67.9% (N = 19) were able to match a picture to its referent and 35.7% (N = 10) were able to match non-identically looking instances of the same picture.

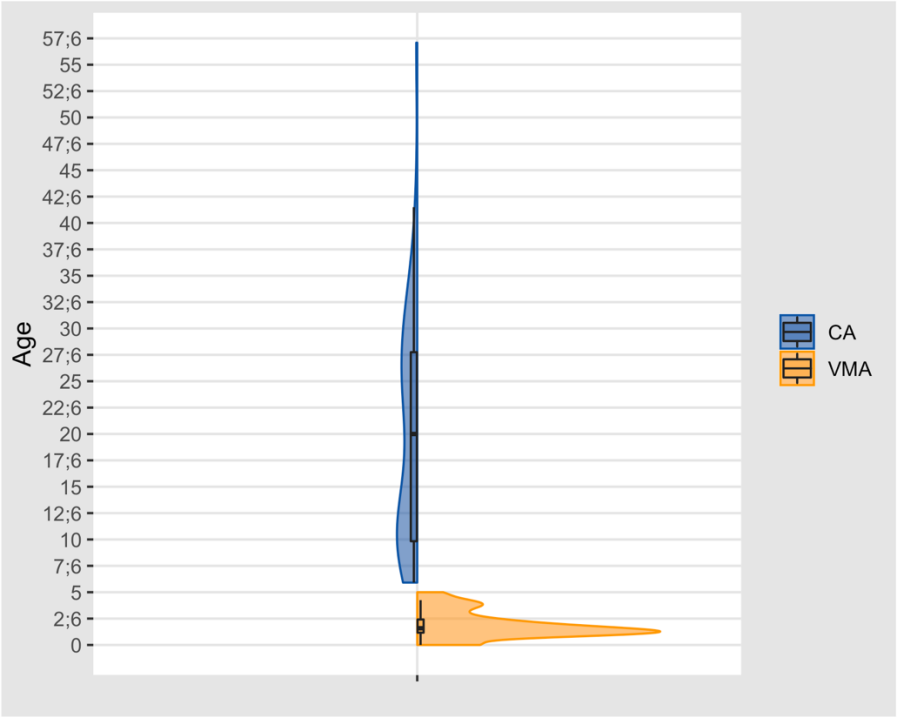
**Table 3.2:** Neuropsychological measures. Summary statistics.

| | | | | | |
|---|---|---|---|---|---|
| **Verbal mental age (VMA)** | | **Mdn** | **IQR** | **Range** | **95% CI of the Mdn (SE)[a]** |
| | N=49 | 1;7 | 1;1 - 2;6 | 0;0 - 5;0 | 1;4 - 1;7 (0;234) |
| | *Youngsters* | *1;3* | *1;0 - 1;11* | *0;0 - 4;3* | |
| | *Adults* | *1;11* | *1;3 - 3;3* | *0;0 - 5;0* | |
| **Expressive language level** | | **N** | **%** | | **95% CI for % (SE)[a]** |
| Few-to-no-words (FNW) | N=49 | 30 | 61.2 | | 51 - 71.4 (6.9) |
| | *Youngsters* | *18* | *75* | | |
| | *Adults* | *12* | *48* | | |
| Some words (SW) | N=49 | 19 | 38.8 | | 26.5 - 51 (6.9) |
| | *Youngsters* | *6* | *25* | | |
| | *Adults* | *13* | *52* | | |
| **Nonverbal IQ (NVIQ)** | | **Mdn** | **IQR** | **Range** | **95% CI of the Mdn (SE)[a]** |
| | N=49 | 53 | 30 - 61 | 30 - 94 | 53 - 53 (4.03) |
| **ComFor sense-making** | N=40[b] | **N** | **%** | | **95% CI for % (SE)[a]** |
| Representation | | 7 | 17.5 | | 10 - 25 (5.9) |
| Developing representation | | 8 | 20 | | 10 - 30 (5.9) |
| Presentation B | | 8 | 20 | | 10 - 30 (5.7) |
| Presentation A | | 6 | 15 | | 7.5 - 22.5 (5.1) |
| Sensation | | 11 | 27.5 | | 17.5 - 40 (6.3) |
| **ADOS-2/A-ADOS score** | | **M** | **SD** | **Range** | **95% CI of the M (SE)[a]** |

| | | | | | |
|---|---|---|---|---|---|
| | N=49 | 19.08 | 3.26 | 12 - 26 | 18.12 - 20.08 (0.47) |

[a]Bootstrapped (BCa) on 10 000 samples.
[b]This sample composition excludes the 9 participants recruited based on their ComFor level (see previous section)

**Figure 3.1:** Relative distributions of CA and VMA across the lifespan




### 3.3.2 Group differences on the neuropsychological measures between youngsters and adults

Group differences in receptive and expressive language between youngsters (N = 24) and adults (N = 25) were computed to explore differences between nvASD adults and youngsters (Figure 3.2). Adults had a higher mean VMA on average (M = 26.08, SE = 3.43; in months) than youngsters (M = 18.92, SE = 2.61; in months), but this difference was not significant $t(47) = -1.65$, BCa 95% CI [-16, 1.97], $p = .109$. Secondly, differences in the binomial proportions of the expressive language levels (FNW and SW) were explored. The difference between the two binomial proportions of .25 was not statistically significant, although it was at the borderline ($p = .052$).

Figure 3.3 explores associations between CA and VMA in youngsters and adults. While the adult subgroup did not show a linear or monotonic relationship, in youngsters CA and VMA followed a monotonic trend. Importantly, however, the data in youngsters formed two clusters with the majority of subjects situated under 2 years of VMA, and 3 data points – constituting outliers – at 3;10 years of VMA and higher. Thus, a Kendall's Tau b correlation was run between CA and VMA in youngsters both with and without including the outliers. There was a moderate correlation between VMA and CA that was significant both with outliers (N = 24), $\tau_b$ = .449, BCa 95% CIs [.13, .72], $p$ = .003, and without outliers (N = 21), $\tau_b$ = .328, BCa 95% CIs [-.06, .68], $p$ = .049. Despite this, the BCa 95% CIs of Kendall's Tau b correlation without outliers intersected with zero, implicating the point-estimate correlation was not statistically significant.

**Figure 3.2:** Group differences in receptive and expressive language between youngsters (N = 24) and adults (N = 25)

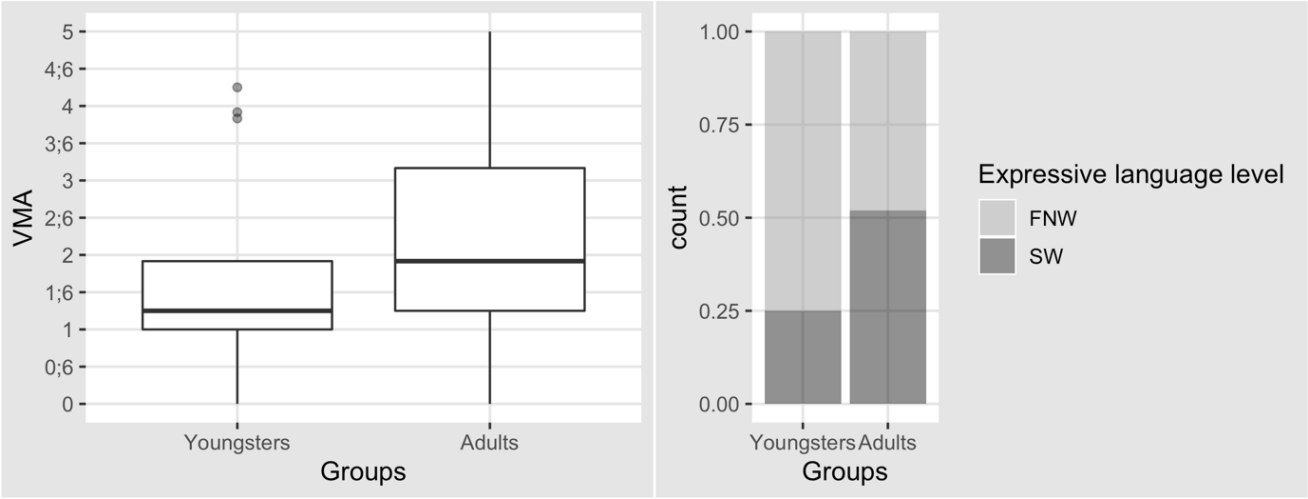
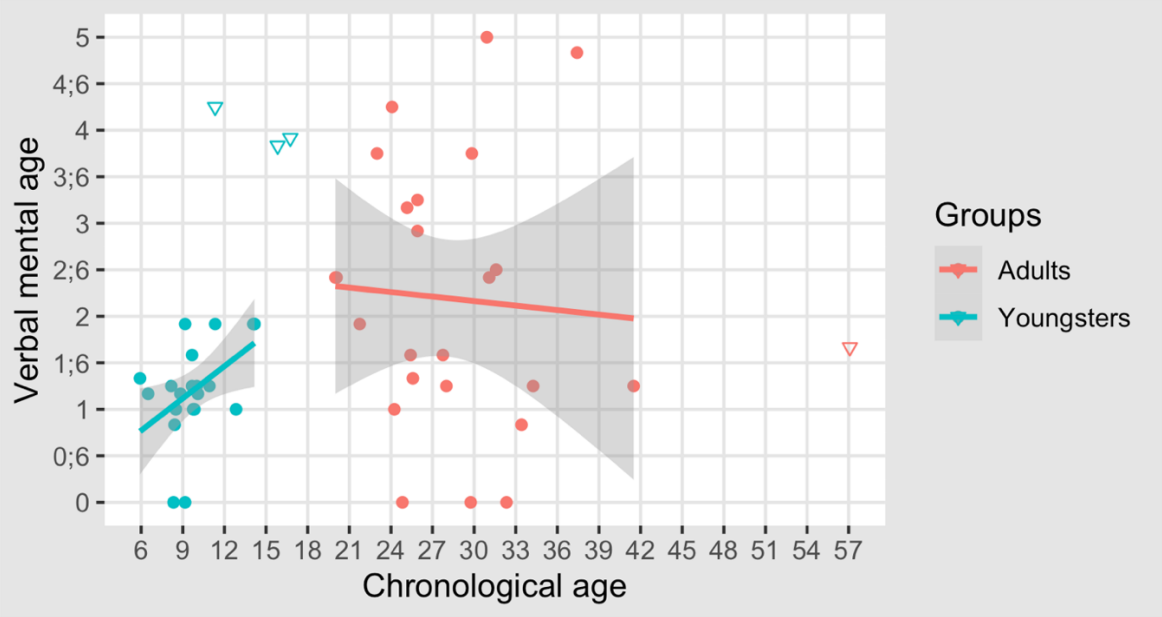



**Figure 3.3:** Association between CA and VMA in youngsters and adults




### 3.3.3 Associations between linguistic and neuropsychological measures

A rank analysis of covariance was run to investigate differences in VMA (PPVT-III) between binarized expressive language levels (i.e., less or more than five words in production during the ADOS session) after controlling for CA. Distributions of CA at each level were similar, as assessed by visual inspection. Participants who produced more than five words (SW, N = 19) had higher Median VMA (*Mdn* = 30; in months) than those who produced less than five or no words (FNW, N = 30, *Mdn* = 15; in months). After adjustment for CA, the difference in VMA between these expressive language subgroups was significant, $F(1, 47) = 17.968$, $p < .0005$, $r = 0.3$.

Subsequently, to estimate the predictive effects of VMA and CA on the likelihood of falling into the FNW or SW subgroups, a binomial logistic regression was conducted. VMA and CA were found to be linearly related to the logit of expressive language level as assessed via the Box-Tidwell procedure ($p = .638$ and $p = .440$, respectively). There were two standardized residuals with a value of -2.622 and 2.504 standard deviations, which were kept in the analysis. The area under the ROC curve was .825, 95% CI [.67 to .95], which is an excellent level of discrimination. The logistic regression model was statistically significant, $\chi^2(2) = 19.019$, $p < .001$, and explained 43.7% (Nagelkerke $R^2$) of the variance in expressive language levels while correctly classifying 85.7% of the sample. Sensitivity was 73.7%, specificity was 93.3%, positive predictive value (having more than 5 words) was 87.5% and negative predictive value (having less than 5 words) was 84.8%. While increasing VMA was associated with an increased likelihood of having more

than five words in production, with an odds ratio of 1.0, 95% CI [1.04, 1.18], $\chi^2(1) = 10.08$, $p = .002$, increasing CA did not significantly predict expressive language level, $\chi^2(1) = .71$, $p = .399$.

There was no linear and/or monotonic association between NVIQ and VMA. Similarly, there was no statistically significant difference in NVIQ, -5.17, BCa 95% CI [-15.05, 4.42], between FNW and SW expressive language levels $t(47) = -1.03$, $p = .303$, $r = 0.149$. BCa 95% CIs intersected with zero, which underlines a statistically non-significant effect.

ComFor/sense-making was strongly correlated with expressive language level, on the one hand, $r_{rb} = .586$, BCa95% CIs [.35, .68], $p < .001$; and VMA/receptive language, on the other hand, $\tau_b = .661$, BCa 95% CIs [.41, .72], $p < .0005$, after controlling for CA. Furthermore, there was a significant moderate correlation between the ComFor and NVIQ, $\tau_b = .330$, BCa 95% CIs [.11, .53], $p = .003$ (see Table 3 for summary). To estimate the joint predictive effect of VMA and NVIQ on the ComFor sense-making, a cumulative odds ordinal regression was run. Both the deviance goodness-of-fit test and the Pearson goodness-of-fit test indicated that the model was a good fit to the observed data, $\chi^2(166) = 88.92$, $p = 1$, $\chi^2(166) = 105.81$, $p = 1$, respectively, but most cells were sparse with zero frequencies in 77.7% of cells. However, the final model statistically significantly predicted the ComFor sense-making over and above the intercept-only model, $\chi^2(2) = 50.32$, $p < .001$. An increase in VMA (expressed in months) was associated with an increase in the odds of reaching a higher ComFor sense-making level, with an odds ratio of 1.187, 95% CI [1.1, 1.28], $\chi^2(1) = 20.99$, $p < .001$. An increase in NVIQ was also associated with an increase in the odds of reaching a higher ComFor sense-making level, with an odds ratio of 1.065, 95% CI [1.02, 1.11], $\chi^2(1) = 9.18$, $p = .002$.

Table 3.3 summarizes the findings of this section. Visualizations of relative associations between the cognitive measures assessed here can be found in Supplementary information (Fig. S1).

**Table 3.3:** Summary of the effect sizes and *p* values of the statistical tests

| | **VMA** | **Expressive language** | **NVIQ** | **ComFor sense-making** |
|---|---|---|---|---|
| **VMA** | – | *r = 0.3*<br>*p*<.0005 | – | *τb* = .661<br>*p* <.0005 |
| **Expressive language** | | – | *r* = .149<br>*p* = .303 | $r_{rb}$ = .586<br>*p* < .001 |

| | | | | |
|---|---|---|---|---|
| **NVIQ** | | | – | $\tau_b$ = .33<br>$p$ = .003 |
| **ComFor sense-making** | | | | – |

## 3.4 Discussion

The first question addressed in this study was the overall variability of conditions under conditions of no phrase speech attainment in nvASD over the lifetime. In this regard, receptive language scores as assessed by VMA fell 2 SDs below the normative sample average, mirroring the finding in children and adolescents reported in Plesa Skwerer et al. (2016). It is also telling that the CELF, which was originally planned to be used for receptive language assessment, proved to be applicable only in three subjects of the entire sample, and was therefore discarded from analyses. To a certain degree, variation in VMA could be observed. Yet, across all subjects at different life stages, variation exceeding 5;0 years of VMA was not observed, and the median fell at 1;7 years of VMA. Expressive language levels ranged from less to more than five words across the sample, though the communicative production of words did never exceed a handful of words at most, regardless of age. In line with previous findings (Bal et al., 2016), a small subgroup of people with nvASD had NVIQ outside of the range of ID. Significant variability was also seen in the ComFor, where more than a half of the sample proved to have the capacity to categorize pictures of objects, and the rest remained at the level 3D object categorization. 10 participants of the entire sample were able to categorize non-identically looking kinds of objects and pictures. Overall, these patterns entail that the adult population with no phrase speech does not represent the most cognitively impaired subjects.

With respect to the question of variability in receptive language in nvASD, binarized grouping of the sample into youngsters and adults also proved revealing. Youngsters corresponded to a CA range frequently sampled in previous studies of nvASD, thus constituting the backbone of our knowledge on receptive language in nvASD so far. The distribution of VMA scores in youngsters found here is consistent with Pickles and colleagues' (2014) longitudinal report on maximal language scores reached in 19-year-old participants with marked language delay, that is, 2- and 5-year equivalents. They are also in line with other studies in children and adolescents with

nvASD (DiStefano et al., 2016; Plesa Skwerer et al., 2016; among others), and young adults (Bal et al., 2020). Such age equivalents, however, do not necessarily imply *understanding* of more complex phrasal language, as would be expected at these VMAs in the neurotypical population. Indeed, as Plesa Skwerer et al. (2016) have shown, the majority of parent reports indicated no understanding of thee-way instructions in children with similar scores on the PPVT.

Interestingly, compared to these youngsters, the adult group showed no significant differences in either average VMA or the proportion of expressive language levels (i.e., more than 5 or less than 5 words in production). This suggests a relatively static cognitive profile in nvASD, which has already alarmed researchers to refocus on this population in ASD research. VMA scores show a striking and deepening recession when viewed from the perspective of ample range of chronological ages. This suggests that age may not play a major role in enhancing receptive language in nvASD through prolonged access to learning opportunities, possibly pointing to a developmental glass ceiling that future longitudinal studies should clarify.

Despite no significant differences between adult and youngster groups, youngsters in the present study showed a positive trend in the relation between VMA and CA, while the correlation of VMA and CA in adults could be equally accommodated within positive or negative confidence bounds. Such a positive trend in youngsters is once again consistent with Pickles and colleagues, who reported a gradual, though slow, growth in language abilities across adolescence. This suggests a certain neural plasticity of the brain at least in youngsters, where central critical periods for language acquisition nevertheless might not have been initiated (Toyoizumi et al., 2013). Importantly, while most youngsters participated in targeted early intervention programs, older adults in our sample did not. It is tempting to see early intervention programs as driving this discrepancy between adults and youngsters, but future studies need to target this issue formally. As no consistent effects of intervention on language capacities were identified in a meta-analysis conducted by Spreckley & Boyd (2009), any gradual gains youngsters might have accumulated over the childhood and adolescent years do not seem to amount to a significant difference with respect to adults, who often without interventions. Conversely, a lack of a clear trend in the adults might indicate a ‘lack of maintenance’, due to their limited exposure to intervention, even when they have continued to participate in life-long educational programs and activities as part of their daily centre infrastructure.

Three subjects in the youngster group were outliers, showing higher sores than the rest of the youngster group, yet not patterning differently from the adult scores. Could these three participants grow their language skills on a higher rate as they enter adulthood, and if so, how would this growth be reflected in their minimally verbal status? Of importance to this question is the relation of receptive language abilities as measured by the PPVT and expressive language in nvASD, which was our second research question above. Results in this regard showed the level of single word production to be correlated with, and predicted by, receptive language across the lifespan, regardless of CA. Specifically, receptive language scores (VMA) alone explained more than 40% of the variance in expressive language level as represented by the cut-off of having either more or no/less than five words in production. More than 80% of the sample was accurately classified as having either less or more than five words based on VMA. This pattern is strong evidence that it would be deficient to see nvASD as a problem residing in motor-speech aspects of language only, and that therapies should not be focused on expressive language alone: Comprehension, though harder to assess and clinically less obvious, is key. Our findings on correlations between expressive and receptive language extend conclusions from Bal et al. (2016), Hartley et al. (2017) and Pickles et al. (2014) to a large nvASD-only group of all ages beyond 6, but they contrast with Chenausky et al. (2018). As noted above, a small sample size and floor level number of words in production might have contributed to a lack of effect in that latter study, as might relating total number of words in production to receptive language as calculated by the PPVT, i.e. according to developmental stages, not total words understood. Here, we instead chose a clinically and behaviourally relevant cut-off point of more or less than 5 words in production, following Bal et al. (2016).

Importantly, the PPVT as a measure of VMA and receptive language is not free of biases, as it is based solely on single-word comprehension assessed through word-picture matching. As such it is not commensurate to comprehension of words as parts of grammatical structures or verbal instructions. In particular, in typical uses of words, the referent of the word obviously need not be present visually. In line with this, Garrido et al. (2015) found that grammatical comprehension in nvASD is not at a level expected from PPVT-based vocabulary scores. Moreover, in assessment, the PPVT requires an understanding of pictures, which our ComFor results demonstrate is by no means a given in nvASD; and as Preissler (2008) shows, grasp of associative relations between words and pictures by no means shows language-like symbolic understanding of such words. For

these reasons the PPVT is limited as an estimate of real receptive language capacities. That said, our results do suggest that the PPVT reflects vocabulary abilities, given that its results mapped onto observed productive language capacities. The exact nature of word comprehension and production in nvASD remains an important open question for future research.

The third and fourth questions that this study addressed concerned the relation between nonverbal cognitive capacities and language measures. In this regard, the relative lack of variation we found in receptive language stands in a stark contrast with variation seen in nonverbal cognition, with absence of ID seen in a substantial minority, despite a most frequently attested minimal NVIQ score (NVIQ = 30, 28,6%), which has been a robust classifier of children that remain minimally verbal after five years of age before (Weismer & Kover, 2015). This contrast and a fundamental lack of association between NVIQ and language across the lifespan suggest a fundamental divergence between verbal and nonverbal cognition in this population, with linguistic cognition being its more defining feature, since cognitive measures other than NVIQ did not show a different profile in the subgroup with normal NVIQ. Interestingly, while Bal et al. (2016) found differences in NVIQ between expressive language levels identified as in our study, our findings are more in line with Chenausky et al. (2019), who reported no predictive effect of NVIQ for expressive language.

It would not be true to conclude, on the other hand, that nonverbal cognition makes no difference: ComFor scores were distributed across all ComFor sense-making levels, thus showing significant variation, which moreover was significantly explained by NVIQ *and* language measures alike. Crucially, the ‘representation’ level of this test requires a more global integration of information when sorting objects according to their kind. To what extent a sorting capacity of this nature truly relies on referential and symbolic understanding, however, and hence reflects capacities also constitutive for language, is unclear. Thus, representational capacities as assessed in the ComFor do not comply with two criteria at the heart of symbolic understanding (Bates, 1979), namely separability (the symbolic item and its referent are not the same thing) and substitutability (use of the symbolic item instead of the referent). Furthermore, the ComFor also only incorporates highly familiar daily-use objects (such as forks, bottles, balls) and pictures, which are often part of the structured learning environments adapted to people with ASD+ID. For these reasons, the result that ComFor scores loaded on both linguistic and non-linguistic factors makes sense: it is a hybrid cognitive measure. Future studies are critically needed that would assess

object conceptualization capacities in nvASD and their relation to the kind of categorization abilities found even in very young, pre-productively verbal neurotypical infants (Vouloumanos & Waxman, 2014). It is worth noting that the primary target and utility of the ComFor lie in determining suitable alternative means of communication in individuals that do not communicate verbally. It is therefore interesting that such abilities may, in fact, still load on a verbal factor, despite directly assessing nonverbal categorization and communication abilities.

## 3.5 Conclusions

NvASD remains one of the fundamental challenges of autism research today, at the most severe as well as neglected end of the spectrum. This study shows that, as youngsters with nvASD turn into adults, they are not likely to become different in their cognitive profiles. While further longitudinal studies and differential cognitive explorations are needed, this study has been the first to provide a lifetime perspective on the development and stability of the cognitive profile of nvASD. It supports directing our research focus on language as a central cognitive system, which as such encompasses both receptive and expressive language, and is not compensated for by non-verbal capacities in this condition. It also shows that even a slight difference in verbal capacities can make an important cognitive difference, while stressing the need to seize developmental opportunities that may otherwise disappear when youngsters turn into adults.

# 4. Functional organization of the core neural language network in nvASD: irregularities along the processing hierarchy

## 4.1 Introduction

As noted earlier, the practical difficulties involved in conducting behavioural assessment in nvASD have often cast doubt upon the exactitude of these measures among the ASD research community (Kasari et al., 2013; Plesa Skwerer et al., 2016). Alternative methods that bypass this challenge by targeting neural processing of language, such as functional magnetic resonance imaging (fMRI), offer a powerful tool to evaluate the language capacity in this context. So far, however, a functional characterization of the language network in nvASD is lacking altogether: fMRI methods require high levels of patient compliance and are particularly sensitive to head motion, which has kept fMRI studies of nvASD out of the horizon. In clinical settings, sedation often has to be used to circumvent these problems, especially in the paediatric population (Bernal et al., 2012). Since task-elicited changes in the blood-oxygen-level-dependent (BOLD) signal have been widely documented to be preserved under sedation, specifically in the case of auditory speech stimuli (Adapa et al., 2014; Davis et al., 2007; Di Francesco et al., 2013; Frölich et al., 2017; Gemma et al., 2016; Liu et al., 2012), this provides opportunities to study the language network in nvASD. The caveat of speech fMRI under sedation, however, lies in reduced responses to semantic content even under light sedation (Adapa et al., 2014; Davis et al., 2007; Gross et al., 2019). Moreover, propofol-induced sedation preserves temporal lobe activations to speech and complex sounds, but suppresses the active involvement of frontal language regions, namely the inferior frontal gyrus (IFG) (Davis et al., 2007; Frölich et al., 2017; Gemma et al., 2016; Plourde et al., 2006; see Adapa, 2017; MacDonald et al., 2015 for review).

Intrinsic brain activity, on the other hand, which results in patterns of synchronized large-scale brain networks, appears more robust to sedation- or sleep-induced states. Thus, even in the context of absent activation of IFG under propofol, the fronto-temporal (François et al., 2016; Liu et al., 2012) and temporal functional interactions (Boveroux et al., 2010; Gómez et al., 2013) remain in place to a considerable extent, although the coupling of IFG and the primary auditory cortex in particular, is dissolved (Liu et al., 2012). The intrinsic coupling of activity between brain regions reveals brain dynamics in the absence of an overt task and has been hypothesized to give

rise to the functional capabilities of the brain circuity (Power et al., 2010; Smitha et al., 2017). As such, the analysis of the functional coupling of neural activity, i.e., functional connectivity (FC), opens an inroad into the organization of the language connectome in severely impaired populations even along higher-order steps in the hierarchical processing of language (de Heer et al., 2017; Friederici, 2020; Sheng et al., 2019).

The functional coupling among brain regions, i.e. the functional connectome, originates in early pre-natal development initially even in the absence of sensory stimuli (Borsani et al., 2019; Bragg-Gonzalo et al., 2021). The long-range connectivity between ipsilateral brain regions gradually increases with gestational age (Thomason et al., 2015), although an early indication of a primordial language network can already be perceived between 20 and 39 gestational weeks (Turk et al., 2019; for review, see Ghio et al., 2021), albeit unilaterally. Close to term, now bilateral interactions between frontal and temporal regions, encompassing the future Broca's and Wernicke's areas, become more integrated and left-lateralized (Thomason et al., 2014). Such early versions of brain functional circuits are thought to set the ground – functionally and structurally – for upcoming stimuli (Bragg-Gonzalo et al., 2021).

Shortly after birth, speech stimuli elicit a propagation of activity that follows a hierarchical temporo-frontal gradient along this circuity (Dehaene-Lambertz et al., 2006) and further fine-tune it (Cusack et al., 2016; Toyoizumi et al., 2013; Zhang & Mu-ming, 2001). Abnormal or absent experience with external stimuli at specific time-limited windows, in turn, can leave long-lasting effects on the cortical circuit assembly (Bragg-Gonzalo et al., 2021; Marín, 2016; Penn & Shartz, 1999), although robust compensatory mechanisms are also at play to guarantee an active maintenance of spontaneous network fluctuations (Blankenship & Feller, 2010).

Extensive evidence suggests that irregularities in the wiring, fine-tuning or conservation of neural circuits is at the core of many neurodevelopmental disorders (Menon, 2011). In high-functioning autism, in particular, an extensive number of FC studies have compiled an ample body of evidence of aberrant functional network organization in ASD, with connectivity altered both within and between networks (see Hull et al., 2017; Vissers et al., 2012 for review). It has been suggested that FC in ASD is characterized by reduced network integration and segregation (Keown et al., 2017; Rudie et al., 2012; Shih et al., 2011), although a consensus has not yet been reached (Holiga et al., 2019; King et al., 2019). On its own account, a single study in low-functioning autism (Lai et al., 2012) similarly suggested a decreased FC in fronto-temporal regions in this

population when processing speech relative to song. The near-absence of studies at the low end of ASD, however, prevents a comprehensive characterization of network organization in ASD, and it is also possible that divergent trajectories may underlie otherwise similar diagnostic profiles (Marín, 2016).

In the present study, I sought to explore FC patterns within and across functional brain networks, with a specific focus on the core language circuity, in 8 children and adolescents with nvASD matched on chronological age and handedness to two comparison groups of 8 typically developing (TD) and 8 verbal ASD (vASD) children. The similarity of FC patterns among subjects of the nvASD relative to the other groups was also assessed, to further address the issue of potential heterogeneity of language comprehension profiles. As I entertain in this thesis, the fundamental lack of phrase speech exerts a stringent barrier on the language faculty, confining its operations to a severely limited space. Thus, despite an ample age range of the sample, higher homogeneity of the FC patterns was predicted. The TD group was chosen to obtain benchmarks of FC that arises along regular developmental paths, while the vASD group exemplifies an irregular circuit assembly as a factor of ASD – a constant between nvASD and vASD, albeit with compensatory mechanisms leading to protracted language acquisition likely at play only in the latter group. Experience-driven neuroplastic mechanisms have been hypothesized to introduce transient delays in development (Meredith, 2015), as manifested in the major portion of ASD where protracted cognitive development is seen. This contrasts with the failure in children with nvASD to meet early milestones and to continue doing so over the course of their lifetime, as well as the lack of malleability of the nvASD phenotype, as has been demonstrated in the previous chapter. The nvASD phenotype, thus, may underline neural changes that incline the brain towards points of vulnerability that intervene with experience-driven neuroplastic malleability. The substrate for such neural changes may reside in the modulation of neural mechanisms such as cell migration, synapse formation, and specialization of structural and functional circuity.

To provide a comprehensive picture on the specialized wiring of the language network in nvASD, FC patterns in the following study were assessed both under resting state and task-evoked conditions, i.e., an auditory verbal stimulation. A sedated brain under complex auditory stimulation has been shown to overcome the relative inhibitory effect on information transfer within brain networks that is characteristic of the resting state. Thus, the connectivity within brain networks is maintained comparable to a wakeful state, but disruptions occur as the information is feed-

forwarded throughout the brain as mirrored in increase of between-network connectivity (Naci et al., 2018). An opposite effect of sedation is observed at rest, when the brain networks remain segregated compared to wakefulness, but individual networks partially lose their internal integrity. Resting state and auditory stimulation therefore represent complementary lenses on functional organization, which might be otherwise blurred under sedation.

In the present study, the auditory stimulation consisted of a bedtime story that was presented both as normal forward speech (FWD) and backward speech (BWD). BWD retains acoustic and phonetic characteristics of speech, but interferes with prosodic, phonological and semantic speech features. Use of this paradigm was inspired by several studies showing differential activation to forward vs. backward speech in neurotypical newborns (Sato et al., 2012; Vannasing et al., 2016), 3-months old infants (Dehaene-Lambertz et al., 2002), and children (Ahmad et al., 2003; Romero-Garcia et al., 2018), including children with ASD (Redcay & Courchesne, 2008). We reasoned that sensitivity of FC to the within-group contrasts between FWD and rest, and FWD vs. BWD, could illuminate the auditory language deficit and its variability in nvASD. Sensitivity in nvASD to the FWD versus BWD distinction would show a way in which their auditory processing of language exhibited patterns already observed in neurotypical newborns. Failure of such sensitivity, on the other hand, could be a remarkable pointer to a very early and basic neural deviation in this population.

With respect to the networks that were the target of the analyses, I first assessed, as in Naci et al. (2018) and previous studies in low-functioning autism, the within-networks and across-networks FC of a set of classical brain networks as extracted from the Human Connectome Project and included as default in FC analysis software (CONN). This was done to obtain a starting point with respect to the overall modular organization of the brain networks (Vasung et al., 2019), which could then be interpreted with regard to sedation effects – following the model of the study by Naci and colleagues. I then zoomed into the canonical language network consisting of the bilateral IFG, superior temporal gyrus (STG), middle temporal gyrus (MTG) and Heschl's gyrus (HG) regions of interest (ROIs), which themselves comprise functionally relevant subregions (i.e., pars triangularis of IFG, parts opercularis of IFG, anterior STG and MTG, posterior STG and MTG). As in the whole-brain analysis, I calculated the average FC across these language ROIs ('across-ROI' FC) and within them ('within-ROI' FC). Within ROIs, FC was extracted as connectivity of the subregions of each ROI bilaterally, that is, average FC between anterior and posterior STG and

MTG, pars triangularis and pars opercularis of IFG and HG bilaterally. Across-ROIs FC, in turn, represents average FC between subregions that constitute distinct ROIs, namely HG, STG, MTG, and IFG. To obtain a more nuanced estimation of the hierarchical organization of the language network, I proceeded to assess FC in the language network between binary pairs of language ROIs, e.g., FC between STG and MTG, taking into consideration their individual subregions. A visual representation of these calculations is found in Methods (see Figure 4.2 below).

## 4.2 Methods

### 4.2.1 Participants

Participants were 8 school-aged children and adolescents with nvASD. Seven originally participated in the larger cognitive-profiling study described in the previous chapter (for recruitment criteria see p. 28). The present sample also included the subject with a co-morbid 22q11 deletion syndrome (subject 5). The remaining participant (subject 7) was recruited solely for the purposes of the MRI exploration based on the same recruitment criteria, although diagnosis was evaluated solely based on the ADI-R, while none of the behavioural measures could be administered due to lack of responsiveness. Subject 5 and 7 were judged not to constitute outliers within the overall sample and were included in the analysis to increase statistical power. Their individual FC profiles can, nevertheless, be found in the Supplement (Figure 4.S4) for transparency. Since all participants live in a bilingual environment (i.e., Spanish-Catalan), the language of the experimental stimuli was determined according to the primary language spoken at home by the participants' parents. The handedness was parent-reported. The sample's demographic data are summarized in Table 1. The sample's individual cognitive profile data (VMA, non-verbal IQ, and expressive language level) are shown in Figure 4.1 below. There were no significant differences in motion ratio between rest and task acquisition data in the nvASD group, $t(7) = 1.079$, $p = .316$.

MRI data from pairwise age, sex, motion and handedness-matched TD and vASD children and adolescents were included to compare FC patterns of the nvASD participants, and their verbal IQ was assessed by Wechsler Abbreviated Scale of Intelligence (Table 4.1). Control data were collected at the University of California San Diego (UCSD) Center for Functional MRI (CFMRI). There was no statistically significant difference between the groups on age, $F(2,21) = .013$, $p =$

.987, or on the motion ratio (number of outlier volumes / total volumes), $\chi^2(2) = 1.661$, $p = .436$. So far, two TD control subjects were recruited specifically for the purposes of this study, i.e., under the same protocol/scanner site conditions. Their TD status was confirmed through a parent-based questionnaire on child and family health history (CFHQ; Linder, 2008).

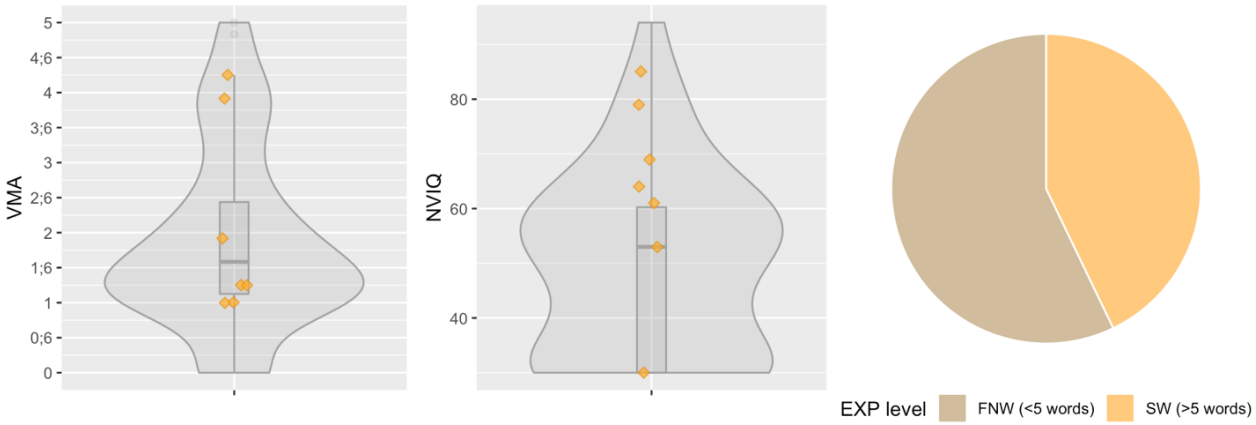
Their FC data are represented on the foreground of UCSD TD group's data (Figure 4.S1, 4.S2, and 4.S3 in the Supplementary information) to address possible protocol/site effects that otherwise might have driven group differences.

This study was approved by an institutional review board (CEIC Fundació Sant Joan de Déu; PIC-99-17) and was in accordance with 1964 Helsinki declaration and its later amendments. Caregivers of all participants gave their informed written consent.

**Table 4.1**: Participant demographics and group matching

| | **nvASD** | | | | **vASD and TD control groups** | | | |
|---|---|---|---|---|---|---|---|---|
| | Age | Sex | Primary language | Handedness | Age (vASD/TD) | Sex (vASD/TD) | Handedness (vASD/TD) | Verbal IQ (vASD/TD) |
| **1** | 8;3 | M | Spanish | L | 9;3 / 8;0 | M / M | L / L | 84 / 99 |
| **2** | 9;6 | M | Spanish | R | 9;6 / 9;2 | M / M | R / R | 95 / 126 |
| **3** | 10;11 | M | Catalan | R | 10;9 / 10;3 | M / M | R / R | 79 / 107 |
| **4** | 11;1 | M | Spanish | R | 11;2 / 11;2 | M / M | R / R | 92 / 102 |
| **5** | 12;3 | F | Spanish | R | 13;4 / 12;3 | F / F | R / R | 91 / 126 |
| **6** | 15;1 | M | Spanish | R | 15;3 / 15;3 | M / M | R / R | 116 /117 |
| **7** | 16;7 | F | Spanish | R | 16;8 / 16;8 | F / F | R / R | 95 / 110 |
| **8** | 17;8 | F | Catalan | R | 17;3 / 18;5 | F / F | R / R | 105 /104 |

**Figure 4.1:** The individual verbal mental age (VMA) and non-verbal IQ (NVIQ) scores of the sample seen against the distribution of VMA and NVIQ scores of the larger representative sample from Study 1. The pie chart represents proportion expressive language levels (EXP level) in the sample.




### 4.2.2 Magnetic Resonance Imaging Acquisitions

MRI data were collected at the Sant Joan de Déu Hospital, Barcelona, on a Philips Ingenia 3T scanner using a 64-channel head coil. A high-resolution T1-w structural image (magnetization-prepared rapid-acquisition, gradient echo sequence; TR = 9.899 msec, TE = 4.6 msec, 8° flip angle, slice thickness = 1 mm, 1 mm in plane resolution, 180 transverse slices, matrix size = 240 x 240) was acquired for each participant. Resting state fMRI sequence (no stimuli were presented during acquisition) consisting of 266 (8 min 52 sec) functional images sensitive to blood oxygenation level-dependent contrast (BOLD; echo planar T2*-weighted gradient echo sequence; TR = 2000 msec, TE = 30 msec, 70º flip angle, acquisition matrix = 68 x 68, 3.5 mm in plane resolution, 3.5 mm thickness, 32 transverse slices aligned to the plane intersecting the anterior and posterior commissures) were then acquired, followed by fMRI language stimulation carried out using the same acquisition parameters. One functional run consisted of 348 (11 min 39 sec) functional images. To reduce acoustic background noise sound-dampening foam cushion was placed inside of the scanner. Total scan time was approximately 50 min as FLAIR, T2-w, T2-w multiecho, and DWI images were also acquired, which are beyond the scope of this study.

### 4.2.3 fMRI experimental design

For the language stimulation, we used a spontaneous narration of a short children story (*The snowman* by Raymond Briggs) recorded in child-directed speech by a female native Spanish-Catalan bilingual speaker. The story was divided in 10 blocks (FWD; forward speech condition). The average block length was 20 sec (range = 18.7 – 21.76 sec). Each block contained short

sentences forming a sequence of complete phrases. The ten blocks containing the original story were then recorded backward (BWD speech condition).

First, the FWD blocks were presented in order, with 15 sec rest periods between blocks where no stimuli were presented (Rest condition). Afterwards, the BWD blocks were presented in order, similarly, with 15 sec rest periods in between. The off-resting periods were set to 15 sec because the BOLD response in children returns to baseline levels faster than in adults (see Richter & Richter, 2003; Blasi et al. 2011). Due to an error in the onset and overall duration acquisition time, however, the duration of some blocks was reduced. Thus, the first 4 seconds of the first FWD block in either language were not acquired shortening the block length to 16.4 seconds instead of 20.2 seconds. Furthermore, the Catalan stimuli (3 participants) had one less BWD block towards the end of the acquisition. Therefore, for the comparison of FWD and BWD conditions, only 9 blocks for each condition were specified to assure comparability. One participant did not complete BWD block due to a complication with scanner headphones and was excluded from any analysis concerning BWD comparisons.

### 4.2.4 Sedation procedure

Anesthesia was induced via a mask with sevoflurane, which is the routine method used in the Sant Joan de Déu Hospital. Immediately after this procedure, the intravenous line was placed, and the participant was transitioned to an intravenous-based anaesthetic with propofol. The initial propofol dose was adjusted to render the patient motionless but able to maintain his or her airway with a laryngeal mask. Propofol dosage for induction was 1 mg/kg and after perfusion, a dosage of 8-10 mg/kg/h was administered. Dosage was gradually decreased to 6 mg/kg/h until the end of the procedure. Two participants did not require initial sedation via mask-induced sevoflurane and directly received a 200 mg propofol dosage for induction. Both sevoflurane and propofol have very small side effects and are drugs routinely used in paediatric neuroimaging. Duration of sedation using both drugs is very small allowing for very fast recovery times (Bernal et al., 2012). Importantly, we took into account the pharmacokinetics of sevoflurane in order to ensure that fMRI scans were carried out exclusively under propofol anesthesia. Because of this, we induced a fast transition to propofol anesthesia after sevoflurane and also ran the first 18 min of the structural imaging first to ensure that the possible effect of sevoflurane on subsequent fMRI was minimal. Indeed, the low solubility in blood of sevoflurane induces a rapidly decreasing alveolar

concentration after cessation of the inhaled agent which is linked to very fast recovery times and wash out (Bernal et al., 2012; Yasuda et al., 1991).

### 4.2.5 Magnetic Resonance Imaging Acquisitions (Control groups)

Data were collected on a GE 3T Discovery MR750 scanner using an 8-channel head coil. A single-shot gradient-recalled EPI sequence (180 whole-brain volumes were acquired (TR = 2000 ms; TE = 30 ms; slice thickness = 3.4 mm; flip angle = 90°; FOV = 22.0 mm; matrix = 64 x 64; in-plane resolution = 3.4 $mm^2$) was used to acquire 6 minutes of resting state fMRI. High-resolution T1-weighted sequences (3D FSPGR; 1 mm isotropic voxel size, NEX=1, TE=min full, TI=600, flip=8°, FOV=25.6cm, matrix=256x256, receiver bandwidth 31.25htz) were collected in each participant. During all resting state functional scans, participants were presented with a white cross on a black screen and instructed to "Keep your eyes on the cross. Let your mind wander, relax, but please stay as still as you can. Try not to fall asleep." Participants' adherence to the instructions to remain awake, with eyes open, was monitored with an MR-compatible video camera.

### 4.2.6 MRI Preprocessing and Analyses

MRI data for FC analyses were preprocessed, denoised and analyzed in Matlab 2019b (Mathworks Inc., Natick, MA, USA) using SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, UK), and the CONN toolbox v19b.

The structural T1-weighted image was converted from DICOM to NIfTI format and was coregistered to the mean functional image, segmented and normalized to MNI space using non-linear registration and the default tissue probability maps included with SPM12. The white matter (WM) and cerebrospinal fluid (CSF) probability maps obtained from segmentation of the structural image for each individual subject were thresholded at 0.9 and eroded by 1 voxel. The WM and CSF time courses extracted from these masks were then submitted to a CompCor (Bezhadi et al., 2007) for subsequent nuisance regression.

*Functional:* EPI images were motion-corrected using rigid-body realignment and slice-timing corrected as implemented in SPM12. The Artifact Detection Toolbox (ART, as installed with conn v19b) was used to identify outliers in the functional image time series from the resulting 6 motion parameters (3 translational and 3 rotational) that had frame-wise displacement (FD) >0.5mm and/or changes in signal intensity that were greater than three standard deviations.

Functional images were directly normalized to MNI space with the same non-linear registration as used for the structural images. Since all analyses were run on averaged voxel time series within pre-defined regions of interest (ROIs), no prior smoothing was applied to the data. Band-pass filtering using a temporal filter of 0.008 to inf Hz was carried out as part of the nuisance regression ('simult' option in the conn toolbox) which also included scrubbing of the motion outliers detected by the ART toolbox, and regression of the 6 motion parameters and their derivatives, as well as the first five PCA component time series derived from the CSF and WM masks. The residuals of the nuisance regression were then used for all subsequent FC analyses.

### 4.2.7 Functional Connectivity Analyses

FC estimates were derived for the rest and auditory stimulation runs separately. BOLD time series were averaged across all voxels comprising the targeted ROIs. Whole-brain network ROIs comprised the classical brain networks (salience network, fronto-parietal network, dorsal attention network, language network, default mode network, visual network, and sensorimotor network) as defined in the default CONN network parcellation (based on Independent Component Analysis of Human Connectome Project dataset), while core language network ROIs corresponded to the Harvard-Oxford atlas included as default in CONN, and their selection was based on a recent meta-analysis of fMRI studies of language comprehension in children (Enge et al., 2020). FC was computed as the degree of similarity of the BOLD time series between ROIs estimated via bivariate Pearson correlation standardized with a Fisher z-transformation. Each ROI pair's correlation magnitude was compared between conditions (rest/FWD and FWD/BWD) in nvASD and to the control groups' FC at rest. A higher FC magnitude reflects more similar time courses between ROIs. Results were corrected for multiple comparisons via false discovery rate (FDR) correction at a threshold of $p < 0.05$.

*Pattern similarity analysis.* The overall FC pattern of all ROI pairs in whole-brain networks and in the canonical language network was compared between participants to assess the pattern similarity (as performed in Linke et al., 2020). First, each 32x32 ROI FC matrix (whole-brain networks) and 14x14 ROI FC matrix (the language network) was triangulated. Pearson correlation with Fisher z-transformation was used to compare the connectivity pattern of each participant with that of every other participant. Then, the average similarity of a nvASD participant to all other nvASD participants and to all TD and vASD controls was calculated. Permutation testing (carried

out in MATLAB, 1 000 permutations with group labels randomly shuffled) was used to assess significant differences.

*Within-networks and across-networks FC of whole-brain networks.* As in the study of Naci et al. (2018), FC estimates corresponding to the rest and forward speech language stimulation were computed. After computing the FC of ROI pairs across all networks, the overall within-networks connectivity and across-network connectivity was estimated. The within-networks connectivity was derived as the average of the FC of ROI pairs within each network. The across-networks connectivity was derived as the average of the FC of ROI pairs between networks. Within-networks and across-networks FC in the nvASD group was compared across conditions (rest/FWD) via a two-way repeated-measures ANOVA. All statistical assumptions were met. Secondly, a two-way mixed ANOVA was run to test for differences across groups (between-subject factor) for within-networks and across-networks FC (within-subject factor) at rest. There was one outlier in the vASD group, who was kept in the analysis as repeating the ANOVA without the outlier yielded similar results.

*Within-ROIs and across-ROIs FC across the core language network.* BOLD time series were derived for the following bilateral canonical language ROIs: HG, anterior STG, posterior STG, anterior MTG, posterior MTG, IFG-pars triangularis, IFG-pars opercularis) as defined in the Harvard-Oxford atlas. The within-ROIs connectivity was derived as the average of the FC within bilateral ROIs (IFG, HG, STG, MTG). The across-ROIs connectivity was derived as the average of the FC of bilateral ROI pairs (i.e., IFG-HG, IFG-STG, IFG-MTG, HG-STG, HG-MTG, STG-MTG). See Figure 4.2 for a visual representation. As in the previous section, a two-way mixed ANOVA was run to test for differences between groups for within-ROIs and across-ROIs FC at rest. All statistical assumptions were met. Secondly, two two-way repeated-measures ANOVAs were applied to test for differences in within-ROIs and across-ROIs FC across conditions (rest/FWD and FWD/BWD). Here, in the FWD/BWD comparison, non-normal distribution was detected for BWD, but non-parametric tests yielded similar results.

*FC of individual ROI pairs of the core language network.* The FC of individual ROI pairs of the language network (i.e., IFG-HG, IFG-STG, IFG-MTG, HG-STG, HG-MTG, STG-MTG; see Figure 4.2) was compared across conditions (rest/FWD and FWD/BWD) and to the control groups' FC at rest.

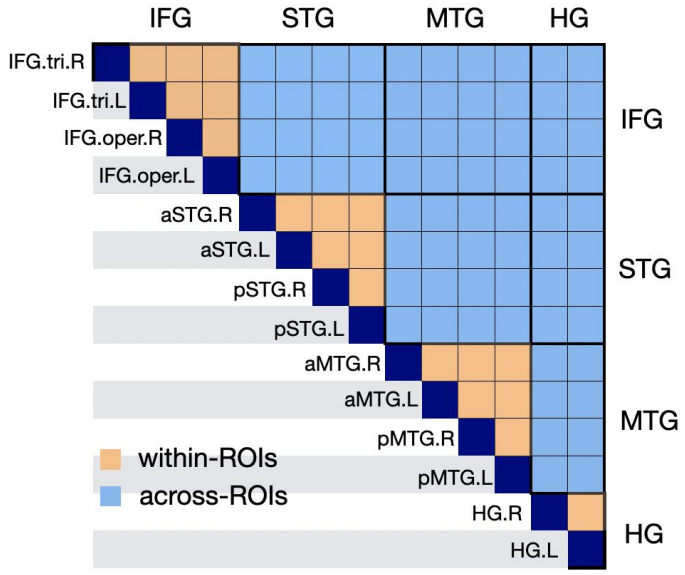
(A) Group comparisons computed via one-way ANOVAs yielded one outlier in the nvASD group for STG-MTG connectivity who was kept in the analysis as similar results were yielded after excluding the outlier. An outlier in IFG-HG was found both for the TD and vASD groups, but data were normally distributed. A non-normal distribution for IFG-STG pair in TD. Again, non-parametric tests yielded similar results.

(B/1) To test for differences between conditions in nvASD, a series of paired-samples t-tests were used. All pairs in both contrasts (rest/FWD and FWD/BWD) followed a normal distribution, except HG-MTG in FWD/BWD, yet non-parametric tests yielded similar results.

(B/2) Additionally, in light of the findings, the STG-MTG pair was compared to HG-STG since both represent spatially adjacent regions, at rest, FWD, and BWD. A series of paired-samples t-tests were used, given normal distributions. FC for STG-MTG was also compared to the connectivity within STG, via a paired-sample t-test, to contrast its functional differentiation to the connectivity found within this functional region.

(B/3) Finally, the preservation of fronto-temporal coupling was assessed by calculating the difference of IFG-HG, IFG-STG and IFG-MTG connectivity from zero, which was done via a series of one-sample t-tests given normal-distributions.

**Figure 4.2:** Visual representation of the core language network connectivity matrix indicating how within/across-ROIs and individual ROI pairs were calculated.




## 4.3 Results

### 4.3.1 Pattern similarity analysis

To evaluate the heterogeneity of FC across subjects of the nvASD group, the pattern of similarity of whole-brain and core language network FC was evaluated, first, by calculating average similarity of one nvASD subject to other nvASD subjects, and then their respective similarity to other TD and vASD control subjects.

The similarity analysis of FC across whole-brain networks showed the nvASD group (mean z = 0.664, SD = .08, range = .56 - .78) to have a more homogeneous pattern than the TD (mean z = 0.61, SD = .05, range = .50 - .67) and vASD groups (mean z = 0.54, SD = .04, range = .48 - .59) had among themselves. This difference in in-group homogeneity was significant between nvASD and vASD (Cohen's $d$ = 1.974, $p$ = .003), but failed to show significance in comparison to the TD group (Cohen's $d$ = .843, $p$ = .169). Additionally, a subject with nvASD was on average significantly less similar to any TD subject (mean z = 0.52, SD = .08, range = .37 - .61) than the TD subjects were to each other (mean z = 0.61), Cohen's $d$ = -1.333, $p$ = .021. However, the dissimilarity of the nvASD group to the vASD group (mean z = 0.51, SD = .06, range = .4 - .61) was not significantly different from that of vASD subjects to each other (mean z = 0.54), Cohen's $d$ = -.565, $p$ = .329.

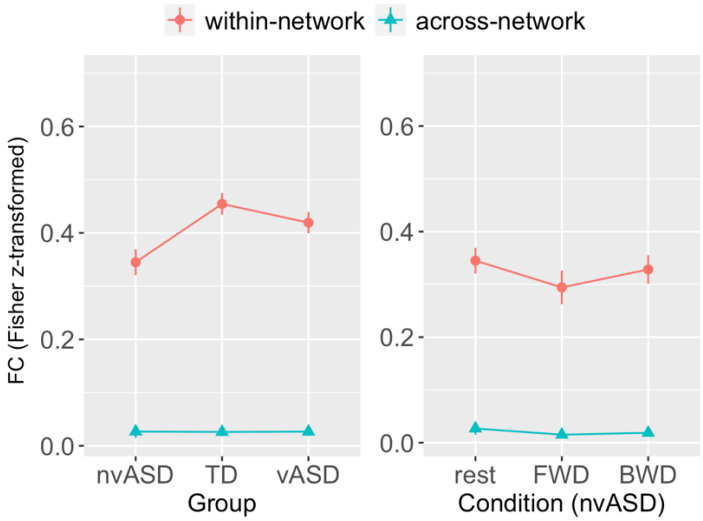
The core language-network FC similarity analysis again revealed that the nvASD group (mean z = 0.87, SD = .11, range = .72 - .99) presented a higher homogeneity of FC pattern than the FC pattern of TD (mean z = 0.6, SD = .08, range = .5 - .71) or vASD (mean z = 0.52, SD = .1, range = .33 - .66) groups. This homogeneity was significantly more pronounced in the nvASD group compared to both TD (Cohen's $d$ = 2.821, $p$ = .003) and vASD (Cohen's $d$ = 3.220, $p$ = .003). Additionally, a subject with nvASD was on average significantly more similar to any vASD subject (mean z = 0.63, SD = .06, range = .54 - .72) than vASD subjects were among each other (mean z = 0.52), Cohen's $d$ = 1.309, $p$ = .021. Importantly, nvASD were not significantly more similar to TD subjects (mean z = 0.64, SD = .06, range = .54 - .74) than TD subjects were to each other (mean z = 0.6), Cohen's $d$ = .440, $p$ = .396.

### 4.3.2 Within-networks and across-networks FC of whole brain networks

To provide a basis for overall functional segregation vs. integration of the networks across the whole brain, patterns of across-networks and within-networks connectivity between groups, and between conditions in nvASD are shown in Figure 4.3. There was a statistically significant interaction between groups and within/across-networks connectivity, $F(2,21) = 7.246$, $p = .018$, partial $\eta^2 = .408$. The difference across groups was significant for within-networks connectivity, $F(2,21) = 6.716$, $p = .018$, partial $\eta^2 = .408$, but not for across-networks connectivity, $F(2,21) = .002$, $p = .998$, partial $\eta^2 < .001$. Data are mean ± standard error, unless otherwise stated. The within-networks connectivity was significantly lower in the nvASD group compared to the TD group (-.11 ± .031, $p = .018$), and while also lower compared to vASD, the difference did not reach statistical significance (-.074 ± .031, $p = .089$). The difference in within-networks connectivity between TD and vASD was also not significant (-.04 ± .031, $p = .556$). Data from the two TD subjects scanned under the same protocol are found in Figure 4.S1 in the Supplement showing higher within-network FC than the SDSU TD mean.

Turning to the FC in the nvASD group across FWD and rest conditions, the interaction between conditions and within/across-networks connectivity was significant before correction, $F(1,7) = 6.399$, $p = .039$, partial $\eta^2 = .478$, but marginally outside of the significance boundary after FDR correction ($p = .07$). While the within-networks connectivity was -.051 (95% CI, -0.97 to -0.004) significantly lower during FWD than rest, $F(1,7) = 6.606$, $p = .037$ (again, $p = .07$ after FDR correction), the across-networks connectivity was comparable between conditions, $F(1,7) = 1.025$, $p = .444$.

**Figure 4.3:** Fisher z-transformed Pearson correlation coefficients (mean + SE) of between-network and within-network connectivity of the whole-brain network across groups, and across conditions in nvASD.



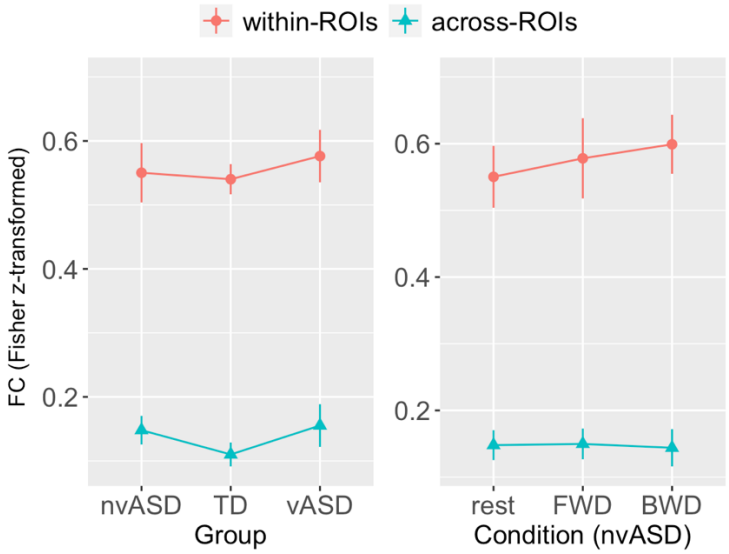

### 4.3.3 Within-ROIs and across-ROIs FC of the core language network

The following analyses zoom in onto the core language network in its overall functional segregation and integration pattern. Figure 4.4 shows across-ROIs and within-ROIs connectivity of the language network between groups, and between conditions in nvASD. No significant interaction was found between groups and within/across-ROIs connectivity, $F(2,21) = .284$, $p = .907$, partial $\eta^2 = .026$, nor any statistically significant main effect of group on within/across-ROIs connectivity, $F(2,21) = .657$, $p = .794$, partial $\eta^2 = .059$. Data from the two TD subjects scanned under the same protocol were comparable to the SDSU TD mean (Figure 4.S2 in the Supplement).

There was no statistically significant interaction between FWD and rest conditions and within/across-ROIs connectivity, $F(1,7) = 1.350$, $p = .752$, partial $\eta^2 = .162$; and no statistically significant main effect of rest and FWD on within/across-ROIs connectivity, $F(1,7) = .894$, $p = .752$, partial $\eta^2 = .113$.

Similarly, no statistically significant interaction transpired between FWD and BWD conditions and within/across-network connectivity, $F(1,6) = .10$, $p = .923$, partial $\eta^2 = .002$; and no statistically significant main effect of FWD and BWD on within/across-ROIs connectivity, $F(1,6) = 3.177$, $p = .75$, partial $\eta^2 = .346$.

**Figure 4.4:** Fisher z-transformed Pearson correlation coefficients (mean + SE) of between-ROI and within-ROI connectivity of the language network across groups, and across conditions in nvASD.




### 4.3.4 FC of individual ROI pairs of the core language network

To estimate the hierarchical organization of the language network, FC between core language ROI pairs was estimated. Figure 4.5 shows the FC matrix of the core language network. Figure 4.6 represents individual ROI-pair FC as mean + standard error. Data (mean ± standard deviation) and test statistics for differences between groups on each ROI pair's FC appear in Table 4.2. Data from the two TD subjects scanned under the same protocol were comparable to the SDSU TD mean (Figure 4.S3 in the Supplement) apart from HG-MTG FC that was considerably higher in the two control subjects.

Secondly, data (mean ± standard deviation) and test statistics for differences between conditions in the nvASD group and, in particular, for FWD vs. rest and FWD vs. BWD contrasts, appear in Table 4.3. Significant group and FWD-rest differences transpired in STG-MTG connectivity and are further elaborated on below. No significant differences in ROI pair connectivity were observed between FWD and BWD conditions.

**Figure 4.5**: BOLD time series Pearson correlations (Fisher z-transformed) between 14 ROIs of the core language network at rest in nvASD, vASD and TD. Warmer colours correspond to stronger positive correlations. IFG = Inferior frontal gyrus, STG = Superior temporal gyrus, MTG = Middle temporal gyrus, HG = Heschl's gyrus.

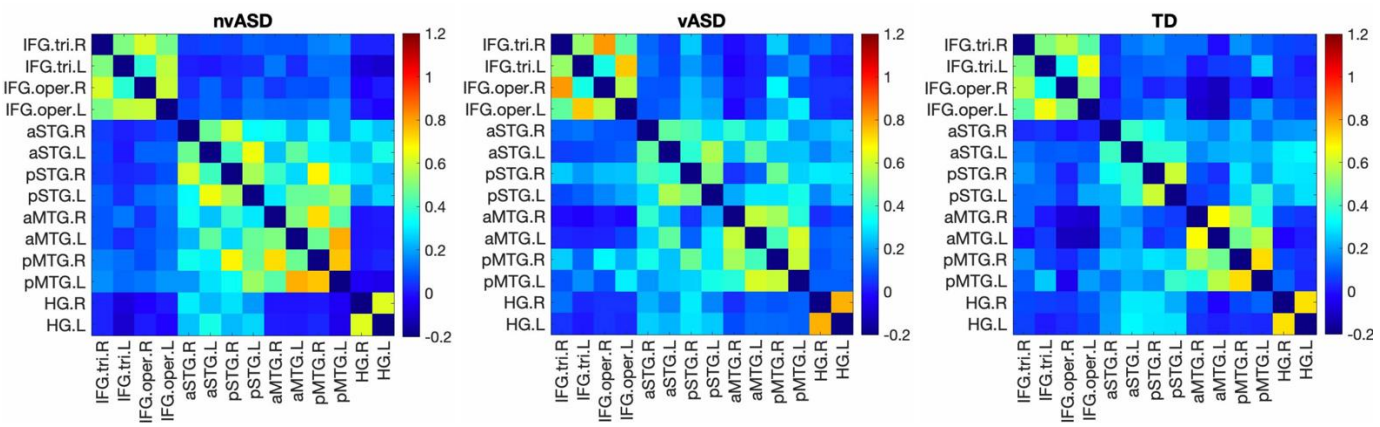



**Figure 4.6**: Mean FC and SE of individual ROI pairs of the language network across groups, and across conditions in the nvASD group.

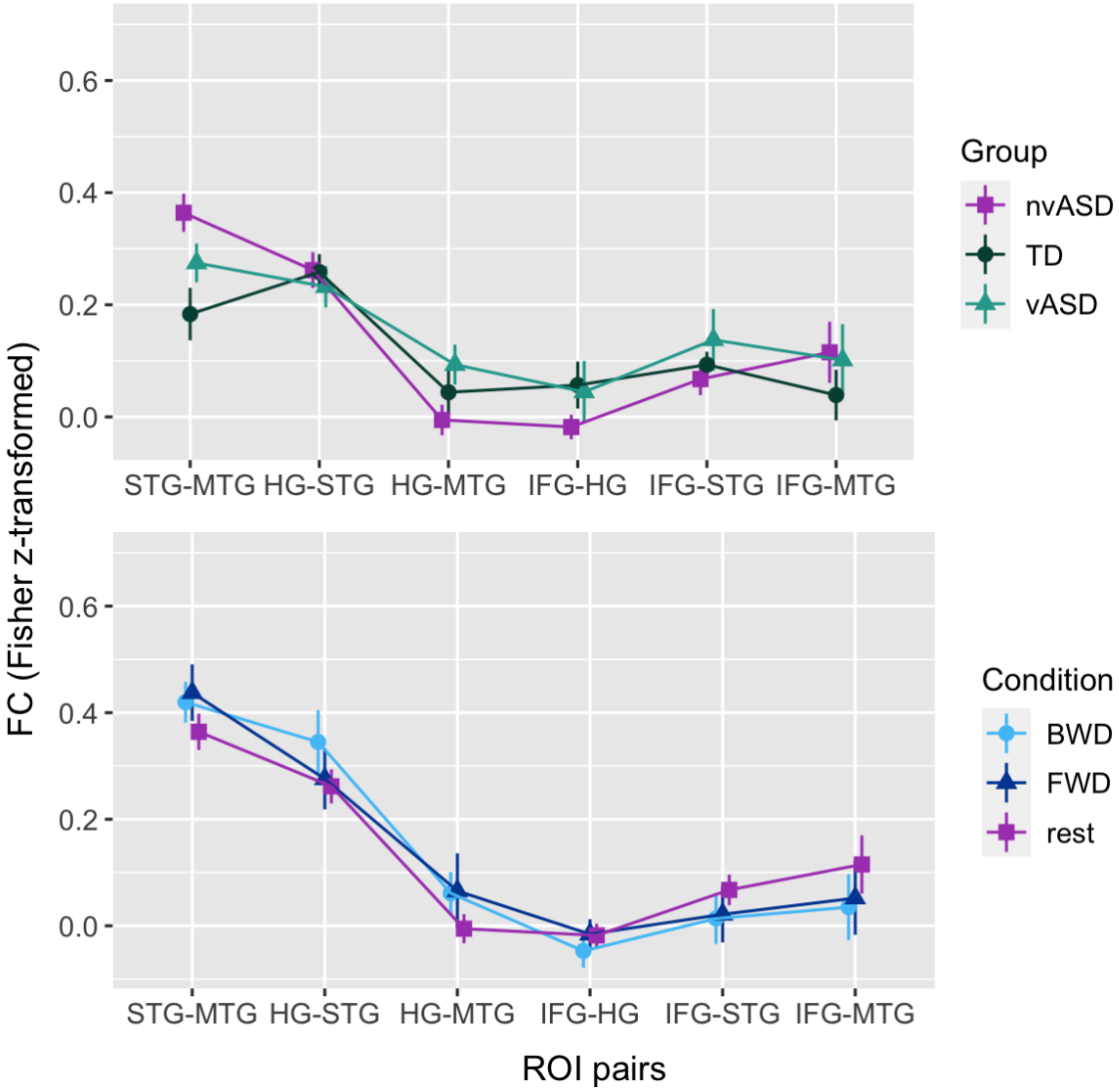


**Table 4.2:** Fisher z-transformed Pearson correlation coefficients (mean ± standard deviation) of individual ROI pairs of the language network across groups at rest, and one-way ANOVA results.

| ROI pairs | Mean ± standard deviation | | | One-way ANOVA | | |
|---|---|---|---|---|---|---|
| | **nvASD** | **vASD** | **TD** | **F (2,21)** | **p** | **partial $\eta^2$** |
| **IFG-HG** | -.02 ± .06 | .04 ± .17 | .06 ± .12 | .914 | ns | .08 |
| **IFG-STG** | .07 ± .08 | .14 ± .16 | .09 ± .07 | .862 | ns | .076 |
| **IFG-MTG** | .16 ± .15 | .01 ± .18 | .04 ± .13 | .542 | ns | .049 |
| **HG-STG** | .26 ± .09 | .23 ± .1 | .26 ± .09 | .232 | ns | .022 |
| **HG-MTG** | -.01 ± .08 | .09 ± .1 | .04 ± .11 | 2.029 | ns | .162 |
| **STG-MTG** | .36 ± .1 | .27 ± .1 | .18 ± .13 | 5.37 | **.013/.052** | .338 |

*p* > .05 (ns)

**Table 4.3**: Fisher z-transformed Pearson correlation coefficients (mean ± standard deviation) of individual ROI pairs of the language network across conditions, and results of paired-samples t-tests for FWD-rest and FWD-BWD contrasts.

| ROI pairs | Mean ± standard deviation | | | Conditions: paired-samples t-test | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | FWD vs. rest | | | FWD vs. BWD | | |
| | **rest** | **FWD** | **BWD** | **t (7)** | **p** | **d** | **t (6)** | **p** | **d** |
| **IFG-HG** | -.02 ± .06 | -.01 ± .09 | -.05 ± .08 | .054 | ns | .12 | .590 | ns | .15 |
| **IFG-STG** | .07 ± .08 | .04 ± .15 | .01 ± .13 | -1.1 | ns | .12 | .457 | ns | .14 |
| **IFG-MTG** | .16 ± .15 | .06 ± .21 | .04 ± .16 | -1.96 | ns | .09 | .446 | ns | .14 |
| **HG-STG** | .26 ± .09 | .31 ± .15 | .35 ± .16 | .373 | ns | .11 | -.648 | ns | .15 |
| **HG-MTG** | -.01 ± .08 | .08 ± .21 | .06 ± .11 | 1.33 | ns | .15 | .196 | ns | .19 |
| **STG-MTG** | .36 ± .1 | .48 ± .11 | .42 ± .1 | 2.43 | **.045**/.565 | .09 | 1.43 | ns | .11 |

*p* > .05 (ns)

*Differences in FC between temporal ROI pairs*

STG-MTG connectivity was statistically different between groups, $F(2,21) = 5.37$, $p = .013$ (although this effect was marginal after FDR correction $p = .052$), partial $\eta^2 = .338$. STG-MTG

connectivity was higher in the nvASD group (.36 ± .1) than in vASD (.27 ± .1) and TD (.18 ± .13). Tukey post hoc analysis showed that a mean difference in STG-MTG FC between nvASD and TD (.181; 95% CI, .048 to .314]) was statistically significant, $p$ = .032. No other group differences were statistically significant. Evaluating the effect of auditory stimulation, STG-MTG connectivity was higher during FWD (.48 ± .11) than rest (.36 ± .1), an increase of .073 (95% CI, .002 to .145), $t(7) = 2.43$, $d$ = .09, significant at $p$ = .045, yet $p$ = .565 after FDR correction. To elucidate such a pattern of hyper-synchrony, further analyses were run.

To test whether this difference was not conditioned mainly by spatial adjacency of STG and MTG, the difference in FC between STG-MTG and HG-STG (another spatially neighbouring ROI pair) was tested. STG-MTG had a significant higher FC at rest (.1 ± .1; 95% CI [.04 to .16]) than HG-STG, $t(7) = 3.923$, $p$ = .006, Cohen's d = 1.4. The difference between STG-MTG and HG-STG, however, disappeared during BWD condition (.07 ± .2; 95% CI [-.11 to .26]), $t(6)$ = .989, $p$ = .364, Cohen's d = 0.2.

I next tested how comparable the hyper-synchrony between STG-MTG in nvASD is to the connectivity within STG. The intra-STG and STG-MTG connectivity at rest were not significantly different from each other (.1 ± .14.; 95% CI [-.24 to .03]), $t(7) = 1.95$, $p$ = .099, Cohen's d = 0.14. Under FWD, however, the intra-STG FC was significantly higher than the one between STG-MTG (.12 ± .1; 95% CI [-.04 to .2]), $t(7) = 3.46$, $p$ = .01, Cohen's d = 0.1.

*Fronto-temporal ROI pairs*

Given results from studies on FC under sedation, the IFG-HG and IFG-STG / IFG-MTG mean connectivity was tested against zero. Mean IFG-HG connectivity (-0.2 ± 0.6) was not significantly different from zero (-0.2, 95% CI -0.7 to 0.3), $t(7) = -.82$, $p$ = .439, d = 0.6. Mean IFG-STG (0.07 ± 0.8) and IFG-MTG (0.16 ± 0.15) connectivity represented a difference of .07 (95% CI, -0.001 to 0.14) and 0.16 (95% CI, -0.01 to 0.24) from zero, respectively. This difference narrowly missed significance $t(7) = 2.36$, $p$ = .05, d = 0.08, and $t(7) = 2.11$, $p$ = .07, d = 0.15, respectively.

## 4.4 Discussion

Here I explored for the first time the brain functional organization in nvASD by assessing functional connectivity (FC) within and across brain networks with a particular focus on the

canonical language network. In particular, I compared FC at rest in 8 nvASD to typically developing (TD) and verbal autism (vASD) control groups, and additionally explored FC in nvASD under auditory stimulation to better ascertain effects of sedation as propofol induces differential but complementary changes in resting and stimulation states.

*Similarity of FC patterns among nvASD individuals*

In line with predictions, the connectivity patterns across whole brain networks and the language network were more homogeneous among the nvASD subjects than they were among subjects of the control groups. This finding has implications for the clinical accuracy of the behaviourally based definition of nvASD: children with 'some words' may pattern similarly along a wider range of language and cognitive abilities (Bal et al., 2016). It also further speaks to the issue of the variability in receptive language in particular. The sample of this study fell at both ends of the range of cognitive abilities reported in the previous chapter, which was determined from a larger cohort of 49 child and adult participants. Yet any variance in the functional organization of the core language network that these cognitive differences may have implied, was less than the variance intrinsic to the vASD group (and perhaps less surprisingly also to the TD group). This may suggest that constraints on the language faculty imposed by a lack of grammatical endowment defies the pervasive heterogeneity that has been so widely noted in other parts of the ASD spectrum. These conclusions are only suggestive, however, since a plausible alternative explanation, namely sedation as driving the higher homogeneity among the nvASD subjects, cannot be excluded based on the current study design. Still, analyses later presented in this thesis of five children with diverse diagnoses and severely impaired language show consistently *diverse* patterns of FC under the same sedation procedure and protocol.

*Similarity of the nvASD FC patterns to the control groups*

The whole-brain functional connectome that transpired in nvASD was, unsurprisingly, significantly dissimilar to the neurotypical one, above and beyond the dissimilarity induced by the more heterogeneous pattern among the TD subjects. Importantly, the whole-brain functional organization of the nvASD group was not significantly dissimilar to the vASD group above and beyond greater heterogeneity in the vASD group. The vASD and nvASD groups may therefore

share atypical functional patterns of whole-brain organization that could underlie the shared behavioural diagnosis.

The exploration of the language network similarity between groups resulted in some unanticipated patterns. Specifically, and strikingly, the nvASD subjects were not significantly more dissimilar to the TD subjects above and beyond the heterogeneity of the TD subjects among each other. While the heterogeneity among the TD group in the core language network remained roughly the same as for the whole-brain networks, the nvASD subjects' language network was slightly more similar to the TD one than was the whole-brain network, resulting in a lack of significant difference. Even more surprisingly, the nvASD subjects and vASD subject were more alike than the vASD group was to each other. This is surprising given the fact that it is the language functioning that strikingly differentiates the nvASD group from both control groups. To clarify where these differences or the lack thereof arise from, the following analyses focused on differences between groups and, additionally, differences between conditions in nvASD, pinpointing particular aspects of the network connectivity.

*Whole-brain networks: the integration and segregation balance*

We have learned so far that whole-brain network organization in nvASD was prominently different from the neurotypical one, but not from the vASD one. This subsection explores average integration inside brain network subregions (within-network FC) and functional segregation of the networks relative to each other (across-network FC). This analysis was primarily used as a starting point to evaluate FC changes in nvASD against the background of alterations that would be expected under sedation.

The average connectivity within brain networks at rest was markedly reduced in nvASD with respect to both control groups (a moderate effect of .04), although a significant reduction transpired only in comparison to the TD circuity. No significant difference was observed for across-network FC. Previous studies of FC in lower-functioning ASD (Gabrielsen et al., 2018; Reiter et al., 2019) have already reported reduced within-network connectivity in several large-scale brain networks at rest. The present results show that there might be a gradual loss of functional coupling within subregions of individual networks in ASD, more prominent at the lower end of the spectrum. The segregation among these networks, on the other hand, was not significantly deviant from neurotypical one in both nvASD and vASD, despite previous findings

in high-functioning autism (Hull et al., 2017) and initial evidence in low-functioning autism (Gabrielsen et al., 2018). In this study, the differences in the across-network FC, if they exist, might not be prominent enough to transpire in a small sample size and after averaging the FC across all networks.

Crucially, decreased within-network FC coupled with preserved across-network FC at rest has been reported in neurotypical adults in Naci et al. (2018) as occasioned by deep propofol sedation – a pattern that would match the one observed here for the nvASD cohort. Yet, a sedated brain under complex auditory stimulation was shown in that study to overcome the relative inhibitory effect on information transfer within brain networks that is characteristic of the resting state. Thus, in Naci et al., within-network FC under auditory stimulation remained comparable during sedation to the wakeful state, but the processing cascade across brain networks was blurred via increasing across-network interaction. In this particular respect, FC in nvASD as observed here diverges from a pattern linked to sedation. Somewhat paradoxically, the within-network integration further *decreased* during forward speech (FWD) relative to rest (a moderate effect of .478, marginally significant after FDR correction), again with no effect on across-network connectivity. This reveals a more specific effect in the nvASD group, as it is not only confined to comparisons between groups, but sensitive to modulation during auditory stimulation in a way unpredicted by sedation effects.

*The segregation-integration balance of the core language network in nvASD*

Turning to the core language network, a particular instance of within-network FC, an altered network integrity would be expected as was observed throughout the brain. Yet, in line with the similarity analysis results reviewed above, no significant difference in average FC within and across language network ROIs was observed between groups. This is highly remarkable as this study involves individuals at the lowest known end of the spectrum of language capacities in humans. Even if this lack of significant difference was a result of sedation that might mask real differences, we might expect a differential effect of this factor under task conditions (Naci et al., 2018) as was the case in whole-brain networks, which was, however, not observed here. One possibility is that uneven differences between pairs of language ROIs would cancel any gross, averaged effects at the whole-network level. Indeed, connectivity between primary auditory cortex (Heschl's gyrus; HG) and the inferior frontal gyrus (IFG) has been found to be abolished in

neurotypical adults (Liu et al., 2012), with FC of other fronto-temporal and temporal ROI pairs reduced, yet preserved. I therefore took a close-up view, where a specific pattern transpired in the hierarchical organization of the language network at the level of individual ROI pairs, to be discussed next.

*The connectivity among pairs of language regions*

Previous studies of the language network in high-functioning ASD have observed specific ROI-pair FC alterations characterized by underconnectivity (Just et al., 2004; Kana et al., 2006; Knaus et al., 2008; Verly et al., 2014a), overconnectivity (Gao et al., 2019), or a mixed pattern of both (Lee et al., 2017). In the present study, the fronto-temporal ROIs connectivity did not result in a significant difference between groups or conditions. The temporal ROIs connectivity, however pointed to an irregularity along the language-processing hierarchy. In particular, among the temporal ROI pairs, STG and MTG showed high correlation of the BOLD signal (hyper-synchrony) in the nvASD group at rest, an unprecedented change under sedation (Boveroux et al., 2010; Gómez et al., 2013). Under auditory stimulation (FWD), this hyper-synchrony once more intensified. Such a hyper-synchrony might have primarily contributed to the fact that language network connectivity diverged from the global loss of within-network integrity at the whole-brain level seen in this study.

Proceeding first with the long-range fronto-temporal connectivity, despite the absence of IFG activations in task-based studies under sedation, intrinsic FC from IFG as seed to STG and MTG (but not HG) has been reported as preserved (Liu et al., 2012). In the present study, in nvASD at rest, IFG-HG was indeed not different from zero, while in the other two fronto-temporal pairs a positive difference from zero equalled a smaller effect size of $d$ = 0.08-0.15 that narrowly missed significance. Out of these pairs, IFG-MTG showed a trend of *increased* connectivity specifically at rest, in nvASD compared to both TD and vASD, although, again, a smaller effect size of $\eta^2$ = 0.049 may have not amounted to a significant difference. A positive trend limited to the resting state is particularly interesting in light of generally deflating effects of sedation particularly on the fronto-temporal FC (Adapa et al., 2014). It also points in the opposite direction of a decrease in FC between Broca's and Wernicke's area as has been previously related to lower language performance in vASD (Verly et al., 2014). Further studies in larger samples are needed to clarify the observed trends especially due to the status of the IFG as a higher-order region for syntactic

and semantic integration of information feed-forwarded from temporal cortices (Adapa et al., 2014; Friederici, 2012). Thus, the structural backbone of the fronto-temporal connections, and specifically the arcuate fasciculus – a long-range tract interconnecting posterior temporal lobes to IFG – has shown a markedly atypical pattern consisting of lateralization reversal in four out of five nvASD children (Wan et al., 2012).

Similarly to the fronto-temporal connectivity, FC under deep propofol sedation in temporal regions decreases, but is not abolished (Gómez et al., 2013; Liu et al., 2012; at rest Boveroux et al., 2010; François et al., 2016). Contrary to an expected sedation-induced decrease of connectivity, in this study, nvASD participants displayed marked hyper-synchrony relative to control groups at rest, which was specifically confined to STG-MTG and not seen between HG and these two regions. Such a hyper-synchrony is remarkable given the lack of sedation in the control groups. STG-MTG FC was significantly higher compared to HG-STG, a pattern that, therefore, transcends the ROIs spatial proximity. The hyper-synchrony of STG-MTG represented a considerable effect size ($\eta^2$ = .338), which remained marginally significant after FDR correction. In fact, a graded increase in STG-MTG connectivity could be observed from TD to vASD to nvASD, although the difference yielded significance only between nvASD and TD.

*The significance of hyper-synchrony at a particular stage in cortical language hierarchy*

Hyper-synchrony between two subsystems may signal a breakdown of the functional differentiation of processes implemented in these regions. A hyper-interaction could disrupt the feed-forward deconstruction and propagation of information along the cortical hierarchy (Naci et al., 2018). Indeed, during forward speech compared to rest, the STG-MTG hyper-synchrony further intensified, although the difference did not survive local FDR significance correction. Speculatively, an increase during FWD might suggest that the particular FC pattern in fact reflects previous histories of co-activation between these two regions. Accordingly, it might reflect limited pruning of initial developmental over-connectivity that otherwise guides functional segregation of information processing (Cao et al., 2017) in the establishment of the cortical hierarchy (Teissier & Pierani, 2021). The FC at any level of cortical language pairs was not significantly distinct for FWD than it was for BWD, in spite of altered lower-level, phonological and prosodic speech characteristics of BWD (Ahmad et al., 2003). Future studies specifically estimating causal neuronal dynamics (Zarghami & Friston, 2020) between regions of the language network in

response to these auditory stimuli should further elucidate the extent of differential cross-regional interactions.

Together, these findings suggest a marked loss of functional differentiation particularly along a specific level of the hierarchy of auditory language processing (Binder et al., 2000; Davis & Johnsrude, 2003; de Heer et al., 2017; Friederici, 2012; Rauschecker, 2018; Sheng et al., 2019), where STG and MTG play a central role. STG hosts the anterior- and posterior-running auditory streams that encode acoustic-phonological aspects of speech (Kumar et al., 2007; Rauschecker & Scott, 2009) coming from primary auditory cortex. Further extending laterally, the superior temporal sulcus responds consistently to speech intelligibility (Friederici et al., 2010; Obleser et al., 2007). Along with the STG, anterior and posterior MTG activation is incorporated to support further semantic and syntactic processing underlying word and sentence formation (Binder et al., 2009; Friederici, 2011, 2012; Sheng et al., 2019; Xu et al., 2015). From the temporal and parietal cortices, information is forwarded to the IFG for further semantic and syntactic processing giving rise to the fronto-parieto-temporal language network (de Heer et al., 2017; Kocagoncu et al., 2017; Tyler & Marslen-Wilson, 2008) as organized along dorsal and ventral processing streams (Hickok & Poeppel, 2004).

The observed STG-MTG hyperconnectivity both at rest and under auditory stimulation thus suggests a blurring of functional differentiation between STG and MTG that could severely disrupt early stages in speech processing, in particular those involved in semantic-syntactic decoding of speech crucial to access and extract meaning. MTG is moreover an important cortical focal point with rich structural and functional connectivity to a distributed set of cortical association areas (Xu et al., 2015, 2019), including the ‘social brain’ network (Xu et al., 2020). On the other hand, the seemingly preserved functional differentiation between HG and STG both during rest and FWD suggests an integration-segregation balance between the two regions that might point to preserved primary auditory processing in nvASD. In line with this interpretation is a recent ERP study of late pre-school nvASD (Cantiani et al., 2016), which reports spared auditory processing alongside altered lexico-semantic processing.

In conclusion, this study is the first to illuminate neural functional organization of the language network at this most severe end of both the language and autism spectra. Taken from this perspective it is noteworthy that the question to be further addressed is not that of *whether* but rather *how*. In other words, severe language impairment need not be characterized by a general

absence of cross-regional functional interactions. Instead, a particular disruption has transpired at a key location of the network: an increased coupling of both the intrinsic and the evoked activity between two regions attributed to higher-level stage of auditory language processing. The neural integration at lower-level stages, between primary and secondary auditory regions, on the other hand, did not show significant alterations. While FC is a rough measure of functional integration, it can nevertheless illustrate a preservation of a basic auditory template for processing whose sensibility to auditory stimuli should further be tested.

## 4.5 Limitations

Several limitations complicate the interpretation of the study findings. The sample size recruited in this study was very limited, which hinders detectability of smaller effects of interest and generalization of the present findings. Moreover, the data from the comparison groups were not obtained under sedation and from a different MRI scanner and site. However, FC analysis is less sensitive to different scanners and sites (Zuo & Xing, 2014) than analysis of functional activation since FC is an inter-subject measure not dependent on BOLD magnitude. Indeed, two TD control subjects scanned under the same site and protocol conditions, generally patterned within the FC ranges of the SDSU TD control group. This was especially the case of the language networks, and in particular of STG-MTG connectivity, which are central to this study's findings. Furthermore, we observed a differentiated pattern of highly specific and functionally confined alterations of brain organization in nvASD, which speaks against a generalized effect of sedation on the FC patterns observed; and the effects observed in nvASD in comparison to the control groups were further accentuated in the comparison across conditions in the nvASD group. Application of MRI scanning under sedation has, furthermore, permitted to obtain high quality data void of confounds associated with short acquisition times (Birn et al., 2013; Lai et al., 2012) or extraction of volumes with high degree of movement (Gabrielsen et al., 2018).

## 4.6 Supplementary information

**Figure 4.S1:** Mean within/across-network FC of 2 TD controls scanned under the same site/protocol conditions as nvASD subjects. Their whole-brain network FC is visualized against the distribution of FC values of SDSU TD cohort. Their within-network FC fell outside two standard deviations of the SDSU TD mean, while the across-network FC was slightly outside the SDSU TD mean in one case.

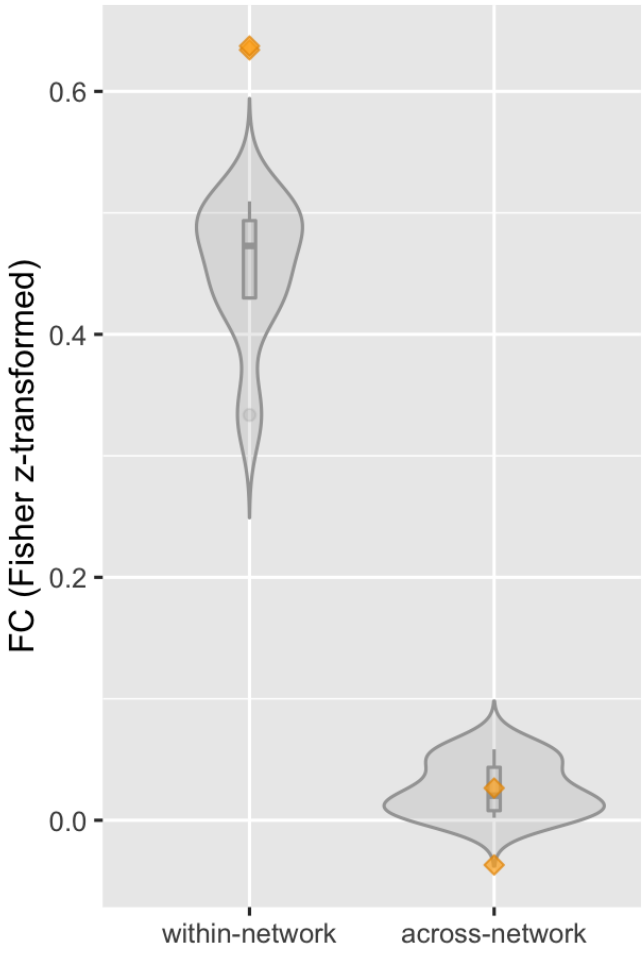


**Figure 4.S2:** Mean within/across-network FC of 2 TD controls scanned under the same site/protocol conditions as nvASD subjects. Their language network FC is visualized against the distribution of FC values of SDSU TD cohort. Their within-ROIs FC was within two standard deviations of the SDSU TD mean, and the across-ROIs FC was slightly above two standard deviations from the SDSU TD mean in one case.

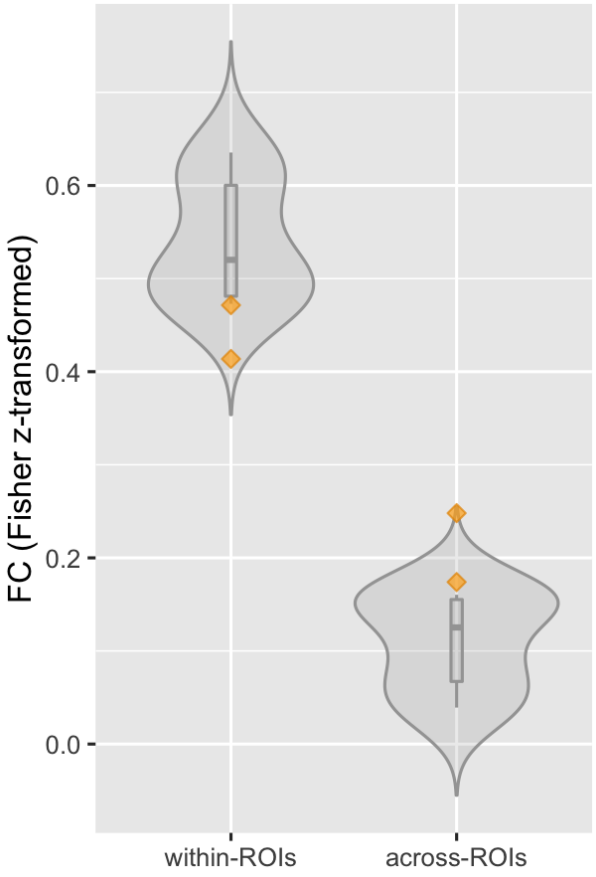
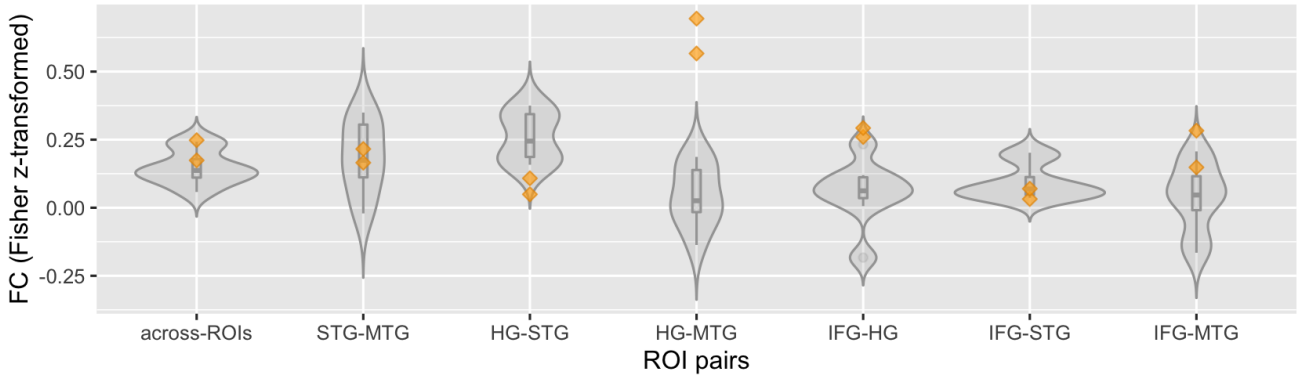


**Figure 4.S3:** Mean FC of the language network ROI-pairs in 2 TD controls scanned under the same site/protocol conditions as nvASD subjects. Their FC scores are visualized against the distribution of FC values of SDSU TD cohort. Their fronto-temporal FC was within two standard deviations of the SDSU TD mean. For the temporal lobe FC, HG-MTG connectivity was far above the SDSU TD mean, but HG-STG and STG-MTG connectivity fell within two standard deviation of the SDSU TD mean.



# 5. Lower-level auditory processing of speech in nvASD

## 5.1 Introduction

When delays in reaching infant language milestones become apparent, a referral for hearing loss screening is the first step to be made. In nvASD, and ASD, serious auditory difficulties such as hearing loss are generally not found. Atypical auditory processing has nevertheless been reported across several neurodevelopmental disorders including DLD or schizophrenia (Damaso et al., 2015; Lense et al., 2021; Roberts et al., 2012), and more extensively in ASD (Schwartz et al., 2018, for review). Even at the highest-functioning end of the spectrum, ASD individuals with no apparent language difficulties, formerly diagnosed with Asperger syndrome, show reduced discrimination and orientation to speech (Kujala et al., 2010; Lepistö et al., 2007). On the opposite site of the ASD spectrum, in nvASD, basic auditory perception remains preserved, albeit with an atypical pattern.

Cantiani et al. (2016) assessed the neural response to a speech event in 10 minimally verbal pre-school and school-aged ASD children. The corresponding electrophysiological potential showed a similar amplitude to neurotypical children, but a markedly delayed latency. In a more complex auditory processing paradigm targeting speech sound discrimination, Matsuzaki et al. (2019) equally demonstrated a preserved, but markedly delayed response latency of the mismatch negativity potential (MNN) in nvASD. This latency delay along bilateral STG gradated significantly from TD to verbal ASD to language-impaired ASD up to nvASD children. In this study, an abnormal rightward lateralization of the MNN potential was also found across all ASD subgroups and correlated with communication skills and general cognitive ability.

On their own behalf, findings from the previous chapter point to a preserved differentiation of the resting state auditory network encompassing the primary auditory cortex and STG in nvASD relative to the control groups at rest. This pattern may likewise serve as a gross indicator of a functional auditory template in place yet falls short of capturing irregularities in downstream processing of auditory information. Against this background, the present study sought to go beyond intrinsic functional connectivity as explored in the previous chapter and examine more concretely the unfolding of auditory processing in response to complex linguistic stimuli. Here the functional properties of the auditory network were examined at the individual level (across 10

nvASD individuals), beyond the group-averaged patterns, to shed light on how the constitution of a functional auditory network relates to the differences in the individual language abilities in nvASD. In particular, I assessed the pattern of evoked activity to speech and its topographical agreement with an intrinsic auditory network that was independently reconstructed from resting-state using independent component analysis (ICA). Both of these functional brain aspects have been shown to be preserved under sedation (for low-level auditory response to language under sedation see Dueck et al., 2005; Liu et al., 2012; Plourde et al., 2006, and for intrinsic resting-state networks, see Bisdas et al., 2016; Doria et al., 2010).

Originating prenatally, spontaneous or intrinsic correlated activity is thought to constitute a template of the characteristic functional circuity for processing of upcoming inputs (see Martini et al., 2021; Teissier & Pierani, 2021, for review). Thus, even before the opening of the sensory channels, endogenous firing of neurons self-organizes into transient circuits (Vasung et al., 2019, for review). Towards the end of the second trimester, a spatially independent component encompassing the STG is already observable (Schöpf et al., 2012). A crucial step towards the emergence of sensory-driven correlated activity is made with the establishment of thalamo-cortical afferents at the beginning of the third trimester (Kostović & Jovanov-Milošević, 2006). Environmental sounds for adjustment of the circuity, in turn, are thought to be available starting from the onset of hearing at the 29$^{th}$ gestational week (Borsani et al., 2019). At this stage, changes in cardiac frequency start to reveal fetal ability to process sound (Kisilevsky et al., 2000).

The emerging intrinsic functional architecture is organized modularly, showing dense connectivity within subsets of neurons, brain regions, and circuits (Edde et al., 2021; Thomason et al., 2014; Vasung et al., 2019). The early version of the resting-state auditory network appears to be circumscribed unilaterally, in either the left or right hemisphere (Schöpf et al., 2012), as the fetal cross-hemispheric connectivity of Heschl's gyrus still needs to be established (Thomason et al., 2013). Unilateral (right-hemispheric) or weaker auditory modules are also visible in prematurely born infants (Doria et al., 2010; Smyser et al., 2010) whose speech-evoked activity is likewise unilateral, extending along the temporal and supramarginal gyrus in the left hemisphere. This suggests a consistency between the early emerging template and its sensitivity to auditory stimulation. The activity then extends towards the frontal lobe and contralateral hemisphere at term-equivalent age (Baldoli et al., 2015). Shortly after full-term birth, new-borns' left temporal cortex also responds more to forward speech (FWD) than it does to backward speech (BWD)

(Bartha-Doering et al., 2019; Peña et al., 2003) or non-native forward speech (Sato et al., 2012; Vannasing et al., 2016; but see May et al., 2011) only a few days after birth. A significant leftward asymmetry in processing of speech-like stimuli has therefore been taken as an early hallmark of the developing brain.

Hemispheric lateralization is a ubiquitous organizational principle of the brain from structure to function, frequently going awry in psychiatric and neurodevelopmental disorders (Kong et al., 2020). Hemispheric asymmetries are thought to exemplify the boosting of brain efficiency and adaptability for specific stimuli (for review, see Güntürkün et al., 2020). In the realm of auditory processing, left-hemispheric predominance of auditory speech processing (Hirnstein et al., 2014) has been linked to efficient decoding of rapid temporal variation in speech (Tervaniemi & Hugdahl, 2003) as opposed to music, which shows right-hemispheric dominance (Perani et al., 2010). The exact mapping between functional and structural aspects of language asymmetry has proven complex (Piervincenzi et al., 2016), although structural white matter asymmetries, particularly in the arcuate fasciculus, have recently been proposed as one of decisive links to functional language lateralization (Ocklenburg et al., 2016). In light of reversed right>left asymmetry reported in nvASD (Wan et al., 2012), this study further sought to assess the hemispheric asymmetry of the evoked activity in response to speech in this population.

Taken together, the aim of this study was to evaluate the actual functionality of the auditory language network in nvASD through two modalities: resting state ICA-based and task-evoked online response to speech. I related these two functional aspects of the auditory circuity to provide a more coherent account of this circuity in nvASD, a bedrock for the study of the language impairment in nvASD. Based on the evidence from the previous EEG studies in nvASD as well as the results from the previous chapter, the presence of an intrinsic auditory network reactive to auditory stimulation in predicted. Yet, irregularities may arise in the degree of lateralization that was previously reported to show a stronger hemispheric reversal (right > left) in nvASD. In an exploratory fashion, the study further examined how functional properties of the auditory network relate to the variation of language abilities reported in chapter three. As I have argued in previous chapters, the absence of grammar as seen in nvASD constrains the linguistic space to a handful of single words, although slight variation in vocabulary persists. With the present study, I set out to explore this slight variability in nvASD in relation to low-level cortical auditory processing. To

this end, the study reports individual patterns of basic auditory processing, alongside the group-level one, across the nvASD sample.

As a first step, the auditory network was independently reconstructed from resting state data via ICA, a data-driven approach for functional connectivity. By analysing signal from all voxels of the brain, ICA parses spatially independent modules with dense internal connectivity, hence enabling to illustrate the extent and integrity of the auditory functional module across individuals. As a second step, the study examined the functionality of this template by quantifying its engagement in processing of speech. First, the evoked activity in response to speech (FWD+BWD) was averaged across the group to identify common activation loci. The stimulus-evoked response was then explored at the individual level. The single-subject responses were subsequently overlapped on individual resting-state auditory networks, to estimate the extent of their topographical alignment. This was done in order to evaluate the correspondence between functionally coherent network identified under resting and task condition. Finally, the study evaluated the hemispheric asymmetry of evoked activity to speech, and to FWD and BWD separately, extracted from the resting-state auditory template averaged across the group.

## 5.2 Methods

### 5.2.1 Participants

Participants were ten school-aged children and adolescents with nvASD. Eight participated in the functional connectivity study reported in the previous chapter. The additional two participants included a) a participant from the larger cognitive profiling study (chapter three) whose fMRI data file was missing at the time of the study completion; b) a participant recruited from the neuropsychiatry unit of the hospital.

The nvASD subject with co-morbid 22q11 deletion syndrome was S7. The subject non-responsive during neuropsychological evaluation was S9. The sample's demographic data are summarized in Table 5.1, and the individual cognitive profile data (based on observation and standardized tests) are included in Table 5.2. More detailed case descriptions can be found in the Supplement. There were no significant differences in motion ratio between rest and task acquisition data, $z = -.98$, $p = .327$. The last volume of S5 resting state data was removed due to a significant movement above 3 mm.

This study was approved by the responsible institutional review board (CEIC Fundació Sant Joan de Déu; PIC-99-17), in accordance with the 1964 Helsinki declaration and its later amendments. Caregivers of all participants gave their written informed consent.

**Table 5.1**: Participant demographics

| | Age (y; m) | Sex | Primary language | Handedness |
|---|---|---|---|---|
| **1** | 8;3 | M | Spanish | L |
| **2** | 9;6 | M | Spanish | R |
| **3** | 10;11 | M | Catalan | R |
| **4** | 11;1 | M | Spanish | R |
| **5** | 11;1 | M | Spanish | R |
| **6** | 11;6 | M | Catalan | ambidextrous |
| **7** | 12;3 | F | Spanish | R |
| **8** | 15;1 | M | Spanish | R |
| **9** | 16;7 | F | Spanish | R |
| **10** | 17;8 | F | Catalan | R |

**Table 5.2**: nvASD subjects' cognitive profile

| | Age of 1st word acquisition | Language regression | NDW | VMA | NVIQ | ADOS score | Response to name | Frequency of directed vocalizations |
|---|---|---|---|---|---|---|---|---|
| **1** | N/A | P | 11 | 1;3 | 85 | 15 | 0 | 1 |
| **2** | N | A | 0 | 1;0 | 53 | 13 | 0 | 1 |
| **3** | 2;0 | P | 0 | 1;0 | 30 | 23 | 3 | 3 |
| **4** | 2;6 | A | 9 | 4;3 | 79 | 18 | 1 | 1 |
| **5** | 1;6 | P | 1 | 1;7 | 69 | 19 | 2 | 0 |
| **6** | 3;0 | A | N/A | N/A | N/A | N/A | N/A | N/A |
| **7** | N | A | 0 | 1;3 | 61 | 14 | 0 | 3 |
| **8** | 8;0 | A | 2 | 1;11 | 64 | 19 | 0 | 2 |
| **9** | 1;0 | A | 0 | N/A | N/A | N/A | N/A | N/A |
| **10** | 3;0 | A | 14 | 3;11 | 69 | 22 | 0 | 1 |

*N/A*: not available | *Age of 1st word acquisition*: based on ADI-R; N (not attained) | *Language regression*: based on ADI-R; A (absent), P (present) | *NDW*: number of different words (based on semi-structured language sample from the ADOS) | *VMA*: verbal mental age (assessed by Peabody Picture Vocabulary Test; PPVT-III) | *NVIQ*: nonverbal IQ (assessed by Leiter-R) | *Response to name*: based on the ADOS item B6 (3 =no eye contact after several calls; 0 = establishes eye contact after first call) | *Frequency of directed vocalizations*: based on the ADOS item A2 (3 = no directed vocalizations; 0 = frequent directed vocalization in several pragmatic contexts).

### 5.2.2 Magnetic Resonance Imaging Acquisitions

The acquisition parameters, experimental design, and sedation procedure are identical to those described in chapter four.

### 5.2.3 MRI Preprocessing and Analyses

MRI data were pre-processed with FMRIB's Software Libraries (FSL v5.0.10; Smith et al., 2004), MATLAB 2020a (Mathworks Inc., Natick, MA), and SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, UK). A standard pre-processing pipeline was implemented for motion correction via rigid-body realignment, slice-timing correction (interleaved slice order, bottom-up), normalization to a 3 mm-isotropic MNI152 template (resized voxels $3 \times 3 \times 3$ mm), spatial-smoothing with a Gaussian kernel of 6 mm at full-width half maximum. Structural T1-weighted images were segmented using the default tissue probability maps. EPI images were corregistered to the structural images. Visual inspection of the pre-processed images was performed to assure quality.

Independent component analysis

Data were analysed using MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version ln(11), part of FSL. EPI images were aligned to anatomical data using FSL FLIRT with six degrees of freedom and a temporal filtering of the signal with high-pass Butterworth filter (0.008 Hz < f) was applied. For S5, the last acquisition volume was discarded due to movement (more than 3 mm), resulting in 265 volumes in total. An automatic dimensionality estimation was applied, and the resulting independent components (ICs) were inspected at individual and group level. An auditory component spatial distribution and time course was visually inspected by 2 raters. Empirical z-thresholding was used on the auditory component, using a threshold of $Z = 3$ ($p < .001$), minimal cluster size of 10.

Functional activation analysis

A block design matrix was specified using the canonical hemodynamic response function. Differential modulation of HRF under sedation was also tested, yet the estimation of HRF via Finite Impulse Response (FIR), with 12 basis functions estimated within a window length of 10 seconds, did not yield contrasting findings (results not shown). Two different conditions were specified: Forward speech (FWD) and Backward speech (BWD). Due to data acquisition errors mentioned in the previous chapter, the Catalan stimuli (3 participants) had one less BWD block towards the end of the acquisition. Therefore, for the analyses where BOLD signal is compared between FWD and BWD conditions, only 9 blocks for each condition were specified to assure comparability. Similarly, the first 4 seconds of the first FWD block in either language were also not acquired shortening the block length to 16.4 seconds instead of 20.2 seconds. Data were high pass filtered (to a maximum of 1/128 Hz). Previously estimated 6 movement parameters were included in the model as part of the nuisance regression. An explicit masking was applied using segmented grey matter images to restrict the analysis to the cortex and reduce the number of comparisons to increase power. 'FWD', 'BWD', and 'Speech' (FWD+BWD) contrasts were estimated.

First, group-averaged activation maps to Speech and its activation coordinates are reported. Second, individual activation maps to Speech are shown and later overlapped on individual auditory resting-state maps. Individual activation maps were reported at an uncorrected threshold of $p < .001$ at the voxel level with a minimal cluster size of 10 voxels. Group-averaged activation map reports voxel-wise uncorrected activation at $p < .01$, cluster-wise FDR-corrected for multiple comparisons at $p < .05$.

The FWD>BWD contrast could not be calculated because the order of FWD and BWD blocks was not interspersed. Thus, all FWD block were presented first followed by the BWD blocks. Quantifying any difference in signal intensity between these two conditions could not be, consequently, reliably discerned from a possible differential effect of sedation between the first and the second half of the acquisition time.

Functional hemispheric asymmetry in response to Speech, and FWD and BWD contrasts

To estimate the functional hemispheric lateralization in response to Speech contrast, the beta weights were extracted from a region of interest corresponding to group-based auditory IC in the

left and right hemisphere separately. This ROI was masked with the brain-extracted MNI152 anatomical image to exclude voxels outside the brain. To directly test whether the BOLD response to speech shows functional hemispheric asymmetry, a paired-sample Wilcoxon signed-rank test was computed, given non-normal distribution of left-right signal differences. Finally, to explore possibly differential contributions of FWD and BWD conditions to the functional asymmetry, the signal difference between LH and RH was calculated for all participants at each block condition using the formula 'difference = LH signal – RH signal'. Positive values therefore indicate higher signal intensity in the LH than RH, negative values indicate a rightward tendency, while zero difference represent a balanced hemispheric signal intensity. A paired-samples t-test was run to assess the difference between FWD and BWD in their respective signal asymmetry pattern.

## 5.3 Results

### 5.3.1 Resting state auditory network at group and single-subject level

The number of ICs reconstructed at the group level was 154. Figure 5.1 shows the group-level auditory resting state network (for anatomical coordinates see Table 5.3). The mean number of ICs per subject was 47.7 ± 8.7. See Figure 5.2 for the auditory IC at the single-subject level. A robust auditory IC was identified in eight subjects. Out of the remaining two subjects, one showed very weak auditory IC with noise signal in the ventricles and around the brain (S5), while in the other this IC was not identified (S6), even after removing movement outliers. Figure 5.S1 in the Supplement shows motor and medial visual ICs of S5. Again, these were not identified in S6, but Figure 5.S1 reports sensorimotor and lateral visual ICs of this subject. A lack of a robust auditory IC as driven by movement artifacts or sedation is thus unlikely. Interestingly, S8 showed two components: (a) one predominantly unilateral auditory IC centred at left superior temporal gyrus (STG; MNI [-60, -43, 14]), and (b) another bilateral auditory IC encompassing right Heschl's gyrus (HG), STG and insula (yet peaking at Rolandic operculum MNI [45, -19, 17]) mainly interconnected with contralateral insular cortex and HG (again peaking at left Rolandic operculum MNI [-48, -22, 17]).

**Figure 5.1:** Group-level auditory independent component thresholded at Z > 3, cluster size > 10 voxels. Underlying anatomical image is a standard template (MNI152).

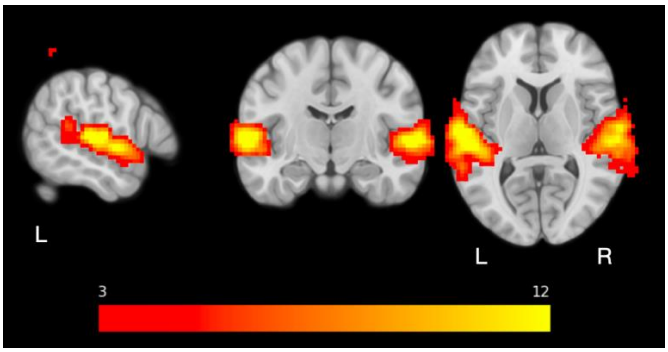
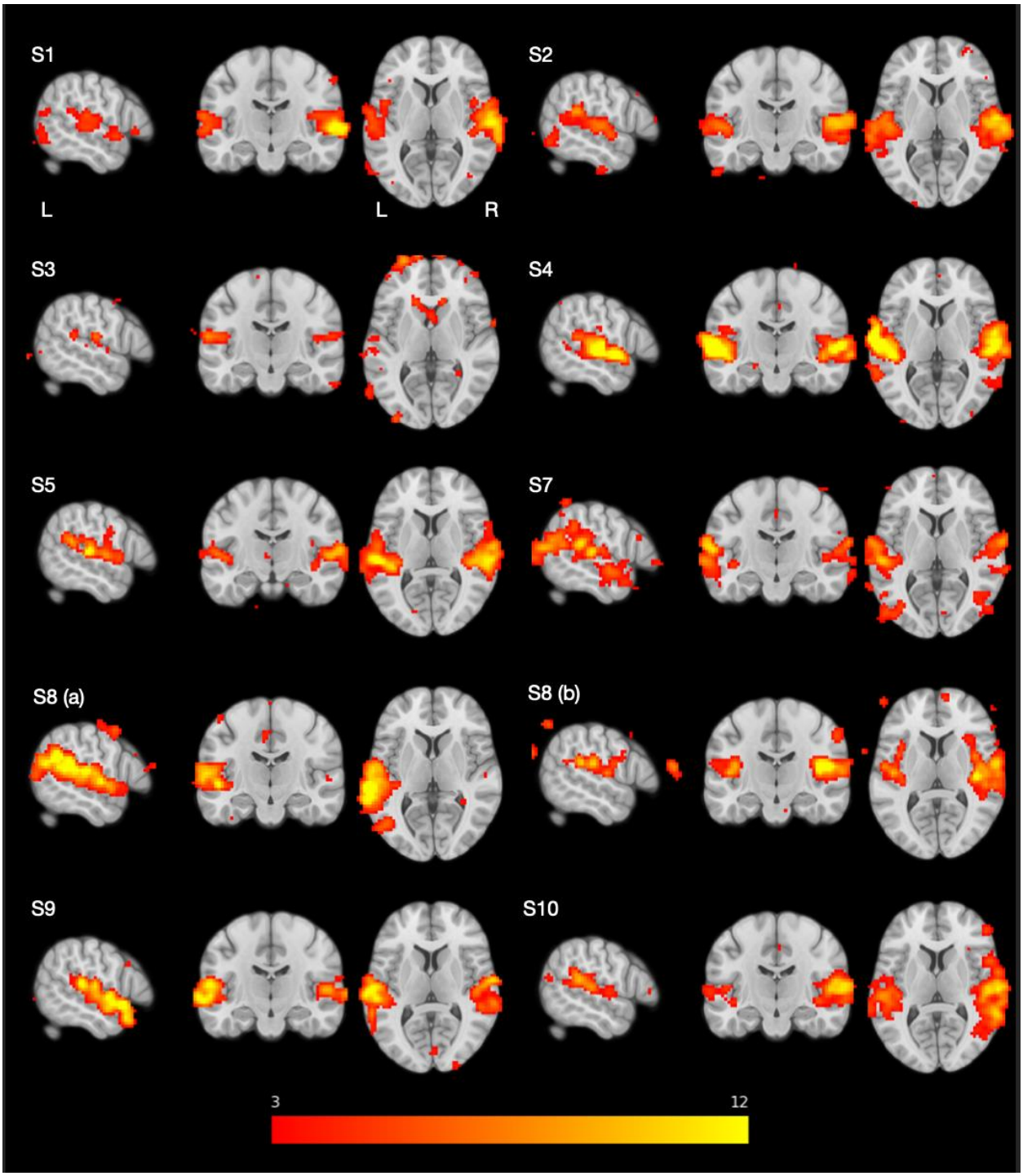



**Table 5.3:** Anatomical areas constituting the group-level resting-state auditory network

| Anatomical area | Cluster size | MNI coordinates (at peak) | | | Z (at peak) |
|---|---|---|---|---|---|
| | | X | Y | Z | |
| left STG, HG, superior temporal sulcus | 1 363 | -60 | -16 | 5 | 16.2 |
| right STG, HG, superior temporal sulcus | 1 347 | 63 | -10 | 2 | 16.2 |
| left angular gyrus | 37 | -36 | -67 | 38 | 3.97 |

**Figure 5.2:** Auditory independent component across the nvASD subjects. The IC was thresholded at Z > 3, cluster size > 10 voxels. Underlying anatomical image is a standard template (MNI152).




### 5.3.2 Grouped-averaged stimulus-induced activation to Speech

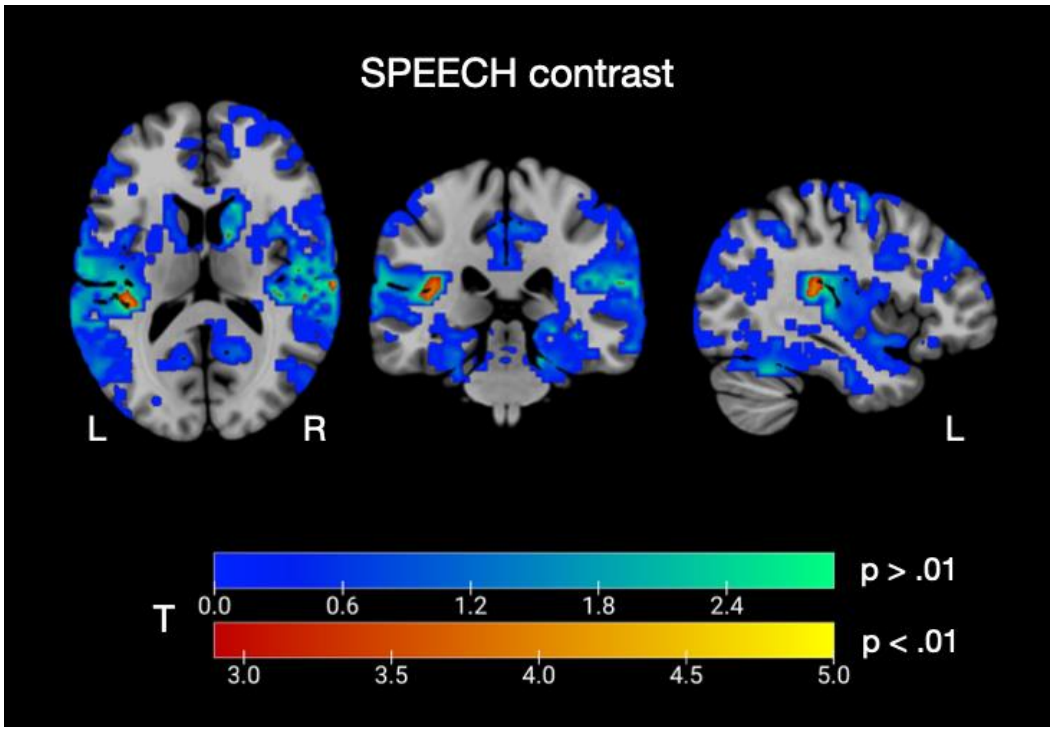
No voxels survived the group-averaged BOLD response to Speech contrast thresholded at T = 4.5 ($p < .001$), uncorrected, minimal cluster size > 10 voxels. Under a more permissive threshold of T

= 2.9 ($p$ < .01), the speech contrast yielded voxel-level activations in the right superior temporal gyrus (STG), and bilateral transverse temporal gyrus (TTG) and cerebellum. However, none of these clusters survived cluster-level FDR correction at $p$ < .05 (see Table 5.4). Figure 5.3 shows voxel-level thresholded activation maps in warm colours, superimposed on un-thresholded activation (in cold colours).

**Table 5.4:** Voxels significantly activated for the Speech contrast. The voxel-wise significance was set at T > 2.9 (p < .01), uncorrected, cluster size > 10 voxels. The resulting clusters were corrected with FDR at $p$ < .05.

| Anatomical area | Cluster size | Cluster-level FDR-corrected *p* value | MNI coordinates (at peak) | | | T (at peak) |
|---|---|---|---|---|---|---|
| | | | X | Y | Z | |
| right STG, right Planum Temporale | 27 | .816 | 66 | -22 | 14 | 4.67 |
| right HG, right CO | 32 | .816 | 48 | -13 | 8 | 4.6 |
| left cerebellum | 51 | .816 | -21 | -49 | -22 | 4.3 |
| right cerebellum | 26 | .816 | 30 | -46 | -28 | 4.24 |
| left HG, left PO | 66 | .816 | -39 | -31 | 14 | 4.2 |

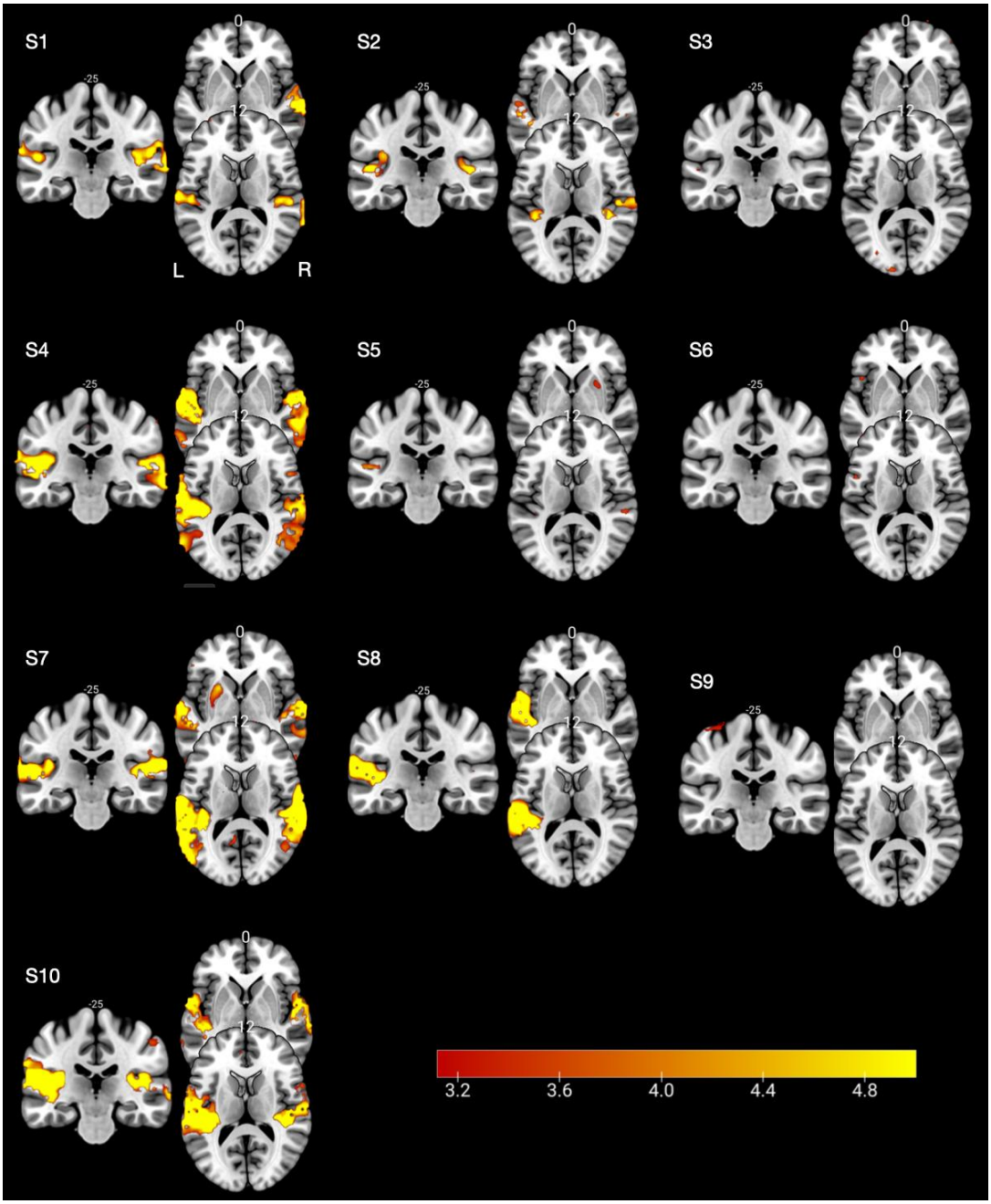
**Figure 5.3:** Group-averaged maps of voxel-level activation to Speech contrast. Significant voxel-wise activation thresholded at T > 2.9 ($p$ < .01), uncorrected, cluster size > 10 voxels, is shown in warm colours. Cold colours represent subthreshold activation. The underlying anatomical image is a standard template (MNI152).




### 5.3.3 Single-subject stimulus-induced activation to Speech

Individual subjects' response to the Speech contrast can be seen in Figure 5.4, thresholded at T = 3.12 ($p < .001$), uncorrected, with minimal cluster size of 10 voxels.

**Figure 5.4:** Single-subject-level maps of voxel-level activation to Speech contrast. Significant voxel-wise activation thresholded at T > 3.12 ($p < .001$), uncorrected, cluster size > 10 voxels. The underlying anatomical image is a standard template (MNI152).




### 5.3.4 Overlap between single-subject auditory IC and stimulus-induced activation to Speech

Figure 5.5 shows topographical overlap between the individual auditory components and the single-subject activations to Speech contrast. The percentage of topographical overlap between the component and the activation is represented in Figure 5.6 along with individual chronological age, verbal mental age and expressive language level represented on a scaled axis.

**Figure 5.5**: BOLD activity maps representing individual auditory IC (green), single-subject BOLD response to Speech contrast (blue) and their topographical overlap (red). All BOLD activity maps were thresholded at Z > 3 ($p$ < .001), uncorrected, cluster size > 10 voxels.

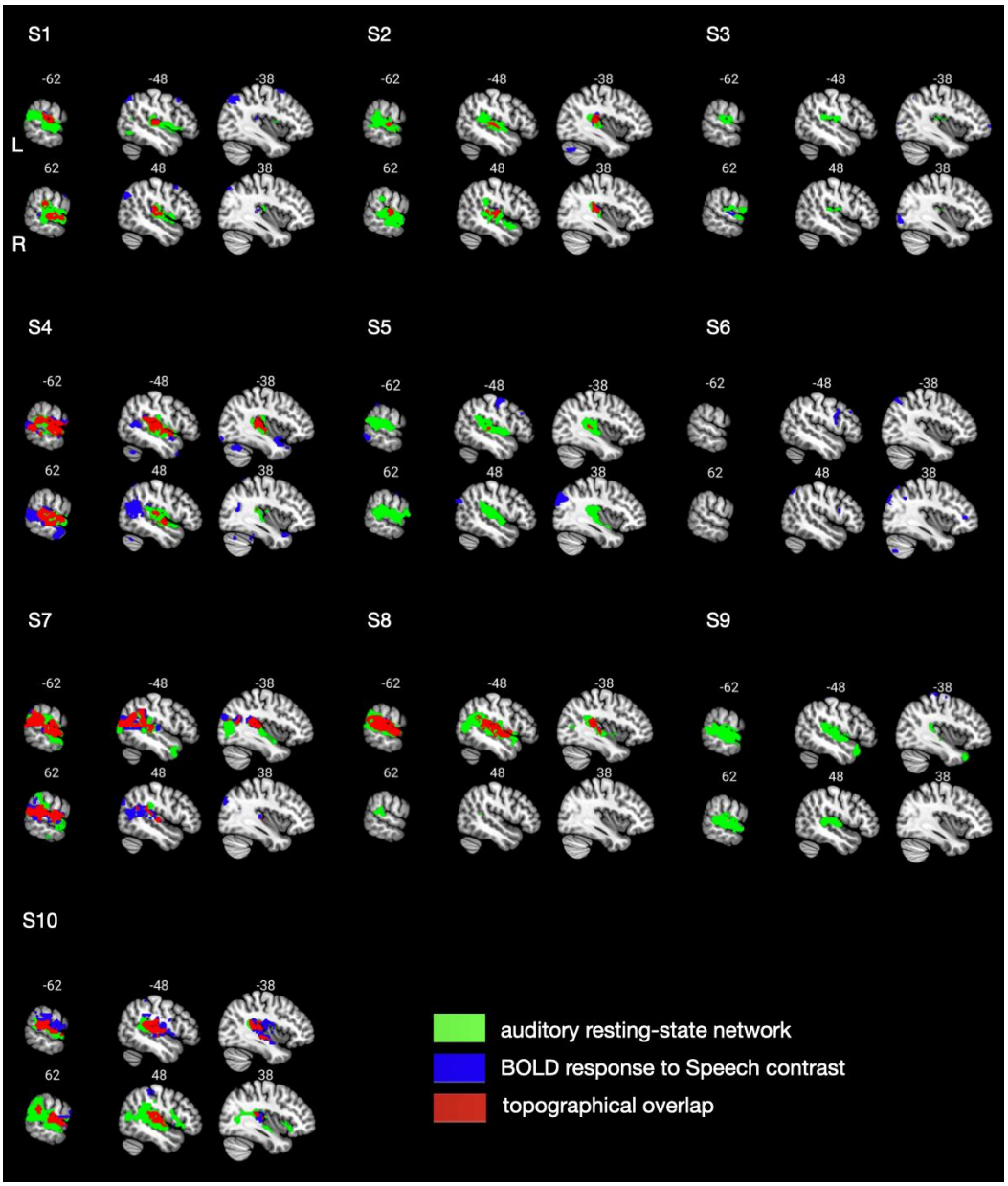
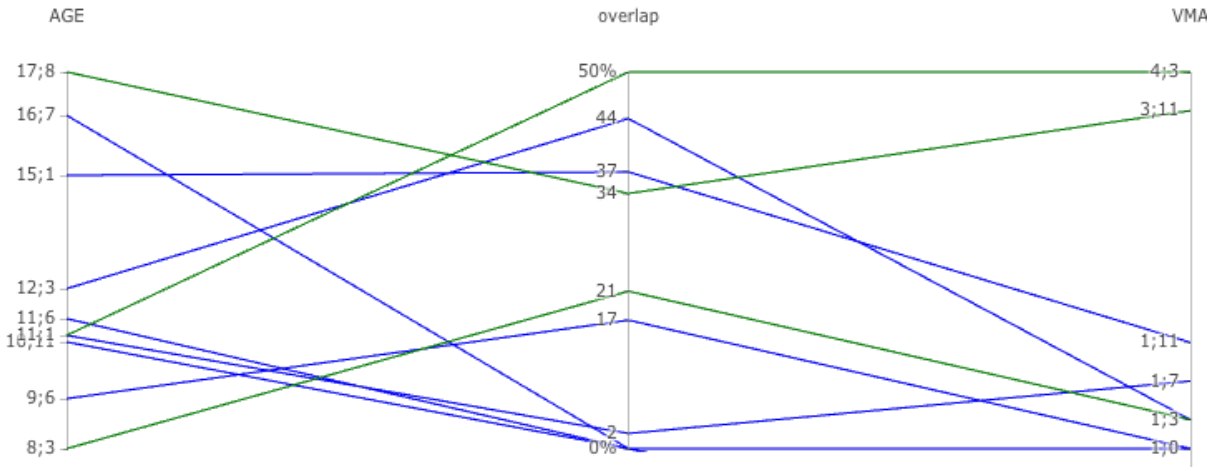



**Figure 5.6:** Percentage of the IC and BOLD response overlap in relation to subjects' chronological age and verbal mental age (VMA) represented on parallel coordinates. Subjects' expressive language level is coloured in blue (FNW) and green (SW).




### 5.3.5 Hemispheric asymmetry of BOLD response to Speech, and FWD versus BWD speech stimuli

Beta weights of the Speech contrast (FWD+BWD) were extracted from a region of interest (ROI) corresponding to group-level reconstructed auditory resting state network in the left (LH) and right hemisphere (RH), separately (Figure 5.7). In case of one participant (S3) the stimulus consisted only of the FWD contrast. The extension of the ROI in the LH and RH was 33 288 $mm^3$ and 29 704 $mm^3$, and the centre of mass left STG [-55.5 -21 4.65] and right STG [58.8 -17.4 2.69], respectively. Out of 10 participants, 9 showed higher activity in the LH than the RH within the selected ROI, and one showed an opposite pattern (S1) (Figure 5.8). The median signal in the LH (.3313) was higher by .4183 compared to the RH (.0187), a difference that was statistically significant at $z$ = -2.497, $p$ = 0.13, with a large effect size of $r$ = -.79.

Beta weights were extracted from the FWD and BWD contrast separately, to assess any differences in signal strength between the two hemispheres for the two speech conditions. No significant differences were observed (not reported).

**Figure 5.7:** ROI extracted from the group-level auditory IC separately for LH (red) and RH (green).

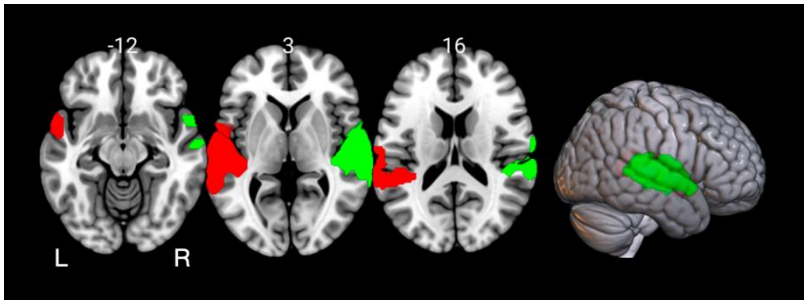
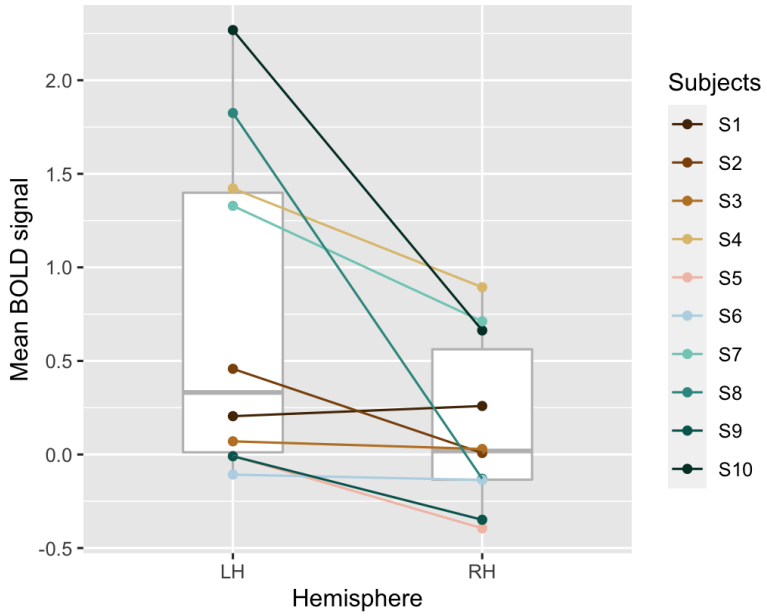



**Figure 5.8:** BOLD signal extracted from LH and RH ROI for Speech contrast. Individual BOLD signal differences between LH and RH are plotted on boxplots representing central tendency of the data




## 5.4 Discussion

The aim of this study was to identify the actual functionality of the resting state auditory network in nvASD. As a first step, an auditory network was reconstructed in correspondence to regions of correlated spontaneous activity at rest, to then, as a second step, assess its sensitivity to speech stimuli. This was done to illuminate the true functionality of this network in nvASD, which may not correspond to the brain's intrinsic connectivity. Individual activation maps were assessed for

the degree of topographical overlap with the resting-state auditory network. The BOLD signal was evaluated in terms of significant group-averaged regions of activation and its hemispheric lateralization.

Results showed that a robust group-averaged resting-state auditory network could be identified characterized by dense internal connectivity centred around primary and secondary auditory cortices. This was expected given the finding of functional connectivity strength between primary and secondary auditory cortices in nvASD relative to neurotypical controls, as assessed in the previous chapter. Here, however, a data-driven approach permitted to explore this auditory network in its anatomical extension as a spatially and temporally independent functional module. In addition to auditory cortices, the extracted auditory network also included a smaller activity cluster in the left angular gyrus, a region independently linked to speech processing in infants (Dehaene-Lambertz et al., 2002).

An exploratory examination of the auditory resting state network at the single-subject level yielded, overall, a robust auditory module in the majority of the sample independently of the variability in nvASD cognitive profile. In one case, a somewhat weak network coupled with noise artifacts was identified. The subject in question was a 10-year-old boy with no speech, no articulated socially directed vocalizations and no response to proper name during diagnostic screening. Another subject did not exhibit an identifiable auditory component. Although a full neuropsychological examination of this subject is not available, his medical records report convulsions during early infancy. Finally, a particularly interesting pattern was noted in a subject with the auditory module split into two – with one component centred around the superior temporal gyrus (STG) predominantly restricted unilaterally to the left hemisphere, and another interconnecting the right STG primarily with left insular cortex. This subject has echolalia and frequently sings Disney songs – with words that he nevertheless fails to use communicatively.

A robust intrinsic auditory network, however, of the kind found in most of our nvASD subjects, says little about the true functionality of these regions, or how sensible these regions are to auditory stimuli. At the group level, the uncorrected stimulus-driven activation maps showed strongly circumscribed clusters of activity within bilateral Heschl's gyrus (HG), cerebellum and left STG – none, however, survived correction for multiple comparisons. This finding contrasts with the robust group-averaged auditory network during the resting state and points to a more heterogeneous pattern of signal augmentation across the nvASD population, as the inspection of

the single-subject activation maps has subsequently also shown. At this single-subject level, three subjects showed extended bilateral activations to speech in primary and secondary auditory cortices, while activations in two participants were limited to the HG. Interestingly, activation to speech of the subject with a unilateral auditory network mentioned above consistently overlapped in topography with his unilateral auditory component despite having a separate second component with a densely interconnected module in the right auditory cortices. This case suggests that the identification of an intrinsic auditory network and its subsequent excitability as part of an active network may indeed show meaningful patterns under sedation.

Continuing with this line of thought, roughly half of the study cohort did not show a significant BOLD signal increase in response to speech stimuli, despite of the fact that all but one subject *had* an identifiable auditory resting-state network. This points to a gap between an intrinsic neural infrastructure of connectivity, as measured by the latter network, and its excitability as part of a functional auditory network. Thus, the inspection of the un-thresholded activation maps of these subjects showed clusters of attenuated activity in the primary auditory cortex not strong enough to exceed the significance threshold. This is unlikely to be due to deep sedation, which has been linked to decreased activation to speech, but confined to frontal cortices. Numerous studies of auditory stimulation have attested significant levels of activation in primary and secondary auditory cortices in both children and adults under sedation (Davis et al., 2007; Gemma et al., 2016; Heinke et al., 2004; Liu et al., 2012; Plourde et al., 2006). At the single-subject level, propofol as a sedative agent has been specifically related to a high probability of detecting significant activation (Bernal et al., 2012) with no significant changes in cluster size and amplitude of activation (Frölich et al., 2017). In line with these observations, Patel and colleagues (2007) found a strong correlation between the number of sound-evoked active voxels in the auditory cortex in sedated hearing-impaired children before cochlear implantation, on the one hand, and improvement in hearing threshold after cochlear implantation, on the other. This reinforces previous comments that sedation need not wipe out the consistency between cognitive performance and the level of activation to auditory stimuli.

In consonance with Patel and colleagues, I estimated the percent of topological overlap between significantly active voxels and voxels of spontaneously correlated activity in the auditory cortices to subsequently relate it, albeit descriptively due to small sample size, to the subjects' cognitive profiles. Subjects with minimal percent of overlap (0% to 20% approximately) also

showed low levels of verbal mental age, while subjects with highest levels of verbal mental age and expressive language showed higher percent of overlap (34% to 50%). Nevertheless, in between these extremes, a lot of variance was observed as well. Even in the best performing nvASD subjects, however, the degree of topographical overlap of the auditory network between spontaneous and evoked activity was relatively low compared to what could be expected in the neurotypical population.

In prenatal development, networks of spontaneous activity are hypothesized to mould a functional template for later processing of upcoming stimuli, insofar as spontaneous activity *precedes* sensory-driven stimulation (Martini et al., 2021) and arises even in dissociated cultures of neurons *in vitro* (Cabrera-Garcia et al., 2021; Pozzi, 2020). In nvASD, this intrinsic auditory network is clearly in place and, in some subjects, significantly reactive to auditory stimuli. To the extent that we found such overlap, then, it points to a certain developmental consistency of functional brain organization between nvASD and the neurotypical population. But this does not necessarily imply neurotypical *processing* of auditory stimuli, and this dissociation is illustrated by our data too. Indeed, both Matsuzaki et al. (2019) and Cantiani et al. (2016) have shown preserved but delayed latencies of neural activity in response to sound in nvASD children compared to neurotypical children.

Matsuzaki and colleagues further observed atypical rightward lateralization of the signal across the ASD population compared to neurotypical controls. The rightward lateralization moreover gradually deepened along decreasing verbal abilities in ASD. Structural and functional lateralization has long been considered a hallmark of brain specialization particularly in the case of the language faculty, whose pattern of lateralization have been extensively researched since the ground-breaking work of Paul Broca in the 19$^{th}$ century (Broca, 1876). Strikingly, the nvASD cohort in this study showed, contrary to Matsuzaki et al. (2019), a significant *leftward* lateralization of the BOLD signal during speech processing, similarly to lateralization observed in neurotypical infants (Dehaene-Lambertz et al., 2002; Sato et al., 2012). Subjects with higher BOLD signal and left>right hemispheric difference were also generally cases with better language performance, suggesting that such laterality pattern may be advantageous even in the nvASD population. Together with the presence of a neurotypical auditory component in most of our subjects, and the relative overlap of responses to auditory stimuli with this component, this left-lateralization pattern

is a third striking indicator of the extent to which even individuals at the lowest end of the spectrum of language abilities in human exhibit neurotypical neural signatures.

No significant difference in the direction or degree of hemispheric predominance in signal intensity was observed between the FWD and BWD speech conditions in nvASD. While some studies have observed significant increase in left-hemisphere activation for FWD compared to BWD especially for native FWD rather than non-native speech (Peña et al., 2003; Sato et al., 2012), others have not (Bartha-Doering et al., 2019; May et al., 2011). The latter authors proposed higher excitability of the left hemisphere for any type of speech-like stimuli from birth onwards, reflecting the ubiquity of this stimulus in the environment (Dehaene-Lambertz et al., 2006). Preserved functional lateralization to speech (and more so in the higher functioning subjects) may therefore signal environmental tuning to speech even in individuals at the lowest end of the language spectrum in humans. The fact that no strikingly atypical pattern in nvASD was observed in this respect is a further noteworthy illustration of how a number of canonical aspects of the language network are preserved even at this end.

In sum, these results highlight a remarkable preservation of spontaneous/intrinsic neural activity, which self-organizes into an auditory circuity. The more variable degree of excitability of this network as measured by the extension of its activity and lateralization in response to auditory speech stimuli, possibly relates to better language performance in nvASD, despite being very limited overall.

## 5.5 Limitations

A frequent limitation common to clinical research on severely impaired uncooperative populations are small sample sizes which, as is the case of this study, limits the detectability of potentially relevant effects and consistent patterns. Furthermore, the absence of data from neurotypical controls has limited the conclusions that could be drawn from the study findings, which must consequently rely on reasonably replicated evidence from the literature. While these are important spheres of concern, this study nevertheless succeeds in complementing the previous EEG-based functional MRI explorations of the population (Cantiani et al., 2016; Matsuzaki et al., 2019) by relating patterns of evoked activity to spontaneous functional organization of the auditory network. Given that spontaneous networks constitute an early template for processing evoked activity in

development, they offer an insight into an early breakpoint and emerging functional properties of the auditory network in nvASD. This is crucial for the effort in reconstructing a basis for the study of language impairment in nvASD.

## 5.6 Supplementary information

### Case descriptions

**S1.** An 8-year-old boy with minimal speech. During the diagnostic session he could produce more than five words such as ‘no’, ‘mama’, ‘more’, ‘agua’ and even onomatopoeia mimicking animal sounds of a cow or a horse while looking at pictures of these animals. He also could use several conventional gestures (e.g., a nod, ‘applause’ during a happy birthday song) and pointing.

**S2.** A 9-year-old boy with absent speech. He has never acquired any words or phrases, but at the age of two used to say inconsistently ‘mama’ and ‘papa’. He can understand simple verbal orders that are embedded in rutinary actions. He can point with his index finger to request an object, although during the diagnostic evaluation he used the gesture inconsistently, that is, pointing to the ceiling to ask for an object in the examiner’s hands. He uses some conventional gestures such as ‘goodbye’ when asked or ‘no’, and some iconic gestures ‘eat’, ‘drink’, ‘sleep’ as requests. He does not accompany the gestures with eye-gaze or vocalizations.

**S3.** A 10-year-old boy with no speech but frequent non-articulated vocalizations that are not socially directed. At two years, he used to produce more than 20 words, point, and establish eye contact, but lost this ability at three years. The language loss was gradual happening over several months accompanied by a loss of appetite. He used to have frequent otitis media and colds. The medical examinations haven’t shown any irregularities in electroencephalogram (EEG) or genetic tests. At the moment of the MRI scanning, he could understand only contextualized simple orders.

**S4.** A 11-year-old boy with minimal speech. He can produce several words and simple phrases that are nevertheless highly stereotyped (‘Now yes’ to select one of two objects while pointing to it). His mother trains him to learn names of objects shown in pictures and he can even say some words in other languages but does not use the words communicatively. He also uses some highly rehearsed gestures, although inconsistently. He does not nod but can shake his head.

**S5.** A 11-year-old boy with minimal speech, not spontaneous but rehearsed to be produced while using an alternative system of communication through pictures, PECS, (in this context, he can use simple 3-word phrases). He frequently vocalizes articulate sounds (such as syllables ‘sa’, ‘si’) but does not direct them socially. He produced his first words at one year of age (‘water’ or

‘bread’). He uses pointing to request, but otherwise have minimal use of gesture, although often hands in pictures from PECS to request.

**S6.** A 11-year-old boy with minimal speech. The mother was diagnosed with hyperemesis gravidarum during pregnancy. The neurological examination of the infant was normal, but the cranial circumference was slightly bigger than average. At roughly one year he started to have convulsions and lost a great part of the acquired skills such as babbling. Several medical examinations through EEG were normal. At three years he could produce some single words or approximations to words. As a younger child, he used to have frequent otitis media. Currently, he uses PECS (level 4 - phrases) as an alternative system of communication.

**S7.** A 12-year-old girl with autism associated to 22q11.2 deletion syndrome and absent speech. Some problems were noted already during pregnancy, such as slow growth, but the girl was born at term. As a younger child, she used to have frequent pneumonias. She never produced any word apart from ‘mama’ and ‘papa’. She can understand simple rutinary orders and uses PECS on a tablet to communicate. She can use some highly rehearsed signs such as ‘goodbye’, ‘drink’ or ‘eat’, and can nod or shake her head.

**S8.** A 15-year-old boy with minimal spontaneous speech limited to single words and occasional echolalia. The boy, nevertheless, can sing Disney songs mainly imitating the song melody with occasional well pronounced words and approximations (‘shark’, ‘sea’, ‘snails’, ‘under’). He does not use these words communicatively. He has acquired his first words (‘eat’ or his proper name) at 8 years. At the moment of MRI examination, he could understand simple orders limited to concrete objects. He can produce some conventional gestures such as ‘goodbye’, ‘no’ or ‘kisses’ when asked.

**S9.** A 16-year-old girl with no speech. As 1-year-old she could produce some single words such as ‘water’ or ‘booger’, yet several months after lost this ability. At the present moment, she does not vocalize at all and appear to understand only some rutinary words of preferred objects or food. She can understand ‘no’ only if one raising their voice. She does not request objects or actions but can use touch to reject them.

**S10.** A 17-year-old girl with minimal speech, although with occasional use of rehearsed three-word phrases used to request and acquired at six years. When producing speech, she repeats the word or phrase two times. She can understand orders embedded in context and uses some

conventional gestures such as ‘goodbye’, ‘silence’, ‘kisses’ when asked. She can nod and shake her head with imperative communicative intent.

**Figure 5.S1:** Motor and medial visual independent component of subject5. Sensorimotor and lateral visual component of subject 6. Thresholded at Z = 3.

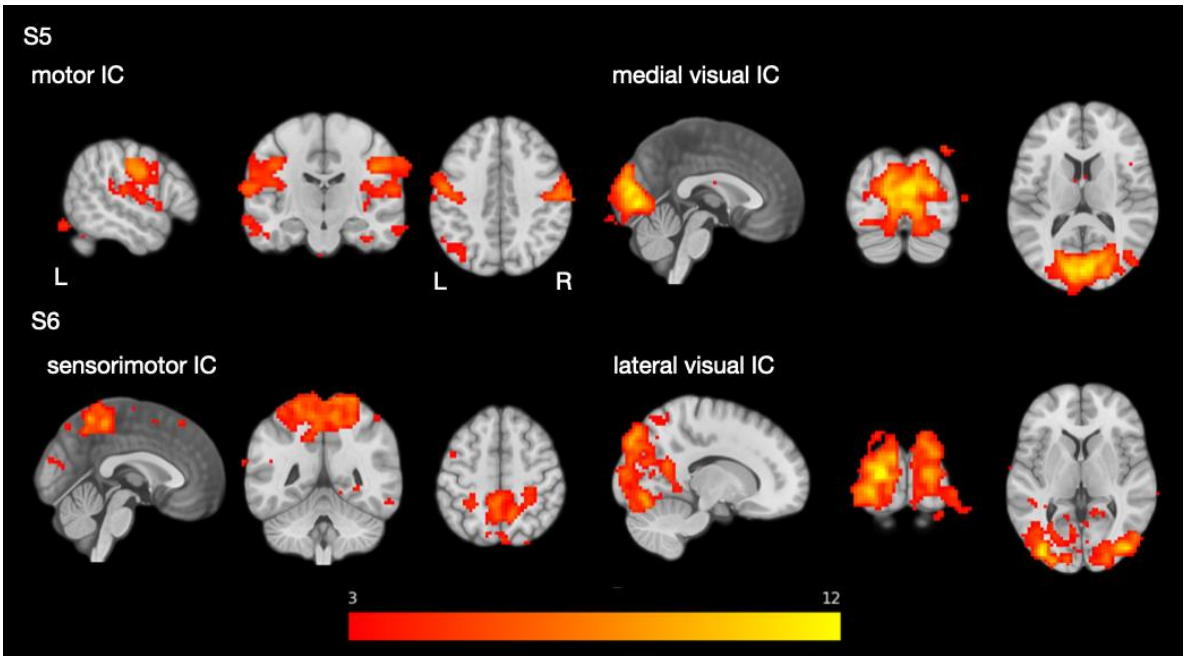
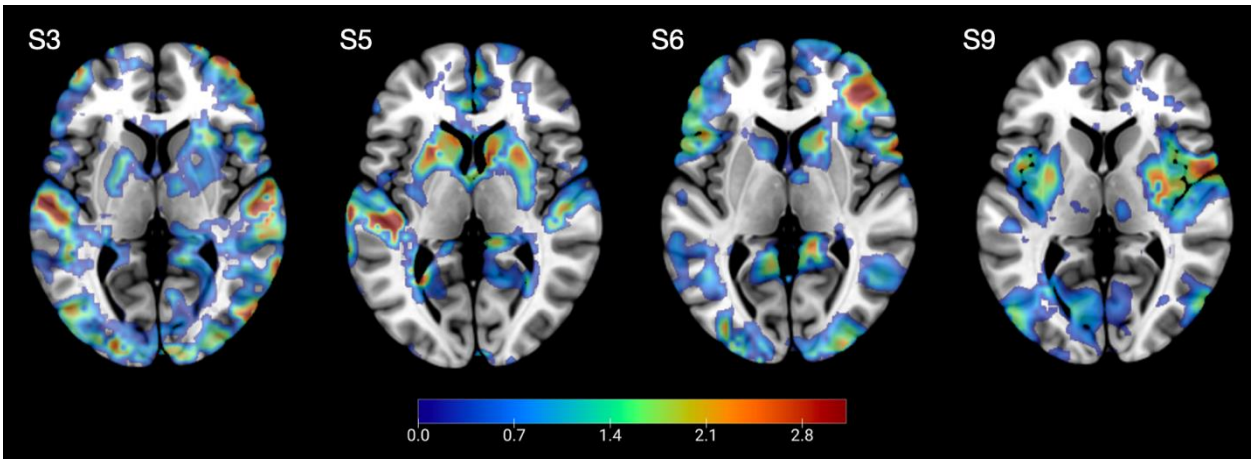



**Figure 5.S2:** Subthreshold activation at T < 3.12 ($p$ > .001) to Speech contrast in four subjects without significant thresholded activation.



# 6. White matter integrity of the dual pathways of the language structural connectome

## 6.1 Introduction

An absence of any gross structural brain anomaly is a common finding in a child with a neurodevelopmental condition that receives a referral for MRI examination. This is a frequent observation even in children with very severe behavioural phenotypes. For example, one third to half of children with absent or minimal speech associated to Snyder-Robinson syndrome (Dontaine et al., 2021), DHX30-associated syndrome (Lessel et al., 2017) or SATB2-associated syndrome (Zarate & Fish, 2017), do not show macroscopic cerebral irregularities.

Interestingly, on the other hand, in a handful of neurodevelopmental conditions absent speech development has been linked to a failure to detect the arcuate fasciculus (AF) bilaterally – including (a) malformations of cortical development such as schizencephaly, focal cortical dysplasia, or bilateral perisylvian polymicrogyria (pPMG) (Paldino et al., 2016), (b) global developmental delay of unknown aetiology (Sundaram et al., 2008) or (c) in genetic diseases such as Angelman syndrome (AS) (Wilson et al., 2011). Absent speech in these cases can be linked to orofacial diplegia as in pPMG, or it occurs with similarly minimal receptive language as in AS (Trillingsgaard & Østergaard, 2004). Relevantly, the AF has been primarily linked to speech-to-motor mapping, that is the mapping the sensory information to articulatory motor representations, although recent findings similarly suggest its involvement in comprehension (Ivanova et al., 2021). In pPMG, furthermore, absent AF has been regarded as a specific and recurrent marker related to some degree of language impairment (Paldino et al., 2015), yet relatively fluent (Kilinc et al., 2015), minimal (Oh et al., 2018), or even absent speech (Bernal et al., 2010). Failure to identify the AF by utilising *in vivo* and non-invasive MRI techniques like Diffusion Tensor Imaging (DTI) in combination with fibre tracking analysis could indicate poor fibre integrity or even AF agenesis, especially in the case of cortical folding abnormalities that severely disrupt the cortical organisation in pPMG (Munakata et al., 2006; Rosen et al., 2000; Zilles et al., 2013).

This study used DTI-based tractography to reconstruct the AF in nvASD while examining the integrity of its corresponding white matter (WM). A study by Wan et al. (2012) has already applied DTI-based tractography to reconstruct the AF in the right and left hemispheres in five completely non-verbal children with ASD. Four nvASD children exhibited a reversal of

neurotypical leftward lateralisation of the AF with respect to its volume, a macroscopic measure of tract integrity calculated based on voxel-wise extent of the tract fibres. The study's control group – five typically developing children – all showed greater AF volume in the left hemisphere than the right, a finding that is in line with numerous studies of the AF lateralization in the neurotypical population (Bain et al., 2019; Eichert et al., 2019; Sreedharan et al., 2015). The AF lateralization is an early emerging process possibly with prenatal onset. Thus, at a microscopic level, leftward lateralization of the AF has been detected in newborns and young infants, based on the WM integrity as indexed by fractional anisotropy (FA) (Dubois et al., 2016; Tak et al., 2016) and on tract myelin content (O'Muircheartaigh et al., 2013). By contrast, from a macroscopic perspective, AF volume at term age has been found to be right-lateralized (Wilkinson et al., 2017). It is unknown whether the macroscopic right>left asymmetry of the AF in nvASD observed by Wan and colleagues is reflected in the tract microstructure, or whether it could be viewed instead as an early-stage characteristic of development parallel to neurotypical newborns.

Hemispheric differences in the tract microstructure have barely been investigated in nvASD, although Chenausky and colleagues (2017) related differences in the microstructure of the AF and the frontal aslant tract (FAT) to intervention-mediated increase in fluency of speech repetition in nvASD. Concretely, greater FA of the AF in the left hemisphere significantly predicted change in the percentage of correct syllable-initial consonants. In the neurotypical population, the FA of the AF exhibits a gradual increase with age from early postnatal days through adolescence, which further leads to a greater consolidation of the AF leftward asymmetry over the years (Tak et al., 2016). The cohorts of both Wan et al. and Chenausky et al.'s studies so far included nvASD children up to approximately nine years. Here I examine a nvASD cohort of a wider age range, namely 8 - 18 years, to assess the FA bilaterally of the AF and its lateralization in relation to age, albeit cross-sectionally. Moreover, while FA is a general proxy of WM integrity, it can globally reflect different integrity aspects from bundle compactness, through degree of myelination, to fibre arrangements. Therefore, to discern the driving factors of tract asymmetry, this study also targets tract microstructure beyond FA by including a proxy measure of the myelin content.

The main focus of the existing DTI literature in nvASD has been placed on the AF. This is consistent with current conceptualizations and definitional characteristics of the population, as mentioned in previous chapters, insofar as the AF has been primarily linked to speech-to-motor

mapping. Thus, the study efforts by Chenausky et al. (2017, 2019) specifically focus on the hypothesis of speech motor deficits as underlying the nvASD phenotype. Yet, as I have argued previously in Chapter 2 and shown with evidence of deeper deficits in Chapters 3 and 4, a motor-speech impairment fails to fully capture the language deficits that can be observed in nvASD. Moreover, such an impairment might similarly be a natural consequence of minimal speech articulation and use during one's life. To characterize the neural correlates of nvASD, research should, therefore, target the entirety of the language connectome: WM tracts along both the dorsal and ventral language pathways, the latter related to sound-to-meaning mapping (for review, see Dick & Tremblay, 2012).

In particular, the fronto-temporal cortical language network distributes information along dorsal and ventral processing streams (for review, see Friederici, 2011; Price, 2012; Skeide & Friederici, 2016). Structurally, both processing streams consist of more than one WM fasciculus, each in charge of particular functions (Dick et al., 2014; Dick & Tremblay, 2012; Friederici, 2015). The dorsal stream incorporates the superior longitudinal fasciculus (SLF) - AF complex, often referred to as SLF/AF, which can be further segregated into one direct and two indirect segments (Catani et al., 2005). The SLF/AF underpins sensorimotor processes during speech production and perception (Hickok & Poeppel, 2007; Rauschecker & Scott, 2009) and is also argued to support higher-level syntactic processes (Friederici, 2015). Within the ventral processing stream, the inferior fronto-occipital fasciculus (IFOF) is regarded as a crucial pathway subserving semantic processes (Duffau et al., 2005; Saur et al., 2008). Running laterally to the IFOF, the inferior longitudinal fasciculus (ILF) have also been hypothesized to aid semantic processing, namely lexical retrieval (Herbet 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 semantic-related processes (Duffau et al., 2009; Harvey et al., 2013).

The principal aim of this study was to characterise the lateralisation of all language-related tracts in individuals with nvASD relative to various microstructural integrity metrics. Concretely, the study complements results from previous studies by analysing a more extended set of relevant tracts (specifically the AF, SLF-III, IFOF, ILF and UF), and by using two microstructure imaging parameters (FA and the myelin water fraction (MWF), a surrogate for the myelin volume, estimated from two MRI modalities: DTI and Myelin Water Imaging (MWI). The study sample consists of nine nvASD children and adolescents, a proportion of which had minimal speech,

contrasting with Wan et al. (2012)'s nonverbal sample. So far, the recruitment and MRI scanning of neurotypical children is ongoing (only three subjects are currently available). Therefore, the results presented here correspond to the nvASD group only.

## 6.2 Methods

### 6.2.1 Participants

Participants were nine school-aged children and adolescents with nvASD. Nine participated in the functional activation study reported in the previous chapter. The remaining subject (S6) included in the previous chapter had unusable DTI data. The nvASD subject with co-morbid 22q11 deletion syndrome was S7. The subject non-responsive during neuropsychological evaluation was S9. The sample's demographic data are reproduced once again in Table 6.1.

**Table 6.1**: Participant demographics

| | **Age (y; m)** | **Sex** | **Primary language** | **Handedness** |
|---|---|---|---|---|
| **1** | 8;3 | M | Spanish | L |
| **2** | 9;6 | M | Spanish | R |
| **3** | 10;11 | M | Catalan | R |
| **4** | 11;1 | M | Spanish | R |
| **5** | 11;1 | M | Spanish | R |
| **-** | | | | |
| **7** | 12;3 | F | Spanish | R |
| **8** | 15;1 | M | Spanish | R |
| **9** | 16;7 | F | Spanish | R |
| **10** | 17;8 | F | Catalan | R |

### 6.2.2 Diffusion MRI: data acquisition, preprocessing and estimation

Diffusion MRI data were acquired for each participant using a 3T Ingenia CX scanner (Philips Medical Systems) located at the Hospital Sant Juan de Déu (Barcelona, Spain) with a standard 32-channel head coil and the following sequence parameters: Field-of-view = 230x230 mm; voxel-size = 2.05x2.05mm2; repetition time (TR) = 10.1s; echo time (TE) = 102ms; flip angle = 90º; number-of-slices = 64; slice-thickness = 2.1mm; number of averages = 1; acceleration factor = 2; number of shells = 2; b-values = 625, and 1250 s/mm$^2$; number of diffusion gradient directions = 36; number of b0 (i.e., b-value=0) images = 3, plus 1 b0 with reverse phase to correct for spatial distortions. For each subject, the diffusion MRI data were corrected to remove susceptibility, eddy-current, and head motion distortions using the topup (Andersson et al., 2003) and eddy (Andersson & Sotiropoulos, 2016) toolboxes in FSL (https://fsl.fmrib.ox.ac.uk/fsl/) (Smith et al., 2004). The FA metric was obtained from the computed diffusion tensors, which were estimated by using the *dtifit* program included in FSL.

### 6.2.3 Multi-echo T2 relaxometry: data acquisition, preprocessing and estimation

Multi-echo T2 (MET2) data were acquired for all participants during the same scanning session described in the previous section by using a gradient and spin-echo sequence (Prasloski et al., 2012b) with the following parameters: Field-of-view = 240 × 230 mm; acquisition voxel-size = 3.5 × 3.5 mm$^2$, reconstructed voxel-size = 1.6 × 1.6 mm$^2$; number of echoes = 32; echo-time spacing (ΔTE) = 8.38 ms; repetition time (TR) = 8620 ms; excitation pulse = 90$^0$; refocusing pulses = 180$^0$; number-of-slices = 40; slice-thickness = 3.5 mm; number of averages = 1; acceleration factor (SENSE) = 2; acquisition time = 8:37 min.

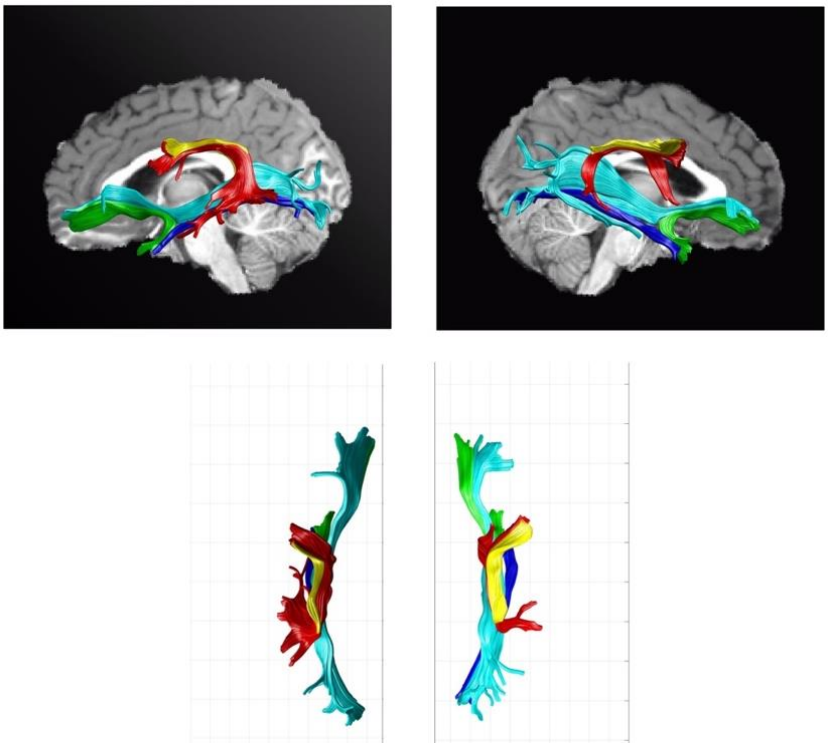
The intra-voxel $T_2$ distribution of relaxation times was calculated by using regularized non-negative least squares (Laule et al., 2007; Mackay & Laule, 2012) with a regularization term to promote smooth solutions that better represent the distribution expected from tissue microstructure (Mackay et al., 1994; Whittall et al., 1997) as described in (Guo et al., 2013). The estimation was carried out using the open-source multi-component $T_2$ reconstruction toolbox (Canales-Rodríguez et al., 2021a; Canales-Rodríguez, Alonso-Lana, et al., 2021b; Canales-Rodríguez, Pizzolato, et al., 2021c) available at https://github.com/ejcanalesr/multicomponent-T2-toolbox. The implementation is based on the extended phase graph (EPG) model (Prasloski, Mädler, et al., 2012) and uses a $T_2$ discrete grid from 10-2000ms (Prasloski, Rauscher, et al., 2012) with $p$=60 $T_2$ logarithmically spaced points.

From the estimated $T_2$ distributions, the MWF was calculated as the area under the curve for $T_2$ times smaller than the myelin water cutoff $T_2$=40ms, normalized by the total area under the curve of the whole $T_2$ distribution (Meyers et al., 2017).

### 6.2.4 Tract segmentation

Bundle-specific tractograms were generated using TractSeg (Wasserthal et al., 2018), an automatic machine learning algorithm that segments the WM into 72 bundles (https://github.com/MIC-DKFZ/TractSeg). It generates tract masks as well as tract orientation maps which enable the creation of accurate bundle-specific tractograms. For each subject, the mean FA and MWF values over the tract masks were estimated for the following language-related tracts: AF, SLF-III, IFOF, ILF and UF. Figure 6-1. shows the spatial distribution of the tracts of interest for a representative participant.

**Figure 6.1:** Language tracts studied in this work of a representative participant: arcuate fasciculus (AF, in red colour), the ventral segment of the superior longitudinal fasciculus (SLF-III, in yellow), inferior fronto-occipital fasciculus (IFOF, in light blue), inferior longitudinal fasciculus (ILF, in dark blue) and uncinate fasciculus (UF, in green).



### 6.2.5 Laterality index

A measure of lateralization was obtained for the tracts of each participant. For each microstructure index (i.e., FA and MWF), the mean value of the left and right regions of interest (i.e., homologous tracts from both brain hemispheres) were extracted, and the following ratio was computed (Desmond et al., 1995).

$$LI = 100\% * \frac{\text{Left} - \text{Right}}{0.5 * (\text{Left} + \text{Right})}$$

which was scaled as a per cent. According to this definition, a positive LI value indicates lateralization to the left side, a negative value to the right side, and a close-to-zero value absence of lateralization.

### 6.2.6 Statistical analysis

A linear regression model was implemented to test for positive or negative correlations between age and each studied microstructure metric for each of the ten tracts of interest (i.e., five tracts, two hemispheres). Only p-values < 0.005 (i.e., 0.05/10) were considered significant after correcting for multiple comparisons using the Bonferroni method.

A two-sided Wilcoxon signed-rank (nonparametric) test was carried out to test the null hypothesis that *LI* comes from a distribution whose median is zero at the 5% significance level. The p-values obtained for each tract were corrected for multiple comparisons (i.e., five tracts). Thus, only p-values < 0.01 (i.e., 0.05/5) were considered significant. Moreover, we also implemented a t-test for the mean equal to zero and verified that results from both tests were similar (result not shown).

## 6.3 Results

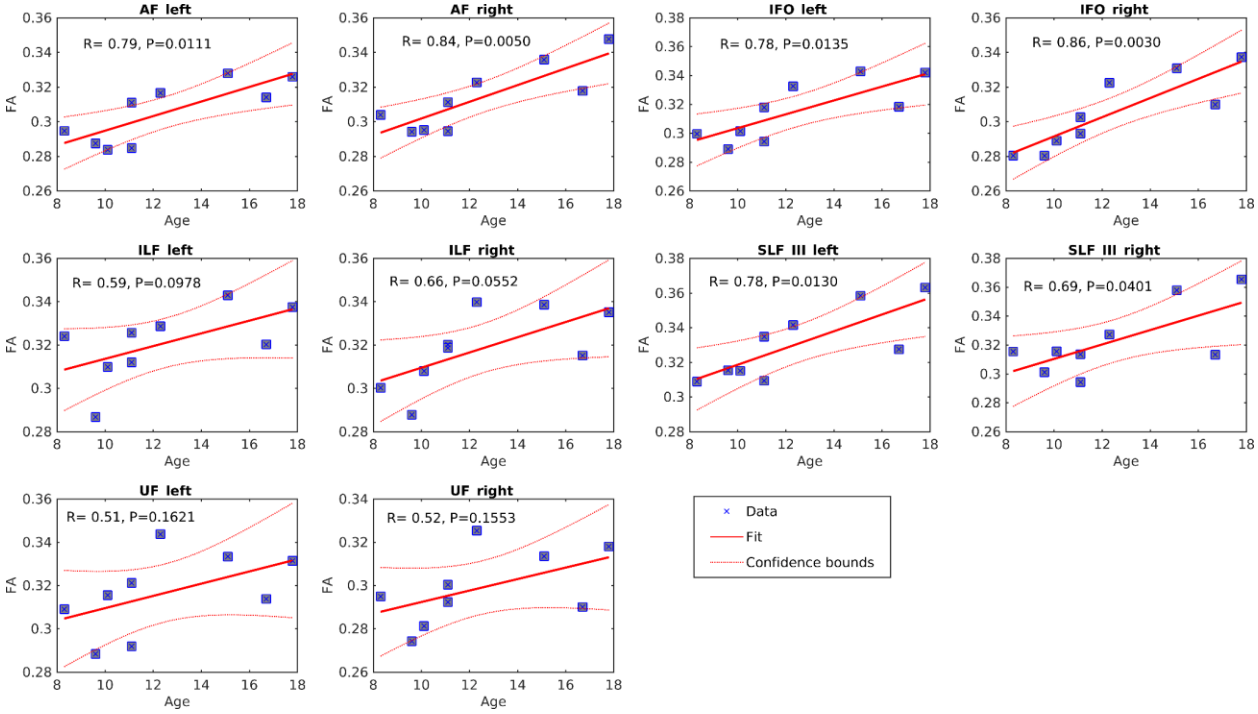
### 6.3.1 Anatomical findings

The individual medical reports of the MRI evaluation (based on T1-weighted and T2-weighted imaging) did not encounter any brain irregularities. Figure 6.S1 in the Supplementary material shows T1-weighted images of all participants in axial plane.

### 6.3.2 Fractional anisotropy

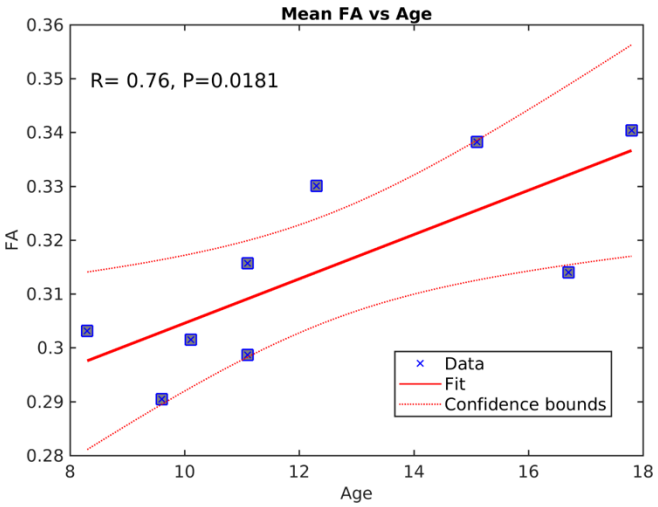
Figure 6.2 shows the relation between FA and chronological age. As can be seen, FA tends to increase with age in all tracts. Indeed, the correlation is significant at the uncorrected level for all the tracts except ILF and UF, both left and right sides, respectively. After correcting for multiple comparisons, only AF right and IFO right survived the correction.

**Figure 6.2**: Correlation between fractional anisotropy (FA) and age for each language-related tract in each brain hemisphere. The correlation coefficients and the p-values are shown.



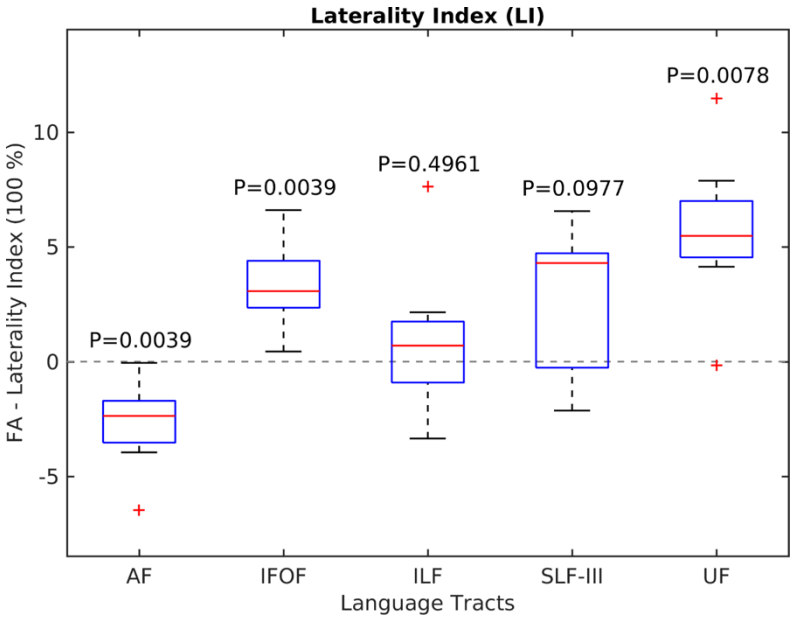

The previous result is summarized in Figure 6.3 by taking the mean FA over all the tracts of interest in both brain hemispheres. The positive correlation was statistically significant ($p$ = 0.0181) with a large effect size of $r$ = 0.76.

**Figure 6.3**: Correlation between fractional anisotropy (FA) and age for all language-related tract in both brain hemispheres.




Results corresponding to the lateralization index relative to the FA for the five tracts of interest are depicted in Figure 6.4. The statistical analyses revealed a significant right-lateralization of the AF ($p$ = 0.0039), and a significant left-lateralization of both IFOF and UF ($p$ = 0.0039 and $p$ = 0.0078, respectively). These tests were significant after correcting for multiple comparisons. No significant lateralization was found in the other two tracts. We verified that the LI was not linearly related with age for any of the studied tracts, even at the uncorrected level (results not shown).

**Figure 6.4:** Lateralization index of five language-related tracts for the fractional anisotropy (FA) microstructure index. The resulting p-value for each test/tract is displayed.




### 6.3.3 Myelin water fraction

Figure 6.5 depicts the dependence between MWF and age. Notably, the correlation was not significant for any individual tract (even at the uncorrected level). The confidence bounds accommodate either positive or negative tendency. Likewise, the correlation was not statistically significant for the mean MWF value for all the tracts combined (see Figure 6.6). The effect sizes of the dependence between MWF and age were within low range (displayed in the figures).

**Figure 6.5**: Correlation between myelin water fraction (MWF) and age for each language-related tract in each brain hemisphere.

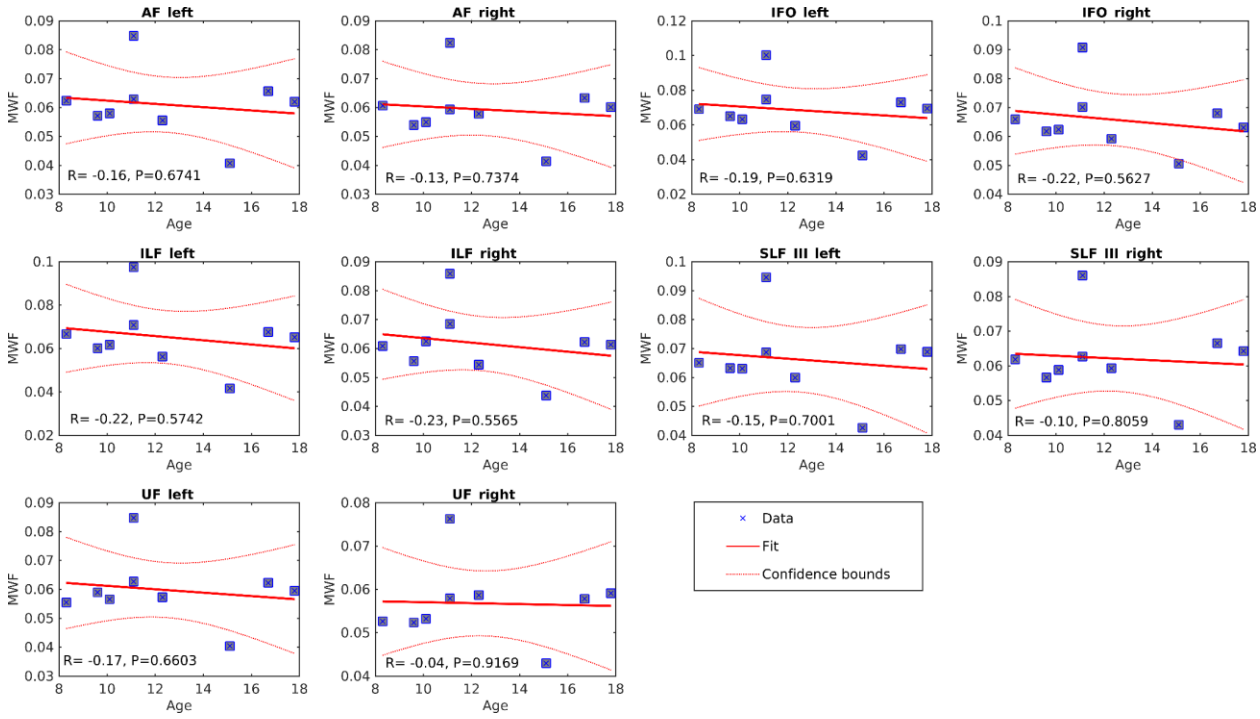



**Figure 6.6**: Correlation between myelin water fraction (MWF) and age for all language-related tract in both brain hemispheres.

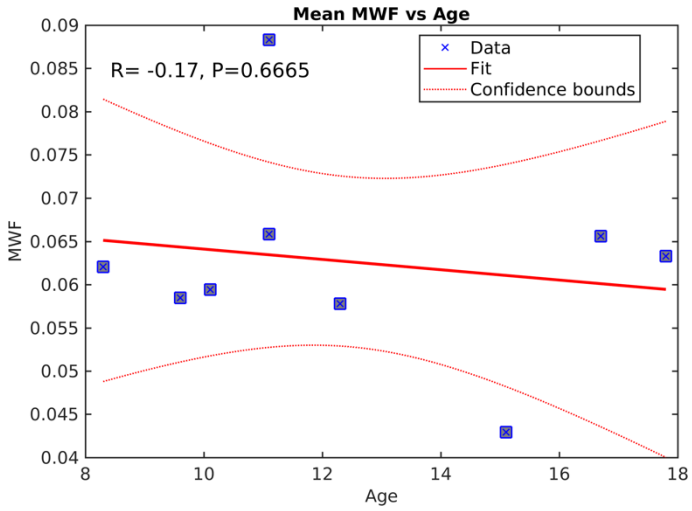
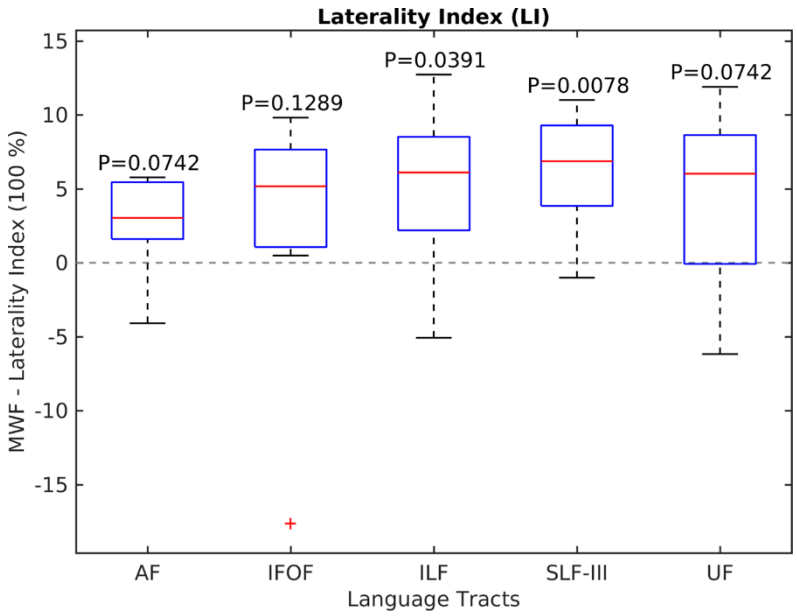


Figure 6.7 shows the laterality index relative to MWF for the five tracts of interest. All tracts showed a leftward trend in lateralization. However, only the lateralization index of SLF-III was significant after correcting for multiple comparisons ($p$ = 0.0078). The *LI* metric was not linearly correlated with age for any of the five tracts, even at the uncorrected level (results not shown).

**Figure 6.7**: Lateralization index of five language-related tracts for the myelin water fraction (MWF) microstructure index.




## 6.4 Discussion

The present study assessed the hemispheric lateralization of five major language-related association tracts with respect to the microstructural white matter (WM) integrity in nine nvASD children and adolescents. The two microstructural measures targeted here – the fractional anisotropy (FA) and myelin-water fraction (MWF) – were further tested cross-sectionally for age-related gradual increase, which has been widely attested in the neurotypical developmental trajectory (Kochunov et al., 2012; Lebel & Deoni, 2018; Morris et al., 2020).

The principal aim of the study was to assess hemispheric asymmetry of the arcuate fasciculus (AF) in nvASD with respect to the tract's WM microstructural integrity.

Macrostructurally, the AF has been found to exhibit a reversed neurotypical asymmetry (left>right) in nvASD (Wan et al., 2012) as indexed by the tract volume. Characterization of the tract microstructure is a crucial objective insofar as volumetric features of the AF had also been found to be lateralized towards the right hemisphere at initial stages in neurotypical development (Wilkinson et al., 2017), despite otherwise adult-like left-hemispheric lateralization of the AF based on its microstructural integrity reported in other newborn studies (Dubois et al., 2016; Tak et al., 2016). The results reported here extend the findings in Wan and colleagues by demonstrating a significant right>left difference in the AF microstructure as indexed by fractional anisotropy (FA) in nvASD, across a slightly larger sample with a broader age range (8;3 – 17;8). In this regard, the AF lateralization in nvASD represents a truly atypical pattern that has not been attested in early developmental stages of neurotypical right-handed infants. As such, nvASD language capacity does not represent an early stage in development but rather a truly atypical wiring of language related brain regions.

The lateralization of the AF with respect to its FA reflects higher WM integrity of the tract in the right hemisphere than the left in nvASD. The lower integrity in the left hemisphere, however, may not be explained by a gradual left-hemispheric WM atrophy, as the FA of the left AF increased cross-sectionally with participants' age, corresponding to a large positive correlation that was, however, not significant after correction for multiple comparisons. The FA in the right AF, too, increased significantly with age, maintaining in place the rightward lateralization of the AF across the entire sample, albeit with no evidence of further gradual consolidation of such lateralization, given that the AF lateralization index was *not* related to chronological age. This shows that the AF tract integrity as indexed by FA, although showing an atypical lateralization pattern, increases with age, and shows a neurotypical developmental pattern in this respect (Kochunov et al., 2012; Lebel & Deoni, 2018). This finding opens queries concerning the pathological or compensatory nature of the reversed pattern.

The leftward lateralization of the AF has been widely reported in the literature across the lifespan of the neurotypical right-handed population (Balezeau et al., 2020; Eichert et al., 2019), and to a lesser extent in the left-handed population (but see Allendorfer et al., 2016). Here all nvASD participants were reported by their caregivers to be right-handed, except one – the youngest participant (8;3 years), who nevertheless did not diverge from the group in either the lateralization or the correlation of FA with age. The lateralization of the second dorsal language tract, the

superior longitudinal fasciculus-III (SLF-III), was not significant, although it was characterized by a tendency of higher FA in the left hemisphere. Likewise, this contrasts with the typically observed prominent rightward lateralization of SLF-III in both volume and FA from adolescence to senescence (Amemiya et al., 2021). These results suggest a disorganization of the entirety of the dorsal language pathway, namely the SLF/AF complex. Nonetheless, it is noteworthy that a reconstruction of the tracts on both hemispheres was possible, unlike in other neurodevelopmental conditions implying absent speech, where the failure to detect the AF has been taken as a specific marker (Paldino et al., 2015). Here, a machine learning segmentation algorithm was employed using the voxelwise fibre orientational distribution function and an atlas of white matter tracts. From a technical point of view, this technique is more reliable and robust than standard fibre tracking analyses, which sometimes cannot detect actual tracts (Wasserthal et al., 2018). Bundle segmentation techniques based on fibre tracking algorithms heavily depend on the chosen algorithm's class (i.e., deterministic or probabilistic) and the intra-voxel method used to estimate the main local fibre orientations (i.e., DTI or spherical deconvolution). For an example of how the results may depend on the tracking method in patients with brain tumours see (Richards et al., 2021). However, in other brain diseases like perisylvian polymicrogyria, the absence of AF may not be related to technical limitations but to genuine agenesis potentiated by a disrupted cortical organisation (Munakata et al., 2006; Rosen et al., 2000; Zilles et al., 2013).

Turning to the ventral language pathway, which has been examined explicitly in the present thesis due to its putative involvement in the sound-to-meaning mapping (Friederici, 2015), two significant lateralization patterns transpired. First, a significant leftward asymmetry of the FA was detected in the inferior fronto-occipital fasciculus (IFOF), a major ventral pathway subserving higher-level semantic processing (Saur et al., 2008). The literature so far has produced conflicting results concerning the lateralization of the IFOF. Thus, rightward lateralization (measured macroscopically or microscopically) of the tract has been reported for children (Banfi et al., 2019) and adults (Arun et al., 2021; Hau et al., 2016), contrasting with the more bilateral pattern seen in Menjot De Champfleur et al. (2013). In foetuses, in turn, higher FA of the IFOF was observed in the left rather than right hemisphere (Mitter et al., 2015). Vassal et al. (2018) targeted this inconsistency by reporting differential leftward and rightward lateralization depending on the termination of IFOF projections (inferior frontal gyrus or superior parietal lobule, respectively).

Thus, an informed interpretation of these patterns will have to be necessarily aided by the inclusion of a neurotypical control group.

The second ventral pathway that was significantly lateralised in nvASD in this study was the UF, showing a leftward asymmetry. In contrast, the remaining ventral tract, the inferior longitudinal fasciculus (ILF), did not exhibit any particular asymmetry pattern. Once again, the literature has so far produced contradictory results regarding laterality of both tracts, reporting either right- and left-lateralized FA (see Arun et al., 2021, for review). Overall, despite differences in lateralisation, both dorsal and ventral language pathways show an expected age-paced increase in FA. It is unclear, however, whether their overall FA is preserved in comparison to the neurotypical population. The interpretation of these findings, thus, crucially depends again on a future analysis of healthy controls that is already underway.

The lateralization patterns based on FA reflect higher integrity of the tracts in one hemisphere over the other, which could be driven by one or more of the microstructural factors that the FA metric represents (e.g., axonal packing, myelin content, fibre dispersion or crossings). In this study, Myelin Water Imaging was used to estimate the myelin content (myelin water fraction; MWF) of the language tracts to determine how this particular aspect of WM integrity contributed to the lateralization patterns that have transpired for individual language tracts reported here. All tracts showed a trend corresponding to higher myelin content in the left hemisphere, although a significant laterality of the MWF was attested only for the SLF-III. A leftward tendency in MWF speaks directly to the overall WM integrity of the AF, as it contrasts specifically with its otherwise rightward lateralization in FA. Therefore, it is unlikely that differences in myelination could be the underlying factor behind the atypical structural organization of the tract. This is particularly interesting because myelination is a time-extensive process dependent on synchronized neural activity from the early postnatal period up to several decades into adulthood. From this perspective, the rightward lateralization of the AF might not represent an experience-driven compensatory reorganization of the structural connectome, of a kind that could have been hypothesized, had the MWF been right-lateralized as well.

On the other hand, it is interesting that leftward lateralization of myelin content (MWF) as found in the present nvASD cohort has also been found in typically developing children from one to six years in the temporal regions overlapping with the trajectory of the AF and ILF (O'Muircheartaigh et al., 2013). Despite the left>right MWF lateralization tendency in nvASD for

the AF and ILF, an expected age-related increase in myelination well attested in the neurotypical population was not observed in nvASD (Deoni et al., 2012, 2015; Dvorak et al., 2021; Morris et al., 2020). Thus, the correlation between the myelin content and chronological age across all the tracts and hemispheres was characterized by a minimal slope that could be accommodated in confidence bounds with either positive or negative tendency. The ongoing efforts in recruiting more nvASD and typically developing subjects for this study are specifically aimed at elucidating the myelination patterns that the present evidence preliminarily outlines. Scrutinizing these findings may hold important insights into targeted interventions in nvASD (Chenausky et al., 2017). These should go beyond productive speech-related regions, not only relating WM integrity as a predictive factor of behavioral training success, but specifically target myelination, perhaps venturing into the realm of neuromodulation (Ortiz et al., 2019).

In sum, the present evidence suggests that the rightward lateralization of the AF volume previously observed in nvASD (Wan et al., 2012) also includes the tract integrity at the microstructural level, rather than merely reflecting the macroscopic characteristics. Such an atypically reversed pattern extends to the whole dorsal pathway, the SLF/AF complex. Bilaterally, the SLF/AF overall FA appears to increase with age in nvASD as would be expected in the general population. Yet, the expected age-related increase of the myelin content was not observed, although further evidence is needed to support this finding. The ventral language pathway showed similar age-modulation in the FA and a lack of significant age-modulation in the myelination. Overall, the ventral pathway was characterized by a leftward trend or significant lateralization towards the left hemisphere. This shows that the AF rightward pattern is unique among the language connectome in nvASD. However, the ‘typicality’ of ventral pathway lateralization remains open to further examination as the literature so far offers contradictory findings. Despite this, the present study importantly demonstrates the detectability of all the ventral and dorsal pathways in nvASD patients of varied ages and cognitive profiles.

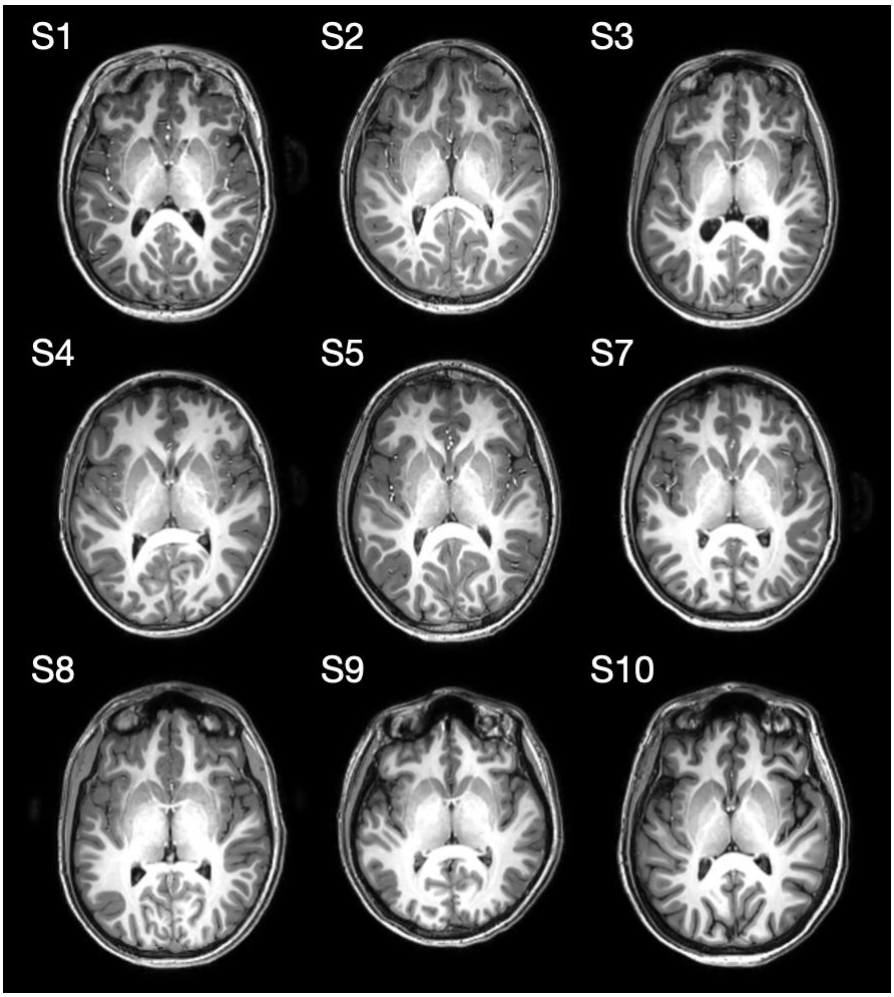
## 6.5 Limitations

This study has various limitations. The sample size of this study is small relative to neuroimaging studies in neurotypical populations, limiting the detectability of potentially relevant patterns. Nevertheless, this study cohort was more extensive than the one employed in the original DTI-

tractography study by Wan et al. (2012). Furthermore, the studied subjects were part of a larger sample involving cognitive profiling, thus may be considered more representative of the larger nvASD population. On the other hand, the absence of data from neurotypical controls has limited the conclusions drawn from the above findings. Thus, the 'atypical' AF lateralization can only be interpreted in relation to previous results from the literature. Although these previous findings have been reasonably replicated, our findings must be validated against controls examined under the same scanning protocol, neuroimaging tools, and statistical analyses. This concern has been addressed in the study design and, so far, data from three neurotypical children matched to three nvASD subjects on chronological age and handedness has been acquired. The lateralization results obtained from the remaining tracts examined here need to be viewed as preliminary. Yet, they have served as a proof of their detectability in nvASD and indicated an apparently differentiated rather than uniform pattern of laterality. A further benefit in using a measure such as the lateralization index is the attenuation of age and gender effects. This makes the findings less dependent on scanner sequences allowing, in this way, for a feasible comparison to healthy controls from other studies. In the near future, I plan to use more advanced diffusion MRI sequences and biophysical models for computing other relevant WM microstructure metrics, including the intra-axonal volume fraction and the orientational dispersion index (Daducci et al., 2015), which may help to better understand the underlying microstructural differences observed here.

## 6.6 Supplementary information

**Figure 6.S1:** T1-weighted structural images of the cohort in the axial plane.



# 7. Contrastive case series: neural markers of severe language impairments beyond ASD

## 7.1 Introduction

It is the norm rather than the exception to encounter language disorders arising as a part of a complex pattern of impairments. Approximately 2-5% of children suffer from a particularly severe neurodevelopmental dysfunction (The Deciphering Developmental Disorders Study, 2017). Language problems linked to a certain 'differentiating condition' (Bishop et al., 2017) – for instance, a genetic syndrome, brain injury or other neurodevelopmental disorders (e.g., intellectual disability) – constitute varied models of language breakdown that surpass the limiting traditional notion of 'pure' neurodevelopmental language disorder. These 'natural language models' span a wide range of heterologous and coincident clinical features with complex aetiologies, yet standardized tests often capture only minimal variability specifically in severely impaired children. Exploration of the neural basis of these disorders by magnetic resonance imaging (MRI), often limited to gross anatomy only, is especially scarce in rare disorders with minimal prevalence. This chapter sought to go beyond investigating the neural basis of this pattern in the nvASD population only, by conducting structural and functional magnetic resonance assessment of seven cases with diverse diagnoses spanning from genetic syndromes to idiopathic disorders, while applying the same MRI protocol and analyses that were used for nvASD above. The syndromes in question are discussed in the remainder of this introduction.

Landau-Kleffner (LKS), a rare acquired epileptic encephalopathy (Hoshi & Miyazato, 2017), is characterized by a severe regression in language between 3 to 8 years of age after normal early development. The epileptiform activity is believed to functionally disrupt central auditory functioning leading to no verbal comprehension frequently accompanied by disturbed or absent speech (Castillo et al., 2008; Fandiño et al., 2011). Problems nevertheless tend to gradually recede before adulthood (Mikati et al., 2009). The agnosia is limited to the auditory modality insofar as children with LKS retain the ability to communicate through written or sign language, lip reading and gesture (Sieratzki et al., 2001).

Intellectual disability (ID), itself oftentimes linked to a genetic disorder (e.g., Down syndrome) or concrete environmental aetiology, also involves a varied scale of difficulties with

language starting with frequent language delay (DSM-5, American Psychiatric Association, 2013), and no speech development in some cases (Bal et al., 2020; Tsoutsoulis et al., 2012). Diagnostically, ID is driven by deficits in general mental abilities and daily adaptive functioning in relation to an age-equivalent developmental threshold. In idiopathic ID, the only pathological manifestation is cognitive deficit with no other concomitant anomalies of organ systems, although persistent efforts in recent years have detected up to 15% of pathogenic copy number variants in previously idiopathic ID cases.

Malformations of cortical development (MCD) in turn manifest as a vast amalgam of neurodevelopmental disorders associated with widely heterogeneous cognitive deficits (Stutterd & Leventer, 2014). Polymicrogyria (PMG), an excessive and minuscule cortical folding, and, in particular, PMG of the Perisylvian cortex (pPMG), is one of the most common MCD (Leventer et al., 2010). Orofacial diplegia causing difficulties in tongue movement, swallowing and speech is the core manifestation of pPMG along with ID and severity of speech impairment ranging from relatively fluent to minimal or absent speech (Bernal et al., 2010; Kilinc et al., 2015; Oh et al., 2018; Paldino et al., 2015).

Rare genetic diseases frequently affect the nervous system with a particularly severe pathology (Lee et al., 2020). The extent of speech delay has been specifically linked to the increased likelihood of having a pathogenic *de novo* mutation in developmentally important genes, above and beyond other severity indicators (The Deciphering Developmental Disorders Study, 2017). Thus, de novo missense mutation in BCL11A (OMIM: 606557) leads to a failure in transcriptional repression of fetal haemoglobin causing a neurodevelopmental disorder termed Dias-Logan syndrome (DLS). Little is known about the exact language difficulties that characterize DLS, apart from a somewhat inexact phenotype of speech and language delay. Yet, a case report in Soblet et al. (2018) details early speech consisting of single words that later develops into inaccurate speech-sound production in school years, limited by praxis problems and oral hypotonia. In turn, Coffin-Siris syndrome (CSS) has been described as multiple malformation syndrome (McCague et al., 2020) often manifesting in severe developmental delay (Coffin & Siris, 1970). Complete absence or speech in single words is observed in the majority of CSS cases especially with ARID1A and SMARCB1 mutations (Kosho et al., 2014; Kosho & Miyake, 2021). Certain understanding of complex language necessary for daily life is present especially in children with phrase speech, while children with no or few words, in turn, were mostly reported to

understand only simple commands (Kosho et al., 2014). Finally, more has been written on the behavioural profile of Fragile X syndrome (FXS) linked to a mutation in FMR1 leading to disturbances in synaptic plasticity and connectivity (Salcedo-Arellano et al., 2020). While almost a half of children with FXS has no or minimal speech as late as up to three years (Brady et al., 2006), phrase speech is ultimately acquired in FXS yet with lower syntactic complexity than expected by mental age (Del Hoyo Soriano et al., 2020).

While individuals with CSS may show a level of severity of language disturbance comparable to the one in nvASD – insofar as CSS may involve absent phrase speech and minimal understanding of verbal commands – other disorders show severe yet more heterogeneous phenotypic outcomes when compared against nvASD. Despite this, absent or no phrase speech is a common developmental outcome across several of the above disorders, yet with important nuances to be considered. Thus, signed, or written language communication remains in place in LKS and language abilities eventually return, the orofacial diplegia is a crucial underlying factor in pPMG, and minimal speech in FXS develops into phrase speech in the pre-school years. As such, all the above disorders constitute an important contrastive basis for models of severe language dysregulation in the brain that speak not only to the model of nvASD yet also go beyond.

Here I first reconstruct the dual pathways of the structural language connectome and assess their hemispheric lateralization based on white matter (WM) microstructural integrity of the tracts as indexed by fractional anisotropy (FA). I then, following the analysis pipelines of previous chapters, move to assess the intrinsic auditory network at rest via the independent component analysis and link it to the cortical activation to auditory speech. Finally, I assess the ROI-to-ROI functional connectivity within the core language network and the whole-brain networks in those cases without cortical malformations that allow for brain normalization to standard space.

## 7.2 Methods

### 7.2.1 Participants

The participants were seven children recruited from a special school in Madrid, Spain devoted to developmental disorders affecting language. They were specifically selected for severe language delays and deviances compared to other children in this school. Individual data and test results

from two standardized language tests are summarized in Table 7.1. T1-weighted anatomical images are shown in Figure 7.1 in axial and coronal planes. Cases were as follows:

**Case 1**: Current diagnosis of Idiopathic intellectual disability (ID): A 15-year-old girl with mild ID and severe difficulties in expressive language as well as reading. Her language comprehension and production is limited to simple sentences with limited vocabulary. She has difficulties in recognizing written words. At the time of study, she had reached a verbal mental age (VMA) of 7 years. In terms of daily functioning, this case shows the highest performance among the reported cases. Radiological examination of brain structure revealed no abnormalities.

**Case 2**: A 4-year-old boy with CSS (CSS_1) linked to a mutation in ARID1B gene. He has no speech and does not use any alternative systems of communication such as signed and written language, pictures, or gestures. He communicates to request mainly through taking adult hand and placing it on objects. His language comprehension is limited to simple orders embedded in rutinary situations. Early examination showed epileptiform activity across both hemispheres (predominant on the right). Currently he is on antiepileptic medication. Radiological examination revealed trigonocephaly, agenesis of corpus callosum (ACC), Hippocampal malrotation, enlargement of the temporal and occipital horns of the lateral ventricles, and probable loss of deep white matter in the posterior region of both cerebral hemispheres.

**Case 3**: A 7-year-old boy with CSS (CSS_2), who shows absent speech production and some, albeit limited language comprehension (VMA of 3 years). He communicates through gestures for everyday rutinary situations, and a communication notebook to structure sentences, yet with great difficulty. He has little sustained attention. Clinical radiological examination revealed dysgenesis of the corpus callosum and Mega cisterna magna consisting in an enlargement of the CSF-filled subarachnoid space in the *posterior* cranial fossa.

**Case 4**: A 5-year-old boy with FXS, who shows difficulties in structuring simple sentences with a VMA of 3 years and production restricted to juxtaposed words and simple phrases of 2-3 elements. Phonological simplification errors appear frequently in his speech production. He has very short attention span. Radiological examination of brain structure revealed no abnormalities.

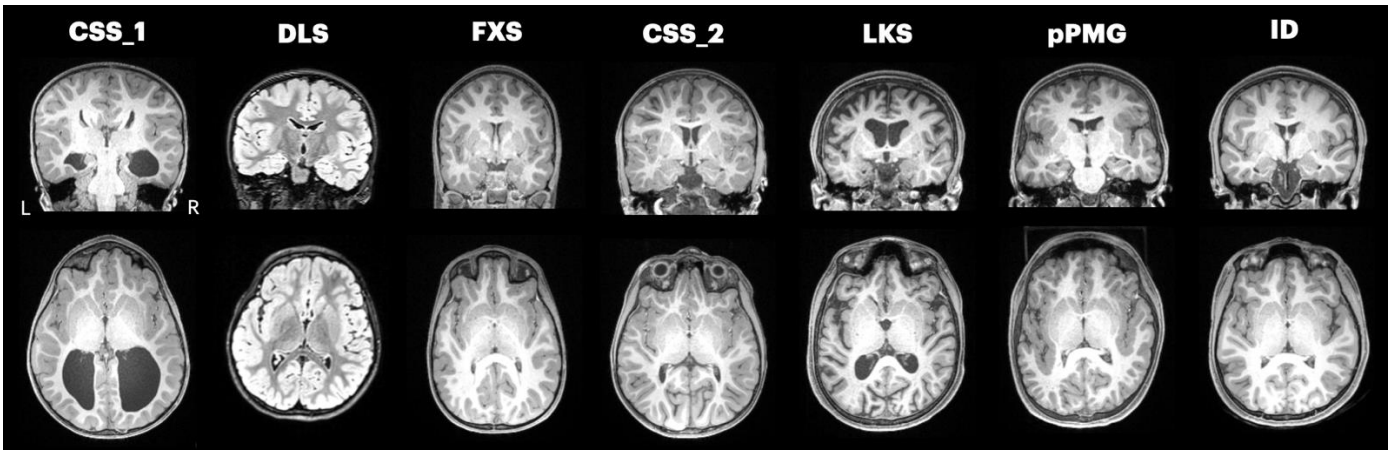
**Case 5**: An 11-year-old boy with LKS, who faced language loss at age 6 but had recovered language capacities by the time of study, though not exceeding 6 years of VMA in receptive

language. He shows difficulties in language production with decreased fluency. Radiological examination revealed an enlargement of the lateral ventricles, particularly on the left.

**Case 6**: A 5-year-old girl with DLS with considerable difficulties in articulation. She has recently started to construct simple sentences with two to three elements (inclusive verbs). She has difficulties to follow verbal orders only that need to be accompanied by visual clues (gestures) and embedded in context. Her vocabulary is limited but comprises semantic categories of animal, tool, house and school, and body parts that she can use declaratively. No alterations were observed in radiological and EEG examination.

**Case 7**: A 12-year-old boy with bilateral perisylvian PMG (pPMG) and absent speech. Genetic tests showed no alteration. He communicates mainly with gestures, text-to-speech application (set exclusively to a non-predictive text mode which enables typing text using a keyboard). He shows morphosyntactic errors in his writing and comprehension, phonological difficulties in distinguishing minimal pairs, and omissions and substitution of syllables and functional parts of speech. Clinical diagnoses include arthrogryposis multiplex congenita, dysgenetic brain malformation with secondary myoclonic epilepsy, light hypoacusis, bilateral hypermetropy, expressive dysphasia, and anarthria.

**Figure 7.1:** T1-weighted structural images from the five cases in coronal and axial planes. Clinical findings are described above.




*In the case of DLS, FLAIR image is shown due to considerable movement artifacts detected in T1-weighted and T2-weighted images.

**Table 7.1:** Subjects' demographics and results of standardized tests (verbal mental age (VMA) based on PPVT: Peabody Picture Vocabulary Test; direct punctuation in CEG: Test de Comprensión de Estructuras Gramaticales [test of grammatical comprehension]).

| Case | Age (y; m) | Sex | Handedness | VMA | CEG |
|---|---|---|---|---|---|
| **CSS_1** | 4;6 | M | R | - | - |
| **DLS** | 5;1 | F | R | 3;7 | 22 |
| **FXS** | 5;11 | M | R | 3;5 | 19/34 |
| **CSS_2** | 7;8 | M | R | 3;2/3;2 | 31 |
| **LKS** | 11;8 | M | R | 4;11/6;3 | 35/52 |
| **pPMG** | 12;3 | M | R | 5;3 | 52 |
| **ID** | 15;6 | F | R | 7;7 | 43/62 |

*VMA (Dunn et al., 2010) and CEG (Mendoza et al., 2005) evaluations were performed twice in two children, as indicated by two values in the same cell. The two tests were performed 5 months before the scan and five months after it, respectively. Children with single values reflect an evaluation at the earlier date.

### 7.2.2 Magnetic Resonance Imaging Acquisitions (Case series)

MRI data were collected at the Ruber International Hospital, Madrid, on a Siemens Prisma 3T scanner using a 64-channel head coil. The acquisition of a high-resolution T1-weighted structural image (magnetization-prepared rapid-acquisition, gradient echo sequence; TR = 2,400 msec, TE = 2.22 msec, slice thickness = .7999 mm, 0.8 mm in plane resolution, 208 sagittal slices, matrix size = 300 x 320) was followed by a T2-weighted structural image (TR = 3,200 msec, TE= 563 msec, slice thickness = .7999 mm, 208 sagittal slices, matrix size = 300 x 320). Diffusion MRI data consisted of 64 slices (FOV = 230x230 mm, voxel size = 1.98 x 1.98 $mm^2$, TR = 4,600 msec, TE = 101 ms , flip angle = 90º, slice-thickness = 2 mm; number of averages = 1; acceleration factor = 2; number of shells = 2; b-values = 625, and 1250 $s/mm^2$; number of diffusion gradient directions = 30; number of b0 (i.e., b-value=0) images = 3, plus 1 b0 with reverse phase to correct for spatial distortions). After structural data was collected, resting state fMRI (no stimuli were presented during acquisition) was acquired. One functional run consisting of 264 (8 min 52 sec) functional images sensitive to blood oxygenation level-dependent contrast (BOLD; echo planar T2*-weighted gradient echo sequence; TR = 2,000 msec, TE = 30 msec, flip angle 80º, acquisition

matrix = 576 x 576, 4.37 mm in plane resolution, 3.5 mm thickness, no gap, 32 axial slices aligned to the plane intersecting the anterior and posterior commissures) was acquired. Finally, after the resting state sequence, a fMRI language task was carried out. One functional run consisted of 358 (11 min 39 sec) functional images BOLD (echo planar T2*-weighted gradient echo sequence; TR = 2,000 msec, TE = 30 msec, flip angle 90º, acquisition matrix = 612 x 612, 2,35 mm in plane resolution, 3.5 mm thickness, no gap, 32 axial slices aligned to the plane intersecting the anterior and posterior commissures).

### 7.2.3 fMRI Experimental Design and sedation procedure

The parameters relative to fMRI design and sedation procedure were identical to the ones in the nvASD study in Barcelona described in chapter four. Sedation was not used in pPMG case.

### 7.2.4 Magnetic Resonance Imaging Acquisitions (controls)

MRI data from typical developing (TD) children and adolescents were included to compare patterns of ROI-ROI functional connectivity to the seven cases. These data were obtained from and collected at the University of California San Diego (UCSD) Center for Functional MRI (CFMRI) on a GE 3T Discovery MR750 scanner using an 8-channel head coil. The acquisition parameters and demographics are reported in Linke et al. (accepted) that used data from the same control cohort to report finding from FC analyses in four out of seven cases included here.

### 5.2.3 MRI Preprocessing and Analyses

Data preprocessing corresponding to Diffusion MRI, Independent components analysis (ICA) and activation to speech stimuli was identical to that applied and reported for nvASD population, apart from normalization of the functional and structural images to a standard space, as the analyses remained in native space. The ROI-to-ROI functional connectivity was computed for images in normalized standard space (3 mm-isotropic MNI152 template), and only in case of those participants without brain malformations (LKS, DLS, CSS_2, pPMG, and ID). The corresponding preprocessing of data was similarly identical to the one reported for nvASD.

<u>Tract segmentation and laterality index</u>

Bundle-specific tractograms were generated using TractSeg (Wasserthal et al., 2018) as in chapter six. For each subject, the mean FA values over the tract masks were estimated for the following language-related tracts: AF, SLF-III, IFOF, ILF and UF. A measure of lateralization (laterality index, LI) was obtained for FA microstructure index for each tract and participant separately, using the same LI computation as in the nvASD population. Thus, a positive LI value indicates lateralization to the left side, a negative value to the right side, and a close-to-zero value absence of lateralization.

Independent component analysis

Resting state data analysis was identical to that in chapter five. In case of pPMG, 52 volumes (25 in the middle of the acquisition) were discarded due to movement (signal intensity changes greater than 5 standard deviations), resulting in 212 volumes in total. An auditory component spatial distribution and time course was visually inspected by 2 raters. Empirical z-thresholding was used on the auditory component, using a threshold of $Z = 3$ ($p < .001$), minimal cluster size of 10.

Functional activation analysis

A block design matrix was specified using the canonical hemodynamic response function. HRF estimation via Finite Impulse Response (FIR) was explored yet has not yielded distinctive results (results not shown). Two different conditions were specified: Forward speech (FWD) and Backward speech (BWD). Each consisting of 10 blocks of stimuli. Data were high pass filtered (to a maximum of 1/128 Hz). Previously estimated 6 movement parameters were included in the model as part of the nuisance regression.

'Speech' (FWD+BWD) contrast was estimated. Individual activation maps to this contrast are shown thresholded at $p < .001$, uncorrected with a minimal cluster size of 10 voxels.

ROI-to-ROI functional connectivity analysis

This analysis FC estimates were derived for the rest and auditory stimulation runs separately. BOLD time series were averaged across all voxels comprising the targeted ROIs. Whole-brain network ROIs, comprising the classical brain networks, and the core language network ROIs were the same as in chapter four. The FC was computed as the degree of similarity of the BOLD time

series between ROIs estimated via bivariate Pearson correlation standardized with a Fisher z-transformation. A higher FC magnitude reflects more similar time courses between ROIs.

The following FC patterns were described for the seven cases. First, individual patterns of functional integration/segregation balance were reported in comparison to those observed in nvASD group in order to compare gross functional organization across different severe language impairments. Thus, the averaged within-network and across-network FC of the whole-brain networks was computed, and individual values of each disorder case were reported superimposed on the overall FC distribution in the nvASD group. The same was done for average within-ROIs and across-ROIs FC of the core language network.

The individual connectivity matrices of the core language network were extracted. Based on the observations from these matrices, data relative to the interhemispheric FC of the Heschl's gyrus (HG) were extracted for each case and superimposed on the HG interhemispheric connectivity distribution in the TD control group, and the mean interhemispheric FC in the nvASD group. FC data from the primary motor cortex were also visualized to exclude the possibility of generalized sedation effect on the observed patterns.

## 7.3 Results

### 7.3.1 Lateralization index of five language-related tracts

Results corresponding to the lateralization index relative to the FA for the five tracts of interest are depicted in Figure 7.2 relative to participants' chronological age in order to account for a possible age-modulation of LI. The AF and SLF-III failed to be reconstructed bilaterally in the pPMG case, in line with recent proposal of the failure to detect the AF as constituting a specific marker of pPMG (Paldino et al., 2015). The anatomical image representing the pervasive convolution of the Perisylvian cortex in the pPMG case can be seen in Figure 7-3. The ILF failed to be reconstructed in the CSS_1 case who shows acute enlargement of the temporal and occipital horns of the lateral ventricles. Overall, the LI patterns across the case series show highly varied patterns of hemispheric dominance, contrary to the nvASD population where rightward lateralization of the AF contrasted with the general leftward tendency in the remaining tracts.

In neurotypical development the AF/SLF-III complex usually shows inverse lateralization respective to each other (Amemiya et al., 2021; Eichert et al., 2019), that is, left-lateralization of

the AF and right-lateralization of the SLF-III. In the disorders targeted in this study, the lateralization of AF and SLF-III shows the same pattern of hemispheric predominance for both tracts, with either right or left tendency. Three cases (DLS, CSS_1 and LKS) show rightward tendency of hemispheric predominance in the AF, otherwise unexpected in right-handed neurotypical population (Eichert et al., 2019). Leftward tendency in the AF, on the other hand, is seen for ID, FXS and slightly so for CSS_2.

In LKS, rightward tendency can be observed for all remaining tracts but the ILF, while being especially profound in SLF-III. Right-hemispheric dominance has been previously reported in LKS for auditory and sign speech processing, reading and sentence generation (Datta et al., 2013; Sieratzki et al., 2001), alongside more pronounced reduction of cortical thickness in the left hemisphere (Linke et al., accepted), arguably suggesting a plasticity-driven hemispheric reorganization. The other two AF-right-lateralized cases were simultaneously the youngest of the sample. Yet, in the neurotypical population, FA-based leftward lateralization of the AF has been shown to be present even in early postnatal development (Dubois et al., 2016; Tak et al., 2016).

ID and DLS cases show roughly constant patterns of lateralization across all language-related tracts – DLS shows rightward tendencies, while ID is consistently left-lateralized. The ventral tracts, UF and IFOF, are strongly left-lateralized in CSS_1, the case with ventriculomegaly and absent ILF contrasting with the inverse tendency of the subject's dorsal tracts. The remaining three cases (pPMG, FXS, and CSS_2) showed more varied patterns of lateralization across the tracts.

**Figure 7.2:** Lateralization index (%) of five language-related tracts for the fractional anisotropy (FA) microstructure index. Positive values indicate a leftward tendency and negative values rightward tendency.

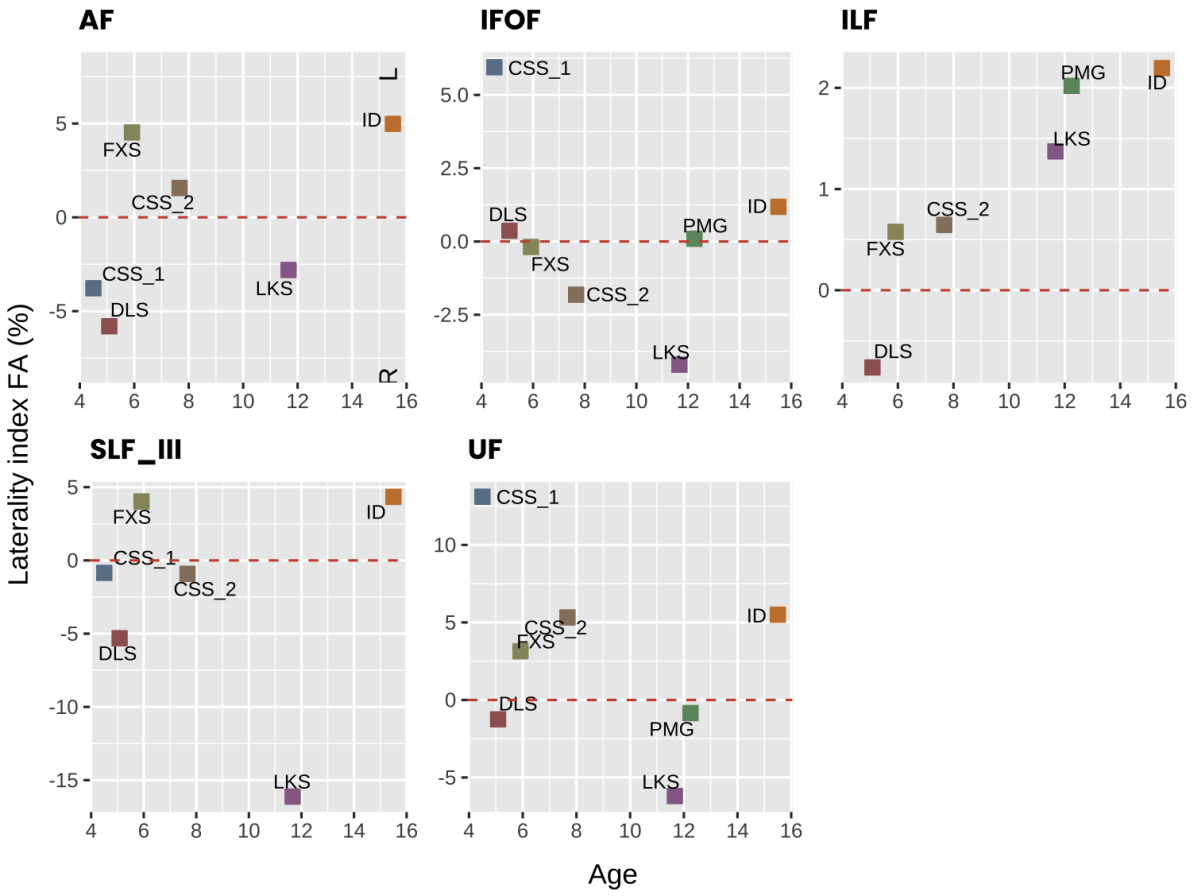
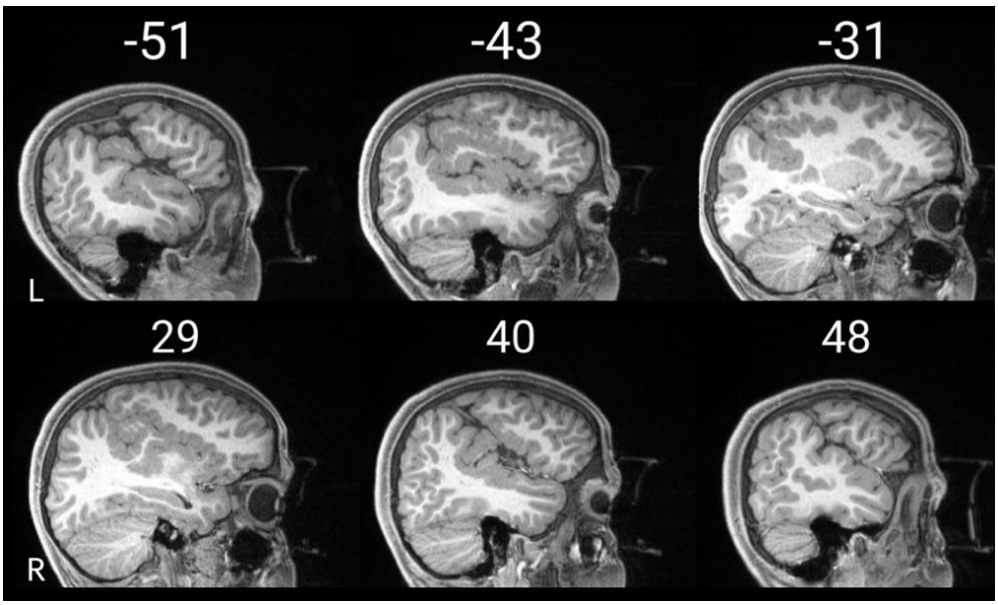



**Figure 7.3:** T1-weighter image of pPMG in sagittal plane. An acute convolution of the Perisylvian cortex can be observed, disrupting the organization of the underlying white matter.

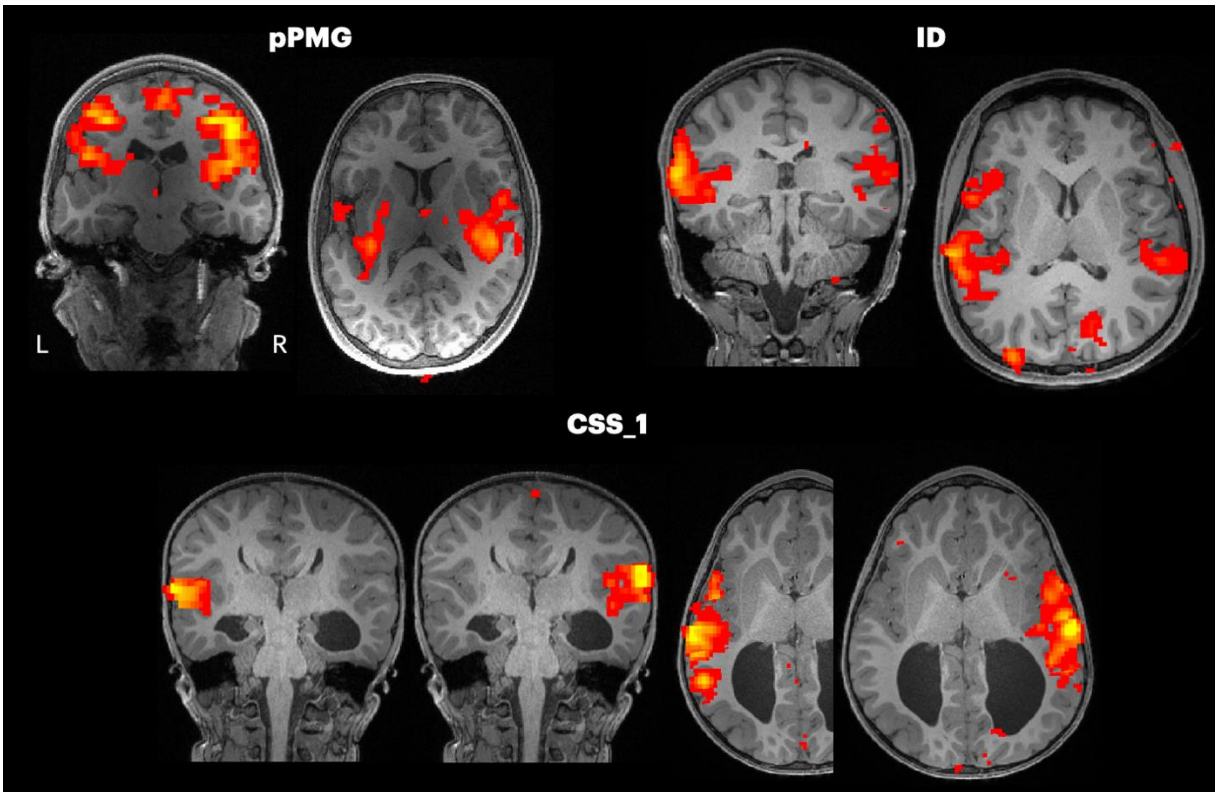


### 7.3.2 Intrinsic auditory network

Mean number of independent components (ICs) per subject was XX ± XX. Figure 7.4 shows individual resting-state auditory networks. An auditory IC could not be detected in 4 cases (CSS_2, LKS, DLS, and FXS). Movement artifacts are unlikely given no movement outliers in two cases, and comparable results after eliminating a few minor outliers in the remaining two.

In pPMG an auditory network arguably displaced dorsally was identified. The consequence of the polymicrogyric cortex on the functional brain organization are currently poorly understood and may include conservation of function in the malformed cortex or relocation of function outside of the affected area (Staudt et al., 2004), with the latter possibility probably reflected in the present pPMG case. Interestingly, the complete agenesis of corpus callosum (ACC) in the CSS_1 case has resulted in a split auditory network consisting of two unilateral components.

**Figure 7.4:** Auditory independent component of pPMG, ID and CSS_1 cases. The IC was thresholded at Z > 3, cluster size > 10 voxels. Underlying anatomical images are individual T1-weighthed images in native space.



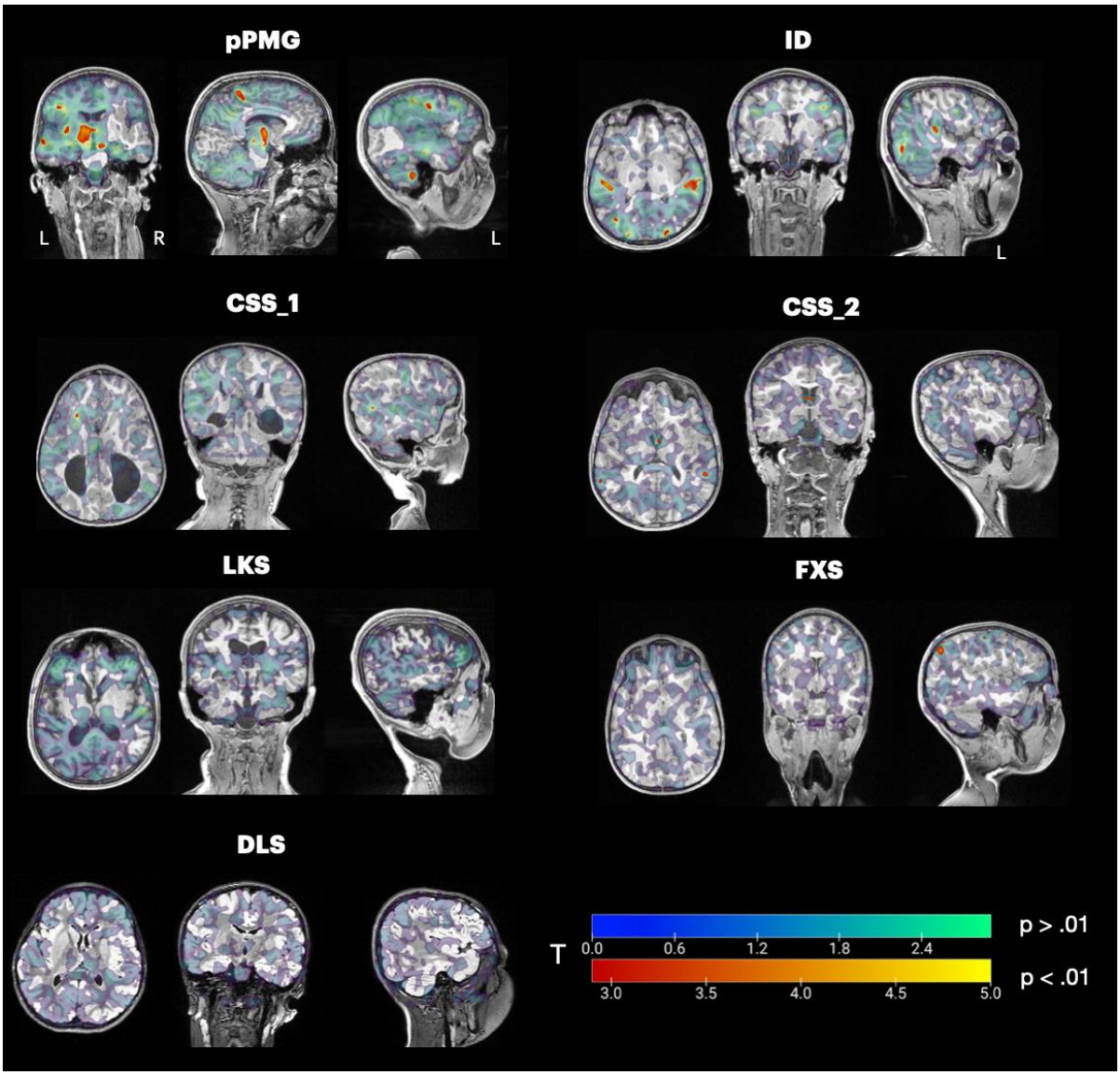
### 7.3.3 Task-evoked activity to Speech contrast

Individual subjects' activation maps to the Speech contrast can be seen in Figure 7.5. Significant activations are represented in warm red colours, voxel-wise thresholded at T = 3.12 ($p < .001$), uncorrected, with minimal cluster size of 10 voxels. Subthreshold activations ($p > .001$, in cold colours) are also represented given minimal or absent activation across all cases. Activations to Speech stimuli in a neurotypical pilot subject under the same scanner site, protocol and analysis, can be seen in Figure 7.S1 in the Supplementary information.

No significant cortical activations were observed in the pPMG case, despite being scanner without sedation. pPMG can indeed disrupt functionality of the malformed cortex leading to either a failure to show cortical activation (Staudt et al., 2004) or a displacement of the activity outside of the affected area (Araujo et al., 2006). Although the present findings are in line with the former possibility in the pPMG case, a significant signal increase was nevertheless observed subcortically, particularly in the thalamus. This finding suggests difficulties in the central auditory processing in the pPMG case as auditory information fails to be significantly relayed from the thalamus to the cortex, although a certain degree of activity over the malformed cortices and beyond could be observed in the unthresholded maps.

Interestingly, the LKS case showed no significant activation despite a previous report of significant bilateral cortical activation to speech despite severely impaired behavioural responses to speech (Sieratzki et al., 2001). A certain degree of cortical activation was instead seen in the ID case, the oldest participant with the highest VMA scores. The BOLD signal failed to increase significantly to speech stimuli in all the remaining subjects of the cohort.

**Figure 7.5:** Single-subject-level maps of voxel-level activation to Speech contrast. Significant voxel-wise activation thresholded at T > 3.12 ($p < .001$), uncorrected, cluster size > 10 voxels, is shown in warm colours. Cold colours represent subthreshold activation. The underlying anatomical images are individual T1-weighthed images in native space.

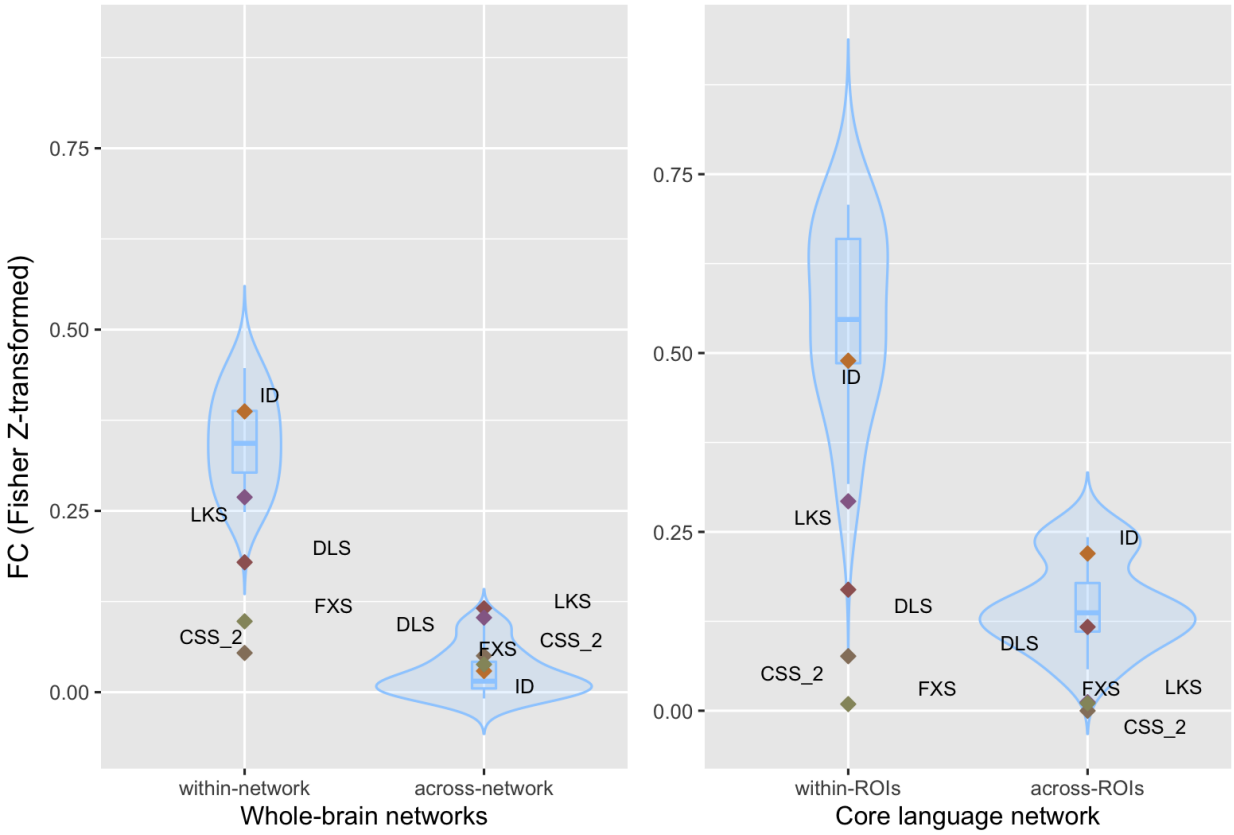



### 7.3.4 ROI-to-ROI within-network and across-network connectivity in the whole-brain and core language networks

Figure 7.6 depicts the average FC within networks and across networks of each of the five disorder cases targeted here, shown in comparison to the average within-network and across-network FC in nvASD. At the whole-brain level, a similar trend of decreased network integration that has been observed in nvASD is equally seen in the non-ASD cases with severe language impairments. Thus, lower or comparable within-network connectivity can be appreciated in in the disorder cases relative to the nvASD mean. DLS, CSS_2 and FXS all showed within-network FC below two

standard deviations of the nvASD mean suggesting greater network disintegration than the nvASD population. At the core language network level, these three cases again showed decreased integration of the network relative to nvASD reflected in the within-ROI FC below two standard deviations of the nvASD mean. This suggests that the decreased FC pattern is consistent throughout the brain and is not specific to the language network in particular.

**Figure 7.6:** A) Within- and across-network FC of the whole-brain networks. The FC values of the five cases are shown superimposed on the FC distribution in the nvASD group. The within-network FC of DLS, CSS_2 and FXS cases was below two standard deviations of the nvASD mean. B) Within- and across-ROI FC of the core language network. The FC values of the five cases is shown on the distribution of FC in the nvASD group. The FC within ROIs was once again below two standard deviations of the nvASD mean for DLS, CSS_2 and FXS cases. DLS and CSS_2 also showed lower FC across ROIs than two standard deviations of the nvASD mean, along with the LKS case.



To illuminate the decreased integration of the core language network across the non-ASD cases, Figure 7.7 shows the FC matrix of the core language network for each case. A strikingly low FC can be observed in the majority of the cross-hemispheric homologues, perhaps most strikingly in the HG across all five cases (Figure 7.8).

In the HG all five cases showed lower FC than both the TD and the nvASD means. FXS and CSS_2, in particular, fell below two standard deviations of these two means, while CSS_2 specifically has shown anticorrelated activity between the homologous auditory cortices. Reduced interhemispheric HG FC has previously been associated with higher ADOS Communication scores, i.e., greater difficulties in communication, in ASD (Linke et al., 2018). While CSS_2 and FXS have particularly low VMA scores relative to other cases, these are somewhat high compared to those that have been observed in the nvASD subjects and the nvASD population in general. Therefore, while strongly reduced interhemispheric coupling of the auditory cortices may be a common factor across severe disorders of language, relating the degree of magnitude to particular language profiles may be less straightforward.

Importantly, on the other hand, FC magnitude was comparable (within two standard deviations of the TD mean) for interhemispheric FC of preCG in four of the five children, suggesting that the lower FC magnitude observed for homotopic auditory regions was not solely due to confounding effects of site or sedation.

**Figure 7.7:** BOLD time series Pearson correlations (Fisher z-transformed) between 14 ROIs of the core language network at rest in the individual cases. Warmer colours correspond to stronger positive correlations. IFG = Inferior frontal gyrus, STG = Superior temporal gyrus, MTG = Middle temporal gyrus, HG = Heschl's gyrus.

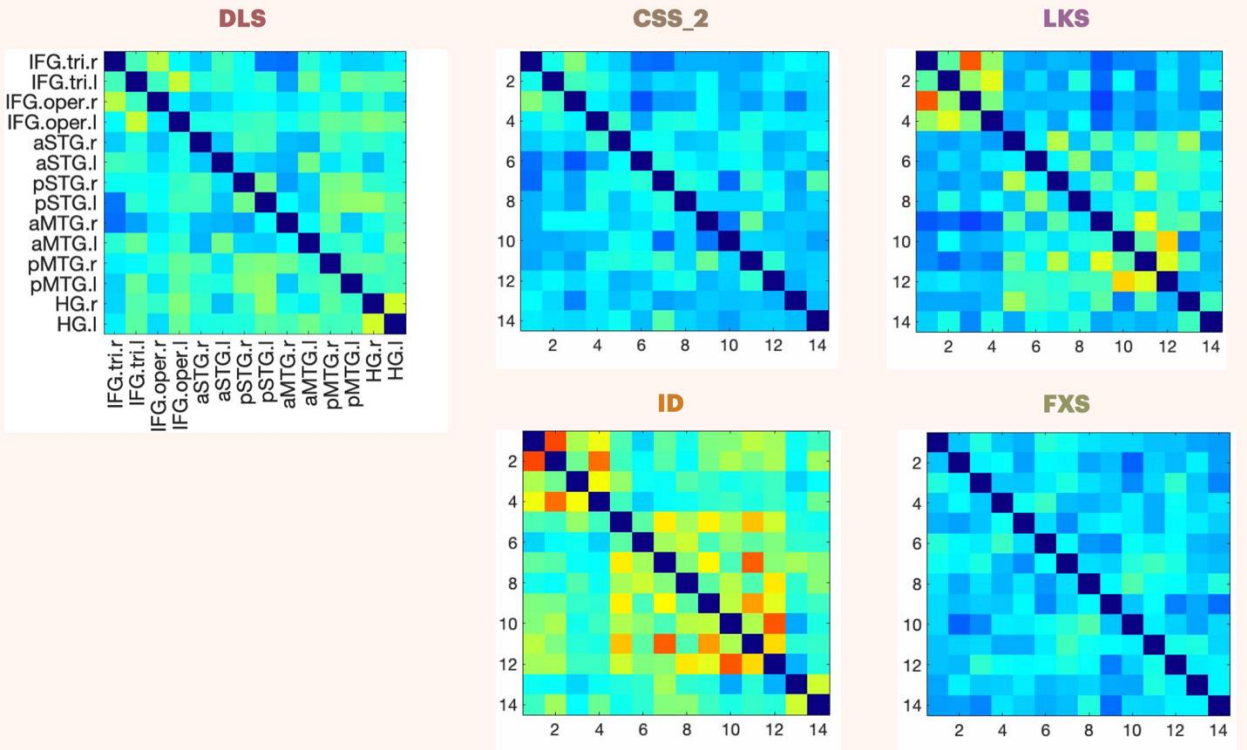
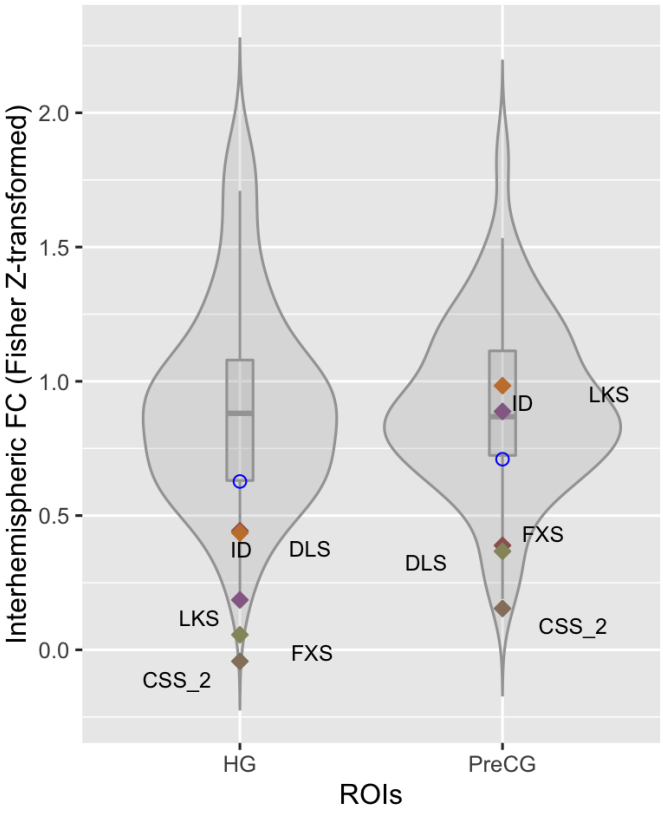



**Figure 7.8:** Interhemispheric FC of the primary auditory cortex (HG) and, as a contrastive control ROI, the primary motor cortex (PreCG). The HG shows reduced interhemispheric FC in the five cases with profound language impairment compared to the control TD group (data shown in gray). The mean FC of the nvASD group is shown in blue. Out of these, FXS and CSS_2 fell below two standard deviations of both the TD and the nvASD mean. FC in preCG fell within two standard deviations of the TD mean for all cases but CSS_2, suggesting that there was no global effect of reduced FC as a result of sedation (note, that interhemispheric auditory FC was not reduced as a function of deep sedation in Naci et al. 2018).




## 7.4 Discussion

In the present case series, I set out to gain a perspective on the neural basis of severe language impairment beyond the nvASD population, within the realm of rare language disorders with complex aetiologies spanning generic diseases, brain malformations and idiopathic disorders. Seven cases with varied diagnoses and severe language impairment were examined following the same protocol that was applied in nvASD, to set a contrastive basis for models of severe language dysregulation in the brain. Unlike in the nvASD cases, there is extensive evidence of anatomical anomalies in cortical folding, tissue loss due to extensive ventriculomegaly and agenesis of white matter tracts such as corpus callosum and possibly language-related tracts. Pervading traces of functional irregularities in brain wiring as indexed by networks intrinsic and evoked activity, could be observed in several of these conditions. Dysregulation od activity-independent mechanisms of cortical wiring such as axonal pathfinding or neural migration often lead to atypical functional

circuity (Penn & Shartz, 1999). It is noteworthy that the pervasiveness of functional atypicalities in these cases seems deeper than that observed in nvASD.

Bilateral perisylvian polymicrogyria (pPMG), in particular, confronted us with a profoundly disturbed brain structural template with overfolded and abnormally layered language-related cortex. Animal models of PMG result in reduction of white matter (WM) afferent and efferent fibre tracts and changes in the functional organization of the cortex (Rosen et al., 2000), aspects that have been similarly reported in human pPMG carriers (Araujo et al., 2006; Paldino et al., 2015). Findings in the present pPMG case are consistent with previous evidence. In particular, we were not able to reconstruct the arcuate fasciculus (AF) here, an observation that has been proposed as a specific marker of pPMG (Paldino et al., 2015). An absent AF has been similarly reported in other cortical malformations (Paldino et al., 2016), Angelman syndrome (Wilson et al., 2011a) or idiopathic intellectual disability (ID) (Sundaram et al., 2008), yet here the AF could be reconstructed in all other cases, including idiopathic ID. In pPMG, the present evidence further expands of previous observations by showing disruption of the entire AF/SLF-III complex, which could not be identified. FMRI studies in pPMG, on the other hand, show variability in the cortical representation of function arguably reflecting the severity of the anatomical alterations. Thus, Araujo et al. (2006) have demonstrated functionality of the malformed cortex specifically in motor tasks, and a displaced activation outside of the PMG cortex for language. Here no significant suprathreshold activation to speech was observed in the cortex (no sedation was used in this case), but enhanced functionality was detected subcortically in the thalamus, a relay point of auditory information flow before ascending to the neocortex (Lee & Sherman, 2010). Interestingly, however, the spontaneous coupling of the activity in the resting state was nevertheless present cortically, possibly corresponding to the auditory network displaced dorsally beyond the malformed cortex.

Language disruption in Landau-Kleffner syndrome (LKS) is regarded as resulting from a functional ablation of the cortex by epileptiform activity. As the verbal aphasia, a core diagnostic feature of LKS, subsides over the course of later childhood and adolescence, a few fMRI studies set out to explore cortical response to language, detecting a right-hemispheric functional reorganization (Datta et al., 2013; Sieratzki et al., 2001). In the LKS case reported here, Linke et al. (in press) earlier similarly observed a greater right-hemispheric preservation of cortical thickness in the language-related regions. The present evidence elaborates on aspects of arguably

reorganized structural wiring in LKS, by showing a right-hemispheric dominance of all the dorsal and ventral language tracts, except the inferior longitudinal fasciculus (ILF). Surprisingly, however, no significant activity to auditory speech stimuli was detected in the cortex in this case, despite previous evidence of activation even in behaviourally severely diminished processing of sound (Sieratzki et al., 2001). Contrary to the previously reported cases of LKS (Hoshi & Miyazato, 2017; but see Huppke et al., 2005), the present LKS case, however, presented macroscopic alterations in the brain structure linked to a considerable enlargement of the lateral ventricles.

Two cases in the current sample consistently yielded strongly reduced functional connectivity (FC) across several loci. Thus, the FC in Fragile X syndrome (FXS) case and the older participant with Coffin-Siris syndrome (CSS_2) fell below two standard deviations not only of the neurotypical mean but also the nvASD mean within whole brain-networks, the language network, and the interhemispheric coupling between the auditory cortices in the Heschl's gyrus (HG). Lower FC within brain networks has been previously observed in ASD, while lower interhemispheric coupling of the HG, in particular, has been linked to greater difficulties in communication (Linke et al., in press). Both the CSS_2 and FXS cases fall among the lowest scores in receptive language of this cohort and have considerable difficulties to produce even simple sentences (in the written or spoken modality, respectively). The low degree of the FC reduction in these cases is striking when compared to what we saw above in the nvASD population, which has both absent phrase speech and lower levels of receptive language.

In this regard, the other CSS case in this cohort (CSS_1) shows a behavioural profile more similar to that of the nvASD population, which is absent speech and minimal understanding of simple orders, and – as in the nvASD cases – shows preserved coupling of spontaneous activity in resting state constituting the auditory network, albeit split between the hemispheres. This case also shows a rightward dominance in the microstructural integrity of the AF, and a more bilateral SLF-III. At the functional level, a degree of subthreshold activity to speech stimuli in the auditory cortices could also be seen, although without a significant signal increase. Such a degree of preservation of the brain wiring is noticeable given extensive loss of tissue due to ventriculomegaly, a mega cisterna magna and agenesis of corpus callosum in this case.

Finally turning to the subject with idiopathic ID, the greatest degree of preservation of the cortical wiring can be observed in this case. It is striking that this greater degree of preservation

was also coupled with the highest receptive language abilities and general functioning in this sample. The conservation is specifically observed in within-network and across-network functional coupling of the whole brain and the core language network, as well as left-hemispheric predominance of the AF integrity.

Despite the aetiological and behavioural heterogeneity of the present cohort, certain commonalities across the individual cases were also observed. These specifically concern reduced interhemispheric coupling of the primary auditory cortices relative to the neurotypical population and the undistinctive lateralization of the AF and the SLF-III tracts, which in the neurotypical population has been reported to show an inverse pattern to each other (Amemiya et al., 2021; Eichert et al., 2019). These findings suggest a possibly highly vulnerable aspect of cross-hemispheric cortical wiring in severe language dysfunction.

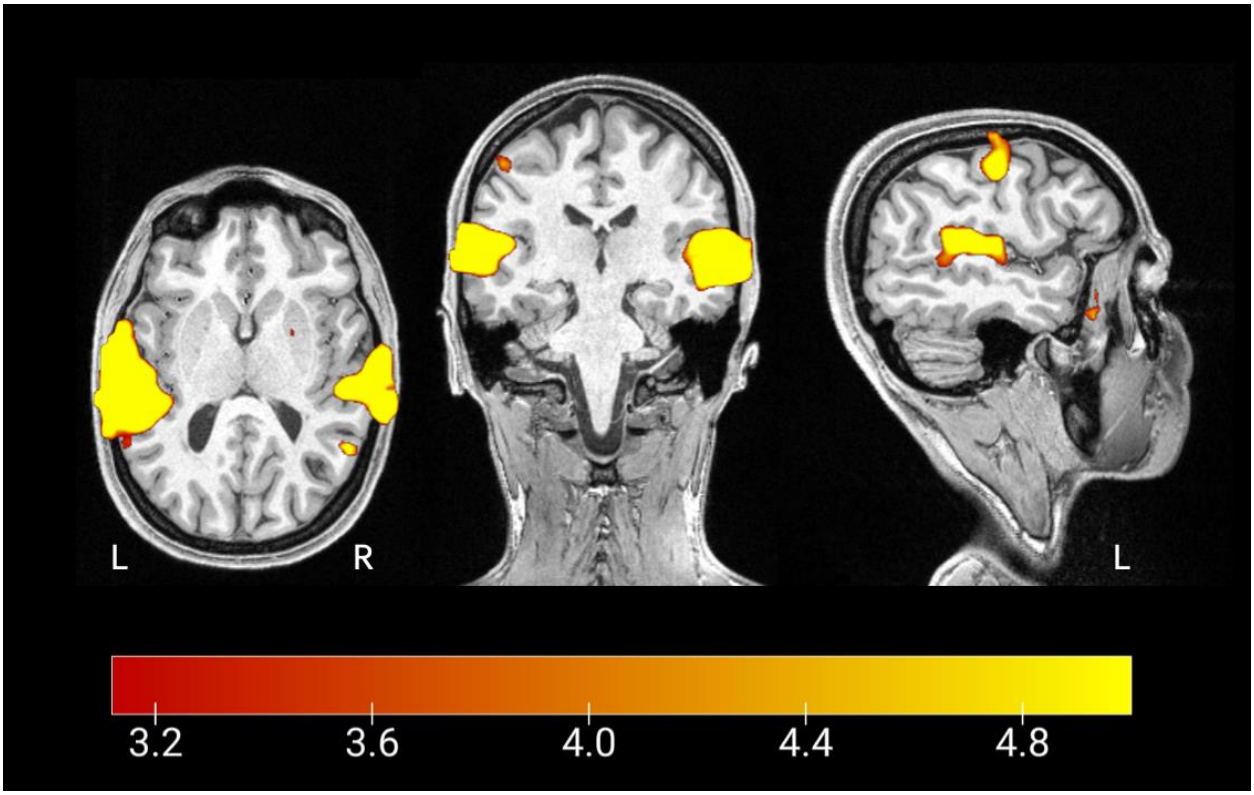
The extent and magnitude of the above findings in diverse and complex disorders implying severe language impairment raises new questions as well as new insights about the nature of brain and language dysfunction in nvASD. Specifically, questions arise about how illuminating current characterizations of the neural basis of language are for severe language impairments – a proposition that is both a question and an insight for future endeavours of understanding the neural basis of language. The following final chapter of this theses opens up to these considerations.

## 7.5 Limitations

The present study has equally suffered from the lack of a control group for the detectability and interpretability of pathological alterations. In this regard, the future endeavours of the research reported here will concentrate on the inclusion of a pool of neurotypical subjects matched on age, sex and handedness. Despite this limitation, the inter-sample comparison of individual cases and their comparison to what has been observed in nvASD, has succeeded in opening up the perspective and future considerations on the neural traces of severe language dysfunction.

## 7.6 Supplementary information

**Figure 7.S1:** A neurotypical pilot subject's (right-handed, female, 28 years of age, non-native speaker of Spanish) map of voxel-level activation to Speech contrast. Significant voxel-wise activation thresholded at T > 3.12 ($p$ < .001), uncorrected, cluster size > 10 voxels. The underlying anatomical image the subject's T1-weighted image.



# 8. General discussion

In some ways, nonverbal autism confronts us with a problem similar to the one faced by researchers in comparative cognition seeking to unearth the mind that does not speak: how do animals or infants *think*? Taking away the language system, if it is our very road of access to the domain of interest, seems to create a paradox and unresolvable problem (Clark & Hassert, 2013). Yet decades of research have not shunned to apply empirical methods to the problem of inferring mental states from behavioural measures, from gaze direction (eye-tracking) to neuroimaging. Much has been learned about from both infants and animals in this respect, yet recalcitrant issues have remained, which seem to accentuate the problem of ultimate undecidability through empiricism. Thus, strikingly, whether animals 'really think', or whether pre-productively verbal infants really form conceptual abstractions, has remained debated, with researchers positioned at both ends of the spectrum. These debates have a philosophical flavor, potentially indicating a limit to empirical inquiry.

Unmoved by this predicament, this thesis has opened yet another window on a population that has a 'language-less' mind as its most striking feature. That was with the promise of gaining unique insight into the fundamental principles of language, as reflected in the neural architecture. Supporting this promise is the severity of the affectation of the language ability in nvASD, which opens doors that traditional perspective on language impairment do not fully reach, in virtue of both the depth of the language and the concomitant cognitive impairment involved. What, then, have we learned from this thesis?

The behavioural profiling of this population from childhood into adulthood in chapter 2 has confirmed the pervasiveness and depth of the impairments in question, which motivated exploring aspects of brain development in this population that we know are already in place, to an extent, in pre-verbal infants. The primal behavioural characteristic that these two populations share is lack of phrase speech, the crucial difference being that development eventually leads to this milestone in the one population but, arguably, not the other. Thus, I explored the brain dynamics in the resting state and in task conditions, which have already been attested in early human development, along with structural connections subserving the brain functional dynamics.

While differences in the wiring of core language regions did emerge, overall, one striking feature of the present findings is the general gross *preservation* of major aspects of both the brain's structural and functional language connectome, and the anatomy of language regions. That preservation stands in a stark contrast with the sheer extent of the dysregulation of language development seen in nvASD, located at the absolute bottom end of language capacities in humans. In particular, the spontaneous coupling of neurons through correlated activity, which subserves their self-organization into networks, has not been erased in nvASD. Topologically, these networks consist of regions and structural and functional connections between them, which are those expected in the neurotypical population. Moreover, the auditory network self-organizing in the resting state shows a certain degree of reactivity and processing sensibility (mirrored in hemispheric specialization) to auditory stimulation. In this very sense, and at this level of inquiry, I have not found what I set out to identify: a neural correlate of the absence of language. Apart from a striking finding of overconnectivity in temporal cortex documented here, major aspects of what we today associate with the neural infrastructure of language is documentable, too, in this population, whose failure of language is, at the same time, its most defining feature.

This profound paradox may lead to different conclusions and future directions. One logical possibility is that what we have come to think of as the neural infrastructure of language fails to be that: if it is present in humans without language, it cannot deserve its name. We are losing our grip of what language is, where to look for it in the brain, and what methods to employ. This would be, however, a hasty conclusion to draw. The present findings about the case series of subjects without ASD are illuminating in this respect: here we *could* observe in many cases a disturbed organization of canonical language cortex at a macroscopic level. We saw cortical malformations and failures of axonal pathfinding, displaced and nonsignificant cortical activation to sound, nondetectable networks of spontaneous activity, or anticorrelations of regions where high correlations are expected. An insightful example is the subject with bilateral perisylvian polymicrogyria and language impairment with considerably less severity than the one of nvASD, who had nevertheless distinctive macroscopical irregularities in brain wiring. The paradox just raised, therefore, is, above all, a paradox about *autism*, at its nonverbal end. This suggest that looking at these aspects of brain wiring can indeed be insightful in diverse severe language impairments, yet fall short of the underpinnings of the kind of impairment that underlies nvASD despite language being its defining feature.

This quandary could evolve into a more full-blown sense of the ultimate inscrutability of the nonverbal mind in this autistic case, echoing similar scepticism as voiced by some in the cases of infants and animals. Yet, short of reaching this dead end, another possible conclusion is that we need to continue on an empirical path, using the results of this thesis that demand further exploration and give us a hint of where to try to go next. In particular, functional connectivity reflects a macroscopic functional organization targeting correlations between active regions, but says nothing yet about their causal interrelations. The task-related activation of these regions amounts to a metabolic increase of only 5% with respect to energy consumed in the resting state (Smitha et al., 2017). Also, the structural connectivity examined here is only a fraction of the language connectome corresponding to long-range associative fibres among the canonical language regions. The insightful irregularities that did transpire from these macroscopical explorations may guide our attention in a more nuanced assessment of brain wiring that appears imperative in the nvASD case.

Brain anatomy and genome as determined from radiological and genetic screening of children with neurodevelopmental disorders are often normal, even with reasonable evidence that brain and genetic anomalies must exist. In such cases, the level of description I have selected here for the case of nvASD may allow only for the tip of the iceberg to be glimpsed. Regardless of where to go next, however, this thesis may provide a healthy reminder of how little we still know, not only about language in the brain at both structural and functional levels, but about autism as well.

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