FACULTEIT PSYCHOLOGIE EN PEDAGOGISCHE WETENSCHAPPEN

THE ROLE OF WHITE MATTER ON LANGUAGE ABILITIES OF CHILDREN WITH AUTISM SPECTRUM DISORDER: A DIFFUSION MRI STUDY

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Preface

Completing this thesis, I have learned invaluable new skills and knowledge, while also deepening my understanding of autism. This project has been a rewarding experience, and I would like to express my gratitude to some special individuals who made it possible.

I want to begin by expressing my appreciation to my promotor Dr. Ellen Demurie for welcoming me into the PIP project and providing me with the opportunity to undertake this thesis. I am thankful for her thorough review of my work and the valuable advice she provided for its enhancement.

I would also like to thank my supervisor Marjolein Mués, whose guidance and support have shaped the content and direction of this thesis. Her continuous feedback allowed me to write this thesis to the best of my capabilities.

Moreover, I would like to thank all the children and parents who participated in this study. Their involvement made it possible for this research to continue. I have immense respect for them and would like to express my special appreciation to these individuals.

Finally, I want to thank my parents for always being there for me during my studies. They have been my biggest supporters. My appreciation also goes out to my other family members, particularly my brother Ersin, for dedicating time to review and proofread my thesis. I am also grateful to my brother Orkun for providing substantial emotional support throughout.

Completing my journey as an Experimental Psychologist and reflecting on this enriching thesis experience in such a captivating field brings me an overwhelming sense of happiness and fulfillment.

Abstract

The heterogeneity of autism spectrum disorder (ASD) renders it a complex and challenging condition to address, especially with regards to language difficulties. The aim of this current thesis was to explore the relationship between language abilities and white matter microstructures in preschool children diagnosed with ASD, by using diffusion MRI. In this study, two language domains were examined, namely 1) expressive language, as assessed by the Mullen Scales of Early Learning (MSEL) and mean length of utterance (MLU) during a play-based interaction between parent and child, and 2) receptive language, also evaluated using the MSEL. The arcuate fasciculus (AF) (i.e., a crucial language-related white matter tract) was quantitatively evaluated by analyzing fractional anisotropy (FA), radial diffusivity (RD), and mean diffusivity (MD). The results revealed significant differences in expressive language measures between children with ASD and typically developing children. However, these differences became non-significant after controlling for nonverbal cognition. Surprisingly, no differences in white matter microstructures were observed between the two groups. Regarding associations between the DTI measures and language scores, a positive relationship was found between FA and an expressive language measure (MLU), while a negative association emerged between RD and MLU, as expected. Overall, some evidence was found for associations between language abilities and white matter microstructures. However, it is essential to exercise caution in interpreting these results, given the small sample size. Exploring the complete sample of the study could further help unravel neural mechanisms behind language abilities in ASD.

Abstract (Nederlands)

De heterogeniteit van autisme spectrum stoornis (ASS) maakt het een complexe en uitdagende aandoening om te benaderen, vooral wat betreft taalmoeilijkheden. Het doel van deze huidige scriptie was om de relatie tussen taalvaardigheden en microstructuren van witte stof te onderzoeken bij kleuters gediagnosticeerd met ASD, door middel van diffusie-MRI. In deze studie werden twee taaldomeinen onderzocht, namelijk 1) expressieve taal, beoordeeld aan de hand van de Mullen Schalen voor Vroegtijdig Leren (MSEL) en gemiddelde lengte van uitingen (MLU) tijdens een op spel gebaseerde interactie tussen ouder en kind, en 2) receptieve taal, ook geëvalueerd met behulp van de MSEL. De fasciculus arcuatus (AF) (een cruciale witte stofbaan gerelateerd aan taal) werd kwantitatief geëvalueerd door de fractionele anisotropie (FA), radiale diffusiviteit (RD) en gemiddelde diffusiviteit (MD) te analyseren. De resultaten onthulden significante verschillen in expressieve taal tussen kinderen met ASS en typisch ontwikkelende kinderen. Deze verschillen werden echter niet-significant nadat er rekening werd gehouden met non-verbale cognitie. Verrassend genoeg werden er geen verschillen waargenomen in microstructuren van witte stof tussen de twee groepen. Wat betreft de verbanden tussen de DTI-maten en taalscores, werd een positieve relatie gevonden tussen FA en een maat voor expressieve taal (MLU), terwijl er een negatieve associatie was tussen RD en MLU, zoals verwacht. In het algemeen werd enig bewijs gevonden voor verbanden tussen taalvaardigheden en witte stof. Het is echter essentieel om voorzichtig te zijn bij het interpreteren van deze resultaten, gezien de kleine steekproefomvang. Het verkennen van de resultaten van de volledige steekproef van de studie kan verder helpen bij het ontrafelen van neurale mechanismen achter taalvaardigheden bij ASS.

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Introduction

Autism spectrum disorder (ASD) is notably a highly heterogeneous developmental disorder (Geschwind, 2009; Amaral et al., 2008; Abrahams & Geschwind, 2008). It is marked by pervasive difficulties with social communication and interaction as well as restricted and repetitive behaviors and interests (American Psychiatric Association (APA), 2013). As the name would suggest, a wide variety of symptoms are exhibited, meaning that no two people with ASD are completely alike. ASD typically manifests itself during infancy and continues throughout the lifespan (Baxter et al., 2015). However, there is evidence that early interventions can yield positive outcomes such as improved language functioning (Dawson et al., 2010).

Language difficulties are a prominent feature in ASD, contributing to significant impairments in communication and social interaction (Baird & Norbury, 2016). However, it is important to note that while these difficulties are prevalent among many children with ASD, they are not present in all individuals (Boucher, 2012; Pickles et al., 2014; Song & So, 2021). Understanding the neural mechanisms behind language abilities in ASD is important for unraveling its complex nature and developing targeted interventions. This thesis aims to investigate the relationship between language abilities and white matter microstructures in children with ASD using diffusion MRI (dMRI). In this introduction, a general overview is given about ASD, including a brief historical overview as well as information on prevalence and etiology. Moreover, the diagnostic criteria are outlined, as described in the Diagnostic and Statistical Manual of Mental Disorders - Fifth edition (DSM-5) (APA, 2013). The difference regarding language criteria between the DSM - Fourth edition (DSM-IV) (APA, 1994) and the DSM-5 is also discussed. This is followed by a comparison of language development in typically developing (TD) children and children with ASD. Furthermore, previous research investigating the neural correlates of language ability in individuals with ASD is examined. The aims and hypotheses of the present study are delineated as a final point.

Autism Spectrum Disorder (ASD)

History

The term “autism” was derived from the Greek word “autos”, which literally means “self”. Eugen Bleuler was the first to mention this word in 1911. He referred to “autism” as a symptom in severe cases of schizophrenia, in which the patients “live in a world of their own” (Bleuler, 1911). A few decades later, in 1943, Leo Kanner was the first to describe autism as a developmental disorder with characteristics that are still implemented today as diagnostic criteria (Kanner, 1943). Autism was eventually first included in the DSM-III (American Psychiatric Association (APA), 1980) as a distinct diagnostic category, under the name of Pervasive Developmental Disorders (PDDs). Apart from the subcategory autism, other subtypes were eventually introduced in the DSM-III-R (APA, 1987) and the DSM-IV (APA, 1994), such as Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), Asperger’s Disorder, Rett’s Disorder and Childhood Disintegrative Disorder (CDD). However, it was not until the DSM-5 that autism was officially acknowledged as a spectrum disorder, emphasizing the wide range of symptoms and challenges experienced by individuals with ASD.

DSM-5 diagnostic criteria

The fifth edition of the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (APA, 2013) is nowadays predominantly used for diagnosing ASD. Due to its heterogeneous nature, ASD is defined as a spectrum disorder (APA, 2013). It is heterogenous in the sense that there is great variability regarding the level of autism characteristics displayed and the developmental trajectory (Wiggins et al., 2011). The symptoms of ASD are placed along two dimensions in the DSM-5, namely domain A and B.

The first broad set of symptoms in the A domain pertains to difficulties in social communication and interaction. Examples of symptoms in this domain are described in three subdomains; deficits in (1) social-emotional reciprocity (e.g., reduced sharing of interests, emotions or affect), (2) nonverbal communicative behaviors (e.g., atypicalities in eye contact and body language), (3) developing, maintaining, and understanding relationships (e.g., absence of interest in peers) (APA, 2013).

The second B domain covers restrictive and repetitive behavior, interests or activities. Examples of symptoms in this domain are described in four subdomains; (1) stereotyped or repetitive motor movements (e.g., lining up toys), (2) insistence on sameness (e.g., extreme distress at small changes), (3) highly restricted, fixated interests (e.g., strong attachment to unusual objects), (4) hyper- or hypo-reactivity to sensory information (e.g., excessive touching of objects) (APA, 2013).

Additionally, these symptoms need to make an appearance in early childhood, cause difficulties in everyday functioning and may not be better explained by intellectual disability or global developmental delay (APA, 2013). In contrast to the subcategories of DSM-IV-TR (APA, 2000), autistic disorder, Asperger's disorder and PDD-NOS are all included in the diagnosis of ASD. Rett's disorder and CDD are no longer considered as autism and therefore are not classified as separate disorders in the DSM-5.

DSM-IV vs DSM-5: language in diagnostic criteria. The introduction of DSM-5 brought with it a transition from using subcategories in the DSM-IV to one single diagnosis using a multidimensional approach in the DSM-5. As a result, there are some notable differences between DSM-IV and DSM-5. In the DSM-IV, autism was characterized by a triad of core symptoms: impaired social interaction, impaired social communication, and restricted behavior patterns, which included specific criteria for language impairments (APA, 1994). Contrarily, language impairments are no longer considered an inherent part of ASD in the DSM-5's dyad of symptoms and therefore are not included as a separate diagnostic criterion. There is, however, the possibility of adding the presence or absence of language impairments as a specifier to the diagnosis and many individuals with ASD ultimately do develop language difficulties (Baird & Norbury, 2016; Pickles et al., 2014). Additionally, there is a distinct category for children with pragmatic language difficulties without difficulties in the B domain of ASD (referred to as Social (Pragmatic) Communication Disorder or SPCD).

Prevalence

Prevalence rates indicate that ASD occurs globally in around 1 in 132 individuals. (Baxter et al., 2015), which makes ASD one of the most common neurodevelopmental disorders. The reported prevalence of ASD has increased in recent years (Maenner et al., 2020). It is often debated whether there is indeed an upward trend of ASD cases in recent years or if the observed increase is due to increased awareness, improved detection methods or changes in diagnostic criteria (Hansen et al., 2015; Russell et al., 2021; Rice et al., 2012). When controlled for methodological aspects, however, Baxter et al. (2015) found that the prevalence of ASD was relatively stable between 1990 and 2010. Regarding gender differences, ASD is predominantly diagnosed in males (Loomes et al., 2017). Whether this is due to actual gender differences or because females are underdiagnosed, is still a question (Rynkiewicz et al., 2016; Loomes et al., 2017).

Etiology

To date, the pathogenesis of ASD is poorly understood. It is thought to be the result of a complex interaction between genetic, environmental and epigenetic factors (Bhandari et al., 2020). In the majority of cases (85%), ASD has an unknown cause (idiopathic autism) (Casanova et al., 2020). The remaining 15% have a known origin (secondary autism), which is either genetic, environmental or a mix of those factors (Casanova et al., 2020). Prior research shows that there is a strong genetic component to ASD (Sandin et al., 2014). However, while it is assumed that multiple genes play a role, it is yet to be determined which genes are specifically responsible for the ASD phenotype. Environmental factors that are putatively related to an increased likelihood for ASD include advanced maternal and paternal age at birth (Croen et al., 2007), maternal fever during pregnancy (Zerbo et al., 2013), oxygen deprivation during birth (Gardener et al., 2011) and extreme prematurity (Limperopoulos, 2009).

Language development

Children are equipped with an innate tendency to acquire language (Miller et al., 1994). This becomes evident when we consider the astonishing speed of how infants develop into competent language users in a time span of just a few years. Therefore, it should come as no surprise that language development is an important aspect of cognitive and social development in humans. Moreover, good language abilities at an early age are significantly associated with later academic and vocational success (Conti-Ramsden et al., 2018; Howlin et al., 2013; Magiati et al., 2014). In addition, there are risks associated with a failure to successfully develop language, such as poor mental health (Law et al., 2009; Sturrock et al., 2022) and poor academic outcome (Beitchman et al., 1996; Conti-Ramsden et al., 2018).

Just as though no child has the same developmental trajectory of language (Fenson et al., 1994), children on the autism spectrum show especially heterogeneity in language abilities (Smith et al., 2007; Baird & Norbury, 2016; Boucher, 2012; Kjelgaard & Tager-Flusberg, 2001; Pickles et al., 2014). In the following paragraphs, typical language development will be compared to language development in ASD and focus will be placed on the heterogeneity of language development in ASD.

Typical language development vs language development in ASD

Regarding the development of language, there is a wide debate between the effects of nature and nurture (Tabery, 2014). In brief, the question is to what extent language develops by exposure to the environment versus by genetic factors that are present at birth. However, there is evidence that the onset of language acquisition emerges even before birth. For example, Moon et al. (2013) showed that fetuses show at least some level of learning when exposed to vowel sounds in the womb. After birth, language develops rapidly during infancy.

Typically developing children exhibit a remarkable growth in vocabulary size during their early stages of life. As they interact with their environment, their vocabulary expands rapidly. In fact, by the age of two years old, most typically developing children possess a vocabulary of around 200 to 300 words, and several thousand words at the age of six years old (Fenson et al., 1994).

One of the key measures used to assess language development is the mean length of utterance (MLU; Scarborough et al., 1991). MLU refers to the average number of morphemes or words in a child's utterance. As language develops in typical development, the MLU increases (Rice et al., 2010), reflecting the progression of expressive language (i.e., expressing one's own thoughts and desires by spoken, written and body language). In ASD, it is observed that there is often a challenge in moving from single-word to multiword utterances (Paul, 2008; Volden & Lord, 1991). Previous work found that children with ASD had significantly lower MLU compared to typically developing children (Volden & Lord, 1991; Eigsti et al., 2007). These observations indicate a lower level of language abilities in children with ASD.

Besides being able to produce language effectively, it is equally important to have the capacity to comprehend it to engage in successful social communication with the environment. The capability to comprehend language is often labeled as receptive language. Typically, receptive language develops prior to expressive language in typical development (Simms, 2007). Evidence shows that language comprehension commonly emerges around the age of 9 months while the onset of language production occurs several months later, by the age of 12 months (Fenson et al., 1994).

In ASD, however, this receptive language advantage is not observed (Luyster et al., 2007). With regard to the expressive and receptive language skills of children with ASD, there is mixed evidence in the literature. While it used to be thought that there is an advantage for expressive language (Hudry et al., 2010), a recent meta-analysis revealed that difficulties are equally present in both receptive and expressive language (Kwok et al., 2015).

Compared to their typically developing peers, children with ASD often show significant deficits in both expressive and receptive language abilities. For instance, Kover et al. (2013) observed that in their sample of male children with ASD, they showed significantly lower scores for both expressive and receptive language compared to TD children. These findings are supported by meta-analyses conducted by Kwok et al. (2015) and Brignell et al. (2018), which have shown that a substantial number of individuals with ASD experience significant deficits in both expressive and receptive language

abilities when compared to their TD peers. Overall, these consistent findings confirm the patterns of language difficulties in children with ASD.

Heterogeneity of language development in ASD

Language impairment is often the first reported parental concern in later diagnosed individuals with ASD (Herlihy et al., 2013). However, there is considerable variability in terms of language abilities in children with ASD (Georgiades et al., 2012). While some children with ASD never acquire language, others have language deficits and yet other children on the autism spectrum have linguistic skills that are intact or even above average (Boucher, 2011). Approximately one third of children with ASD remain minimally verbal[1] after the age of 5 (Anderson et al., 2007; Billstedt et al., 2007; Rose et al., 2016). Among the remaining two third that do develop language, many exhibit grammatical and lexical errors to some extent in their spontaneous speech (Boucher, 2012; Wittke et al., 2017). However, it is worth noting that the degree and prevalence of these errors varies greatly among individuals (Groen et al., 2008; Rapin et al., 2009). In addition to the heterogeneity observed in expressive language abilities, there is also significant variability in receptive language skills among individuals with ASD (Kjelgaard & Tager-Flusberg, 2001). This implies that language impairment is not universal in ASD and shows that there is great heterogeneity in language abilities. It is poorly understood what causes this heterogeneity, but several risk factors seem to play a role (Tager-Flusberg, 2016).

Even though there is considerable debate about ASD being related to developmental language disorder (Kjelgaard & Tager-Flusberg, 2001; Georgiou & Spanoudis, 2021), the language impairments in ASD cannot solely be explained by a comorbid language disorder (Özyurt & Eliküçük, 2018; Georgiou & Spanoudis, 2021). This is because language deficits and their underlying mechanisms cannot be assumed identical in ASD and developmental language disorder (Luyster et al., 2011).

[1] In the literature, there is little consistency in the definition of being 'minimally verbal'. Some classify children as minimally verbal when their expressive language consists of a minimal set of spoken words (the precise quantity of words may vary, usually around 20) (Kasari et al., 2013; Koegel et al., 2020), while others define it as lacking all spoken language (Tager-Flusberg & Kasari, 2013).

Language in the brain

Language is a complex process which involves specific brain areas that are connected to each other via certain pathways. Through these connections, language areas – which are generally more pronounced in the left hemisphere in the majority of right-handed people (Knecht et al., 2000) – work together in a coordinated manner in order to process language. Wernicke's area is a region located in the temporal lobe of the language-dominant hemisphere and is broadly associated with receptive language (Chertkow et al., 1997; Okada & Hickok, 2006). Another region that is involved in language, the Broca's Area, is located in the frontal lobe and is in part associated with language production (Geschwind, 1970; Amunts et al., 2010).

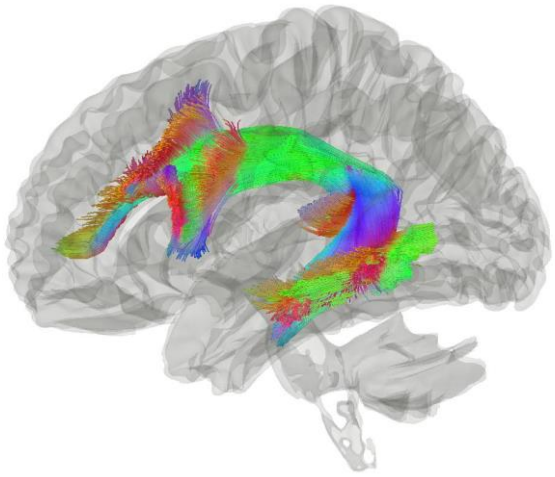
Language and white matter

In addition to the gray matter, which is mainly found in the outer cortex, there is another type of brain tissue, namely white matter. This is the tissue underneath the cortex and it is made up of bundles of nerve fibers (i.e., axons). It ensures the exchange of information between different brain areas by connecting gray matter regions to one another (Nave & Werner, 2014). This exchange is rapid due to the myelin that coats the axons (Nave & Werner, 2014), hence the white color of the tissue (Fields, 2010).

A white matter pathway that is crucial for language processing, called the arcuate fasciculus (AF, Figure 1), connects Wernicke's and Broca's areas directly and carries information between them. It has been suggested that the AF is involved in several components of language processing, namely in articulation, speech perception and phonological processing (Vandermosten et al., 2012). Apart from this direct pathway, an indirect pathway running parallel and lateral to the AF was also observed (Catani et al., 2004). The anterior segment of this indirect pathway connects Broca's area with the inferior parietal lobe, while the posterior segment connects the inferior parietal lobe to Wernicke's territory. In other words, this indirect pathway provides an alternative route for the flow of information between language-related regions in the brain (Catani et al., 2004).

Figure 1

Illustration of the arcuate fasciculus tract



Note. Reprinted from "Population-Averaged Atlas of the Macroscale Human Structural Connectome and Its Network Topology", by Yeh et al., 2018, *Neuroimage, 178*, 57–68.

It is indeed shown by previous work that the AF is associated to language outcomes in typically developing children and adults (Powell et al., 2006; Catani et al., 2007). Unsurprisingly, the observation of the AF being associated to language is particularly the case in the left hemisphere (Catani et al., 2007; Lebel & Beaulieu, 2009). This leftward asymmetry of the AF is already present by the age of two years (Reynolds et al., 2019).

Neural bases of linguistic abilities in ASD

Individuals with ASD have, on average, distinct brain development and structure compared to individuals without ASD (Hazlett et al., 2017; Thompson et al., 2020; Van Rooij et al., 2017). One of the most robust findings regarding brains of individuals with ASD is the presence of enlarged brain volumes at early ages, which diminishes later on (Redcay & Courchesne, 2005). Surprisingly, this brain enlargement seems to be mainly the result of an increased white matter volume (Herbert et al., 2004). This observation suggests that abnormal white matter might be one of the factors underlying ASD.

In recent years, researchers have tried to clarify the neurobiological mechanisms behind language abilities in ASD. For example, Rojas et al. (2005) showed that there is an abnormal asymmetry in the planum temporale (i.e., a language-related area involved in auditory processing and receptive language (Nakada et al., 2001)) in children with ASD. Typically developing individuals have larger language-related areas, such as the planum temporale, in the language-dominant left hemisphere. However, in the study of Rojas et al. (2005), the researchers observed a smaller planum temporale in the left hemisphere, by using MRI with children with ASD. In pars triangularis and pars opercularis, which are language-related regions located in Broca's area, a rightward asymmetry was observed only in children with ASD who have language impairments (De Fosse et al., 2004).

Research regarding functional lateralization of language cortices have found that children and adults with ASD showed reduced or reversed functional lateralization of language compared to TD individuals (Eyler et al., 2012; Kleinhans et al., 2008). When examining the associations between language abilities and functional language lateralization in ASD, however, inconsistencies arise. While some studies find positive associations (Redcay & Courchesne, 2008), others find negative (Dawson et al., 1989) or no associations (Knaus et al., 2010).

However, connections between brain regions are as crucial as the brain regions themselves for language (Ivanova et al., 2016). Therefore, investigating white matter connectivity to better understand the neural underpinnings of language ability is important. The next paragraphs will emphasize the evidence derived from diffusion magnetic resonance imaging (dMRI), a technique utilized to examine white matter tracts.

Evidence from diffusion MRI (dMRI)

dMRI is an important non-invasive imaging technique that enables us to trace the trajectory of white matter. This technique allows to assess the orientation of white matter fibers by measuring the direction and degree of microscopic water diffusion. By using different diffusion tensor imaging (DTI) parameters derived from dMRI data, such as fractional anisotropy (FA), radial diffusivity (RD), and mean diffusivity (MD), it is possible to quantitatively evaluate the AF.

FA is used to measure connectivity and it reflects water diffusion directionality. It is believed that a higher FA represents a greater white matter integrity (Madden et al., 2009). Radial diffusivity (RD) is another important parameter derived from dMRI. It represents movements of water molecules perpendicular to axonal fibers. Increased RD values suggest decreased white matter integrity (Jeong et al., 2011). Mean diffusivity (MD) reflects average diffusion of water molecules within the tissue, regardless of direction. Increased MD values indicate decreased integrity (Winklewski et al., 2018).

MRI studies on the contribution of white matter on language abilities of children with ASD are scarce (Di Martino et al., 2013). This is in part due to difficulties in image acquisition in very young children (Gilmore et al., 2018), who usually have a limited ability to cooperate with scanning procedures (i.e., lying still and minimizing motion). This poses difficulties in obtaining high-quality brain images. Additionally, for the choice of sequence parameters and scanner equipment (i.e., age-appropriate head coils) in MRI studies, the developmental stage of participants needs to be taken into account (Turesky et al., 2021). This often leads to trade-offs regarding quality of images and comparability across age ranges (Turesky et al., 2021).

Prior research established an association between the AF and language outcomes in TD by using dMRI. Powell et al. (2006) combined functional MRI and dMRI in their study to investigate the connectivity between language areas in the brain. They found significant correlations between activation in language regions and mean FA in language-related white matter tracts, for different language measures (i.e., word generation and reading). This suggests an important relation between white matter microstructure and language function.

The limited amount of research regarding white matter microstructure of the AF in ASD rendered mixed results. To explore differences in white matter between TD children and children with ASD, Zhang et al. (2018) conducted a dMRI study in which they looked at different DTI parameters of the AF in children with ASD with language regression (i.e., decline in previously acquired language skills). They reported a reduced FA in ASD relative to TD children. Joseph et al. (2014) have found that there was – in their sample of children with ASD aged four to seven – a reduced left-lateralization of

RD in the AF. In addition, they found no difference in FA and MD when compared to TD children. A recent meta-analysis by Minteer et al. (2022) examined irregularities in language-related white matter tracts in ASD, in a sample of five- to 33-year-old individuals. In line with the results of Zhang et al. (2018), the meta-analysis revealed a reduced FA in a sample consisting of children and adults with ASD. In addition, the results also showed increased MD in the AF of individuals with ASD (Minteer et al., 2022).

Naigles et al. (2017) explored the relationship between white matter microstructures and language abilities in ASD. They used DTI on 108 male participants with ASD to test whether the degree of their language development was associated to variability in their brain structure. The results of the study revealed that the FA in the inferior longitudinal fasciculus (i.e., a white matter tract between the occipital and temporal lobes) correlated positively with language scores (i.e., expressive and receptive language). In a study investigating children with ASD aged six to 18 years, elevated MD in both the right and left arcuate pathways was found to be linked to language difficulties in individuals with autism (Nagae et al., 2012). Taken together, we expect that differences in white matter microstructures might contribute to the heterogeneity in language abilities in ASD.

Current study

Considering that early language levels are one of the best predictors of outcomes in adulthood (Magiati et al., 2014) and that early interventions can improve language functioning (Dawson et al., 2010), it is important to shed light on the neural bases of language ability in children with ASD. Due to the inherent difficulty in scanning children aged one to five, there is a limited number of imaging studies in this age group. However, this is a crucial period with language expansion due to rapid neurodevelopment (Dehaene-Lambertz et al., 2002; Aslin & Schlaggar, 2006). Hence, elucidating neural mechanisms of language – especially during this period of development – is crucial.

This study aims to clarify the role of white matter microstructure in language abilities in preschool children with ASD. More specifically, we will examine the following research questions:

1. Is there a difference in language abilities between typically developing (TD) children and children with ASD?

We hypothesize that there will be an advantage for the three language outcome variables analyzed in this thesis (i.e., receptive language, expressive language and Mean Length of Utterance (MLU)) in the TD relative to the ASD group, as previously observed (Eigsti et al., 2007; Kyvelidou et al., 2021; Olson et al., 2020).

2. Is there a difference in white matter microstructures in children with and without ASD?

Given the observations that the Arcuate Fasciculus (AF) plays a key role in language and that it shows altered diffusion variables in children with ASD (Zhang et al., 2018; Minteer et al., 2022), we expect a decrease of the fractional anisotropy (FA) of the AF in the ASD group. Regarding the radial diffusivity (RD) and mean diffusivity (MD) of the AF, we expect an increase in the ASD group.

3. Are fractional anisotropy (FA), radial diffusivity (RD) and mean diffusivity (MD) of the arcuate fasciculus (AF) significantly associated with language abilities in preschoolers with and without ASD?

We believe that there will be a positive correlation between FA of the AF and the three language outcome variables, while negative correlations are expected between RD and mean MD of the AF and the three language outcome variables. This hypothesis is based on previous research on associations between different white matter properties and language abilities (Naigles et al., 2017; Nagae et al., 2012).

Methods

Participants

This master's thesis was part of the European Preschool Brain Imaging and Behaviour Project (PIP), which is a longitudinal study conducted at five different research facilities in Europe. The aim of the PIP is to investigate the development of children with and without ASD in order to identify prognostic biomarkers for ASD. To this end, each child participated at three time points in total and each timepoint consisted of either two or three institute visits. The analyses presented in this thesis focused on data collected during the first time point at the Ghent site. This thesis comprises data from 26 children, including 11 children with ASD and 15 TD children. The sample size is smaller than initially targeted due to ongoing data collection. The participants' ages ranged from 2.5 to 4.5 years.

This study was approved by the Ghent University Hospital Medical ethical committee. A written informed consent was signed by the parents. Families received €50 for their participation and were reimbursed for their travel expenses. The child received a small gift and parents received an MRI picture of their children's brain (if the scan was successful) and a short report about their child's results on the developmental test.

Inclusion and exclusion criteria

For all groups, participants had to meet the following criteria. All had a non-verbal mental age of at least 18 months, as assessed by the Mullen Scales of Early Learning (MSEL) (Mullen, 1995) and had no known genetic conditions such as Fragile X Syndrome, Tuberous Sclerosis Complex, Neurofibromatosis 1, Down's Syndrome, or Rett Syndrome. They had no uncorrected vision or hearing difficulties and no known developmental or medical conditions that could affect brain development or behavior. In the TD group, participants did not have any first-degree relatives with ASD. Participants in the ASD group had an established clinical diagnosis of ASD prior to participation according to DSM-5 criteria.

Participants were excluded if any of the following criteria applied: vision or hearing impairments were present without the correction by glasses or hearing aids; any contraindication to MRI (e.g., ferromagnetic metal implants) was present; no dMRI data was available.

Regarding participant characteristics in this sample, significant differences were observed between the ASD and TD groups in terms of nonverbal cognition and autism traits. The ASD group exhibited lower scores in nonverbal cognition compared to the TD group, indicating differences in cognitive abilities in this sample. Additionally, individuals in the ASD group demonstrated higher levels of autism traits compared to the TD group. A summary of participant demographics and characteristics is included in Table 1.

Table 1

Participant demographics and characteristics for autism spectrum disorder (ASD) and typically developing (TD) children

Characteristics		**ASD (n=11)**	**TD (n=15)**	**Test statistic**	**p-value**
Gender (male/female)		8/3	12/3	$X^2 = 0.00$	1
Age in months (mean ± SD)		45.21 ± 6.03	41.74 ± 7.52	$X^2 = 26.00$	0.219
Age in months (range)		37.17 - 54.57	31.07 - 54.10	-	-
Nonverbal cognition (mean ± SD)		22.06 ± 4.18	25.07 ± 3.02	t = 2.14	0.0424
Autism traits (mean ± SD)		90.82 ± 28.26	35.36 ± 18.45	t = - 5.93	<0.001
Maternal education (%)	1 2 3 4	1 (9) 2 (18) 6 (55) 2 (18)	0 (0) 2 (14) 7 (50) 5 (36)	$X^2 = 2.81$	0.589

Note. In the group of typically developing children, the sample size for the autism traits and maternal education scores consisted of 14 participants instead of 15. Nonverbal cognition: calculated as the sum of MSEL visual reception and fine motor skills, divided by two. Autism traits: raw scores on the Social Responsiveness Scale-second edition (SRS-2). Maternal education: mothers provided information on their highest level of education, which was categorized into four levels: level 1 represented lower

education (equivalent to primary or middle school), level 2 indicated secondary education (equivalent to high school), level 3 was non-university higher education, and level 4 represented university-level higher education (i.e., bachelor's degree or higher).

Apparatus and materials

The Mullen Scales of Early Learning (MSEL)

The MSEL is a test battery used to assess development in infants and preschoolers from the ages of 0 to 68 months (Mullen, 1995). Consisting of 124 items, it measures various developmental domains including visual reception, fine motor skills, receptive language, and expressive language. Scores on 4 cognitive scales (i.e., visual reception, fine motor, receptive language, and expressive language) are combined to get an indication of the developmental index. The scores on expressive and receptive language scales were implemented to characterize standardized language abilities in this thesis. Raw scores were converted to age equivalent scores which were then divided by the child's chronological age.

Despite being a relatively outdated measure, the MSEL is still commonly used in research due to being user-friendly, quick to administer, applicable to a wide age range and translated in many languages, including Dutch. Furthermore, Swineford et al. (2015) have found an indication for convergent and divergent validity for the MSEL in both children with and without the diagnosis of ASD. However, the different translated versions of this test – including the Dutch version – are based on old, American norms (Mullen, 1995).

Social Responsiveness Scale (SRS-2)

The Social Responsiveness Scale-2nd Edition (SRS-2; Constantino, 2012) was used to describe the sample but was not included in the main analyses. This well-validated tool has demonstrated strong reliability and validity in assessing the severity of autistic traits (Bölte et al., 2008). It consists of 65 items that are rated on a 4-point Likert scale, ranging from 'Not True' to 'Almost Always True', yielding a total score as an overall measure of social responsiveness.

Parent-Child Interaction (PCI)

Parent-child interaction (PCI) involved a 15-minute play session during which the parents were instructed to play together with their children, just as though they were playing at home. This free-play session took place in a room which was equipped with four cameras, including two mounted on tripods, one fixed to the wall, and an additional camera. A standardized set of toys was provided for the session. This measure is ecologically valid as it reflects the natural interactions experienced by the child in their everyday context. Despite the study taking place during times of the COVID-19 pandemic, the parent was allowed to take their face masks off during the entire play session (the child did not wear a mask for the entirety of the testing session).

These interactions between parent and child were recorded and thereafter transcribed. The transcriptions were carried out by three master's students who were blind to the diagnosis of the children. These transcribers underwent training to ensure consistency in transcription. The Mean Length of Utterance (MLU) was calculated as a measure of expressive language, based on the average number of morphemes in each utterance. This was done by someone who was not involved in transcribing. The utterances of a 10-minute recording were analyzed for this investigation.

MRI data acquisition

DTI data were acquired on a 3T Siemens MAGNETOM Prisma MRI scanner with a 64- channel head coil. Children wore double layers of hearing protection and their head position was stabilized using sponge support within the coil. Single shell dMRI was acquired for 48 directions with a b-value of 1000 s/mm^2. In addition, a minimum of six b = 0 images using opposite phase encodings were acquired to allow for image correction. Other dMRI acquisition parameters were as follows: 2 mm isotropic voxel slice, 70 slices, a field of view (FoV) of 212 mm and the repetition time (TR) and echo time (TE) were set to 3700 ms and 71 ms, respectively.

MRI data acquisition procedure. Participants were scanned during their nocturnal sleep, without the use of sedation. If children already took medication to sleep, specifically melatonin (in the case of participants with ASD), they were allowed to do so for the sleep scan too. Parents were instructed to mildly sleep deprive their children on the scanning day so that they would fall asleep more easily. If the child fell asleep, there was a waiting time of approximately 15-20 minutes before initiating the scanning protocol. Subsequently, ear plugs were inserted onto the child's ears, MRI hearing protectors were placed on the ears and the child was repositioned in order to place their head inside the head coil. The parent could either stay inside or leave the scanner room during the scan, depending on their preference. One or two of the researchers remained in the scanner room at all times to observe the child during the entire scan and stop the scan in case the child woke up. The scanning protocol lasted approximately 55 minutes, while the entire scanning session spanned the entire evening.

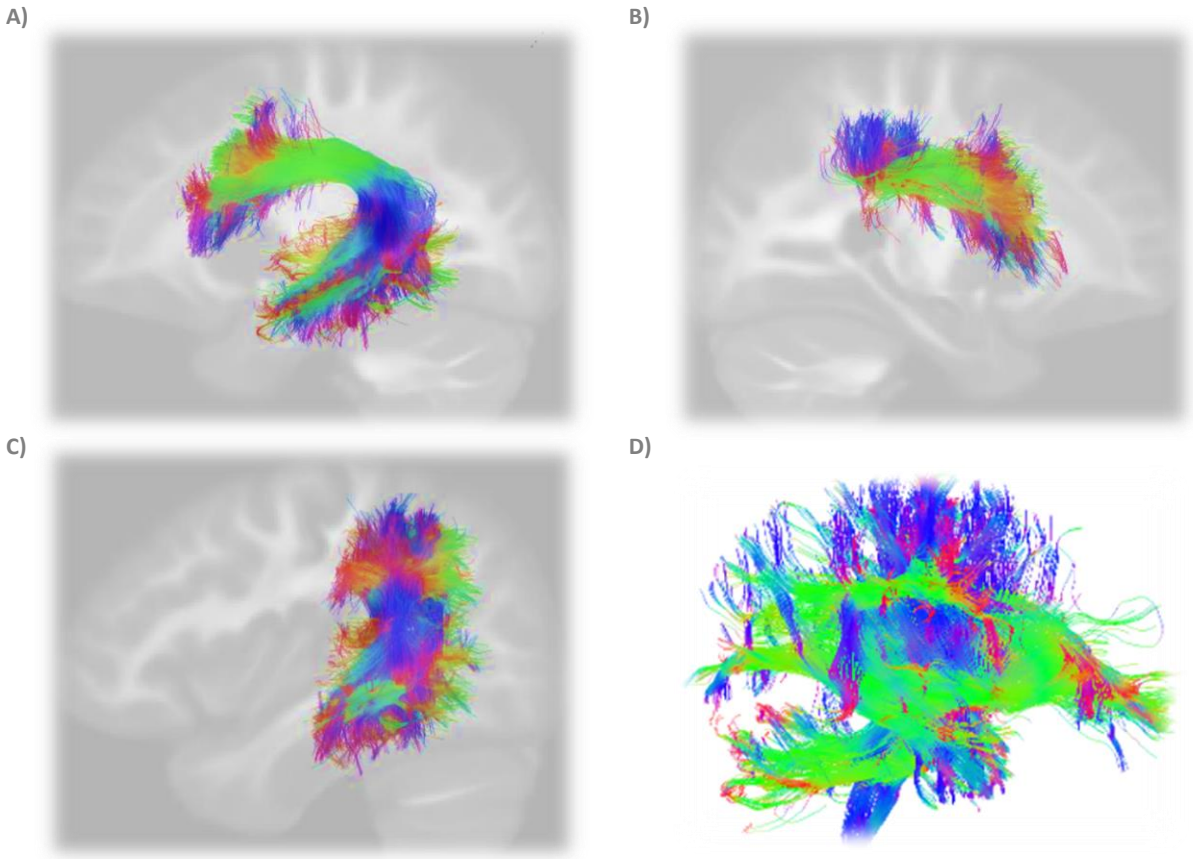
Diffusion preprocessing and tractography

The preprocessing of dMRI data involved several steps, including denoising, top up, motion distortion correction, removal of Gibbs ringing and correction of eddy current distortions. Prior to tractography, all images were normalized against the FA Montreal Neurological Institute (MNI) template. For tract dissection, a novel method called MegaTrack, developed by the Natbrainlab, was employed (Dell'Acqua et al., 2015). With MegaTrack, the normalized images from all participants were concatenated into a single tractography dataset, allowing for group-level dissections. Using this approach, the final dissections exhibit not only comparable outcomes to classical individual manual dissection but also demonstrate increased inter-class correlation and improved inter-rater reliability scores compared to individual dissections. Deterministic tract dissection using ROIs on the three segments of the arcuate fasciculus was carried out on this "Mega" dataset. This process generated average values for tract volume, fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) for each segment of the AF (classic long segment, anterior segment and

posterior segment) per participant per hemisphere. In this thesis, only the data of the long segment were analyzed. See figure 2 for a visualization of the reconstructed AF segments in this study.

Figure 2

Tract reconstruction of AF segments



Note. Reconstructions of the Arcuate Fasciculus (AF) segments utilized in the current study. The four images depict the A) long segment, B) anterior segment, C) posterior segment, and D) a whole-brain reconstruction, which served as the basis for tractography. The data analyzed in this thesis specifically focused on the long segment of the AF.

Procedure

This study consisted of 3 institute visits: two visits at the university and one visit for the MRI scan. The MRI scan visit often took place on the same day as the second visit. Prior to the visits, an eligibility screening was administered over the phone. Subsequently, during the first visit, several behavioral tasks were administered, which are outside the scope of this current thesis. Parent-Child Interaction (PCI) was also recorded during this visit. Additionally, tools with the purpose of preparing the children for the MRI scans (e.g., ear plugs and MRI sounds) were also given to the parents.

In between the first and second visits, the child was prepared for the MRI scan by the parent. This preparation happened at home and involved: 1) sleeping with earplugs; 2) sleeping on their back (since the child has to lay on its back inside of the scanner); 3) letting the child listen to MRI scan noises and playing these while the child is asleep; and 4) watching a "Pip and the brain explorers" video clip. The purpose of this video clip was to habituate the children to the scan procedure and the sounds made by the scan.

During the second institutional visit, MSEL was administered together with other measures which are unrelated to this thesis. MRI scans were scheduled in the evening of this second visit due to the fact that it was carried out during the natural nocturnal sleep. The parents and child were invited at the scan center approximately between 6.30 and 8.00 pm, depending on the bed time of the child. Everyday bedtime routines of the child were carried out as much as possible and preference for sleeping conditions was taken into account (e.g., lights completely dimmed or some light in the room). The scanner room was decorated in an effort to make it more appealing for the children, as depicted in Figure 3. Children were laying on a scanner bed with soft toys and a blanket. The bed was also surrounded by a 'space tent' to the left of the child. To the right, a bed was provided for one of the parents, allowing one parent to remain present throughout the entire scanning procedure. The lighting in the scanner room was adjusted depending on the child's preference.

Figure 3

Image of the scanner room

Note. An image of the scanner room setup. The room decoration included a 'space tent' positioned to the left of the scanner bed and an additional bed for parents to accompany their child during the entire scan. Soft toys and a blanket were also placed on the scanner bed.

Data analysis

All statistical analyses in this thesis were carried out using RStudio (version 4.0.5). Normality of the data was first assessed with the Shapiro–Wilk test. If the data were found to be normally distributed (i.e., a p-value higher than 0.05), an analysis of variance (ANOVA) was performed. In the case of non-normally distributed data (i.e., a p-value lower than 0.05), the Mann-Whitney U test was used. As displayed in table 2, the p-values for the left and right Fractional Anisotropy (FA) and the left Mean Diffusivity (MD) of TD children were below the significance level of 0.05, suggesting non-normal distribution. All hypothesis tests were performed at a significance level of $\alpha = 0.05$ and a Bonferroni correction was used to correct for multiple comparisons.

Table 2

Results of the Shapiro-Wilk normality tests

	ASD		TD	
Variables	**W**	**p-value**	**W**	**p-value**
MSEL-RL	0.88	0.0979	0.91	0.168
MSEL-EL	0.96	0.752	0.90	0.0892
MLU	0.94	0.526	0.96	0.636
FA left	0.97	0.898	0.87	**0.0326**
FA right	0.98	0.971	0.84	**0.0132**
RD left	0.94	0.541	0.95	0.524
RD right	0.96	0.774	0.90	0.109
MD left	0.90	0.176	0.86	**0.0208**
MD right	0.95	0.623	0.95	0.591

Note. This table displays the outcomes of the Shapiro-Wilk normality test conducted on the language measures and different microstructure DTI parameters utilized in this thesis. MSEL-RL: scores on the Receptive Language subscale of the Mullen Scales of Early Learning (MSEL). MSEL-EL: scores on the Expressive Language subscale of the MSEL. MLU: Mean Length of Utterance. FA: fractional anisotropy. RD: Radial Diffusivity. MD: Mean Diffusivity. Significant p-values are presented in bold. The p-values for the left and right Fractional Anisotropy (FA) and the left Mean Diffusivity (MD) of TD children were found to be less than the significance level of 0.05, indicating non-normal distribution.

Behavioral data

An ANOVA was conducted to analyze the three language outcome variables between the two groups, with age as a covariate. To account for multiple comparisons, a Bonferroni correction was applied to the three separate ANOVAs, leading to an adjusted significance level of $\alpha = 0.0167$ to control for familywise error rate. After applying the Bonferroni correction, statistical significance was determined using an adjusted p-value threshold of $p < 0.0167$.

MRI data

To investigate potential differences in DTI measures of the AF between children with and without ASD, three Mann-Whitney U tests were conducted for both left and right AF. After applying the Bonferroni correction, the adjusted significance level was set at $\alpha = 0.017$.

Additionally, the relationship between white matter microstructures and language across the entire sample was explored through nine Spearman correlations, assessing the associations between three DTI measures (FA, RD, and MD) and three language outcomes (EL, RL, MLU). The tests were performed separately for both the left and right AF. Again, a Bonferroni correction was applied, resulting in an adjusted significance level of $\alpha = 0.006$.

Results

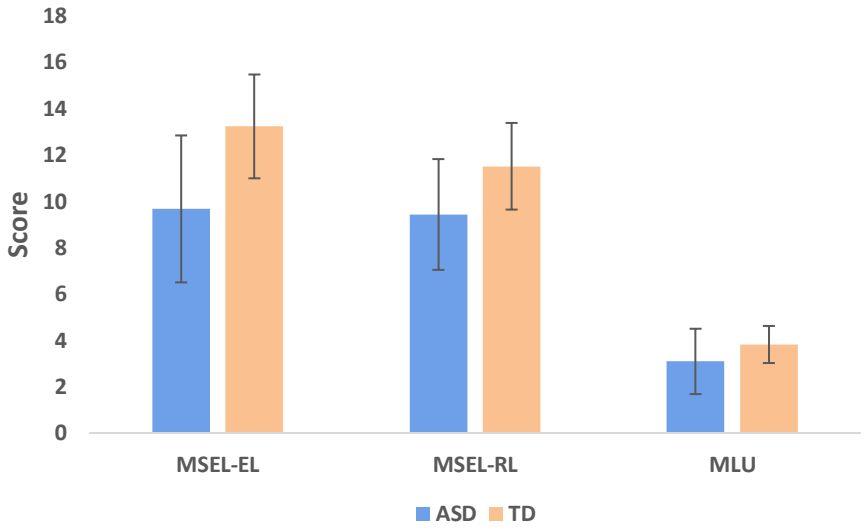
Language abilities

As expected, the ANOVA results indicated significant group differences for EL ($F(1, 23) = 10.57$, $p = .0035$, partial $\eta^2 = .31$) and MLU ($F(1, 23) = 8.83$, $p = .0068$, partial $\eta^2 = .28$), while controlling for age. The typically developing group demonstrated higher scores compared to the ASD. No significant group differences were observed for RL ($F(1, 23) = 5.50$, $p = .028$, partial $\eta^2 = .19$), though there was a trend in the expected direction.

For exploratory purposes, additional ANOVAs were performed with nonverbal cognition as covariate, since it has been previously observed that there are associations between nonverbal cognition and both expressive and receptive language (Nevill et al., 2017). After controlling for nonverbal cognition, none of the results remained statistically significant. This suggests that the previously observed group differences in language measures (figure 4) can be attributed, at least in part, to variations in nonverbal cognitive abilities in this sample.

Figure 4

Overview of Language Measure Scores between the ASD and TD group



Note. MSEL: Mullen Scales of Early Learning; EL: expressive language; RL: receptive language; MLU: Mean length of Utterance.

White matter microstructures: ASD vs TD

As shown in Table 3, the results indicated that there were no significant group differences in FA (*W*= 73, *p*= 0.646), RD (*W*= 89, *p*= 0.760), or MD (*W*= 91, *p*= 0.683) for the left. Median values for left FA were comparable in children with ASD (*Mdn*= 0.51) and TD children (*Mdn*= 0.51), as were the median values for RD (ASD: *Mdn*= 0.49; TD: *Mdn*= 0.51) and MD (ASD: *Mdn*= 0.74; TD: *Mdn*= 0.73). Regarding right AF, there were no group differences in FA (*W*= 77, *p*= 0.799), RD (*W*= 94, *p*= 0.574), or MD (*W*= 89, *p*= 0.760). The DTI measures of right AF showed similar median values of FA between children with ASD (*Mdn* = 0.51) and TD children (*Mdn* = 0.50), as well as for RD (ASD: *Mdn* = 0.49; TD: *Mdn* = 0.50) and MD (ASD: *Mdn* = 0.72; TD: *Mdn* = 0.72). Contrary to our expectations and previous studies (Minteer et al., 2022), none of the diffusion measures in the AF exhibited statistically significant group differences, suggesting that there is no difference in white matter microstructures between ASD and TD children in this sample.

Table 3

DTI Measures comparison of left and right Arcuate Fasciculus between the ASD and Typically Developing (TD) group

		ASD (n=11)			TD (n=15)			Mann-Whitney U test	
		Range	Median	SD	Range	Median	SD	W	p-value
FA	left	0.47 – 0.54	0.51	0.024	0.48 – 0.57	0.51	0.023	73	0.646
	right	0.47 – 0.53	0.51	0.018	0.35 – 0.57	0.50	0.052	77	0.799
RD	left	0.47 – 0.53	0.49	0.020	0.45 – 0.54	0.51	0.025	89	0.760
	right	0.46 – 0.54	0.49	0.020	0.44 – 0.58	0.50	0.042	94	0.574
MD	left	0.69 – 0.75	0.74	0.020	0.71 – 0.76	0.73	0.022	91	0.683
	right	0.70 – 0.75	0.72	0.016	0.69 – 0.77	0.72	0.024	89	0.760

Note. This table presents the mean values of fractional anisotropy (FA), radial diffusivity (RD), and mean diffusivity (MD) for the left and right arcuate fasciculus in ASD and TD groups.

Additional correlational analyses were performed to explore the possibility that we did not observe differences in white matter structures due to effects of nonverbal cognition. Spearman correlations were calculated between nonverbal cognition scores and the DTI parameters. No corrections for multiple comparisons were applied due to a small sample size. As shown in table 4, the results indicated a significant negative correlation between left RD and nonverbal cognition (r= - 0.79, p= 0.0061) in the ASD group. Moreover, there was a significant negative correlation between right MD and nonverbal cognition (r= - 0.64, p= 0.040) in the ASD group. These negative correlations suggest that higher left RD and right MD values, indicative of decreased white matter integrity, were associated with lower nonverbal cognitive abilities specifically in the ASD group. Surprisingly, in the TD group, a significant positive correlation was observed between left RD and nonverbal cognition (r= 0.52, p= 0.049). This positive correlation indicates that higher RD values were associated with higher nonverbal cognitive abilities in the TD group.

Table 4

Spearman correlations between nonverbal cognition and the DTI measures in the left and right arcuate fasciculus

	ASD		TD	
	Rho	**p-value**	**Rho**	**p-value**
Nonverbal cognition				
AF left	0.527	0.100	- 0.421	0.119
AF right	0.509	0.114	- 0.289	0.295
RD left	- 0.791	**0.00606**	0.521	**0.0488**
RD right	- 0.600	0.0561	0.157	0.575
MD left	- 0.491	0.129	0.314	0.254
MD right	- 0.636	**0.0404**	0.200	0.474

Note. This table represents Spearman correlation analysis results between nonverbal cognition and each DTI measure separately for the Autism Spectrum Disorder (ASD) and Typically Developing (TD) groups. AF: Arcuate Fasciculus. RD: Radial Diffusivity. MD: Mean Diffusivity. Significant p-values (< 0.05) are presented in bold. Note that these results have not been corrected for multiple comparisons.

Relation between white matter microstructures and language measures

The results of the Spearman correlation analyses revealed statistically significant associations between the DTI measures and MLU. More specifically, a significant positive correlation was found between left FA and MLU (r= 0.64, p < 0.006), indicating that higher FA values in the left AF were related to higher mean lengths of utterance. Furthermore, a significant negative correlation was observed between left RD and MLU (r= -0.54, p < 0.006), suggesting that lower RD values in the left AF were associated with longer mean lengths of utterance. No significant correlations were found between any of the other DTI measures and the language outcomes, as shown in Table 5.

Table 5

Spearman correlations between the DTI parameters in the left and right arcuate fasciculus and language outcome measures

	Left hemisphere		Right hemisphere	
	Rho	**p-value**	**Rho**	**p-value**
FA				
MSEL-RL	- 0.220	0.278	- 0.352	0.0786
MSEL-EL	- 0.0174	0.933	- 0.0721	0.726
MLU	0.640	**0.000453**	0.319	0.112
RD				
MSEL-RL	0.272	0.1776	0.282	0.162
MSEL-EL	0.117	0.567	0.123	0.549
MLU	- 0.543	**0.004705**	- 0.322	0.109
MD				
MSEL-RL	0.141	0.4919	0.0242	0.907
MSEL-EL	0.119	0.562	- 0.00512	0.981
MLU	- 0.259	0.1998	- 0.292	0.148

Note. This table displays the results of Spearman correlations between the DTI parameters and language outcome measures across the whole sample. DTI parameters include Fractional Anisotropy (FA), Radial Diffusivity (RD), and Mean Diffusivity (MD) of the left and right Arcuate Fasciculus (AF). The language outcome measures assessed in this study include the two subscale scores of the Mullen Scales of Early Learning (MSEL): Receptive language (RL) and Expressive Language (EL). Further, Mean Length of Utterance (MLU) was also used to assess expressive language. Significant p-values are presented in bold.

To gain further insights into the data, additional correlational analyses were conducted separately for the two groups. It is important to note that the results represented in table 6 were not corrected for multiple comparisons due to a limited sample size. Therefore, interpretations were carried out with a significance level set at $\alpha = 0.05$. This analysis revealed several significant correlations between white matter microstructure and language measures. In the ASD group, a positive correlation was found between left FA and MLU ($r = 0.63$, $p = 0.044$). Further, negative

correlations were found between left RD and MLU (r = -0.62, p = 0.048), and between right RD and MSEL-EL scores (r = -0.71, p = 0.019). In the TD group, a positive correlation was found between left FA and Mean Length of Utterance (MLU) (r = 0.71, p = 0.0043). Interestingly, there was a negative correlation between right FA and MSEL-RL scores (r = -0.54, p = 0.042), suggesting that higher FA values in the right AF were associated with lower RL abilities of TD children.

Table 6

Spearman correlations between the DTI parameters in the left and right arcuate fasciculus and language outcome measures in the ASD and TD groups

	ASD				TD			
	Left		Right		Left		Right	
	Rho	p	Rho	p	Rho	p	Rho	p
FA								
MSEL-RL	- 0.0454	0.903	0.200	0.558	- 0.243	0.382	- 0.535	**0.0422**
MSEL-EL	0.200	0.558	0.582	0.0655	- 0.182	0.515	- 0.429	0.113
MLU	0.627	**0.0440**	0.582	0.0655	0.707	**0.00431**	0.175	0.532
RD								
MSEL-RL	- 0.0727	0.839	- 0.236	0.486	0.461	0.0861	0.375	0.169
MSEL-EL	- 0.318	0.341	- 0.709	**0.0187**	0.396	0.145	0.364	0.182
MLU	- 0.618	**0.0478**	- 0.364	0.273	- 0.539	**0.0407**	- 0.311	0.259
MD								
MSEL-RL	- 0.291	0.386	- 0.318	0.341	0.318	0.248	0.0750	0.793
MSEL-EL	- 0.309	0.356	- 0.573	0.0706	0.214	0.442	0.189	0.498
MLU	- 0.327	0.327	- 0.264	0.435	- 0.379	0.165	- 0.450	0.0944

Note. Results of Spearman correlations between the DTI parameters and language outcome measures. DTI parameters include Fractional Anisotropy (FA), Radial Diffusivity (RD), and Mean Diffusivity (MD) of the left and right Arcuate Fasciculus (AF). The language outcome measures assessed in this study include the two subscale scores of the Mullen Scales of Early Learning (MSEL): Receptive language (RL) and Expressive Language (EL). Further, Mean Length of Utterance (MLU) was also used to assess expressive language. Significant p-values (< 0.05) are presented in bold. Note that these correlations have not been adjusted for multiple comparisons.

Discussion

The present study aimed to investigate the relationship between white matter microstructure and language abilities in preschool children diagnosed with autism spectrum disorder (ASD). Our hypotheses were that 1) the typically developing (TD) group would demonstrate an advantage over the ASD group in terms of the three language outcome variables examined in this thesis, namely receptive language (RL), expressive language (EL), and Mean Length of Utterance (MLU), as this was already observed in previous research (Eigsti et al., 2007; Kyvelidou et al., 2021; Olson et al., 2020). Furthermore, we expected that 2) children with ASD would exhibit a reduction in white matter integrity, as measured by three white matter microstructure parameters of the left arcuate fasciculus (AF), namely fractional anisotropy (FA), radial diffusivity (RD), and mean diffusivity (MD) and that 3) there would be significant associations between these parameters and the three language outcome variables.

Regarding language abilities, no significant differences were observed in the RL scores between the two groups, though there was a trend in the expected direction. Consistent with our expectations and previous studies (Kyvelidou et al., 2021; Eigsti et al., 2007), we found that the TD group exhibited higher scores in EL and MLU compared to the ASD group. This aligns with the common observation that children with ASD often experience language difficulties (Baird & Norbury, 2016; Pickles et al., 2014). However, these differences might have been related to effects of nonverbal cognition since the results were no longer significant after controlling for nonverbal cognition. This highlights the importance of taking sample characteristics, such as nonverbal cognition, into account.

In contrast to our expectations, we found no evidence for differences in white matter microstructures between the two groups, which contradicts the findings reported in the literature. For instance, Zhang et al. (2018) observed differences in white matter microstructures. However, it is important to note that their sample specifically consisted of children with language deficits (i.e., language regression). In contrast, our sample did not specifically include individuals with language impairments, which might explain the disparity in results. Furthermore, ASD is a highly heterogeneous

disorder, with individuals exhibiting a wide range of language abilities (Boucher, 2011; Georgiades et al., 2012). It is possible that in this sample, some individuals in the ASD group had higher levels of language skills compared to the typical profile seen in ASD populations. As a result, this could have contributed to less prominent group differences, leading to null results. This suggests that including a sample with language impairments may be essential to gain deeper insights into the neural mechanisms underlying language abilities in ASD.

The lack of differences in white matter microstructures between the two groups may also be attributed to the aforementioned effect of nonverbal cognition. Indeed, interesting patterns emerged when exploring the correlations between nonverbal cognition and DTI measures. Specifically, there was a significant negative correlation between MD of left AF and nonverbal cognition in the ASD group only, aligning with a previous study indicating that higher white matter integrity was associated with increased cognitive abilities (Schmithorst et al., 2005). Surprisingly, a positive correlation between left RD and nonverbal cognition was observed in the TD group, in contrast to the negative correlation found in children with ASD. These correlations in the opposite directions might have prevented to detect significant differences in white matter microstructures between children with ASD and TD children. Matching participants based on nonverbal cognition could offer a more comprehensive understanding of the white matter differences between the two groups. Summarized, the null results regarding white matter microstructures found in the current thesis are not in line with the literature. Therefore, further investigation is needed to explore this in depth.

In line with our hypothesis, there were significant correlations between white matter microstructures and language measures across the whole sample. More specifically, we observed a positive association between FA and MLU and a negative association between RD and MLU. This is consistent with the literature and indicates that abnormal white matter microstructure might be associated with language difficulties. In contrast to our expectations, however, no significant associations were found between MD and MLU, and all of the DTI measures and EL and RL. This might be because we combined the two groups for the correlation analyses due to a small sample size. This

might bring certain limitations in terms of interpretation. By combining the two groups, it becomes challenging to look at possible distinct characteristics that could influence the associations between white matter microstructures and language function. Each group might have different type of associations which may have overshadowed certain effects.

Therefore, additional correlation analyses were conducted to explore potential differences in correlations between the groups. This analysis revealed interesting patterns. In the ASD group, expressive language showed associations with white matter microstructure, in the sense that children with a higher left FA and lower RD had higher MLU scores. Further, children with lower right RD showed higher MSEL-EL scores. The TD group also showed a positive correlation between left FA, as well as a negative correlation between left RD and MLU. These observations were in the expected directions (Naigles et al., 2017; Nagae et al., 2012). Although not significant, interesting trends emerged from our observations, indicating that higher white matter integrity in the right AF of children with ASD might be associated with improved performance on language measures. In the TD group however, there was a pattern in the opposite direction, especially in the right AF since there was a negative correlation between right FA and MSEL-RL. A highly tentative interpretation of these results is that they might indicate a reversed asymmetry in ASD, as was previously observed (Wan et al., 2012). In sum, these findings suggest that white matter microstructure differences could play a role in language abilities, with distinct patterns seen in ASD compared to typical development. Important to note is that these results should be interpreted with caution since this analysis was exploratory in nature and the sample size was too small.

Furthermore, it is important to note that the structural connectivity of language in the brain does not only consist of the AF. Previous research showed two distinct pathways, namely the ventral and dorsal pathways (Hickok & Poeppel, 2007; Friederici, 2009). While the dorsal pathway (i.e., AF and superior longitudinal fasciculus), is involved in transforming sound into speech (Giampiccolo & Duffau, 2022), the ventral pathway (i.e., uncinate fasciculus, inferior longitudinal fasciculus (ILF), inferior frontal-occipital fasciculus) is mostly associated with language comprehension and meaning

processing (Friederici & Gierhan, 2013). In the study of Naigles et al. (2017) for example, they observed significant correlations between two language measures (EL and RL) and FA of the ILF, which is part of the ventral pathway. This emphasizes the need to consider the differential contributions of these two pathways to language abilities in ASD.

This thesis has several limitations that need to be acknowledged. Firstly, the targeted sample size of this study was not met due to ongoing data collection. It is important to note that null effects observed in this thesis do not necessarily imply the absence of true effects or relationships. The small sample size might have prevented to identify meaningful effects, as a result of reduced power to detect significant differences.

Another potential limitation is that only data of the long segment of the AF were investigated. This is because data from the anterior and posterior segments of the AF were not yet preprocessed at the time of writing this thesis. However, these different segments of the AF are thought to be involved in distinct aspects of language processing, as observed in the study of Ivanova et al. (2021). They demonstrated that the long segment is associated with naming abilities, while the anterior segment contributes to fluency and naming and the posterior segment to comprehension. For instance, it is plausible that we failed to find a relationship between the DTI parameters and RL scores due to the focus solely on the longitudinal segment, as the posterior segment is more prominently associated with comprehension. Investigating the anterior and posterior segment of the AF at the end of this study will provide a more comprehensive understanding of the relationships between white matter microstructures and language abilities in ASD.

A methodological limitation is the utilization of the MSEL as a measure of EL and RL. It has been reported that the MSEL might pose difficulties in children with ASD, particularly in terms of engagement and motivation (Akshoomoff, 2006), which might influence their scores. Furthermore, the MSEL may not fully capture all aspects of receptive and expressive language skills as it does not focus on more nuanced aspects of language, such as pragmatics or higher-order language abilities (Bruyneel et al., 2019). The lack of correlations between DTI parameters and language scores as assessed by the

MSEL could be the result of this. Using more comprehensive language measures could provide a more thorough understanding of the linguistic abilities in children with ASD.

Lastly, handedness is a crucial participant characteristic to consider when studying language. Some left-handed individuals exhibit right language dominance, as evidenced by research conducted by Mazoyer et al. (2014). However, it is important to note that previous studies have indicated that a significant number of children do not develop a hand preference until approximately the age of 6, as highlighted by Ozturk et al. (1999). Handedness was therefore not taken into account in this thesis. Nevertheless, it is worth emphasizing that examining handedness could be important in understanding language-related processes.

Conclusion

Concluding, this thesis has provided insights into language abilities and white matter microstructures in preschool children with autism spectrum disorder (ASD) and typically developing (TD) children. First, we observed significant group differences for mean length of utterance (MLU) and expressive language (EL), which diminished after controlling for nonverbal cognition. Surprisingly, results indicated no significant differences in white matter microstructures between the ASD and TD groups, contradicting previous literature. Correlations between white matter microstructures and language measures revealed significant associations between fractional anisotropy (FA), radial diffusivity (RD), and MLU. However, none of the other language measures were significantly correlated with any of the DTI measures. Future research should take into account variations in participant characteristics such as severity of language impairments and nonverbal cognition, to get a deeper understanding of the findings. Furthermore, it is advisable to approach the interpretation of these findings with caution, considering the limitations associated with the small sample size. The results of the complete sample might further help to better understand the relationships between white matter microstructures and language functions in children with ASD.

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