Sex differences in coordinated brain activity in clinical child populations

by
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Abstract

A disruption of normal brain development during early stages of life has been associated with higher male vulnerability expressed by male preponderance among affected individuals and/or more severe impairments in males for developmental disorders. Although this phenomenon is frequently acknowledged by the scientific community, its neurophysiological underpinnings remain largely unclear. In this thesis I investigate male vulnerability in very preterm children and individuals with Autism Spectrum Disorder (ASD). Both clinical child populations entail early developmental adversity leading to behavioural and cognitive alterations, believed to be elicited, in part, by disrupted communication between brain areas. Therefore, I examine resting state whole-brain connectivity and its developmental changes in these clinical populations using fMRI and MEG and test the hypothesis of sex-specific connectivity differences between males and females resulting in male disadvantage.

In the first study I investigate sex differences in interhemispheric homotopic connectivity and its developmental trajectories in participants with ASD as well as in typically developing individuals. Our findings demonstrate differences in developmental trajectories rather than connectivity. Both females and males with ASD deviate from typical female trajectories while expressing similar developmental trajectories to typical males. In the second study I examine local connectivity and its age-related changes using a similar cohort of participants. Group and sex differences are observed in both local connectivity and its developmental trajectories. Females with ASD are characterised by more robust alterations. Lastly, in the third study I test the hypothesis that male vulnerability in very preterm children can be detected as more pronounced alterations in inter-regional connectivity in boys compared to girls. Our results confirm this hypothesis suggesting that connectivity alterations might contribute to male disadvantage reflected in long-term behavioural and cognitive outcome.

Overall, this thesis highlights that disruptions in brain connectivity and/or its developmental trajectories differ between males and females with altered early development supporting the existence of female protective features preventing females from developing pathological outcome.

Keywords: brain connectivity; sex differences; autism spectrum disorder; very preterm children; fMRI; MEG

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List of Acronyms

ASD	Autism Spectrum Disorder
BOLD	Blood Oxygenation Level Dependent
EEG	Electroencephalography
EMB	Extreme Male Brain
fMRI	Functional Magnetic Resonance Imaging
fNIRS	Functional Near-Infrared Spectroscopy
FPE	Female Protective Effect
GI	Gender Incoherence
MEG	Magnetoencephalography
ReHo	Regional Homogeneity
TD	Typical Development
VMHC	Voxel-Mirrored Homotopic Connectivity
SAT	Spontaneous Activity Transients

Chapter 1.

Introduction

In this chapter I will present a review of current knowledge regarding coordinated brain activity and male vulnerability. First, I describe how the male vulnerability is expressed in various clinical populations. Then I review sex differences in fetal development that could account for such male bias. In the following subsections I present how the male vulnerability is expressed in the clinical populations central to this thesis – individuals with autism spectrum disorder (ASD) and very preterm children. This will include a review of multiple hypotheses which were generated to explain male bias in ASD population. After that I focus on the large-scale connectivity as a way to investigate such sex disparity given that alterations in these clinical child populations involve behaviour and cognition. Latter subsections review previous findings on developmental changes of large-scale connectivity, sex differences in normal population and reports of connectivity alteration in ASD and very preterm children. I conclude this chapter outlining aims and hypothesis of the current thesis.

1.1. Male vulnerability or female resiliency in early life

There is mounting evidence of certain sex differences in pathological outcome of early developmental disorders and pathological conditions. For example, numerous adverse prenatal and neonatal factors such as very preterm birth, low birth weight, intrauterine growth restriction lead to worse long-term health consequences in males compared to females such as higher mortality, morbidity and impaired behavioural and cognitive abilities (Hindmarsh et al. 2000; Johnson 2007; Tyson et al. 2008; Torrance et al. 2010; Binet et al. 2012). Such male vulnerability is not limited to the neonatal period but perseveres to childhood. Multiple reports show that the neuropsychiatric disorders with onset in early childhood, commonly referred as neurodevelopmental disorders, also show substantial male preponderance together with higher severity in males (Rutter et al. 2003; Thapar et al. 2017). These include ASD, Attention Deficit/Hyperactivity Disorder (ADHD), intellectual disabilities, specific learning, communication and motor disorders (Yairi and

Ambrose 1999; Freeman et al. 2000; Strømme and Diseth 2007; Reigosa-Crespo et al. 2012; Willcutt 2012; Halladay et al. 2015; Quinn and Wagner 2015). The female/male ratios among clinical child populations are provided in the Figure 1.1 to exemplify a strong male prevalence.

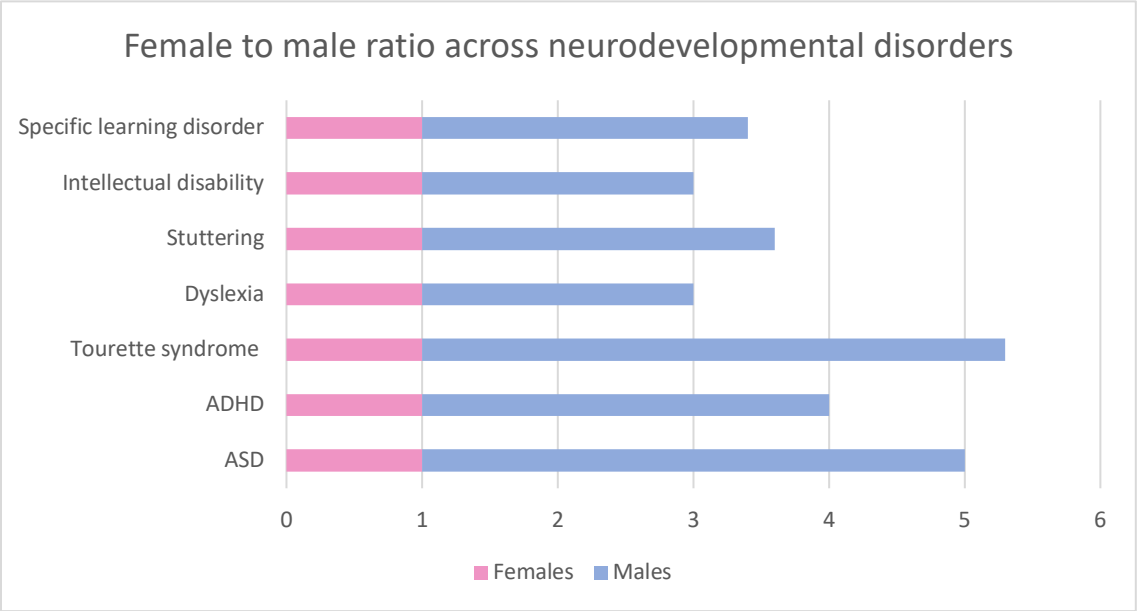

Figure 1.1. Female to male ratio across neurodevelopmental disorders

The blue bar represents a number of males diagnosed per one diagnosed female (a pink bar).

The majority of neurodevelopmental disorders are characterized by high heterogeneity of clinical expression within a disorder, yet the symptoms of neurodevelopmental disorders greatly overlap with each other (Thapar et al. 2017) and the examples of co-occurrence of neurodevelopmental disorders are frequently reported (e.g. ASD and intellectual disabilities, ADHD and specific learning disorder) (American Psychiatric Association 2013). Also, the majority of them share similar risk and prognostic factors and male sex is one of the most common.

Such evidence of differential impact of maturational disturbances in males and females might indicate that there are particular features in female early neurodevelopment which have a protective effect during childhood. In contrast, the absence of those in males causes higher vulnerability.

1.1.1. Impact of fetal sex on brain development

The timing of disturbance in normal development appears to be central to the phenomenon of male and female vulnerability in clinical populations. While pathological alterations in early life maturation tend to affect males more than females resulting in more frequent and/or severe neuropsychiatric and neurologic conditions, females are more likely to suffer from them in adulthood (McCarthy et al. 2017). According to this, it is reasonable to suggest that sex differences in early brain maturation processes could provide a physiological foundation of male vulnerability phenomenon.

There are two major factors responsible for sex differences in brain development. Cells of male and female individuals have different sets of sex chromosome genes and are regulated in a temporally distinct manner by different combinations of gonadal hormones. At conception, the sex of a fetus is determined by the presence of the SRY gene on the Y chromosome. Its expression triggers the development of testes and male external genitalia, and if SRY gene is not present the fetus will develop a female reproductive system. The deficiency of the SRY expression leads to a female phenotype in an individual with XY genotype even if the rest of the Y chromosome remains intact (Berta et al. 1990; Lovell-Badge and Robertson 1990). Conversely, a translocation of the SRY gene to the autosomal chromosome in a male mouse and subsequent breeding with normal female results in mice with male phenotype and female genotype XX among the offspring (Becker et al. 2005). Such animal models helped to disentangle the contribution of the sex-linked genes and gonadal hormones to sexual differentiation (Arnold et al. 2004).

In both sexes the development of sex gonads and external reproductive organs is completed by 13th gestational week (McCarthy 2008). Around that time the testes start producing testosterone that due to its steroid nature passes freely through brain-blood barrier and sets sex-specific hormonal milieu in male brain, while female ovaries remain mostly quiescent through gestation. Thus, from the very early developmental stages, male and female brains maturate in different hormonal environments starting from neural proliferation (third - fourth months of gestation) and neuronal migration (third - sixth months of gestation) to three - eight weeks after birth (birth-related testosterone surge in males). After that hormonal differences get smaller until puberty when gonads resume production of sex hormones (Knowles et al. 2012; Clarkson and Herbison 2016). It has been demonstrated that sex-specific hormonal milieu in the foetus brain leads to volumetric

(through regulation of cell apoptosis) and connective (by regulating neurites growth, branching, synaptic patterning, ion channels modulation) sex differences in the brain, some of which potentially underlie future predisposition to pathological outcome in males (McCarthy 2011; Sellers et al. 2015). Such extensive impact of sex hormones on brain reconfiguration is not limited to early development but continues throughout the lifetime (Sheppard et al. 2019).

Following subsections describe in detail two clinical child populations which are the primary focus of this thesis due to profound male disadvantage and various interlinks between them.

1.1.2. Male bias in Autism Spectrum Disorder

ASD is a neurodevelopmental condition characterized by social communication and interaction deficits, and restricted, stereotypic behaviours (American Psychiatric Association 2013). ASD has always been of particular interest to the scientific community studying sex differences due to one of the largest and the most reliably documented ratios of male prevalence (4 males to 1 female) (Christensen et al. 2018). This led to the introduction of various hypotheses to explain the disparity between sexes in ASD incidence and symptomatology (Halladay et al., 2015). The majority of them suggested that the same level of predisposing factors causes pathological disruptions in males but not in females, thus the number of affected females is smaller. Many of them also posit existence of some biological characteristics of female sex which protect females from developing autistic phenotype. Such hypotheses can be grouped under umbrella Female Protective Effect (FPE).

The earliest references of FPE were introduced through the hypothesis of differential genetic loading in males and females (Tsai et al. 1981; Tasi and Beisler 1983). The relevance of genetic factors in ASD etiology was confirmed by studies showing ASD to be highly heritable with high concordance rate among monozygotic twins and little or no concordance in dizygotic twins implying that genetic factors might prevail the environmental influence (Colvert et al. 2015; Tick et al. 2016). It suggested that the higher ‘dose’ of pathogenic genetic variants is necessary for females to manifest ASD phenotype. Recent large-sample studies demonstrated that siblings of females with ASD had

significantly more autistic traits then siblings of males with ASD confirming that females require greater familial etiologic load to be affected (Robinson et al. 2013; Jacquemont et al. 2014). However, the exact mechanism responsible for such female resilience was not discussed in the genetic loading hypothesis.

The obvious candidate to cause such protective effect is the X chromosome (Skuse 2000). Relative to other chromosomes, the X chromosome contains the largest number of genes that are highly expressed in brain tissues (Nguyen and Disteche 2006). Evidence of higher ASD occurrence in such genetic conditions as Turner syndrome (females with only one X chromosome [45,X0 genotype]) and Fragile X chromosome (faulty FMR1 gene on the X chromosome) suggest that the X chromosome plays an important role in ASD etiology (Belmonte and Bourgeron 2006; Barnett et al. 2017). Similarly, the higher female resilience was hypothesized to be originated by absence of the Y chromosome. Again, there is supporting evidence of higher prevalence of autism in the population with sex chromosome aneuploidy. It has been shown that males with XYY and XYYY genotypes have 4.8 times higher risk of developing ASD compared to males with XXY genotype and 20 times higher risk of developing ASD compared to males with standard set of sex chromosomes (Tartaglia et al. 2017). However, the majority of linkage studies targeting sex chromosomes failed to identify genes located on the sex chromosomes to account for such male bias (Schaefer 2016).

Male preponderance in ASD was also explained through the hypothesis of the sex difference in brain plasticity (Mottron et al. 2015). It builds on four scientific observations: (i) males are more vulnerable to genetic mutations that affect genes related to synaptic plasticity (Huber et al. 2002; Kang et al. 2011); (ii) evidence that normal sex differences are often registered within higher order cortical regions which are more prone to plastic changes than primary sensory or motor areas (Lopez-Larson et al. 2011; Zhang et al. 2018); (iii) ASD related alterations mostly involve higher-order areas (Müller et al. 2011; Rane et al. 2015; Hull, Jacokes, et al. 2017); (iv) brain recovery due to brain plasticity after pathological events displays sex-specific pattern (Kirkness et al. 2004; Girijala et al. 2017). This hypothesis suggests that the disproportionate male-female ratio in ASD can be explained as following: any pathological factor is more likely to elicit plastic brain changes that lead to ASD manifestation in males compared to females. In this way, it shares similar basic concept with FPE that identical etiological risk will produce more alterations in males than females (Figure 1.2, A).

The idea of the FPE was also extended in the theory of 'three hits' proposing that ASD development is elicited by superposition of three predisposing factors: genetic risk variants, environmental stress and testosterone modulation of the stress reaction that results in pathological outcome (Pfaff et al. 2011). Thus, according to the authors the absence of negative impact of testosterone in early life and the existence of protective agents such as oxytocin in females prevent them from developing ASD. Accordingly, to manifest ASD phenotype females have to be confronted with higher number of pathological factors compared to males (Figure 1.2, A).

Neuroimmune mechanisms were also hypothesized to be involved in the ASD etiopathology and used to explain male bias. Such evidence came from animal research that highlights the role of inflammation in normal sex differentiation (McCarthy and Wright 2017). It has been shown that estrogenic metabolites of testosterone trigger activation of inflammatory signaling molecules (Hisasue et al. 2010). Normally, they are necessary for the masculinization of specific brain areas. However, in case of infection such neuroimmune activation increases fetus' susceptibility to pathological outcome. Consistently with this hypothesis, maternal infection is a well-known risk factor for developing ASD and evidence of inflammatory-like markers was reported in postmortem ASD brains (Patterson 2011). Plausibly, less reactive neuroimmune system due to the absence of testosterone modulation, prevents female fetuses from developing ASD.

Other group of hypotheses emphasises the direction of ASD-related alteration in respect to typical sex differences. One of them is the Extreme Male Brain (EMB) theory. Its main idea is grounded in typical sex differences in cognitive styles where females on average tend to empathize and males on average are inclined to systemize (Baron-Cohen 2002; Baron-Cohen et al. 2011). Respectively, females tend to score higher on the Empathy Quotient than typical males and the opposite is true for the Systemizing Quotient. Individuals with ASD demonstrate an extreme male profile scoring very high on Systemizing Quotient and very low on the Empathy Quotient. It is important to keep in mind, however, that the EMB theory does not imply that all psychological sex differences have an exaggerated form of maleness in individual with ASD (Baron-Cohen et al. 2011). In search of physiological bases for the EMB theory, its supporters focused on the endocrinological explanation of male bias in ASD linking it to fetal testosterone. The exposure to testosterone in early development is shown to affect brain development leading to its masculinization (Auyeung et al. 2009; Nguyen et al. 2013). The hypothesis

links elevated levels of testosterone to hypermasculinization that leads to autistic-like phenotype based on EMB theory (Auyeung et al. 2010). The relation between elevated levels of testosterone and ASD was supported by studies of elevated autistic traits in females with polycystic ovaries syndrome and increased risk of autism among their children. Polycystic ovaries syndrome is a condition when ovaries produce excessive amount of androgens including testosterone (Cherskov et al. 2018). Similar findings of increased autistic traits were reported for another hyperandrogenic condition – congenital adrenal hyperplasia (Knickmeyer et al. 2006). Despite this evidence, increased foetal testosterone levels alone cannot explain the etiology of ASD. For example, a large longitudinal study demonstrated that testosterone concentrations from umbilical cord blood are not related to autistic-like traits in the general population (Whitehouse et al. 2012).

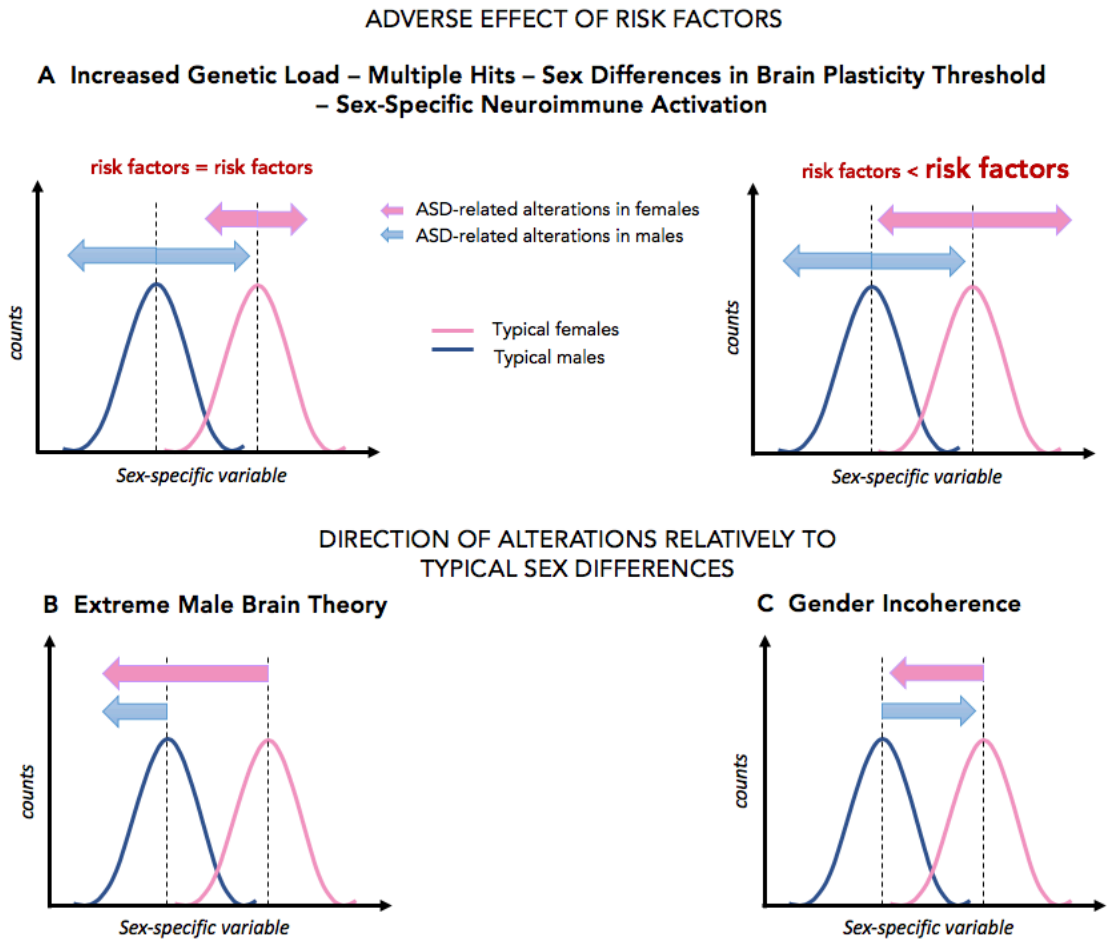

Figure 1.2 Schematic illustration of hypotheses explaining male bias in ASD

The most common hypotheses of the origin of male bias in ASD are schematically explained relatively to the typical sex differences. In each graph two Gaussian curves represent distribution of some trait (e.g. brain connectivity) in typical males (in blue) and in typical females (in pink). The alterations from typical male or female distributions related to ASD phenotype are represented as arrows starting from the distribution means (blue arrow for ASD males and pink arrow for ASD females). A – the hypotheses relying on existence of female protective mechanisms; B – the extreme male brain theory; C – the gender incoherence hypothesis.

There is another hypothesis that interprets ASD-related alterations within the context of the typical sex differences in the set of traits which are modulated by exposure to sex hormones. It is called Gender Incoherence (GI) hypothesis (Bejerot et al. 2012). Originally, its authors aimed to test the EMB theory on anthropological features which were reported to be affected by testosterone. Partially the EMB theory was confirmed and their findings revealed masculinization of some specific traits in females with ASD. However, males with ASD surprisingly displayed feminization of the anthropologic traits rather than masculinization. The principal difference between the EMB theory and GI hypothesis are illustrated on Figure 1.2, B and C. It is important to mention that unlike other hypotheses discussed above, the GI hypothesis does not attempt to explain the male bias in ASD but only rationalizes the results of the conducted study (Bejerot et al. 2012).

There is an alternative explanation for the higher incidence of ASD in males. It posits that the disproportion of male/female ratio might be caused by the imperfections of diagnostic tools which were developed and perfected on mostly male samples. Consequently, they might not be sensitive enough to female-specific variation of the ASD symptoms. For this reason female phenotypes of neurodevelopmental disorders often fail to be properly diagnosed leading to the male preponderance among diagnosed population (Bargiela et al. 2016). Research focussed on clarifying the particularities of ASD symptoms manifestation in females reported the existence of a female ASD phenotype. The female ASD phenotype is characterized by more internalizing problems (anxiety and depression) and less externalizing problems (hyperactivity/impulsivity, conduct problems), higher social motivation and ability of camouflaging the symptoms (Mandy et al. 2012; Kirkovski et al. 2013; Hull, Mandy, et al. 2017). The latter is of particular interest, because constant camouflaging of difficulties in social interaction requires considerable cognitive effort, induces stress, anxiety and depression and even problems with self-identification (Lai, Lombardo, et al. 2017).

Such distinctions in ASD manifestation between females and males disregarded in the standard tools used for ASD diagnostics could systematically leave females undiagnosed resulting in the inflated male-to-female ratio. However, recent genetic,

immunological and endocrinological studies provide evidence of a different threshold for genetic or environmental 'hits' needed to introduce the pathological alterations in males and females as well as important role of sex hormones in brain development (McCarthy 2008; Robinson et al. 2013; Bilbo 2018; Cherskov et al. 2018). Thus, the adjustment of diagnostic tools to account for sex specific variations of neurodevelopmental problems might potentially reduce the male bias but will not eliminate it.

1.1.3. Effect of sex on long-term outcomes of very preterm birth

Children born very preterm (≤ 32 weeks gestational age) is another population that is characterized by a profound male disadvantage. The causes of premature birth are highly heterogeneous and not limited to fetus vulnerability. Multiple pregnancies, genetic factors, infections, placental insufficiency and chronic maternal conditions (e.g. hypertension, diabetes) might lead to interruption of normal intra-uterus development ("WHO | Preterm birth" 2017). The prevalence of male infants born very preterm is just slightly higher than females (male/female ratio = 1.06 in a population of 549,048 births), however, male sex is recognized as a risk factor leading to lower survival rates and worse long-term outcomes (Ingemarsson 2003; Nyman et al. 2017). Thus, the better ability to cope with premature birth consequences is the most important factor that differentiates females from males and leads to male disadvantage in preterm population. Notably, similar proportion of premature birth in girls and boys excludes the possibility of underrepresentation of one sex due to imperfections in assessment tools as in case of neurodevelopmental disorders.

The male disadvantage comprises a higher risk of neurodevelopmental impairment including moderate to severe cerebral palsy, Bayley Mental and Psychomotor Developmental Indices < 70, and deafness or blindness in extremely preterm (<28 weeks gestation) boys compared to girls (Hintz et al. 2006a). Multiple lines of evidence suggest that extremely preterm male infants have higher risk of respiratory complications (e.g. bronchopulmonary dysplasia) and associated morbidities compared to the same gestational age female infants (Binet et al. 2012; Shim et al. 2017). Also, very preterm birth results in largely increased risk of neurodevelopmental disorders: ASD, ADHD, learning difficulties, and motor disorders. And again, male sex increases the likelihood or severity of these disorders (Marlow et al. 2005; Johnson et al. 2010; MacKay et al. 2010;

Edwards et al. 2011; Lindström et al. 2011; Uccella et al. 2015; Young, Morgan, Powell, et al. 2016). Such interlinks between very preterm birth and neurodevelopmental disorders might imply a similar origin of male vulnerability.

The course of development of very preterm infants is affected by multiple factors aside from low gestational age at birth and subsequent immaturity of multiple organ systems to cope with external environment. To ensure life support, very preterm babies are placed into neonatal intensive care unit and undergo numerous procedures to which full-term babies are never exposed. Some adverse experiences during this period are associated with harmful long-term outcomes. For instance, increased exposure to pain (usually through skin breaking procedures) was linked to lower IQ at school age (Vinall et al. 2014). Another source of neonatal pain is mechanical ventilation to which preterm babies are subjected due to pulmonary insufficiency (Bellù et al. 2010). The use of morphine-based medication to mitigate the pain induced by ventilation was shown to adversely affect internalizing behaviours at school (Ranger et al. 2014). Interestingly, that pain sensitivity and adverse consequences also have sex-specific character (Burke and Trang 2017; Mogil 2020). Thus, lower gestational age, prolonged ventilation and higher exposure to pain could be considered as risk factors that contribute to worse long-term outcomes.

Overall, very preterm birth and ASD are interrelated due to adverse alterations in behaviour and cognition, early disruptions of neurodevelopment which is interrelated by male disadvantage in both clinical populations. Very preterm babies are also at higher risk of developing ASD (Johnson et al. 2010; Johnson and Marlow 2011). Yet there are some distinctions between two populations which could be considered helpful to male vulnerability investigation. The fact that ASD and very preterm children are different in terms of clinical gradation – a disorder and ‘at risk’ population enables to examine the male vulnerability phenomenon on distinct levels of clinical severity. Another advantage of investigating very preterm children is that while the male bias in diagnostic tools could be partially responsible for male preponderance in ASD, it is highly unlikely that male vulnerability in very preterm children can be explained in that way.

Adverse consequences of early neurodevelopmental disruptions affect behaviour and cognition in both clinical populations. Male vulnerability could be investigated from multiple perspectives. Endocrinological and genetic research is very informative since sex bias is likely to be modulated by genetic and hormonal differences between males and

females. However, since in both clinical populations examined in this thesis male vulnerability is expressed through alterations in behaviour and cognition the investigation of brain function directly could be promising in revealing the mechanisms that mediate such discrepancy between males and females. The availability of neuroimaging modalities enables in vivo non-invasive investigation of brain activity even in challenging participant groups, such as children and participants with ASD. That being said, before plunging in investigating group differences in large-scale brain activity and connectivity it is important to understand the nature of the brain signal collected by neuroimaging modalities.

1.2. Coordinated brain activity as bases of behaviour and cognition

Whole brain activity registered by neuroimaging tools reflects a complex and highly dynamic pattern of multiple groups of neurons firing together (Schnitzler and Gross 2005). It is generally accepted that behavior and cognition are maintained through functional specialization and integration of brain areas (Friston 2011). Functional segregation implies that various cortical areas can be attributed with specific functions. While such kind of organization is well observed in some areas (e.g. right fusiform face area is highly specialized in face processing, Wernicke area is specifically activated during language processing), the majority of cortical areas do not express such specificity. Functional integration helps to solve this inconsistency suggesting that many specialized areas are interconnected to accommodate efficient performance of specific functions. In this way, functional segregation could be also considered on the network level with distributed networks being characterized by certain spatial pattern and activation to empower certain functions and cognitive states. Overall, functional integration and segregation provide the bases of network organization of the brain which has been extensively studied as intra- (short-distance or local) and interregional (long-range or distributed) connectivity over the last three decades using neuroimaging techniques. Short-distance connectivity reflects local information processing within functionally segregated areas, whereas long-range connectivity is used by large-scale networks to coordinate their activity and share relevant information reflecting functional integration. On the structural level, communication between neurons is supported through abundant synaptic connections locally as well as distantly facilitated by white matter tracks. Functionally, it results in local oscillation of neural activity and the synchronization of such oscillations between distributed areas

define a network (Schnitzler and Gross 2005). Temporal characteristics of rhythmic oscillations are very important to functional purpose that they conduct (Fries 2015). Fast oscillations (30-90 Hz) also known as gamma band rhythm facilitate local synchronization and information processing within a neural group. Such communication is thought to represent bottom-up information flow. Essentially, it includes receiving an input from sensory systems, information processing and propagation of the outputs towards higher order cortical areas. Such fast communication is ideal for synchronization of small closely located groups of neurons and allows to balance their excitation and inhibition cycles facilitating efficient information processing. Slower oscillations in alpha and beta bands (8-13 Hz and 13-35 Hz, respectively) promote top-down information flow encompassing distant regulation imposed by higher order areas to the regions involved in primary information processing. In this way, through inhibition and modulation of neural resources higher order areas prioritize processing of the most salient stimuli. This mechanism has been proposed to underpin attention and working memory (Fries 2015; Miller et al. 2018). The oscillation in theta frequency band (4-8 Hz) also has been shown to modulate gamma synchronization reflecting attentional shifts required for exploratory behavior (Fries 2009). Theta-gamma modulation in hippocampus was shown to be involved in memory consolidation and action planning (Buzsáki and Llinás 2017). There is an evidence that disrupted balance between alpha, theta and gamma oscillatory activity underlies numerous neurological and neuropsychiatric conditions (Ribary et al. 2017).

Apart from temporal features, networks can be characterized by the connectivity strength between its nodes. For example, the strength of connections within particular brain networks could be potentially used as a signature of cognitive processes, as it has been recently shown with sustained attention (Rosenberg et al. 2016). There are other ways to characterise connectivity using various network properties derived employing graph theory such as modularity (an extend of network's division into modules), centrality (node's importance according to its connections), efficiency (information propagation capacity) (Bullmore and Sporns 2009). Such approach offers a comprehensive theoretical framework for understanding neurophysiological underpinnings of brain function and can provide insights into cortical architecture, evolution, development and clinical disorders.

The communication between different brain regions is not limited to task performance but also happens during rest. Multiple resting-state networks have been identified in humans (van den Heuvel and Hulshoff Pol 2010). These networks incorporate

distributed brain areas (nodes and hubs) with synchronized activity that can be detected even if a subject is not engaged in any task. It is believed that such coordination between distant areas at rest promotes efficient information processing when specific goal-directed behaviour is required. For example, sensory networks are interconnected to efficiently process incoming sensory information (e.g. visual network comprising occipital cortical regions (Beckmann et al. 2005) and auditory network localized in temporal lobes (Andoh et al. 2015)). Sensorimotor network is responsible for body representation, control and initiation of body movements and involves areas which surround central sulcus (Yeo et al. 2011). Attention network is engaged in reorganisation of neural activity to modulate processing of relevant stimuli. Usually it is subdivided in dorsal and ventral attention networks which are located in frontal and parietal areas (Yeo et al. 2011). Executive network including dorsolateral prefrontal and posterior parietal cortex mediates successful task accomplishment when cognitive control and working memory is needed (Seeley et al. 2007). Default mode network is unique in being highly active during rest which has been related to spontaneous thoughts, self-introspection and thinking about others (Buckner and DiNicola 2019). The biggest hubs of default mode network include posterior cingulate cortex, medial prefrontal cortex and temporoparietal junction. These networks were shown to possess high test-retest reliability within participants and, at the same time, their spatial properties vary across participants (Mueller et al. 2013; Zuo and Xing 2014). Application of multiple modalities and development of various connectivity estimation methods have supported a rapid progress in resting-state functional connectivity investigation.

1.2.1. Approaches to investigate brain connectivity

Brain connectivity can be studied on multiple scales using variety of invasive (local field potentials and electrocorticogram) and non-invasive (functional Magnetic Resonance Imaging [fMRI], functional Near-Infrared Spectroscopy [fNIRS], electroencephalography [EEG] and magnetoencephalography [MEG]) modalities. Functional MRI and MEG have proven to be gold standards in the research of brain functional connectivity due to their non-invasiveness, whole brain coverage and an excellent spatial resolution of fMRI and temporal resolution of MEG (Matthews and Fair 2015; Baillet 2017). fMRI and MEG are costlier compared to their counterparts fNIRS and EEG but it comes with considerable

advantages. More specifically, fMRI outmatches fNIRS in capturing activity of deep brain sources while MEG has much higher spatial resolution compared to EEG. fMRI and MEG provide similar information about brain function essentially reflecting the activation of large groups of neurons. However, their signals reflect very distinct physiological processes.

The fMRI signal (blood oxygenation level dependent - BOLD) measures changes in blood oxygenation level caused by increased metabolic demands of firing neurons as the result of phenomenon called a neuro-vascular coupling (van den Heuvel and Hulshoff Pol 2010). The fluctuations of the BOLD signal are quite slow and lie below 0.1 Hz (Cordes et al. 2001). BOLD signal is measured for each voxel within subject's head and typically gets averaged within all voxels of a functionally or anatomically defined area to increase a signal-to-noise ratio and reduce dimensionality of the fMRI data. With an improvement in computational power, however, voxel-wise connectivity analysis becomes more common. Functional connectivity is defined as statistical temporal dependence between BOLD signals of two brain areas or voxels. Among connectivity estimates, the most common are Pearson's correlations, partial correlations, Kendal's coefficient of concordance (Jiang and Zuo 2016; Sala-Llonch et al. 2019).

Unlike fMRI, MEG collects the information on neuronal activity in a more direct way by measuring tiny magnetic fields generated by electrochemical currents within and between neurons (Baillet 2017). These magnetic fields are detected by an array of sensors called SQUIDs (Superconductive Quantum Interference Device) which surround subject's head. Various approaches have been developed to reconstruct neuronal activity in a brain space – inverse modelling – using magnetic signal measured by sensors and their geometry relative to head location. In resting state research the most common inverse modelling methods are beamforming, minimum norm estimate, LORETA (Hämäläinen and Ilmoniemi 1994; Pascual-Marqui et al. 1994; Nunes et al. 2020). Despite advances in these approaches, the estimation of source-space brain dynamics remains an ill posed problem that significantly restraints the spatial resolution of MEG (Baillet 2017). In contrast, MEG does not suffer from temporal restrictions on the measured signal which is considered to be among of its main advantages over fMRI. MEG captures brain dynamics on the level of milliseconds. It is similar to the temporal scale on which brain is believed to operate. Consequently, the signal acquired by MEG is more complex and can be studied in different frequency domains. There are five standard frequency bands that are often investigated in MEG and EEG studies: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta

(13-35 Hz), gamma (>35 Hz). It also results in different approaches of measuring connectivity between two brain sources based on MEG signal. The most common connectivity metrics estimate amplitude coupling (e.g. envelope correlation), stability in phase difference (e.g. phase-locking value), cross-spectral properties (e.g. coherence) or directional relationships (e.g. Granger-causality) of two signals (Colclough et al. 2016).

Overall, both modalities – MEG and fMRI – provide complementary information to aid the overall understanding of brain functional organization and have been successfully applied to study how brain connectivity is affected by sex and age in typical population. Both modalities were crucial for examining alterations in brain communication in neurodevelopmental disorders and particularly in ASD, suggesting disrupted brain connectivity to be a potential neural signature of ASD (Maximo et al. 2014).

1.2.2. Sex differences in large-scale brain connectivity

Within- and inter-regional brain communication is believed to underlie behaviour and cognition and those diverge in multiple aspects between males and females (Schnitzler and Gross 2005; Miller and Halpern 2014). Such sex differences are related to social behaviour, memory, emotions and were formed over the course of human species evolution and likely augmented by specialization of male and female sociocultural roles (Morrow 2015; Choleris et al. 2018). On the other hand, it was hypothesised that not all organizational differences between male and female brain lead to differences in function. The dual-function hypothesis proposed that different mechanisms employed by male and female brains may help to prevent sex differences in behaviour and cognition by compensating for sex differences in physiology (De Vries 2004). It is very plausible that such sex-specific variation in brain function underlies the sex biases reported in neuropsychiatric and neurodevelopmental conditions.

Within recent decades research that aims to elucidate differences in brain function between males and females gained more attention in scientific community. Multiple studies have shown that male and female brains differ in some aspects including connectivity and significant portion of confirming evidence came from machine-learning studies. Female and male participants can be discriminated based on functional connectivity using machine learning classification with accuracy over than 80% (Pervaiz

et al. 2020). Similar findings of accuracy as high as 87% were reported by another research (Zhang et al. 2018). The accurate prediction of sex described in this study relied mostly on functional connectivity within default mode, fronto-parietal control and sensorimotor networks. Another work employing machine learning approach demonstrated that personality traits could be decoded based on functional connectivity pattern in a sex-specific manner (Nostro et al. 2018). It suggests that functional connectivity is not just distinct in males and females but the way connectivity facilitates individual behavior and cognitive traits can be sex-specific.

Previous connectivity studies report sex-specific variation in the strength connectivity and network organization in human brain, although most of them far from being dimorphic and display a high degree of overlap between males and females (Joel and Fausto-Sterling 2016). The balance of within- and between-module (basically, segregation vs integration) functional connectivity has been shown to be distinct in males and females with males having more between-module connectivity and *vice versa* (Satterthwaite et al. 2015). Similar findings of higher local connectivity in females were reported when investigating regional homogeneity and functional connectivity density (Lopez-Larson et al. 2011; Tomasi and Volkow 2012). Sex differences in distributed networks were found in sensorimotor network (higher in males) and default mode network (higher in females) (Ritchie et al. 2018).

Another subject of particular interest in sex-specific brain organization is laterality of brain connectivity. Such attention is triggered by the well-established fact of the male prevalence in left-handedness and evidence of sex hormones' impact on brain lateralization (Papadatou-Pastou et al. 2008; Nguyen et al. 2013). A recent structural connectivity study with a sample size of nearly 1000 individuals demonstrated higher within-hemispheric connectivity in males whereas females were characterized by higher between-hemispheric connectivity (Ingalhalikar et al. 2014). Another study that included participants from 5 to 18 years old demonstrated that reliance on interhemispheric connectivity was increasing with age in girls but not in boys (Schmithorst and Holland 2007).

In summary, there is converging evidence of sex differences in functional connectivity in healthy population. Those involve differences in local connectivity, distributed networks and interhemispheric communication. The evidence of sex-specific

variations in functional connectivity implies its potential relevance in studying male vulnerability reported in clinical groups.

1.2.3. Age-related changes in brain connectivity

Brain connectivity is not constant and changes throughout life (Benasich and Ribary 2018). Resent methodological advances in fMRI made possible to study functional connectivity *in utero* (van den Heuvel and Thomason 2016). The studies conducted on human fetuses suggest the presence of bilateral functional connectivity between distributed brain regions even before birth and its rapid increase with gestational age (Thomason et al. 2013). A study on preterm infants similarly found that interhemispheric functional connectivity between homologous medial cortical regions was evident as early as on 26 weeks of gestational period, whereas homotopic connectivity between more spatially distant areas developed later on (Smyser et al. 2010). Apart from interhemispheric homotopic connections, there is evidence of lateralised networks in fetal brain (Schöpf et al. 2012). A precursor of default mode network in full-term infants was detected based on fMRI signals within 2-3 days after birth (Smyser et al. 2010). Such findings suggest that the bases of resting state networks are established even before the term age potentially due to a rapid neural growth during the last trimester of pregnancy. The strengthening of such functional connectivity is the most likely to reflect the process of myelinization in the infant brain which soon after birth is combined with extensive pruning of axonal branches and connections (Low and Cheng 2006).

Similar developmental changes during first years of life can be observed in EEG or MEG studies. The electrophysiological brain activity of extremely preterm infants consists of large amplitude, long-lasting events called spontaneous activity transients (SATs) (Vanhatalo and Kaila 2010). Those are believed to result from immaturity of GABAergic inhibition circuits and tend to coincide in homologous interhemispheric regions (Vanhatalo et al. 2005; Vanhatalo and Kaila 2006a). With increase in gestational age SATs occurrence diminishes and EEG activity becomes higher in frequency and more continuous which is believed to be associated with GABA-inhibitory signalling development and establishment of thalamocortical and cortico-cortical connections (Vanhatalo and Kaila 2010). At the same time the developmental studies of event-related potentials report age-related increase in amplitude and decrease in latency of evoked

potentials in children traditionally accounted for by increase in myelination, synaptic efficacy and neural synchronization (Kushnerenko et al. 2002).

Later development in childhood (5-8 years) is characterized by adult-like functional connectivity in sensory and motor networks but lower within-network functional connectivity and higher proportion of aberrant connections between distributed areas in associative cortex compared to adults (de Bie et al. 2012). Numerous evidence suggested a general pattern of connectivity development in which local connectivity decreases with age while long-range connectivity increases (Fair et al. 2009; Kelly et al. 2009). In other words, the inter-regional communication in children is arranged by anatomical proximity whereas adult brain represents a more 'distributed' connectivity architecture. Such reorganization is thought to reflect a balance shift from feed-forward to feedback regulation strategies leading to more mature and controlled cognition (Rubia 2013). Similar evidence comes from EEG studies that report age-related reduction in information processed locally and increase in information processed through distributed communication (Vakorin et al. 2011). Electrophysiological studies also report profound age-related changes in spectral characteristics of resting state activity expressed through reduction in low frequency oscillations and increase higher frequency power (Somsen et al. 1997; Clarke et al. 2001). Another developmental signature is a resting state frequency shift to a faster rhythm which cooccur with reorganization of alpha band cortical networks (Miskovic et al. 2015). Thus, age-related changes of functional connectivity underlie complex reconfiguration of the brain organization that is likely to be disturbed as a result of neurodevelopmental disorders and premature birth complications.

1.2.4. Brain connectivity is altered in autism spectrum disorder

Functional connectivity alterations have been considered to be intrinsic to ASD pathophysiology. One of the earliest hypothesis of connectivity alterations in ASD suggested that autistic brain is characterised by local overconnectivity and long-range underconnectivity (Belmonte et al. 2004; Courchesne and Pierce 2005). Since then, multiple reviews consolidated findings provided by a large number of fMRI studies testing this hypothesis (Müller et al. 2011; Vissers et al. 2012; Rane et al. 2015; Hull, Jacokes, et al. 2017). Similar evaluation was conducted merging conclusions from MEG and EEG studies (Vissers et al. 2012; O'Reilly et al. 2017). They reported mixed findings regarding

local overconnectivity, however, the suggestion of long-range underconnectivity in ASD was confirmed to a certain extent. One of the most consistent findings of reduced communication between distributed cortical areas in ASD involved default mode network (Weng et al. 2010; von dem Hagen et al. 2013; Padmanabhan et al. 2017). It was also suggested that alterations in default mode network are not stationary across age pinpointing distinct developmental trajectories in ASD and TD (Padmanabhan et al. 2017). The only study that focused on sex differences in default mode network connectivity in ASD confirmed the presence of underconnectivity in the default mode network in both males and females with ASD (Ypma et al. 2016). Despite, the pattern of long-range underconnectivity received general confirmation, connectivity research still suffers from discrepancy in findings that cannot be simply explained by differences in methodology and study design.

Recently hypothesis suggested that inconsistencies in connectivity alterations in ASD research can be explained by atypical developmental trajectories of functional connectivity in participants with autism (Uddin et al. 2013). The following studies confirmed this assumption by showing aberrant age-related trajectories for large-scale and local connectivity in ASD population (Nomi and Uddin 2015; Dajani and Uddin 2016; Vakorin and Doesburg 2016). Similar findings of altered developmental curvatures of network modularity and global efficiency were found in ASD group (Henry et al. 2018). Interestingly, they also reported marginal significance of age-by-sex-by-group interaction that suggests an important role of sex in developmental changes of connectivity in ASD.

Relatively few neuroimaging studies explored resting state connectivity alterations in males and females with ASD. The investigation of whole brain seed-based connectivity aiming to test the EMB theory revealed patterns of typical female-like connectivity (hypoconnectivity) in males with ASD and typical male-like connectivity (hyper connectivity) in females with ASD (Alaerts et al. 2016). While the EMB theory was not confirmed, such pattern was hypothesized to reflect the Gender incoherence (GI) hypothesis. Very similar results were obtained when investigating cortico-cerebellar connectivity (Smith et al. 2019). In contrast, when the resting state functional connectivity was investigated only in mentalizing cortical regions, the opposite pattern was observed: overconnectivity in males and underconnectivity in females (Yang and Lee 2018). Later, another research group decided to test both the EMB and the GI hypotheses in males with ASD measuring shift-towards-femaleness and shift-towards-maleness in network

connectivity (Floris et al. 2018). They found both effects were present in males with ASD. Shift-towards-maleness was the most evident in default mode network supporting the EMB theory, whereas shift-towards-femaleness was observed in somatomotor network providing arguments towards the GI hypothesis. A recent study that tested the FPE hypothesis investigated ASD-associated risk alleles of the oxytocin receptor gene and connectivity in the reward network (Hernandez et al. 2020). They found that females and males with ASD had different modulation of increased genetic risk on brain connectivity. Connectivity profile of amygdala with the rest of the cortex exhibited sex differences in typical children but not in ASD (Lee et al. 2020). Overall, most of the studies show altered patterns of sex differences in ASD population compared to typical individuals (Beacher et al. 2012; Alaerts et al. 2016; Ypma et al. 2016; Yang and Lee 2018). Importantly, there is a lack of neuroimaging studies exploring the developmental trajectories of brain function in males and females with ASD, whereas epidemiological and psychological findings suggest that differences in symptom expression between males and females with ASD arise due to the time mismatch of symptoms coming into sight (Halladay et al. 2015).

1.2.5. Evidence of altered brain connectivity in very preterm children

Altered interregional connectivity is characteristic of very preterm population. Multiple disruptions in structural connectivity have been reported in individuals born preterm that suggests possible alterations in functional connectivity as well (Karolis et al. 2016; Young, Morgan, Whyte, et al. 2016; Keunen et al. 2017). The study with the largest up to date sample including extremely and very preterm children have shown altered connectivity in frontal areas and decreased spectral power (Kozhemiako, Nunes, et al. 2019). However, further analysis identified that only connectivity alterations were associated with both adverse neonatal experience and long-term behavioural outcome.

In the case of very preterm population, the knowledge of sex differences in brain coordinated activity is scarce. There were few EEG studies aiming to unravel the causes of male vulnerability in preterm population. Most of those were interested in auditory brainstem responses (ABR) in preterm infants. Three studies demonstrated shorter latencies and higher amplitudes in ABR waves in females compared to preterm male suggesting more mature brain response in females (Eldredge and Salamy 1996; Morlet et al. 1999; Li et al. 2013). Similar studies came to the same conclusion, observing a larger

number of high amplitude bursts and less cyclicity in background activity in males compared to females born very preterm (Olischar et al. 2013; Griesmaier et al. 2014; Reynolds et al. 2014). However, it's not unique to preterm population, the same pattern of earlier female maturation has been shown in full-term infants (Li et al. 2013). The study on older children (5 years) using the oddball paradigm found larger amplitudes of the P3 component (large positive event related potential that occurs around 300 ms after stimulus presentation with the highest amplitude over frontal/parietal electrodes that is believed to reflect stimulus evaluation and categorization) in full-term boys compared to girls, whereas there were no differences in extremely preterm children mostly due to diminished amplitude in extremely preterm boys (Lavoie et al. 1998).

A slightly larger number of neuroimaging studies aimed to clarify the underlying mechanism of male disadvantage searching for sex differences in brain structural connectivity in preterm population. One example of such study on the preterm population reported lower regional and mean fractional anisotropy (FA) and higher medium diffusivity in males indicating less organized white matter microstructure (Constable et al. 2008; Liu et al. 2011; Thompson et al. 2018). Similarly, other studies have reported male sex to be associated with reduced corpus callosum (CC) microstructural growth trajectory during the first 6 month of life as well as being a risk factor for diffuse white matter injury (Barnett et al. 2017; Teli et al. 2018). The white matter features had different associations with long-term outcome in males and females (van Kooij et al. 2011). Importantly, mean FA was shown to increase in preterm females but not in males in response to erythropoietin treatment, indicating sex-specific medical therapy effectiveness in the very preterm population (Phillips et al. 2017). Konties et al., found that preterm females had lower mean diffusivity in CC than full-term females but no differences between males (Kontis et al. 2009). These multiple evidences of white matter alterations suggest that exploration of brain connectivity is a promising direction to get closer to the origin of sex differences in the preterm population.

1.3. Thesis outline and specific aims

The overarching goal of this thesis was to examine if functional brain connectivity and its age-related changes can provide valuable insights on the nature of male

vulnerability during early life. I focused specifically on two clinical child populations: ASD and very preterm children for the next reasons: i) both of them are characterized by well reported male vulnerability, ii) both conditions are caused by disruption of normal development at very early stages of life and iii) are related by the higher risk of ASD in very preterm children. Moreover, the combination of these child populations provides the unique opportunity to investigate the sex differences on two levels: a disorder with well-established symptomatology (ASD) and relatively healthy population 'at risk' of having cognitive and behavioural difficulties (very preterm children). Importantly, while in the ASD cohort the assessment of risk factors that played role in the pathological outcome was not possible, the data quantifying neonatal adverse experience was available for the very preterm cohort. The comparison between very preterm boys and girls showed no differences in adverse neonatal experience in our cohort and this enabled us to investigate how sex effects connectivity alterations when the risk factor burden is approximately the same. Additionally, in very preterm population we could exclude the possibility of artificial male bias due to male-oriented diagnostic tools since the accidence of very preterm birth is similar in girls and boys.

Given the abovementioned commonalities between ASD and very preterm children and literature observations I presumed that in both populations the nature of male disadvantage had a similar origin and was based on typical sex differences in early development. In the projects described in the following three chapters of this thesis I **tested if brain connectivity alterations in ASD and very preterm cohort have a sex-specific pattern that can support any of the existing hypotheses which attempt to explain the male disadvantage**. Based on the previous reports advocating the developmental approach in ASD research, I also investigated differences in age-related changes in connectivity with similar intent to **test if the pattern of typical sex differences in connectivity trajectories is altered in ASD**.

I also specifically tested the common hypothesis of FPE in very preterm children cohort exploiting the fact that common risk factors such as gestational age, number of skin breaking procedures, the total duration of mechanical ventilation, cumulative morphine dosage were not different between very preterm girls and boys. I hypothesised that based on FPE in the case of equal number of complication-predisposing factors, very preterm males would express more pronounced alterations in connectivity than females.

The aims and hypothesis specific to the studies which were conducted under the framework of the current thesis:

Aim 1 (**Chapter 2**) Using resting-state fMRI data I aimed to investigate sex differences in interhemispheric homotopic connectivity and its developmental trajectories in ASD population. I also examined if there was a significant association between revealed alterations and ASD symptoms severity separately in males and females with ASD.

I hypothesized that that sex-specific alterations in ASD might be better characterized as differences in developmental trajectories rather than connectivity differences per se. Also, it was hypothesized that alteration in developmental trajectories of homotopic connectivity would be associated with ASD symptomatology.

Aim 2 (**Chapter 3**) Using fMRI data at rest I aimed to examine sex-specific alterations in local connectivity and its association with age within the context of established resting-state networks (RSNs) in ASD population. I also investigated if the alterations revealed by our analysis were relevant to ASD symptomatology.

Although being similar to Chapter 2 hypothesis of finding sex-specific alterations in developmental trajectories of local connectivity, I also expected to find alterations in local connectivity especially within default mode network boundaries. I also hypothesized that such alterations, if present, will correlate with ASD symptomatology.

Aim 3 (**Chapter 4**) Using MEG resting state data I aimed to test the hypothesis that connectivity alterations were more pronounced in very preterm boys as compared to very preterm girls as reflection of higher male vulnerability or female protective effect. I also investigated how such alterations were linked to adverse neonatal experience and long-term behavioural outcome.

For Chapter 4 I hypothesized that very preterm boys have more pronounced alterations in interregional connectivity than girls potentially reflecting the male vulnerability. I also expected that the alteration in connectivity in very preterm children would be associated with long term behavioural and cognitive outcome and adverse neonatal experience.

Chapter 2.

Sex differences in interhemispheric connectivity and its developmental trajectories in ASD

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2.1. Abstract

It has been proposed that autism spectrum disorder (ASD) may be characterized by an extreme male brain (EMB) pattern of brain development. Here, we performed the first investigation of how age-related changes in functional brain connectivity may be expressed differently in females and males with ASD. We analyzed resting-state functional magnetic resonance imaging data of 107 typically developing (TD) females, 114 TD males, 104 females, and 115 males with ASD (6–26 years) from the autism brain imaging data exchange repository. We explored how interhemispheric homotopic connectivity and its maturational curvatures change across groups. Differences between ASD and TD and between females and males with ASD were observed for the rate of changes in connectivity in the absence of overall differences in connectivity. The largest portion of variance in age-related changes in connectivity was described through similarities between TD males, ASD males, and ASD females, in contrast to TD females. We found that shape of developmental curvature is associated with symptomatology in both males and females with ASD. We demonstrated that females and males with ASD tended to follow the male pattern of developmental changes in interhemispheric connectivity, supporting the EMB theory of ASD.

2.2. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which is diagnosed behaviorally based on deficits in social communication and restricted and repetitive behaviors and interests (American Psychiatric Association 2013). ASD has a

heterogeneous genetic basis (Muhle et al. 2004), and lacks clear objective biomarkers. Altered connectivity between brain areas is increasingly understood to be a characteristic feature of ASD (Geschwind and Levitt 2007). There is considerable evidence that brain connectivity in ASD is associated with cognitive and affective alterations in this group (Rane et al. 2015; Picci et al. 2016; Hull, Jacokes, et al. 2017; O'Reilly et al. 2017). Both functional overconnectivity and underconnectivity, as well as normal levels of connectivity have been reported in ASD population (Monk et al. 2009; Vissers et al. 2012; Di Martino et al. 2014; Cerliani et al. 2015; Cheng et al. 2015). However, due to a significant number of contradictory findings, it has been argued that alterations in connectivity strength do not consistently explain ASD pathogenesis (Picci et al. 2016).

A number of recent studies have also indicated that rather than being overconnected or underconnected per se, atypical brain development in individuals with ASD can be characterized by an altered developmental trajectory of brain rhythms and connectivity (Uddin et al. 2013). Atypical maturational trajectories in ASD have been described in studies on brain connectivity estimated from functional magnetic resonance imaging (fMRI) (Nomi and Uddin 2015; Dajani and Uddin 2016). Also, a recent study based on neurophysiological recordings has reported complex spatial–temporal profiles of age-related changes in neuromagnetic rhythms and spontaneous network synchrony in ASD, whereas no overall group differences were observed (Vakorin et al. 2017). Similar findings were reported using other imaging modalities and their derivatives, such as cortical thickness and brain volume estimated from structural MRI (Ecker et al. 2015). A particularly promising recent study showed that the rate of change in infants' brain volume and surface areas was sensitive enough to predict ASD in the brains of individual participants using machine learning tools (Hazlett et al. 2017).

There is compelling evidence emerging that altered maturational trajectories in brain structure as well as functional network connectivity are hallmarks of ASD. However, whether atypical maturation of structural and functional features of the brain are expressed differently in males and females with ASD remains poorly understood. The prevalence of ASD is significantly greater in males (Werling and Geschwind 2013). Epidemiological studies on ASD report the ratio of males to females to be between 1.9 and 16 males diagnosed for every female (Fombonne 2009). This suggests that the female brain may have protective features, or conversely that the male brain may confer heightened vulnerability, or that ASD may manifest differently in the female brain (Lai, Lerch, et al.

2017). Our study aims to investigate sex-related and clinical group differences in functional connectivity in ASD and typically developing (TD) populations under the framework of developmental trajectories. The autism brain imaging data exchange (ABIDE), a large repository of both structural and functional MRI data for ASD research, allowed us to form a large pool of both males and females for ASD and TD populations (Di Martino et al. 2014).

Our study tested the hypotheses that males and females with ASD would show distinct alterations in developmental trajectories of functional connectivity. Specifically, we focused on interhemispheric homotopic connectivity, and investigated voxel-mirrored homotopic connectivity (VMHC) which reflects the similarity between fMRI time series of two symmetrical voxels in the right and left hemispheres. This measure previously was shown to have a potential to distinguish various neurological and neuropsychiatric populations, including ASD (Anderson et al. 2010; Hahamy et al. 2015), schizophrenia (Lang et al. 2016), depression (Hou et al. 2016), Parkinson's disease (Zhu et al. 2016), Alzheimer's disease (Wang et al. 2015), amyotrophic lateral sclerosis (Zhang et al. 2017), and stroke (Yang et al. 2017). In addition, it has been reported to change throughout the lifespan in typical brains (Zuo et al. 2010). Another rational to explore interhemispheric connectivity was the evidence of great impact of sex hormones in brain lateralization, which makes VMHC particularly interesting to explore in terms of sex differences (Nguyen et al. 2013).

We studied age-related changes in VMHC in 115 males and 104 females with ASD, 114 TD males, and 107 TD females, selected from the ABIDE database and aged between 6 and 26 years old. Using a data-driven approach, we applied a multivariate technique to decompose the entire data into latent variables (LVs), each associated with a group contrast and z-scores of how this contrast is expressed across specific brain regions. We found no overall group differences for VMHC. However, we observed significant differences between the groups in terms of the rate of age-related changes in VMHC.

2.3. Methods

2.3.1. Cross-sectional participants

Structural and resting-state fMRI data were provided by the ABIDE I and II releases initiative, which combines data across multiple centers (Di Martino et al. 2014).

First, to investigate sex differences in interhemispheric homotopic connectivity in ASD, we created four cross-sectional groups of subjects: 104 females with ASD, 115 males with ASD, 114 TD males, and 107 TD females. Detailed phenotypic information is provided in Table 2.1 Characteristics of the participants (due to limited phenotypic data available on ABIDEs I and II, not all the subjects included to this study had intelligence quotient (IQ) and Autism Diagnostic Observation Schedule (ADOS) measurements which we admit being a limitation of this study).

Table 2.1 Characteristics of the participants

Group characteristic	Typical Development (TD)		Autistic Spectrum Disorder (ASD)	
	Males	Females	Males	Females
Number of subjects	114	107	115	104
Age (Mean±STD)	12.9±4.04	12.7±4.16	13.0±3.76	12.8±4.31
Full IQ (Mean±STD)	110.7 ±11.87 (98 subj)	113.5±14.89 (93 subj)	104.8±19.22 * (101 subj)	105.2±17.45 ** (92 subj)
Verbal IQ (Mean±STD)	112.4±12.20 (79 subj)	113.7±16.47 (75 subj)	105.8±21.13* (83 subj)	104.4±17.17** (79 subj)
Performance IQ (Mean±STD)	107.4±12.87 (88 subj)	108.9±19.93 (84 subj)	104.9±18.7 (89 subj)	101.4±18.09** (83 subj)
ADOS total (Mean±STD)			12.0±4.25 (56 subj)	11.6±3.66 (54 subj)
ADOS communication (Mean±STD)			3.7±1.69 (56 subj)	3.5±1.48 (54 subj)
ADOS social interaction (Mean±STD)			8.0±3.08 (56 subj)	7.7±2.32 (54 subj)
Framewise Displacement (Mean±STD)	0.11±0.107	0.12±0.097	0.13±0.121	0.12±0.127

*- significant differences between ASD groups and TD groups of the same sex;

** - significant differences between ASD groups and TD groups of the same sex which survived Bonferroni correction;

There were no significant differences between males and females within the same diagnostic group.

Due to limited number of female participants, we formed the female groups first. With the preponderance of males in ASD studies, and to alleviate a possible bias due to unbalanced sample sizes, we took a random subsample of male subjects from the larger TD and ASD cohorts of males, under the condition that all four below-mentioned inclusion criteria were satisfied. There were no significant group differences in age in the final sample, as confirmed by six two-sample *t* tests comparing all four groups on a pair-wise basis. Our inclusion criteria were: (a) participants between 6 and 26 years of age (considering our research question we aimed for the widest age range as possible). As the number of participants available on ABIDEs I and II drops considerably after 30 years, we initially defined the age range 6–30 years, which was adjusted after preprocessing procedures to 6–26 years); (b) similar number of selected subjects for all four groups per center and total number of selected subjects per center more than 10 (please see Table A1); (c) acceptable quality of structural MRI scans based on visual inspection; and (d) successful preprocessing using the Configurable Pipeline for the Analysis of Connectomes (C-PAC).

2.3.2. Longitudinal participants

With the primary goal of investigating potential abnormalities of developmental trajectories of interhemispheric homotopic connectivity in ASD, we also analyzed longitudinal data available through the ABIDE II release, wherein subjects were scanned twice with an interval varying between 1 and 4 years. We applied the same inclusion criteria, as in the case of the cross-sectional data, and generated two age- and sex-matched groups of participants (aged between 9 and 18 when the first scan was obtained): $n = 19$ for ASD (16 males and 3 females, mean 12.5 ± 2.3 year of age) and $n = 13$ for TD (11 males and 2 females, mean 13.5 ± 1.8 year of age). Two-sample t-tests found no significant group differences in age, wherein age was determined either by the first scan or the second one or both.

2.3.3. Data acquisition and preprocessing

Data acquisition parameters varied across sites. Detailed descriptions of the scan parameters and site-specific protocols are available at **fcon_1000.projects.nitrc.org/indi/abide/**. All data were preprocessed using the C-

PAC (Craddock et al. 2013). Preprocessing steps included the removing of the first four volumes to ensure magnetic stability was reached. All volume slices were time corrected and head motion was regressed on 24 parameters (Friston et al. 1996). Sources of spurious signal were removed, regressing out the global, linear, motion and quadratic signals, CompCor was applied calculating five components derived from nuisance signals from the white matter and cerebrospinal fluid (Behzadi et al. 2007). Then, the functional images were temporally filtered between 0.1 and 0.01 Hz. Structural and functional images were aligned into Montreal Neurological Institute (MNI) standard space, and then a spatial smoothing using a Gaussian filter of 6 mm was performed.

2.3.4. VMHC calculation

Interhemispheric homotopic connectivity was computed by applying a standard implementation of C-PAC where structural and functional images were fitted to MNI symmetrical template and the Pearson's correlation between each pair of symmetrical voxels in different hemispheres was calculated (Craddock et al. 2013). To assess the spatial features of interhemispheric homotopic connectivity, we used population-level resting-state fMRI atlas of intrinsic connectivity of homotopic areas (AICHAs) which clusters the cerebrum into 192 homotopic regional pairs (Joliot et al. 2015). To compute region of interest (ROI)-specific VMHC, we averaged voxel-specific VMHC values across all the voxels within a given ROI. A number of previous studies reported the multicenter variability in MRI-based measures (Haar et al. 2016). We removed the variability of homotopic connectivity across centers in ABIDE with a linear regression analysis, modeling the presence of centers with dummy (binary) variables. To avoid any issues caused by variability of scan parameters or recording procedures, we did not merge individuals from the same centers in ABIDE I and II. The schematic illustration of the analysis pipeline is displayed in Figure 2.1.

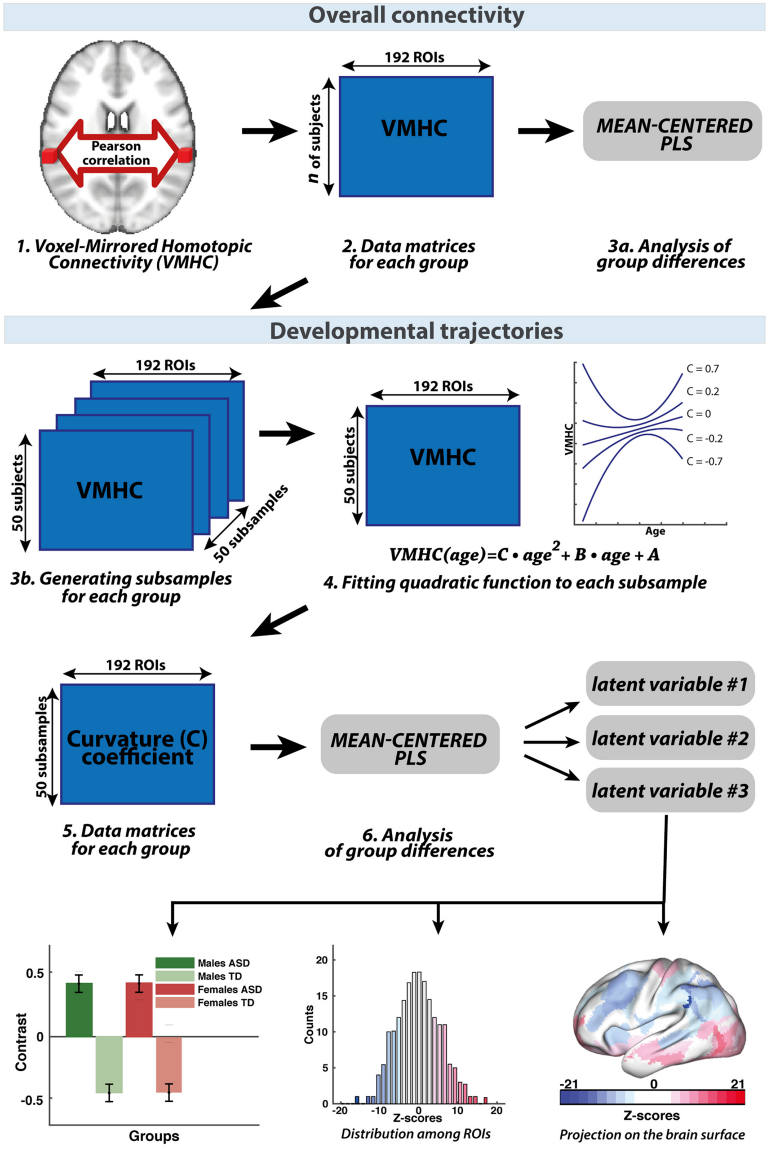

Figure 2.1 Schematic illustration of the data analysis.
To investigate overall interhemispheric homotopic connectivity, we used C-PAC to calculate the VMHC (1). We applied AICHA atlas and obtained data matrix (number of subjects × ROIs) for each of the group (2) which we explored the statistical differences by mean-centered PLS (3a). We used the same data matrices to build developmental trajectories. First, we generated 50 subsamples for each group. Each subsample contained VMHC values of 50 subjects for 192 ROIs (3b). Then we fitted quadratic function to each subsample (4) and formed new data matrices of curvature coefficients (5), which define curvature shape. We compared these new data matrices by using PLS analysis (6). PLS analysis provides the LVs, for each of them we obtained between group contrast and z-score distribution within ROIs which was projected back to the brain surface

2.3.5. Developmental trajectories of VMHC

A number of studies have reported U-shaped, or inverted U-shaped patterns for the age-related changes in gray matter density within the age range which includes the onset of puberty, possibly reflecting synaptic reorganization (Giedd et al. 1999). Also, it has been shown to be a reasonable compromise between nonlinearity and complexity. Quadratic model was previously found optimal for exploring age-related changes in VMHC when compared to cubic and linear models (Zuo et al. 2010) and was successfully applied in neuromagnetic and structural neuroimaging studies in ASD population (Vakorin et al. 2017).

To further support our choice of the quadratic model, we tested goodness of fit for VMHC as a function of age, using both linear and quadratic models. This was done for each ROI, separately for each group, by computing the Akaike information criterion (AIC) and Bayesian information criterion (BIC). We found that the model that best fit the data could be either linear or quadratic, depending on which ROI was being investigated. In addition, we performed paired t tests, separately for each group, comparing AIC or BIC values for linear and quadratic models across ROIs within a specific group, and found no significant differences (*p*-values for AIC criterion were ranging from 0.44 to 0.84, and for BIC—from 0.2 to 0.95) indicating that there is no ultimate winner among the models. This would also support our choice of considering only quadratic functions, taking into account that a linear model represents a specific case of a quadratic function.

One unique property of quadratic functions is their curvature, which is mathematically defined as the second derivative, and represents how the rate of change of a variable is itself changing. The quadratic functions have constant curvatures, which can be either positive (concave upward or U-shaped functions), negative (concave downward or inverted U-shaped functions) or zero (linear function). Previously, curvature values were found to be a sensitive neurophysiological marker to differentiate ASD and TD groups (Vakorin et al. 2017). To test group differences in terms of the curvatures of age-related changes, we need to introduce variability in the curvature values. Instead of calculating the curvature for the original groups which would result in one estimate per group, we used subsampling to generate a distribution of the curvature estimates per group. This was done by randomly choosing subsamples of subjects, separately for each group, subsequently estimating the trajectories of VMHC for each subsample. This

method was adapted from a previous study on atypical developmental trajectories in ASD brain network connectivity (Vakorin et al. 2017).

One limitation of the ABIDE database is that subjects are not uniformly distributed across age. To mitigate this, we generated a large number of subsamples (10,000) of 50 subjects for each group (TD/ASD for males/females), but ultimately chose only 50 subsamples with the flattest age distribution. We used a criterion based on calculating the entropy of distributions, which is highest for uniform distributions Then, for each subsample, we fitted a quadratic function, estimating its curvature. Thus, each group was associated with 50 estimates of the curve parameter for each of 192 ROIs. Analysis of group differences was then performed in a multivariate manner, for all groups and ROIs simultaneously (see Analysis of group differences), resulting in three individual *p*-values, each associated with a data-driven group contrasts and ROI-specific z-scores. To increase the robustness of the results, we performed all these procedures 100 times including generation of subsamples (thus in total 100 × 50 subsamples were selected for each group), curve fitting, and group analysis), subsequently averaging the *p*-values and z-scores. In Section **3**, we report the averaged three group contrasts, their *p*-values, and z-scores.

To assess group differences in the curvature parameter reflecting the trajectory of development of connectivity strength for each interhemispheric region pair from the longitudinal data, we applied a similar procedure. First, for each subject, we estimated the rate of changes in VMHC values computed as the ratio of VMHC changes between two longitudinal points to the corresponding age difference. Thus, each subject was associated with the 192 ROI-specific first derivatives of the developmental trajectories, and these estimates were used for further analysis. For each round of trajectory analysis (out of total 100), we generated, separately for each group (TD and ASD), 10 randomly chosen subsamples of 10 subjects. For each subsample, we fitted a linear function, estimating its slope. Note that the slope parameter computed from the rate of changes in VMHC (from the first derivatives) represents how the rate of change in VMHC is changing, which is equivalent to the curvature estimated from the cross-sectional data, as described above for the cross-sectional data. Thus, each group was associated with 10 estimates of the curvature parameter for each of 192 ROIs.

We then performed an analysis of group differences, estimating the global contrast between the two groups (one *p*-value), and assessing how the contrast is expressed

across ROIs (a 192-dimentional vector of z-scores). The procedure that includes subsample generation, linear regression, and group analysis, was repeated 100 times to increase the robustness of the results. We report the group differences in the curvatures computed from the longitudinal data, as *p*-value and ROI-specific z-scores averaged across the rounds of trajectory analysis (Figure 2.5).

2.3.6. Analysis of group differences

The analyses of group differences, both for homotopic connectivity and its trajectories, were performed with partial least squares (PLS) analysis. PLS is a multivariate statistical technique designed to extract LVs that account for the variance in the data, which is similar to principal component analysis (Lobaugh et al. 2001; McIntosh and Lobaugh 2004). Two versions of PLS are often applied in the neuroimaging literature: mean-centered and behavioral PLS (Krishnan et al. 2011). Mean-centered PLS is designed to derive data-driven contrasts between two or more groups or conditions. Behavioral PLS investigates the significance of correlations between imaging data and continuous variables such as behavioral scores (ADOS generic scores in our case (Lord et al. 2000)).

For both versions of PLS, the entire data structure is considered at once, and the data are organized into matrices: subjects within groups by ROIs. Mathematically, PLS is based on singular value decomposition (SVD). SVD is a factorization of the original data matrix into three matrices: left matrix with vectors of dimensionality equal to the number of groups (overall group contrast), diagonal matrix showing the strength of each component (which is ultimately related to the amount of explained variance), and right matrix with vectors of dimensionality equal to the number of ROIs (robustness of overall group contrast).

Routinely, the PLS method includes one global test and a series of local tests. The global test is based on permutations and assesses the significance of the effect represented by the overall data-driven contrasts or overall correlation between imaging and clinical data by measuring how it is different from random noise. Thus, PLS generates one *p*-value for each LV associated with a group contrast or overall correlation mitigating the multiple comparison problems. Also, each LV is associated with local tests based on bootstrap procedures, which is performed separately for each ROIs, exploring how the overall contrast or correlation is expressed across ROIs. As a result, each ROI is

associated with a bootstrap ratio value. Under the assumption of a Gaussian distribution, the bootstrap ratio value of 2.5 or −2.5 approximately corresponds to the limits of the 95% confidence interval. In this study, we use the terms of z-scores and bootstrap ratio values from PLS interchangeably.

We applied the following PLS analyses: mean-centered PLS for exploring group differences for VMHC and their curvatures, and behavioral PLS for exploring correlations between VMHC and ADOS as well as between the VMHC curvatures and ADOS (see Section 2.3.7). In each PLS analysis, we used 1,000 permutations for the global test, and 1,000 bootstrap samples (for local tests). For the longitudinal data, we used 500 permutations for the global test and 500 bootstrap samples for local tests. In each case, we report the significance of group contrast or over-all correlation (*p*-value), and if the *p*-value is less than 0.05, we report the distribution of z-scores across ROIs. The z-scores that are largest in magnitude (positive or negative), indicate the most robust effects, which was identified as 5% tails of the overall distributions of z-scores. ROIs with positive z-scores directly support the given contrast or sign of the overall correlation. ROIs with negative z-scores also support the same contrast or correlation, but in a reverse direction. Thus, for ROIs with negative z-scores, we have to invert the overall group contrast to have an idea about the directionality of changes in the curvature values across the groups. Group differences in demographic, psychometric characteristics were explored by series of two-sample *t* tests which were further corrected by Bonferroni method. The results are presented in Table 2.1.

2.3.7. Brain-behavior correlations

We performed behavioral PLS analysis to investigate associations between VMHC values and ADOS-Generic scores for communication, social interaction and total (social interaction and communication). In addition, we applied behavioral PLS analysis to investigate potential associations between ADOS with VMHC trajectories. We included only those participants who had information on all three ADOS modules: $n = 56$ for males with ASD, and $n = 54$ for females. There were no significant differences in any of the three subscales between females and males with ASD, as confirmed by two-sample *t* tests.

Our approach for investigating potential associations between trajectories and ADOS scores was based on recent findings regarding slowing of cortical thinning in

individuals with ASD (Nunes et al. 2019). Specifically, the study demonstrated that the shape of developmental curvature is related to ASD symptomatology. Following these methods, we investigated the relationship between curvature shape of fMRI-based connectivity in ASD participants and mean ADOS score, separately for males and females.

First, we generated a set of 50 subsamples separately for males and females with ASD and calculated mean ADOS score for each subsample. Each subsample contained 20 subjects (the number of subjects was reduced in order to increase the impact of single subject on curvature shape and mean ADOS score). Then, we computed the ROI-specific VMHC curves as functions of age, fitting a quadratic function to each subsample. In result, we obtained curvature coefficient for each ROI and mean ADOS score per subsample.

Finally, we performed six PLS analyses to correlate VMHC with ADOS scores (communication, social interaction, and total), separately for males and females. We also performed six PLS analyses to investigate correlations between three ADOS scores and the curvature coefficients (for males and females). In all cases, we used 1,000 permutations to assess the significance of an overall correlation between ADOS and VMHC or their curvatures (one *p*-value from a global test), and 1,000 bootstrap samples to explore the robustness of the correlation effects across ROIs (ROI-specific z-scores from local tests, see group-level analysis).

2.4. Results

2.4.1. No group differences for overall connectivity

The multivariate group analysis, which we performed using mean-centered PLS with all four groups included, did not find any significant group differences in interhemispheric homotopic connectivity.

2.4.2. Three significant group contrasts for age-related changes

We thus tested the hypotheses that age-related changes in homotopic connectivity would be atypical in ASD and differentially expressed in males and females with ASD. We

characterized the development of functional connectivity in both TD and ASD in terms of anatomical loci, and tested for group differences in the curvature of maturational changes, including all four groups into the PLS analysis. PLS revealed, in a data-driven manner, three significant group contrasts (LVs), which represent contrasts between TD females and all other groups (Figure 2.2; LV1), between TD and ASD (Figure 2.3; LV2), and between males and females in ASD (Figure 2.4; LV3). Note that each contrast is associated with a single P-value, resulting from one global test for group differences in the multivariate PLS analysis. In turn, each contrast is associated with a vector of saliences representing contributions of individual ROIs to the overall group contrast. In our study we report the stability of those saliences, quantified as bootstrap ratio values (or z-scores) from a series of local tests, each associated with a ROI, as described in the Methods section.

2.4.3. Group differences between TD females and all other groups

The first group contrast (LV1) explained 57% of the total variance in the data, and represents overall group differences between TD females and three other groups, as illustrated in Figure 2.2a ($p < .001$). It essentially decomposes the variability in developmental trajectories of functional connectivity into two clusters: one cluster uncovering similarities between TD males with males and females with ASD, and the other one representing TD females. Figure 2.2b shows the corresponding distribution of z-scores, each associated with one ROI, which reflects the robustness of the effects specified by the group contrast in Figure 2.2a. The largest absolute values of z-scores, which in general, can be either positive (here shown in red) or negative, are associated with the most robust effect. The same distribution of z-scores is illustrated in Figure 2.2c as a color map within a template of the brain in the MNI space, using the Computerized Anatomical Reconstruction Toolkit (Caret) (Van Essen et al. 2001).

To identify the most robust effects across ROIs specified by a given group contrast, we typically explore 5% left and right tails of the distribution of z-scores (Vakorin et al. 2013, 2017). Note, however, that the distribution in Figure 2.2b is highly skewed. Thus, for the group differences in developmental trajectories of homotopic connectivity, which reflected TD females contrasted with all three other groups, we focus only on the largest positive z-scores (shown in dark red in Figure 2.2b,c). Specifically, these group differences in the shape of trajectories of connectivity were expressed prominently in the middle frontal

gyrus, paracentral lobule, intraparietal sulcus, and superior and middle temporal gyri. We report that, on average, the developmental trajectories were concave upward (an inverted U) to a larger degree in the group of TD females than in the other three groups. In other words, the trajectories of connectivity for TD males, ASD males, and ASD females were flatter, in comparison to TD females. To further illustrate these effects, we identified two ROIs with the largest positive z-scores (angular and parahippocampal gyri). We then computed the mean fitted quadratic functions, which represent age-related changes in homotopic connectivity, averaging across all ensembles of trajectories, separately for each group. The averaged trajectories for the two brain areas are plotted in Figure 2.2d.

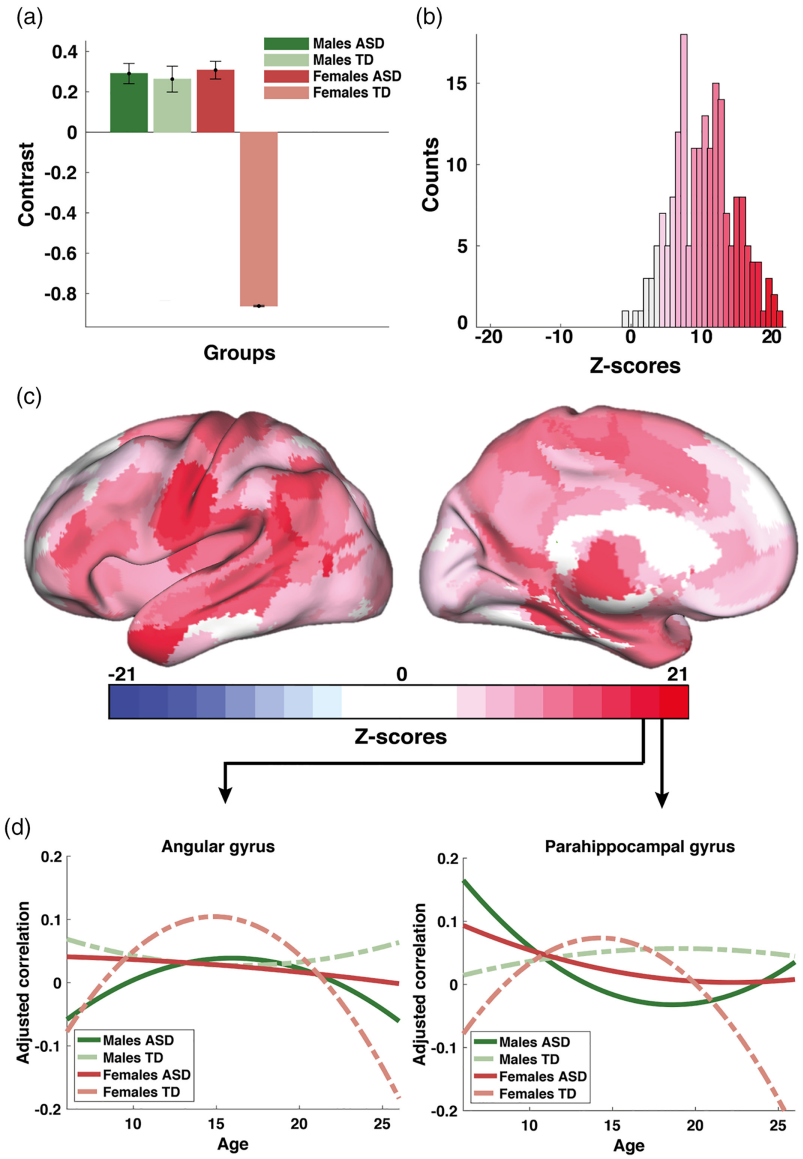

Figure 2.2 Male patterns of development of functional brain connectivity in ASD (cross-sectional sample)

(a) first significant data-driven contrast (57% variance explained, LV1); (b) a corresponding distribution of z-scores showing how the contrast is expressed across 192 ROIs from the AICHA atlas; (c) same z-scores shown as a topographic map in the MNI space; and (d) estimated group-specific trajectories exemplified for two ROIs (brain areas G Angular-1 and G ParaHippocampal-1 according to the nomenclature of the AICHA atlas). The contrast in (a) represents a similarity between two ASD groups and TD males in contrast TD females. Note that the PLS analysis of group differences revealed three significant contrast; the other two are described in Figure 2.3 and Figure 2.4

2.4.4. Altered development of homotopic connectivity in ASD

Explaining 26% of the total variance, the second group contrast (LV2) in PLS ($p < .001$) represents a difference in the VMHC trajectories between ASD and TD populations, with the differences between ASD and TD males being stronger than those between ASD and TD females, as shown in Figure 2.3a. The robustness of these effects is represented by the distribution of z-scores in Figure 2.3b, which shows how the group contrast is expressed across individual ROIs. Figure 2.3c illustrates the same z-scores as a topographic map on the template of the brain in the MNI space, similar to Figure 2.2c.

The largest absolute values of z-scores define the most robust effects. ROIs within the 5% positive tail of the distribution in Figure 2.3b (shown in red) include orbitofrontal cortex, middle and inferior temporal gyri, and the lateral occipital gyrus. On average, the developmental trajectories were concave upward (negative curvature) in both TD and ASD males; however, the curves tend to be flatter for males with ASD. ROIs belonging to the 5% negative tail of the distribution of z-scores (shown in blue in Figure 2.3b,c) were mostly located in the middle frontal gyrus, intraparietal sulcus, and inferior parietal lobule. Quadratic functions describing the age-related changes in VMHC for these ROIs tend to be concave downward (positive curvature for a U-shaped function) for TD males, whereas they tend to be concave upward for ASD males (negative curvature for an inverted U function). To illustrate the contrast in Figure 2.3a, we chose two ROIs with the largest absolute values of z-scores (one positive and one negative). Similar to Figure 2.2d, the averaged trajectories in VMHC for these ROIs are plotted in Figure 2.3d.

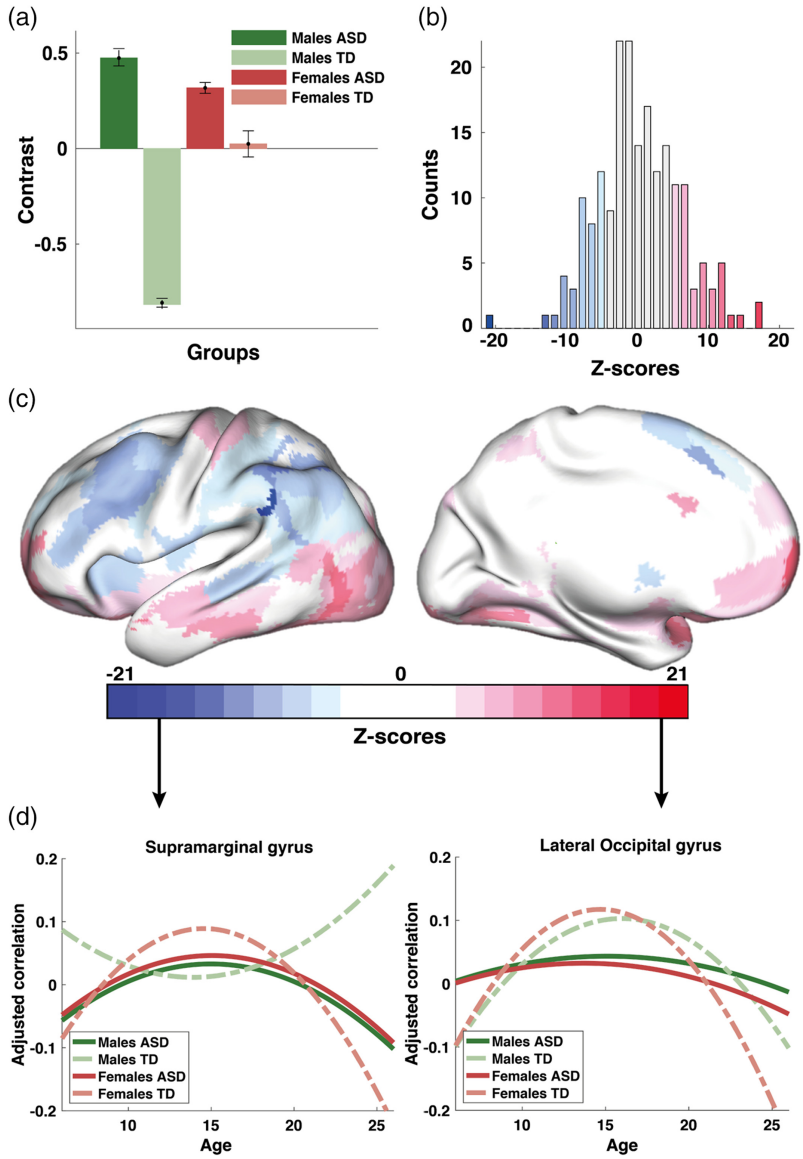

Figure 2.3 Altered developmental trajectories in ASD (cross-sectional data set)

(a) second significant contrast (26% variance explained, LV2); (b) a corresponding distribution of z-scores demonstrating the robustness of the overall group contrast across ROIs; (c) topographic distribution of the same z-scores in the MNI space; and (d) group-averaged trajectories for two ROIs with the largest positive and negative z-scores (brain areas G SupraMarginal-5 and G Occipital Lat-2 in the AICHA atlas). The contrast in (a) represents differences between ASD and TD populations, driven mostly by males. Note that the contrast is supported by both highly positive and negative z-scores. Positive z-scores (shown in red) directly support the contrast in (a) whereas for interpretation purposes, the contrast has to be flipped to interpret its expression across ROIs with negative scores

2.4.5. Group differences between males and females with ASD

The third group contrast (LV3, $p = .003$, 17% variance explained) identified significant differences in developmental trajectories between females and males with ASD as shown in Figure 2.4a. The corresponding distribution of ROI-specific z-scores is shown in Figure 2.4b as a histogram, and in Figure 2.4c as a topographic map. The most robust effects expressed by the group contrast in Figure 2.4a are supported by both positive and negative z-scores.

Positive z-scores represent a scenario wherein the estimated curvatures are higher in ASD males in comparison to ASD females. ROIs with the highest positive z-scores were included the middle frontal gyrus, paracentral lobule, middle, inferior temporal and fusiform gyrus, and the precuneus (red areas in Figure 2.4c). For these ROIs, the trajectories in the ASD female group were concave downward (negative curvatures) and relatively flat for ASD males (small negative or positive values of the curvatures).

The same contrast is supported by negative z-scores (curvature is higher for ASD females). ROIs with the most robust effects were observed in the prefrontal cortex, premotor cortex and inferior temporal gyrus (blue areas in Figure 2.4c). The fitted quadratic functions are concave upward for ASD males, whereas for ASD females they tend to follow the opposite U-shaped pattern.

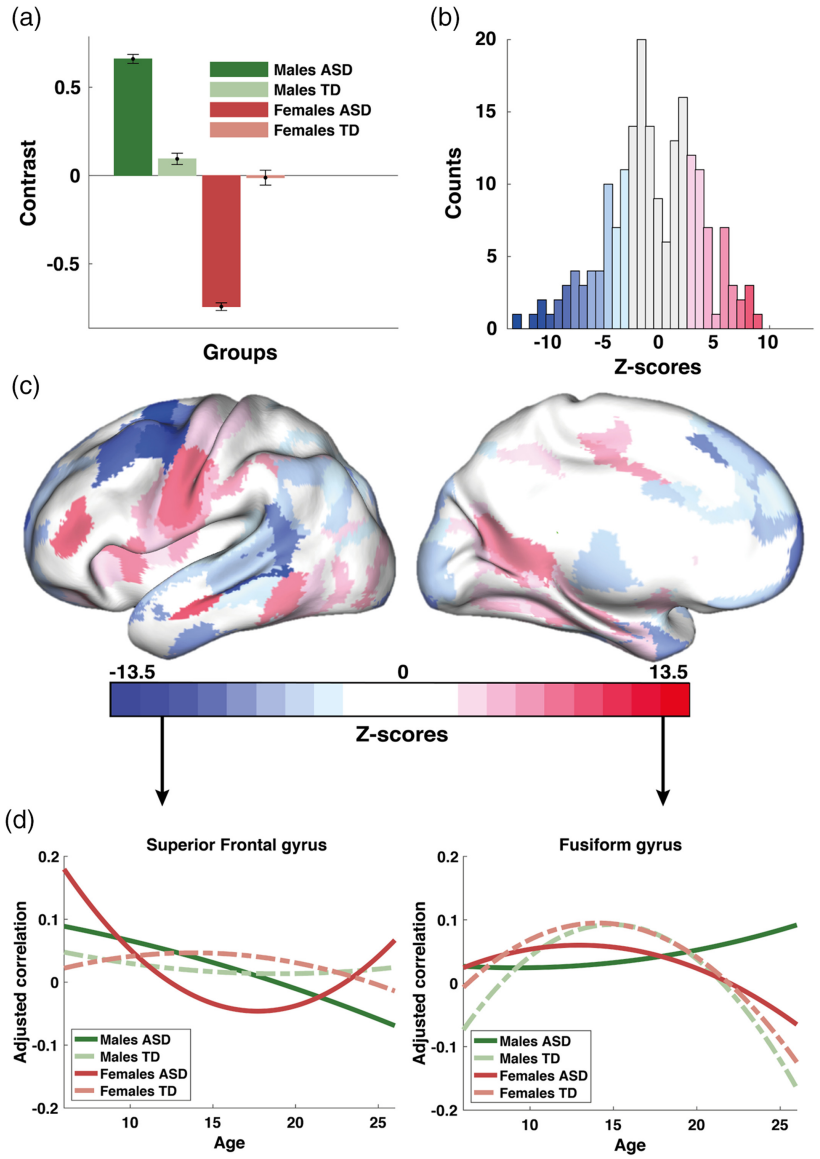

Figure 2.4 Sex differences in developmental trajectories of brain connectivity in ASD (cross-sectional sample)

(a) third significant contrast (17% variance explained, LV3); (b) a corresponding distribution of z-scores each associated with ROIs from the AICHA; (c) same z-scores shown topographically in the MNI space; and (d) group-specific trajectories for two ROIs with the largest positive and negative scores (brain areas G Frontal Sup-1 and G Fusiform-6 according to the nomenclature of the AICHA atlas). The contrast in (a) represents differences between ASD males and ASD females. The contrast in (a) reflect mainly differences between males and females in ASD

2.4.6. Longitudinal sample

The smaller longitudinal sample was used to test the hypothesis that atypical trajectories reported in the cross-sectional analysis were also observed in a longitudinal analysis. Due to the small sample size, however we did not have that ability to further

stratify differences between ASD and TD participants according to their sex. Our results based on the longitudinal sample were qualitatively similar to those obtained from analysis of the cross-sectional data. Specifically, PLS analysis did not find significant overall group differences in VMHC between ASD and TD groups when either the first scans or second scans or both were used. However, group differences were significant in terms of the curvatures of the maturational trajectories. Note that we computed the normalized difference in VMHC between the two scans to estimate the rate of change in VMHC. These changes were further used to estimate the curvatures of the trajectories in VMHC, which were the foci of our analysis based on the cross-sectional sample.

PLS analysis revealed an association between VMHC and age, which was measured in terms of the rate of changes in VMHC (curvatures), was significantly different between ASD and TD groups. The overall group contrast ($p < .001$) is illustrated in Figure 2.5a. The distribution of z-scores associated with this contrast is shown in Figure 2.5b. The topographic map in Figure 2.5c shows the same distribution in anatomical space. Similar to the effects shown in Figure 2.3, the group contrast contributed to by brain areas showing expressing both positive and negative z-scores. ROIs with the largest positive z-scores (5% tail), which reflect the U-shaped patterns for ASD as opposed to the inverted U-shaped ones frequently observed for TD, include lateral prefrontal cortex, inferior temporal gyrus, and cingulate gyrus. These patterns are reversed for negative z-scores. The 5% negative tail of the overall distribution of z-scores in Figure 2.5b are composed of ROIs localized in medial prefrontal areas, intraparietal sulcus, inferior parietal lobule, and precuneus, as shown in Figure 2.5c.

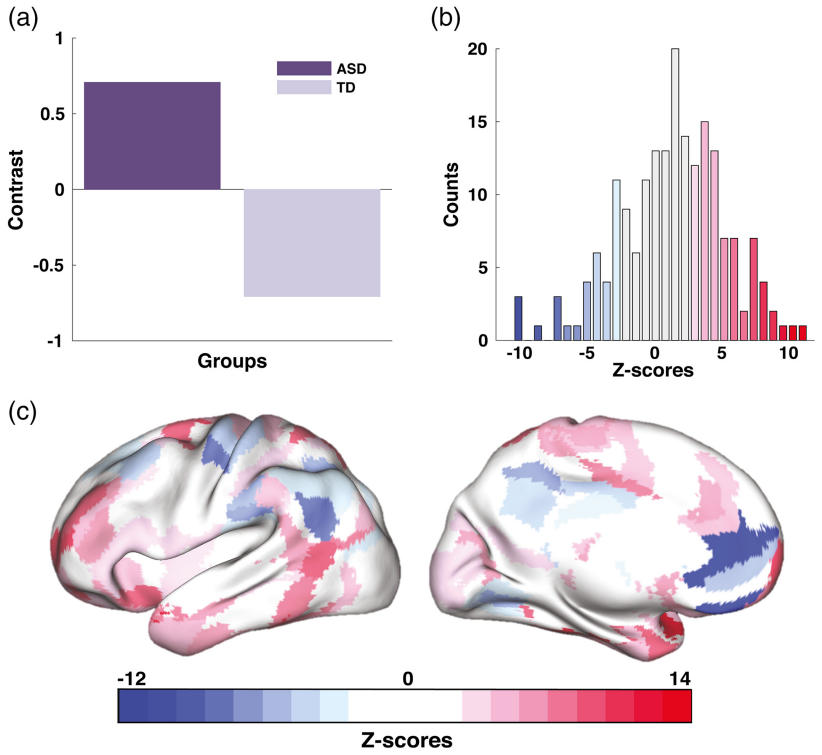

Figure 2.5 Group differences between ASD and TD in the curvature of developmental trajectories in interhemispheric homotopic connectivity (group analysis based on longitudinal sample)

(a) group contrast ($p < .001$); (b) corresponding distribution of z-scores showing how the contrast is expressed across 192 ROIs; and (c) same z-scores shown as a topographic map in the MNI space

2.4.7. Correlation with ASD symptomatology

Associations between VMHC and ASD symptomatology were analyzed only for the cross-sectional data as the number of subjects in the longitudinal data set with ADOS scores was small. Our multivariate group analyses, which were performed with behavioral PLS separately for males and females did not find any significant correlations between VMHC and any of the ADOS scores. Significant correlations, however, were observed in the ASD groups between the mean social ADOS scores and shape of developmental trajectories. The latter was quantified as curvature coefficient (see Section 2.2.7). Correlations between the curvature shape of individual ASD participants and ADOS social interaction scores were positive (Figure 2.6a). More specifically, the significance of correlations was $p = .004$ for the ASD males and $p = .02$ for the ASD females. Essentially, the bigger curvature coefficient (the more curvature was concave upward), the higher severity of symptoms.

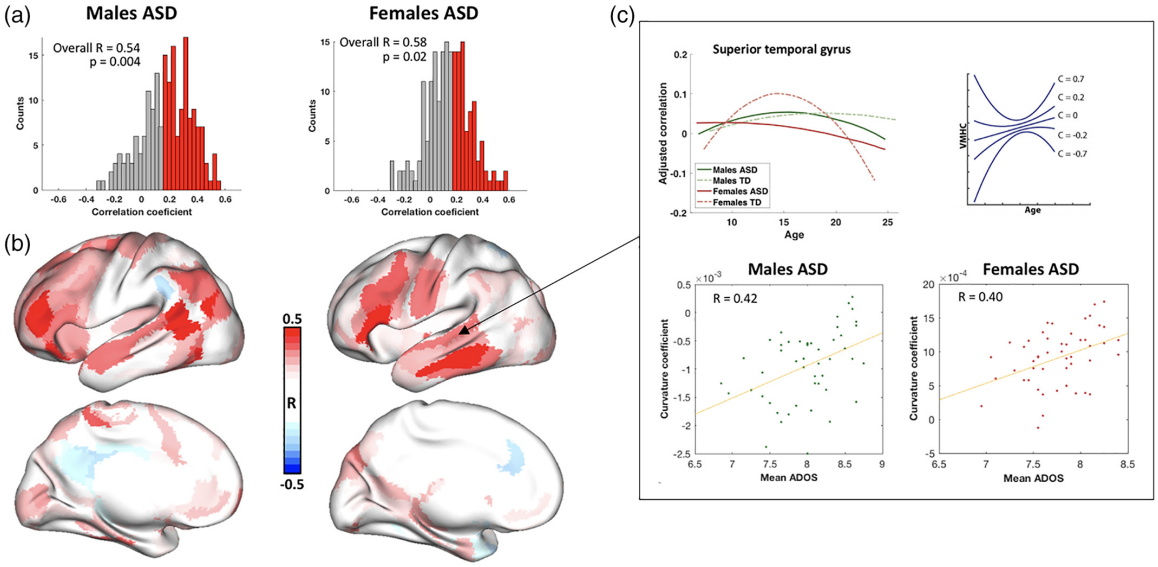

Figure 2.6 Association of curvature shape and ADOS (social subscale)
(a) distribution of correlation coefficient among ROIs for ASD males (left) and ASD females (right); (b) ROIs where curvature shape correlated the most with ADOS scores for ASD males (left) and ASD females (right); (c) the association between ADOS (social subscale) and developmental trajectories exemplified in superior temporal gyrus. The top left plot illustrates developmental trajectories of interhemispheric homotopic connectivity in all four groups. The top left plot is a schematic illustration of curvature coefficient and curvature shape. The scatterplots in the bottom demonstrate the association between curvature coefficient and ADOS social scores. The bigger deviation from TD female down concaved trajectory to "U" shape correlates with higher ADOS score in both ASD males and ASD females

Interestingly, the spatial distribution of ROIs with highest correlation between curvature shape and mean ADOS scores (middle frontal gyrus, paracentral lobule, inferior parietal lobule and superior temporal sulcus [Figure 2.6b]) in both males and females with ASD were similar to the ones with highest z-scores in the first group contrast (LV1). Note that the main difference revealed by LV1 was highly concaved down developmental curve (negative curvature coefficient [Figure 2.6c, top right]) for TD females, whereas ASD groups and TD males tended to have flatter (close to zero) or concaved up curvatures (positive curvature coefficient [Figure 2.6c, top right]). In other words, the higher curvature coefficient (the more the trajectory curved upward in contrast to the downward curving typical female trajectory) was associated with higher mean social interaction score on the ADOS (Figure 2.6c).

2.5. Discussion

The present study has yielded several novel insights into sex differences in ASD, unified under the framework of developing trajectories of functional brain connectivity. First, based on a large cross-sectional cohort of males and females, robust differences in interhemispheric homotopic connectivity (quantified as VMHC) between ASD and TD populations were identified by estimating the curvatures of age-related changes. This analysis identified robust differences in the developmental trajectories of functional brain connectivity where no overall group differences were observed. Importantly, these results were replicated with a similar analysis on a smaller longitudinal sample. Thus, our results can be incorporated into the growing body of recent evidence which supports the view that atypical development of functional brain connectivity may be a cardinal feature of ASD (Uddin et al. 2013; Nomi and Uddin 2015; Dajani and Uddin 2016; Vakorin et al. 2017). The present study extends this line of research by delineating the critical role of sex differentiation in the development of brain network connectivity in ASD.

Previous studies reported both decreased interhemispheric homotopic connectivity in ASD compared to typical population as well as no differences between groups (Anderson et al. 2010; Di Martino et al. 2014; Hahamy et al. 2015). Our findings indicate no differences in VMHC between groups while differences in developmental changes of VMHC were very pronounced. This indicates that alterations found in VMHC might be highly dependent on the age range of participants and have to be taken into consideration (note that age ranges of participants in the studies mentioned above differ).

Importantly, our results found similarities between ASD populations and TD males, in contrast to TD females, in terms of the curvatures of age-related changes in interhemispheric connectivity. This finding supports the extreme male brain (EMB) theory which considers many autism traits as an extreme profile of “typical male” strengths and challenges (Baron-Cohen et al. 2011). It explains ASD as an extreme manifestation of a male inclination to perceive the external world through systemizing rather than the female inclination toward empathizing (Baron-Cohen et al. 2005). Within a biological context, the most popular explanation of EMB theory is an alteration of the level of sex hormones, particularly fetal testosterone as it has been shown to have a considerable effect on prenatal and postnatal brain development (Auyeung et al. 2009).

Previously, the EMB theory has been supported by behavioral (Baron-Cohen et al. 2005), endocrinological (Auyeung et al. 2009), and genetic (Chakrabarti et al. 2009) lines of research. Recent studies of brain morphometry are also consistent with the EMB theory (Beacher et al. 2012; Lai et al. 2012). Particularly, it has been shown that females with male-typical cortical thickness pattern more likely to have ASD than biological females with a characteristically female brain phenotype (Ecker et al. 2017). An fMRI study showed that brain activity pattern in females with ASD was shifted to neural masculinization; however, at the same time, males with ASD demonstrated a shift toward the neuronal feminization (Alaerts et al. 2016). Another study got closer to EMB theory by focusing on default mode network (DMN) intraconnectivity. It was shown that among four groups, TD females had the highest connectivity between DMN nodes whereas ASD females had lower connectivity than TD males but higher connectivity than ASD males (Ypma et al. 2016). Our study provides one of the first robust neuroimaging evidence in support of the EMB theory with a large sample size.

Results from our investigations of associations between developmental trajectories of homotopic connectivity are also concordant with observed patterns of group differences. LV1 demonstrates that the curvature coefficient in TD males, males and females with ASD was on average more positive in contrast to negative curvature coefficient of TD females. Thus, the positive association between curvature coefficient and mean ADOS score is also consistent with the EMB theory. In other words, the more distinct is the shape of the curvature in ASD groups from that of the typical female trajectory the more severe the ASD symptomatology. This result is partially congruent with recent studies on ASD idiosyncrasy (Hahamy et al. 2015; Vakorin et al. 2017; Nunes et al. 2019). Although these prior studies demonstrated that ASD symptomatology increases with deviation from typical pattern, they did not investigate the impact of sex differences in this association.

Although the second group contrast explained only 26% variance in the data, it captured significant differences between TD males and ASD groups. Mapping of age-related deviations from typical brain development has the potential to predict ASD from individual scans, which has thus far proven difficult using only estimates of overall connectivity. Although a number of studies reported relatively high predictive power of abnormal brain connectivity in ASD, in particular in adults (Yahata et al. 2016), other studies, especially those covering a wide range of ages during development are more

conservative regarding the performance of machine learning tools, reporting accuracies indistinguishable from chance (Katuwal et al. 2015; Haar et al. 2016). A recent study, however, found that the rate of change in surface area and brain volume in infancy can predict, with high accuracy, the diagnosis of ASD in high risk siblings of children with ASD at 2 years of age (Hazlett et al. 2017). Thus, adopting a developmental perspective provides a promising framework to further clarify the nature and extent of atypicalities in ASD and is more likely to yield success in the search for imaging biomarkers for ASD than focusing on neural alterations which are expected to remain consistent throughout development.

Our results indicate that a developmental trajectories approach is effective when exploring sex differences in ASD. We show that although both males and females with ASD tend to follow typical male brain trajectory they still are significantly different from one another. We suspect that sex differences in ASD may be better characterized as differences in developmental trajectories of brain function in ASD rather than absolute differences which remain static throughout the course of development. Behavioral research shows a similar pattern of findings. Few consistent sex differences in ASD behavioral symptoms have been identified, particularly when IQ is in the average range. However, different developmental trajectories of symptom presentations are more commonly found for boys and girls with ASD. For example, boys were more impaired on social and communicative behavior at a young age, whereas females had more social deficits as adolescents and adults, specifically in reciprocal interaction and peer relationships (McLennan et al. 1993) and more lifetime sensory issues (Lai et al. 2011).

2.5.1. Conclusion

Using multivariate data-driven techniques from computational neuroscience and neuroimaging, we uniquely demonstrate that the developmental trajectories of homotopic connectivity variable across young age with different typical or atypical patterns, providing a new framework for studying pathological alterations and sex differences. Moreover, our findings indicate that age- and sex-specific alterations in connectivity might better characterize this group rather than specific overconnectivity or underconnectivity per se. Importantly, we provide one of the first direct evidences in support of the EMB theory in terms of developmental trajectories in homotopic functional connectivity. Finally, our

results demonstrate the association between developmental trajectory shape and symptoms severity in both males and females with ASD.

Chapter 3.

Effect of sex on local connectivity and its developmental trajectories in ASD

This paper has been accepted for publication in Cerebral Cortex as Kozhemiako N., et al. Alterations in local connectivity and their developmental trajectories in autism spectrum disorder: Does being female matter

3.1. Abstract

Autism Spectrum Disorder (ASD) is diagnosed more often in males with a ratio of 1:4 females/males. This bias is even stronger in neuroimaging studies. There is growing evidence suggesting that local connectivity and its developmental trajectory is altered in ASD. Here we aim to investigate how local connectivity and its age-related trajectories vary with ASD in both males and females. We used resting-state fMRI data from the ABIDE I & II repository: males (n=102) and females (n=92) with ASD, and typically developing (TD) males (n=104) and females (n=92) aged between 6 and 26. Local connectivity was quantified as regional homogeneity.

We found increases in local connectivity in participants with ASD in the somatomotor and limbic networks and decreased local connectivity within the default mode network. These alterations were more pronounced in females with ASD. In addition, the association between local connectivity and ASD symptoms was more robust in females. Females with ASD had the most distinct developmental trajectories of local connectivity compared to other groups. Overall, our findings of more pronounced local connectivity alterations in females with ASD could indicate a greater etiological load for an ASD diagnosis in this group congruent with the female protective effect hypothesis.

3.2. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication, interaction and stereotypic behaviours and restricted interests (American Psychiatric Association 2013). The ratio of 4 males to 1 female is reported in the ASD population but not well understood (Halladay et al. 2015). This gender bias is even higher in neuroimaging studies with frequent underrepresentation of females with ASD (Hull, Jacokes, et al. 2017). For example, according to the ABIDE I data set description, 25% of the sites excluded females participants by design (Di Martino et al. 2014). In the largest dataset with neuroimaging data on participants with ASD (ABIDE I & II) the approximate ratio of males to females with ASD is 6:1. Thus, most of the findings on brain alterations in ASD are based on male-skewed samples and not necessarily applicable to females with ASD.

There is growing evidence that brain connectivity alterations are intrinsic to the ASD population (Wass 2011). Multiple studies have reported disrupted connectivity in distributed networks in ASD, suggesting impaired long-range communication between brain areas (Weng et al. 2010; von dem Hagen et al. 2013; Cerliani et al. 2015). Such alterations are often interpreted without taking into account the local connectivity within the regions which belong to these distributed networks. It has been shown, however, that local connectivity alterations might induce long-range connectivity changes underscoring the importance of understanding both together with their relationship (Deco et al. 2014). Investigation of local connectivity within borders of distributed networks might provide additional information facilitating the interpretation of the reported differences in long-range communication previously reported in ASD.

One of the most reliable and commonly used fMRI metrics to measure local connectivity is regional homogeneity [ReHo] (Zuo and Xing 2014; Jiang and Zuo 2016). ReHo measures the concordance of time-series of neighboring voxels, and thus is designed to represent local synchronization of spontaneous neural activity on approximately one-centimeter scale depending on the voxel size and the number of neighboring voxels included in the computation. ReHo typically decreases with age (Lopez-Larson et al. 2011), and is associated with cognitive control and inhibition (Wang et al. 2014), intelligence (Wang et al. 2011), the signaling hierarchy of information processing in the brain (Jiang, Xu, et al. 2015). Sex differences in ReHo distributed across

various cortical regions were reported across healthy populations. Using a multivariate-pattern analysis a recent study was able to predict sex based on ReHo with 79% specificity and 88% sensitivity (Wang et al. 2012). This study also reported higher ReHo in males in cortical regions located primarily in the right hemisphere, while ReHo in the left hemisphere was increased in females. Another study investigated sex differences in ReHo within twelve resting-state networks (RSNs) and found males had higher ReHo in primary visual network whereas females had marginally significant increase in ReHo within left attention network (Xu et al. 2015).

ReHo has been previously studied in people with ASD and was shown to be altered across multiple brain regions, mostly in the right hemisphere (Paakki et al. 2010; Shukla et al. 2010; Maximo et al. 2013; Di Martino et al. 2014; Jiang, Hou, et al. 2015). Some of these studies also investigated associations between ReHo and age, showing how local connectivity age-related changes differ between ASD and typical individuals (Shukla et al. 2010; Jiang, Hou, et al. 2015; Dajani and Uddin 2016). These studies support the view that the developmental approach can be useful in studying ASD alterations (Uddin et al. 2013). Importantly, male to female ratios in the previous studies investigating ReHo ranged from 2.5:1 (Paakki et al. 2010 with 20 males & 8 females) to 25:1 (Shukla et al. 2010 with 25 males & 1 female). The studies with the largest sample sizes did not include females at all (Jiang et al 2015, Maximo et al. 2013).

Based on previous reports of sex differences in ReHo in normative populations (Wang et al. 2012; Xu et al. 2015), together with findings of altered ReHo in ASD (Paakki et al. 2010; Maximo et al. 2013; Jiang, Hou, et al. 2015b; Dajani and Uddin 2016) it could be expected that local connectivity would be disrupted in a different manner in males and females with ASD. Due to the relationship between local and long-range brain connectivity, and the established disruption of large-scale brain network connectivity in ASD, we tested our hypothesis of sex-specific ReHo alterations in ASD population within the context of established RSNs. This approach enables us to relate our results to the vast findings reported in the context of long-range connectivity alterations. In particular, we expected to see alterations in default mode network based on the persistent evidence of underconnectivity in participants with ASD within this network (Padmanabhan et al. 2017). Moreover, there are also indications that such hypoconnectivity is expressed differently in males and females suggesting that we could expect to see similar patern in the present study (Ypma et al. 2016). Our previous investigation in interhemispheric

connectivity indicated that sex-specific alterations in ASD might be better characterized as differences in developmental trajectories rather than connectivity differences per se (Kozhemiako et al. 2018). More specifically, our previous findings indicated that males and females with ASD were following the typical male developmental trajectories in interhemispheric homotopic connectivity (Kozhemiako, Vakorin, et al. 2019). A similar male-like pattern of developmental trajectories was recently shown for modularity in males and females with ASD (Henry et al. 2018). Given the indications that ReHo reflects functional segregation and modularity of cortical areas (Jiang and Zuo 2016) we hypothesized that males and females with ASD would display more similarities in trajectories in local connectivity with typical males than with typical females. Accordingly, we used cross-sectional data to investigate associations between ReHo and age and specifically compared the developmental trajectories of ReHo between males and females with and without ASD.

3.3. Methods

3.3.1. Participants

Resting-state fMRI and anatomical T1 scans were obtained from the ABIDE repository (releases I and II), a publicly available repository of neuroimaging data from multiple data acquisition centers (Di Martino et al. 2014). To investigate the effect of sex and age on ReHo, four groups of subjects were formed: males (male TD, n = 104) and females (female TD, n = 92) with typical development (TD), males (male ASD, n = 102) and females (female ASD, n = 92) with an ASD diagnosis. Table 3.1 provides detailed phenotypic information of the cohorts. Data for some variables are only available for a subset of participants (some information was missed across the centers).

Table 3.1 Demographic characteristics of the cohort

Group characteristic	Typical Development (TD)		Autistic Spectrum Disorder (ASD)	
	Males	Females	Males	Females
Number of subjects	104	92	102	92
Age (Mean±STD)	13.1±4.14	12.6±4.08	13.0±3.56	12.9±4.13
Full IQ (Mean±STD)	110.8 ±12.23 (89 subj)	113.1±15.33 (81 subj)	103.9±19.82 ** (89 subj)	103.7±17.22*** (83 subj)
Verbal IQ (Mean±STD)	112.7±11.83	113.2±16.76	104.9±21.12**	103.2±16.56***

	(71 subj)	(66 subj)	(75 subj)	(71 subj)
Performance IQ (Mean±STD)	107.6±13.22 (80 subj)	108.2±14.47 (73 subj)	104.1±19.12 (80 subj)	101.1±17.88** (72 subj)
ADOS total (Mean±STD)			12.1±4.40 (50 subj)	11.4±3.57 (49 subj)
ADOS communication (Mean±STD)			3.8±1.75 (50 subj)	3.3±1.36 (49 subj)
ADOS social interaction (Mean±STD)			8.0±3.17 (50 subj)	7.6±2.29 (49 subj)
ADOS restricted & repetitive behaviours (Mean±STD)			2.1±1.80 (42 subj)	1.9±1.6 (42 subj)
SRS total (Mean±STD)	18.4 ±11.09 (34 subj)	17.4 ±16.72 (31 subj)	91.6 ±27.03*** (32 subj)	95.5 ±28.60*** (34 subj)
SRS awareness (Mean±STD)	4.1 ±2.19 (34 subj)	4.0 ±3.20 (31 subj)	12.8 ±3.37*** (32 subj)	12.4 ±4.52*** (34 subj)
SRS cognition (Mean±STD)	2.7 ±2.21 (34 subj)	2.6 ±2.45 (31 subj)	15.7 ±6.44*** (32 subj)	17.7 ±5.40*** (34 subj)
SRS communication (Mean±STD)	5.8±4.79 (34 subj)	5.8 ±6.20 (31 subj)	30.6 ±9.65*** (32 subj)	32.1 ±10.73*** (34 subj)
SRS motivation (Mean±STD)	4.2±2.52 (34 subj)	2.9 ±3.87 (31 subj)	14.5 ±5.92*** (32 subj)	15.4 ±5.66*** (34 subj)
SRS mannerism (Mean±STD)	1.7±2.73 (34 subj)	2.1 ±2.91 (31 subj)	18.0 ±6.86*** (32 subj)	18.0 ±6.60*** (34 subj)
Framewise Displacement (Mean±STD)	0.18±0.145	0.17±0.143	0.20±0.122	0.20±0.133

** - $p < 0.01$, *** - $p < 0.001$, STD – standard deviation, IQ – Intelligence Quotient, ADOS – Autism Diagnostic Observation Schedule, SRS – Social Responsiveness Scale

The female TD and female ASD groups were formed first because the dataset included few females. To avoid any possible bias due to unbalanced sample sizes, random subsamples of male subjects were selected from the total cohorts of TD and ASD male cohorts to form age- and center-matched male TD and male ASD groups. All groups were formed with the following inclusion criteria: (i) similar representation of subjects per center and the total number of subjects per center more than ten (Table A2); (ii) acceptable quality of structural MRI scans and acceptable coverage of cerebellum on fMRI scans confirmed by visual inspection (since cerebellum ROIs were included in our subsequent analysis); (iii) successful preprocessing using Configurable Pipeline for the Analysis of Connectomes (C-PAC, see more details below); (iv) age range 6 – 26 years and no significant group differences in age between groups tested by six two-sample t-tests. Due to our intention to investigate developmental trajectories, we aimed to use the sample with

an age range as wide as possible. Initially we focused on participants with ages 6 to 30 years. As the number of subjects available through the ABIDE I & II database drops significantly after 30 years, because additional scans were not available for analysis due to unsuccessful preprocessing or failure of visual scan inspection, we restricted our analysis to participants 6 – 26 years of age.

3.3.2. Data acquisition and preprocessing

Since ABIDE I and II is a multi-center dataset, with data acquisition parameters which were specific to each data acquisition site. The scan parameters and acquisition protocols are provided at http://fcon_1000.projects.nitrc.org/indi/abide/. C-PAC was used to preprocess anatomical and functional MRI scans (Craddock et al. 2013). Structural MRI scans underwent brain extraction and segmentation into gray matter, WM, and CSF. For the functional MRI, the following standard preprocessing steps were applied: slice timing correction and spatial realignment. Sources of spurious signals were removed by regressing out linear and quadratic trends, Friston 24 motion parameters signals and 10 CompCor components derived from nuisance signals from the cerebrospinal fluid (CSF) and white matter (WM) (Friston et al. 1996; Behzadi et al. 2007). Subsequently, the temporal band-pass filter of 0.1 – 0.01 Hz was applied. Then, functional and anatomical images were normalized by aligning to the Montreal Neurological Institute (MNI) standard space and co-registered together.

3.3.3. Brain-behaviour correlation

For a subset of the original ASD sample, three scores of the Autism Diagnostic Observation Schedule (ADOS) were available: reciprocal social interaction, communication, and stereotyped behaviors and restricted interests. The majority of those participants were assessed with module 3 (33 males with ASD and 32 females with ASD). However, there were a few participants assessed with module 4 (9 males with ASD and 8 females with ASD) and module 2 (1 male with ASD and 2 females with ASD). Scores for participants who were assessed with module 2 and 3 were taken only if the ADOS version was ADOS-Generic. Module 4 assessment scores were used from both ADOS-Generic

and ADOS2 since changes introduced to computational algorithms with the ADOS update did not include module 4.

For a different subset of individuals from all groups scores of the Social Responsiveness Scale (SRS) five sub-scales were available, including awareness, cognition, communication, motivation, and mannerism. Table 1 describes the sample with available ADOS and SRS scores. We used these scores to investigate correlations between ReHo and ASD symptomatology.

3.3.4. Regional Homogeneity calculation and analysis

The standard implementation of C-PAC was used to calculate ReHo, a voxel-based measure of brain activity, which represents the similarity between the time series of a given voxel and the surrounding 26 voxels. This was accomplished by computing Kendall's coefficient of concordance (Zang et al. 2004). Obtained individual ReHo maps were spatially smoothed using a Gaussian filter of 6 mm. To explore changes in ReHo in terms of functional networks, we applied cerebral gray matter 7 Network Liberal Mask for selecting cerebral gray matter voxels and 7 network mask of the cerebellum (Buckner et al. 2011; Yeo et al. 2011). Each voxel from these masks has a label indicating its membership in one of the seven RSNs: visual, somatomotor, dorsal attention, ventral attention, limbic, fronto-parietal control and default mode network. To correct for center-specific variability in ReHo, for each voxel a linear model was fitted to estimate the variance explained by centers while preserving the variance explained by group, sex, age, group-by-age, and sex-by-group interactions:

$$\boldsymbol{ReHo}(\boldsymbol{age}) \sim \boldsymbol{Intercept} + \boldsymbol{\alpha_o Center} + \boldsymbol{\alpha_1 Age} + \boldsymbol{\alpha_2 Group} + \boldsymbol{\alpha_3 Sex} + \boldsymbol{\alpha_4}(\boldsymbol{Group} \times \boldsymbol{Age}) + \boldsymbol{\alpha_5}(\boldsymbol{Group} \times \boldsymbol{Sex}),$$

where $Center$ is a binary matrix (the number of subjects by the number of centers – 1) and α_o explains the center variability for a given voxel. The term $\alpha_o Center$ was removed for ReHo values of each voxel. Further, individual ReHo values were standardized by dividing the ReHo value of each voxel by the global mean across all voxels for a given individual similarly to previous ReHo studies on ASD (Paakki et al. 2010;

Shukla et al. 2010; Dajani and Uddin 2016). We use this normalized ReHo for all subsequent analyses.

3.3.5. Statistical analysis

We applied a series of Partial Least Squares (PLS) analysis to test for group differences and associations between ReHo scores and ASD symptomatology (Lobaugh et al. 2001; McIntosh and Lobaugh 2004). There numerous advantages of PLS. First, it is a multivariate technique that considers ReHo values for all voxels at once when testing for group differences (so called mean-centered PLS) or associations with behavioural variables (so called behavioural PLS). Second, PLS allows comparison of several experimental groups or conditions at once. Third, there is no need to specify group contrast when using PLS.

PLS is a multivariate approach that aims to extract the latent variables (LVs) which express the most variance in the data. The LVs are based on the singular value decomposition of the ReHo data organized as one matrix of size number-of-subjects by number-of-voxels. Subsequently, permutation and bootstrap tests are performed to test the significance of individual LVs, and to investigate the robustness of contribution of individual voxels to overall group differences (mean-centered PLS) or correlations (behavioral PLS). The permutation test is based on randomly permuting subjects across groups. It renders a single p-value for each LV, reflecting its statistical significance (it can be considered as a 'global' test). The bootstrap test is based on randomly sampling the subjects with replacement within the groups. As a result, each voxel is associated with a bootstrap ratio value (original saliences divided by the standard deviation of the saliences in the bootstrap samples), which is equivalent to z-scores.

In our study, we used two types of PLS analyses: mean-centered and behavioral PLS. Mean-centered PLS is used to test the significance of group differences whereas behavioural PLS is used to investigate the significance of associations between 'imaging' data (such as ReHo) and 'behavioral' variables (continuous such as behavioural scores or age). For both types of PLS, a LV is composed of three elements: (i) singular vector that represents a group contrast in mean-centered PLS (e.g. Figure 3.2, A) or overall correlation in behavioural PLS (e.g. Figure 3.4, A). (ii) Singular value that indicates total variance of the data expressed by this LV. We report total variance of the data explained by a LV in percentage next to groups contrast or overall correlations (e.g. Figure 3.2, A).

(iii) Singular vector with bootstrap ratios for each voxel that demonstrates the contribution of each voxel to the groups contrast or overall correlations and can be interpreted as a z-score. In the results section, we report general distribution of the z-scores (e.g. Figure 3.2, B) and spatial distribution of z-scores on the brain surface (e.g. Figure 3.2, C) using the z-score threshold of -2.5 and +2.5 since it is approximately corresponds to 99% confidence interval (McIntosh and Lobaugh 2004). For all PLS analyses 3000 permutations and 3000 bootstrap resampling were run. We ran PLS both including ReHo for all voxels as well as separately for each resting state network (n=7). In the latter case, the p-values were corrected for multiple comparisons using Bonferroni correction. Figure 3.1 schematically illustrates the workflow of the analyses. We tested differences in demographic and psychometric characteristics between groups by a series of two-sample t-tests (Table 3.1).

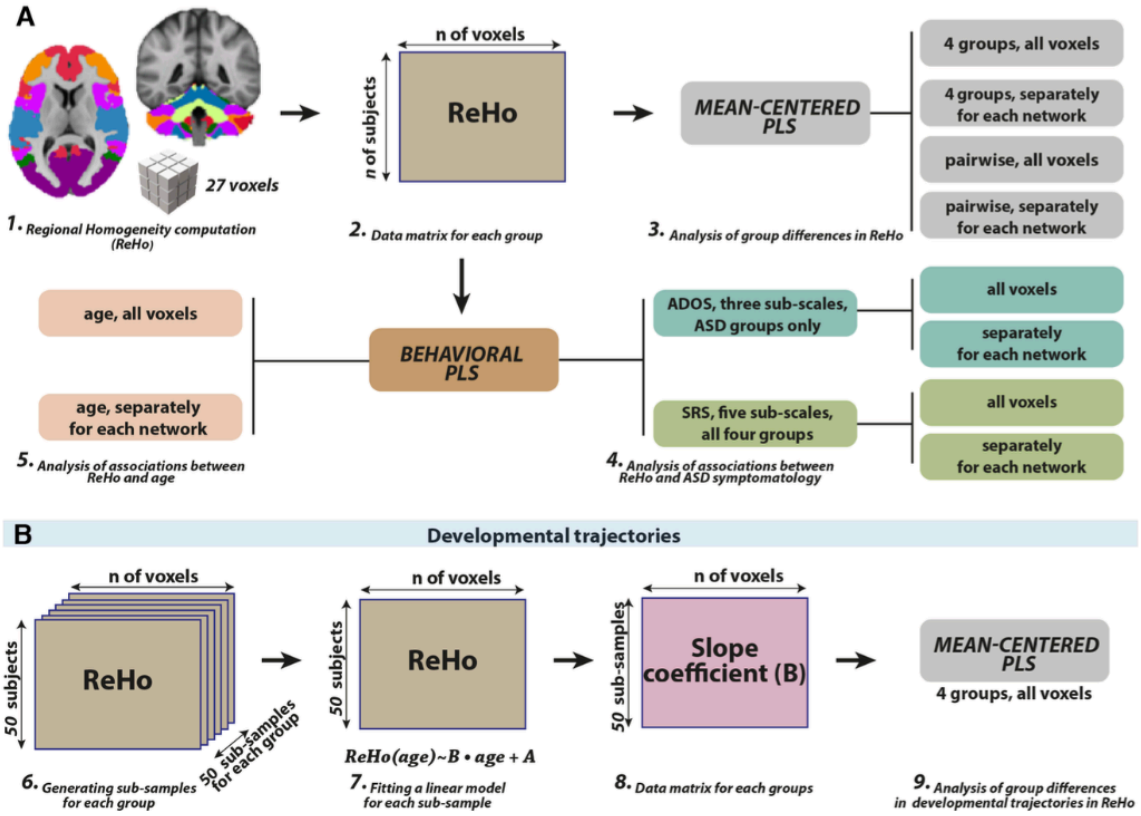

Figure 3.1 Analysis workflow

A – Group analysis of regional homogeneity (ReHo): We used C-PAC to compute ReHo as Kendell's Concordance Coefficient (KCC) between time series of 27 voxels and applied seven resting state networks (RSNs) liberal gray matter mask of cortex and cerebellum (1). Four ReHo matrices were obtained – one for each group (2). To test if there were group differences in ReHo we employed mean-centered Partial Least Squares (PLS) analysis (3): one global PLS was run with four groups across all voxels and seven PLS analyses were run separately for voxels within particular RSN. We also ran four pairwise PLS analyses (males ASD vs males TD, females ASD vs females TD, males ASD vs females ASD and males TD vs females TD)

across all voxels as well as separately for each RSN. We investigated the association between ReHo and ASD symptoms severity using behavioral PLS (4). For ASD groups, we used three subscales of the ADOS to investigate associations with ReHo with all voxels and for each RSN separately. Five SRS subscales were used to investigate the association with ReHo in all four groups (across all voxels and separately for each RSN) (4). We also used behavioural PLS to investigate the association between ReHo and age for all four groups across all voxels and separately for each RSN (5). B – Group analysis on developmental trajectories of ReHo: first we generated 50 subsamples of 50 subjects separately for each group (6), then we fitted a linear function to each subsample (7) and formed new data matrices of slope coefficients (8) which were compared across four groups using mean-centered PLS (9).

3.3.6. Developmental trajectories of ReHo

To investigate age-related changes in ReHo using cross-sectional data we used a linear model to fit ReHo as a function of age for each group separately. To compare the slope coefficients of developmental trajectories between the groups, subsampling was used to generate a distribution of the slope estimates per group. This was done by randomly choosing subsamples of subjects, separately for each group, and fitting the linear model for each subsample (Vakorin et al. 2017; Kozhemiako, Vakorin, et al. 2019). Assuming that the procedure for removing site-specific effects worked reasonably well, we did not apply any restriction on the sub-samples center representation. The distribution of ABIDE subjects across age was not uniform. To alleviate these effects, we generated a large number of subsamples (10,000) of 50 subjects for each group and selected 50 subsamples with the flattest age distribution based on the entropy of the distribution (the higher entropy would be characteristic for more uniform distributions). The slope coefficients were estimated for each subsample and each voxel by fitting a linear polynomial model of age. This resulted in four matrices (one for each group) with dimensions 50 subsamples times the number of voxels. In other words, for the purpose of testing for group differences, the subsamples were equivalent to the observations (subjects), whereas the features (attributes) were represented by the slope estimates of the fitted trajectories across voxels, instead of ReHo values across voxels.

3.4. Results

3.4.1. Differences in ReHo between ASD and TD groups

PLS analysis performed to test for group differences across the four experimental groups (ASD/TD males/females) revealed two significant LVs. The first significant LV ($p < 0.001$) expressed 40% of the data variance (Figure 3.2). Data-driven group contrast associated with this LV essentially expressed group differences in ReHo between ASD and TD groups (Figure 3.2, A). As shown in Figure 3.2, B, the z-score distribution is not skewed dramatically to the right or left, indicating that there were areas with higher (right tail highlighted in red) ReHo, as well as areas with lower (left tail highlighted in blue) ReHo in both males and females with ASD groups compared to TD groups. The spatial distribution of z-scores on the brain surface (Figure 3.2, C) indicates that higher ReHo in the ASD groups was most pronounced in the right primary motor cortex, left and right supplemental motor areas, left operculum, posterior cerebellum, and bilateral temporal poles. Lower ReHo in ASD groups was observed bilaterally in the medial prefrontal cortex, middle frontal gyrus, posterior cingulate cortex and precuneus, right supramarginal area. We computed the percentage of significant voxels for each resting state network (Figure 3.2, D). ASD groups had higher ReHo in the limbic and somatomotor resting state networks, whereas lower ReHo was observed for the ASD groups in the default mode network.

In addition, two pairwise PLS analysis were run separately for male and female groups (males ASD vs males TD [Figure 3.2, E] and females ASD vs females TD [Figure 3.2, F]) to determine if group differences between ASD and TD participants were present in both male and female groups if compared separately. Both analyses confirmed the differences revealed by the main PLS analysis.

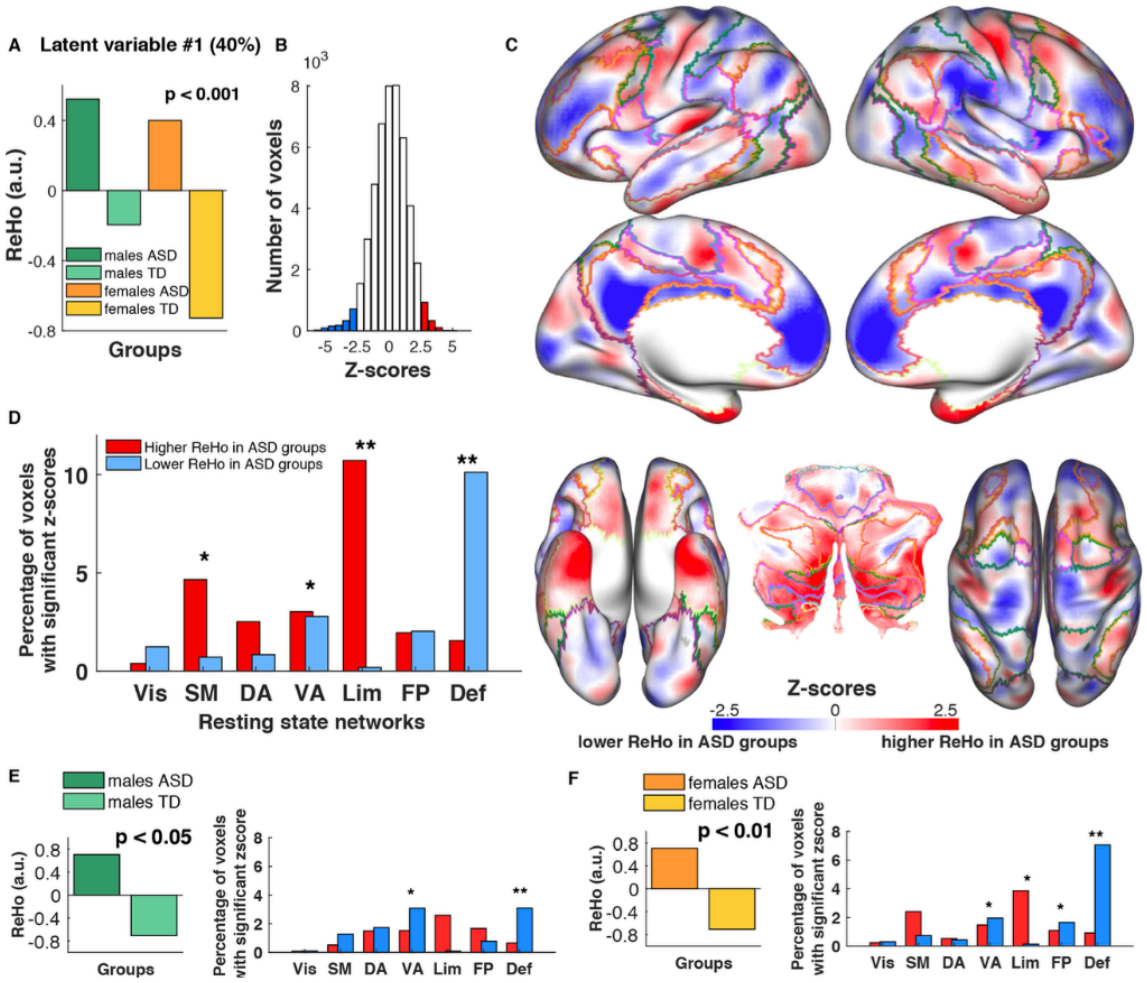

Figure 3.2 Group differences in ReHo between ASD and TD participants

A – significant PLS contrast ($p < 0.001$); B – distribution of z-scores across ROIs where red and blue tails represent number of voxels with z-score > 2.5 or <-2.5 respectively; C – spatial distribution of z-scores where areas in blue represent regions with lower ReHo in ASD groups and areas in red map the regions with higher ReHo in ASD groups compared with TD participants; D – the graph represents the percentage of voxels with z-score >2.5 (red) or <-2.5 (blue) of each of the seven resting state networks; E – group contrast between ASD and TD males and a bar graph of the significant voxels within resting state networks; F – group contrast between ASD and TD females and a bar graph of the significant voxels within resting state networks.

* - significant group differences that were revealed by a series of PLS analyses run for each resting state network separately; ** - significant differences after Bonferroni correction.

3.4.2. Differences in ReHo between male and female groups

The second data-driven LV ($p < 0.05$) expressed 33% of the variance in the data. The corresponding group contrast (Figure 3.3, A) represented group differences in ReHo

between males and females. According to the z-score distribution, there were slightly more significant voxels with negative z-score than with positive z-scores. Given the group contrast, this indicates that that, on average, female groups had lower ReHo compared to the male groups. Decreased ReHo in the female groups was located bilaterally in the inferior parietal cortex, anterior insular cortex, temporooccipital and supramarginal areas (Figure 3.3, C). On the RSN level, the highest percentage of voxels with z-score < -2.5 were observed in the somatomotor, default mode networks (Figure 3.3, D). The areas with higher ReHo in female groups included the parieto-occipital sulcus, inferior temporal cortex bilaterally and anterior cerebellum. The highest percentage of significant voxels with positive z-scores were registered in the ventral attention network. To determine if sex differences revealed by the second LV we ran two separate PLS analyses: (i) males ASD vs females ASD; and (ii) males TD vs females TD. Both PLS analyses did not reveal statistically significant differences, for TD groups where was a statistical trend with the p-value was approaching significance. Speculatively, this might suggest that sex differences in ReHo observed in the second LV were driven mostly by differences between the TD groups.

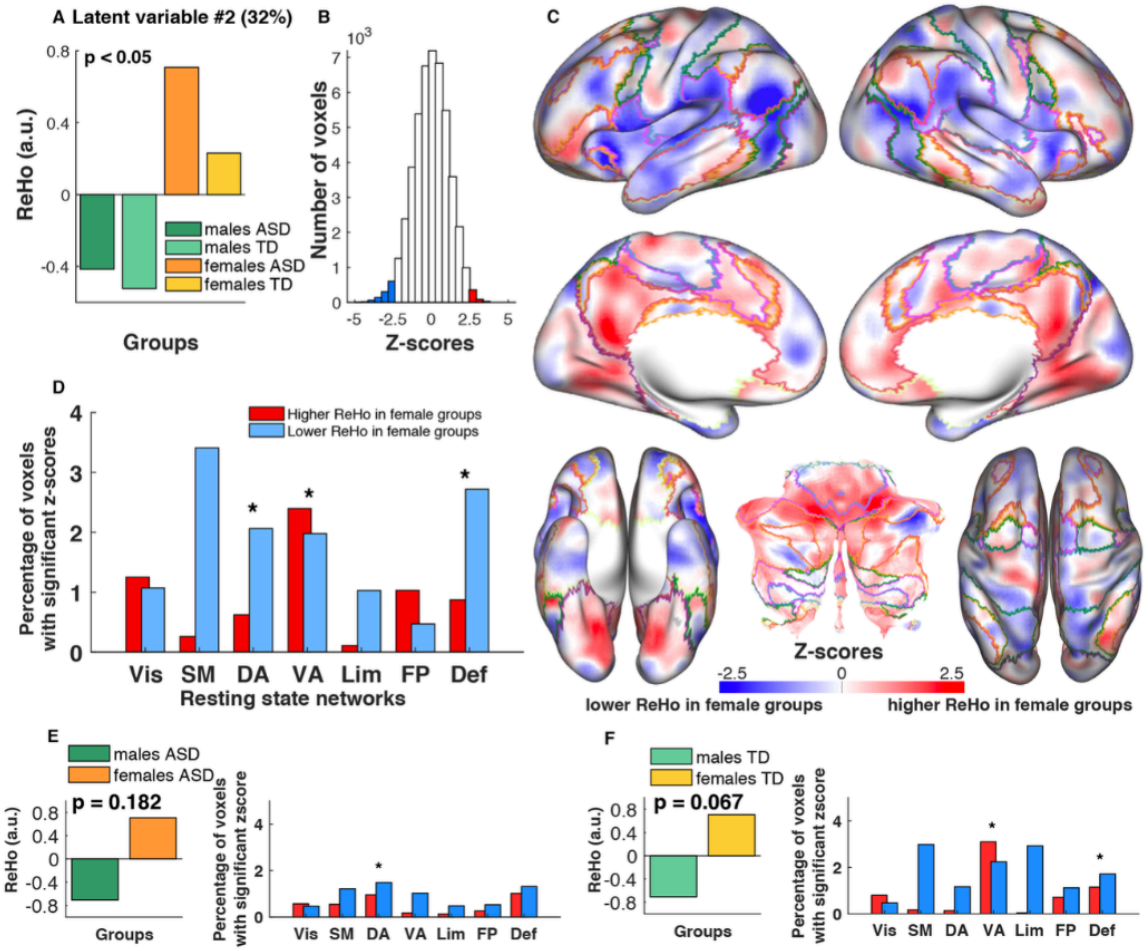

Figure 3.3 Sex differences in ReHo between groups

A – significant PLS contrast associated with second LV ($p < 0.05$); B – distribution of z-scores across ROIs where red and blue tails represent number of voxels with z-score > 2.5 or <-2.5 respectively; C – spatial distribution of z-scores where areas in blue represent regions with lower ReHo in female groups and areas in red map the regions with higher ReHo in female groups compared to male participants; D – graph represents the percentage of voxels with z-score >2.5 (red) or <-2.5 (blue) in each of the seven resting state networks; E – group contrast between males and females with ASD and the bar graph of the percentage of significant voxels across resting state networks; F – group contrast between TD males and females and the representation of group differences across resting state networks.

* - significant group differences revealed by series of PLS analyses run for each resting state network separately (but none of them survived Bonferroni correction).

3.4.3. ReHo association with ASD symptomatology

We ran two behavioral PLS analyses, separately for males and females with ASD, to test if there was an association between three subscales of the ADOS and overall ReHo across all voxels. We found no significant correlation between ADOS and ReHo on the global level (ReHo in all voxels) for both groups. Subsequent analyses on the network

level, however, revealed significant correlations between the ADOS scores in three subscales and ReHo within the limbic network in females (Figure 3.4, A). The distribution of the z-scores across voxels was positively skewed, indicating the presence of overall positive correlations between ADOS scores and ReHo within the limbic network (Figure 3.4, A).

SRS scores were also available for some subjects in each group (see Table 3.1). To investigate their association with ReHo, we ran four separate PLS analyses across all voxels (one for each group). No group expressed significant correlations between the SRS scores and global ReHo. The analyses at the network level, though, revealed a few significant correlations. Females with ASD demonstrated significant correlations between ReHo in the ventral attention network and all five subscales of the SRS (Figure 3.4, B). From the z-score distribution across voxels we can see that it was mostly positive correlations. For males with ASD, ReHo in the somatomotor network was positively correlated with the SRS cognition score (Figure 3.4, C). Among TD groups, only females had significant associations between SRS scores and ReHo. Similarly to males with ASD, TD females showed significant correlations between SRS subscales and ReHo within the somatomotor network (Figure 3.4, F). However, whereas in males with ASD ReHo was significantly linked to SRS cognition sub-scale only, the association for TD females involved all five sub-scales of the SRS. Another significant positive association between ReHo and the SRS in TD females was registered in the limbic network (Figure 3.4, D). This association shares some similarity with correlations we found for females with ASD, where higher ReHo within the limbic network was also associated with ASD severity (but measured by ADOS scores). ReHo in the ventral attention network in typical females was positively correlated with all SRS sub-scores, similarly to females with ASD (Figure 3.4, E). We ran seven PLS analyses for each group (one for each RSN) to investigate the association between ReHo and ASD symptoms severity. After correcting for multiple comparisons with a Bonferroni method, only the association between ReHo within the limbic network and SRS sub-scales in TD females survived the correction.

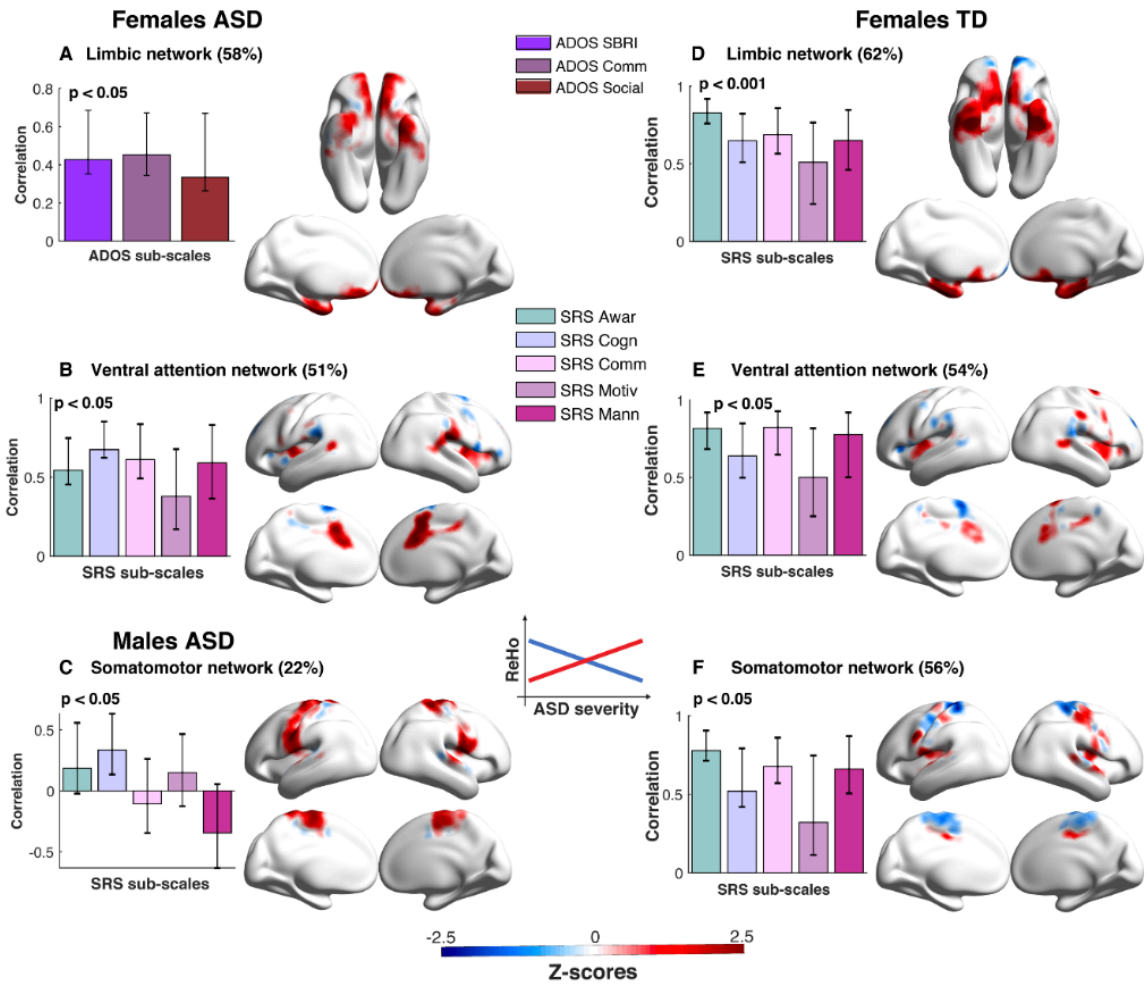

Figure 3.4 Association between ReHo and severity of ASD symptoms
A – Overall correlations between ADOS sub-scales and ReHo within limbic network in ASD females. Overall correlation between SRS sub-scales and ReHo within B – ventral attention network in females with ASD; C – somatomotor network in males with ASD; D – limbic network, E – ventral attention network, F – somatomotor network in TD females. ADOS SBRI – stereotyped behaviors and restricted interests, ADOS Comm – communication, ADOS Social – reciprocal social interaction, SRS Awar – awareness, SRS Cogn – cognition, SRS Comm – communication, SRS Motiv – motivation, SRS Mann – mannerism.

3.4.4. Association between ReHo and age

We performed four behavioral PLS analyses to investigate an association between ReHo across all voxels and age of participants, one for each group. We found significant correlations between age and global ReHo for all four groups (Figure 3.5). In general, there were both positive and negative correlations between age and ReHo for all groups. In the visual network, ReHo was positively correlated with age in all four groups. Similarly,

positive correlations were seen in the ventral attention network but only in ASD groups. ReHo within other resting state networks expressed negative correlations with age.

We also checked for similarities in the spatial distribution of the correlations between age and ReHo among all groups by computing correlations between z-scores obtained from PLS analyses (Figure 3.5, E). The highest similarity was observed between TD groups (r = 0.58). Male groups also had a similar pattern of ReHo association with age (r = 0.51). Interestingly, ASD females were more distinct in the spatial pattern of age association with ReHo with TD groups (r = 0.25 with males TD and r = 0.27 with females TD) compared to ASD males. While all other groups had z-score distributions slightly skewed to the negative side, females with ASD had it more symmetric. Visual analysis indicates that correlations between ReHo and age in the cerebellum, medial occipital cortex were more positive compared to TD groups, while the parietal and central areas had more negative correlations with age. Such results suggested that investigating developmental trajectories could be considered a sensitive biomarker for sex-specific alterations in ASD. We thoroughly tested this hypothesis in the next section in more detail. Since our results indicate significant linear associations between age and ReHo in all four groups we decided to focus primarily on linear age effects. However, in the appendix B we also provide developmental trajectories analyses using quadratic and cubic functions to model the age-related changes in ReHo.

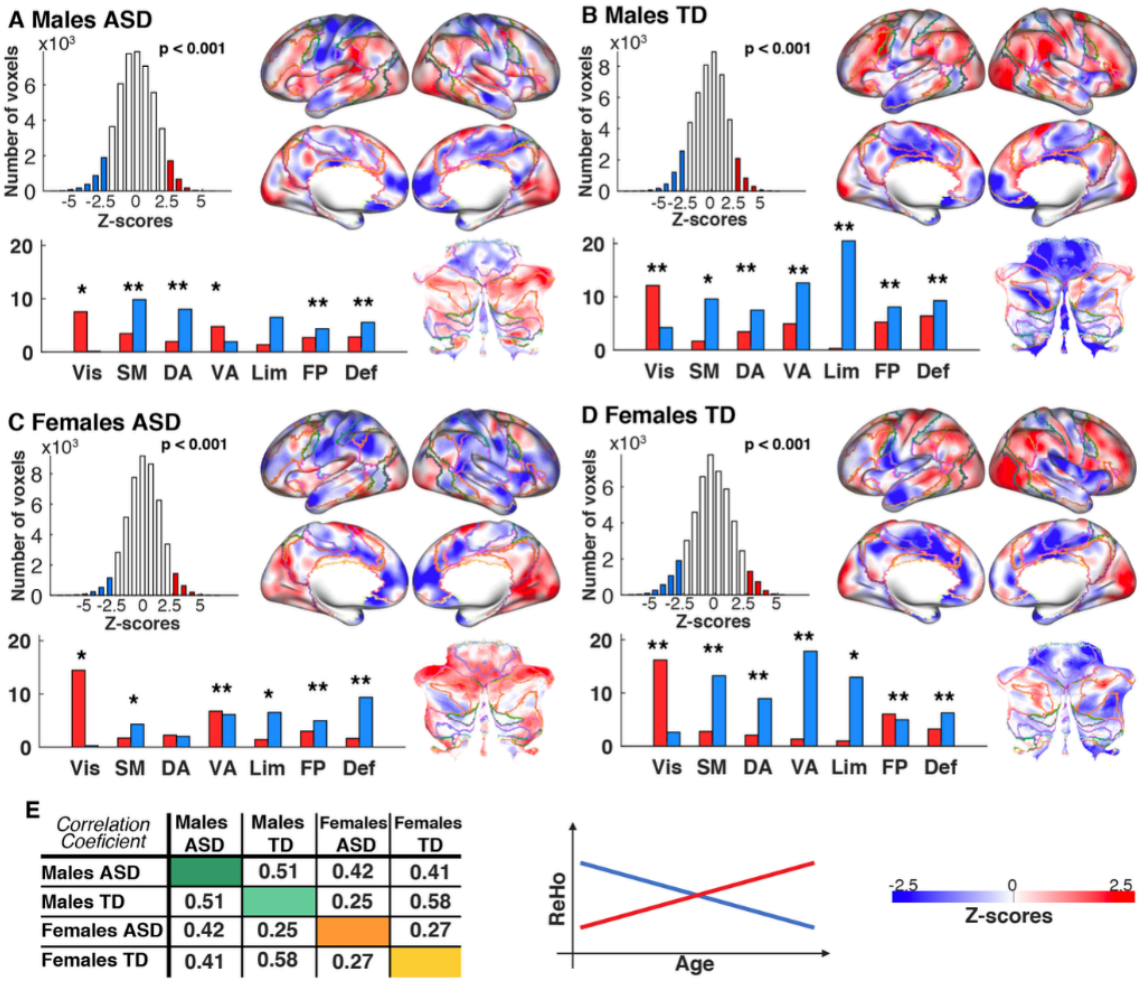

Figure 3.5 Association between ReHo and age, separately for each group

A - males ASD; B – males TD; C – females ASD; D – females TD. For each group there is z-score distribution histogram, the spatial distribution z-score on brain surface (in the red-colored areas ReHo increases with age and in the blue-colored areas ReHo decreases with age relative to the mean ReHo); E – table representing a spatial similarity between age-related changes in ReHo.

* - significant group differences revealed by a series of PLS analyses run for each resting state network separately; ** - significant differences after Bonferroni correction.

3.4.5. Group differences in ReHo developmental trajectories

We compared the rate of changes in ReHo, which was quantified as the slope coefficients estimated by fitting a linear model to sub-samples generated for each group. The PLS analysis revealed three data-driven significant LVs. The group contrast associated with the first LV explained the largest portion of data variance (41 %, Figure 3.6, first row). Based on dissimilarities in ReHo developmental trajectories, this contrast separated females with ASD from three other groups (and in particular, from TD females).

Differences in developmental trajectories were distributed across all RSNs with visual, limbic, ventral, and dorsal attention networks consisting of mostly positive z-scores and somatomotor, frontoparietal control, and default mode network consisting of negative z-scores. Note that in this analysis, we compare the slope coefficients and positive z-score in general means a bigger slope coefficient in one group compared to the other. Thus, a positive z-score can mean i) stronger positive correlation (stronger association with age) in one group compared to others; ii) correlation closer to zero (weaker association with age) in one group when other groups have negative correlations. Similarly, a negative z-score implies a smaller correlation coefficient indicating either i) stronger negative correlation in one group compared to others or ii) a correlation closer to zero in one group when other groups have positive correlations. For this group contrast, females with ASD demonstrated stronger positive correlations between ReHo and age in the visual network, weaker negative correlation in the ventral attention and limbic networks, as compared to other groups. At the same time, females with ASD expressed stronger negative correlation between ReHo and age within frontal-parietal control and default mode networks.

The second LV explained 32% of the variance in the data and identified developmental trajectories in ReHo that were distinct in males with ASD, relative to the other three groups. In comparison with other groups, males with ASD had stronger negative correlations between age and ReHo within the somatomotor and dorsal attention networks, together with weaker negative correlations in the default mode network. The last (third) statistically reliable LV (27% of variance explained) associated with group contrast (Figure 3.6) revealed differences between TD males and all other groups. ReHo within the ventral attention, dorsal attention, and somatomotor networks had weaker negative correlations with age in TD males, whereas in the visual system there were weaker positive correlations with age as compared to other groups.

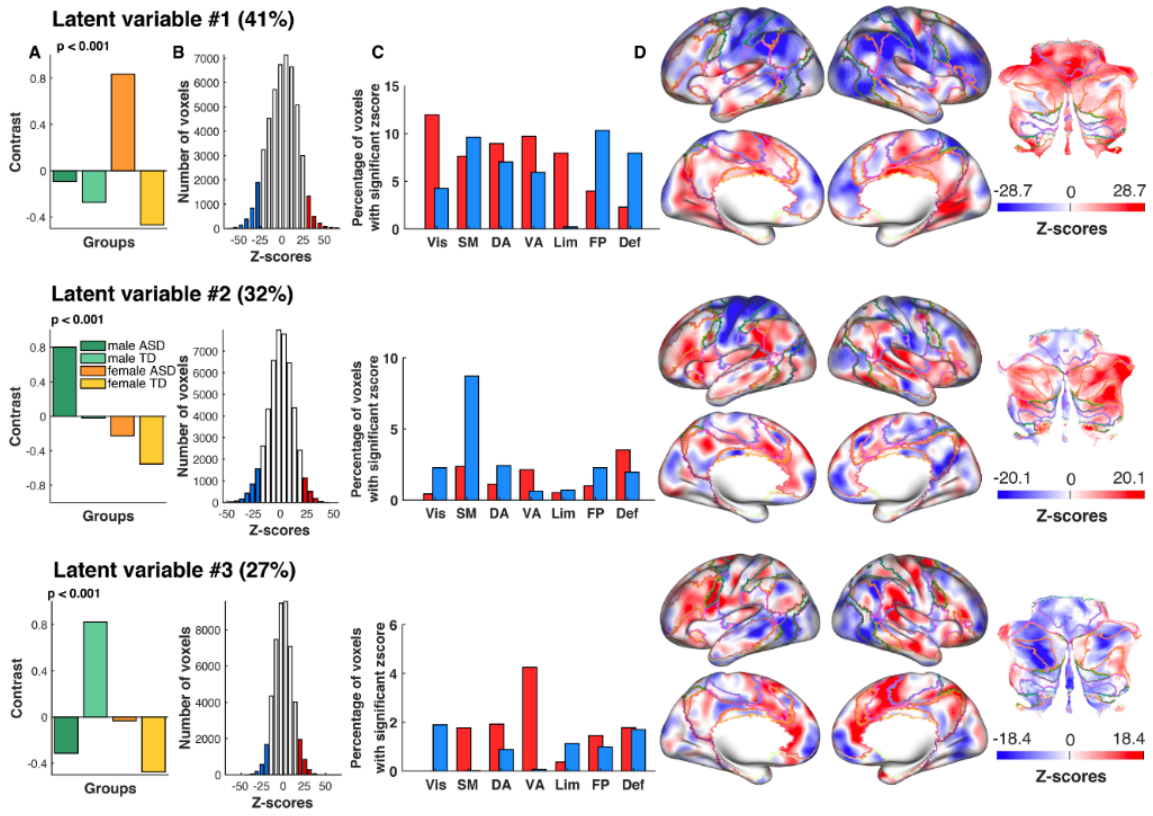

Figure 3.6 Group differences in ReHo developmental trajectories
the first row is LV #1 representing 42% of variance in the data that separates females with ASD from the other three groups (in particular from TD females); the second row represents LV #2 that expresses 32% of variance in the data and revealed different developmental trajectories in ASD males as compared to other groups; the third row illustrates LV #3 that represents 27% of variance in the data and shows differences in developmental trajectories between TD males and other three groups. Column A – group contrasts showing group differences; column B – distribution of Z-scores with tails representing 5% of voxels with highest (in red) and lowest (in blue) z-scores; column C – a bar graph illustrating percentage of voxels that belong to the 5% tails for each RSN; D – spatial distribution of z-scores associated with each group contrast on the brain surface.

3.5. Discussion

In this study, we used a large publicly available dataset to test the hypothesis of sex-specific alterations in local connectivity and its developmental trajectories in participants with ASD. We found that both males and females with ASD had substantial alterations in ReHo within the somatomotor, limbic, and default mode networks as compared to participants without ASD. Moreover, increased ReHo in the somatomotor network in males with ASD and the limbic network in females with ASD positively correlated with ASD severity. We also found that ReHo was significantly associated with

age in all groups but followed distinct developmental trajectories across groups. In particular, females with ASD had the most divergent age-related changes in ReHo compared to the other groups. Importantly, it is the first study focusing on ReHo that investigates females with ASD as a separate group and our results indicate that alterations in ReHo, their association with ASD symptomatology, and distinct developmental changes of ReHo are not only present in females to the same extent as in males but are even more robust.

3.5.1. The overall findings in the context of existing hypotheses explaining male bias in ASD

Existing hypotheses attempting to explain male preponderance in ASD could provide some help in interpreting our findings. The Female Protective Effect (FPE) hypothesis (Lai, Lerch, et al. 2017) suggests that there are innate protective features of the female sex (particular genetic and/or hormonal features) that protect females from developing ASD. This theory stipulates that the etiological load has to be greater for females to manifest the same ASD symptoms as compared to males. This hypothesis has been supported by multiple genetic studies (Robinson et al. 2013; Jacquemont et al. 2014). In the present study, both females and males had demonstrated a sufficient level of behavioural impairments to receive an ASD diagnosis. Speculatively, the level of alterations in ReHo and its developmental trajectories could be higher in females due to the confirmed presence of ASD symptomatology despite protective features of the female brain as suggested by the FPE hypothesis. It also implies that more pronounced disruptions in females with ASD may be triggered by greater etiological load (e.g. genetic risk factors, environmental perturbations) faced by them compared to males. Alternatively, the Extreme Male Brain (EMB) theory considers many autism traits as an extreme profile of 'typical male' cognitive style (Baron-Cohen 2002). It has been supported by multiple behavioural, endocrinological and genetic studies (Baron-Cohen et al. 2005; Auyeung et al. 2009; Chakrabarti et al. 2009) as well as some neuroimaging findings (Ypma et al. 2016; Kozhemiako, Vakorin, et al. 2019). Based on this theory, there should be more similarities between participants with ASD and typical males as compared to typical females. Our findings of more robust differences in ReHo and its developmental trajectories between female groups are consistent with this hypothesis. However, there was no indication of similarity between ASD-related alterations and typical male local connectivity pattern in our results to support EMB theory entirely. Another possible

explanation for the more pronounced alterations in females could arise from imperfections in diagnostic tools and procedures which more often fail to identify ASD symptomatology in females. There is evidence that females are better in camouflaging the social and communication deficits (Lai, Lombardo, et al. 2017). Consistent with this view, a recent study demonstrated that to be diagnosed with ASD girls need to exhibit more severe behavioural impairments compared to boys (Dworzynski et al. 2012). Accordingly, more robust disruptions in connectivity in females with ASD could potentially reflect the difference in the symptomatology between male and female participants due to the male bias in diagnostic tools. However, our results showing a high correlation between ReHo and symptomatology in females are not consistent with this interpretation. Overall, while being partially compatible with multiple hypotheses aiming to explain the disproportionate male-to-female ratio in ASD, our overall results support the FPE hypothesis as the most viable interpretation.

3.5.2. Group differences in local connectivity

Our comparison of ReHo across groups revealed both increased and decreased ReHo in participants with ASD. These results are topologically congruent with previous studies showing differences between participants with and without ASD (although mostly from male-composed samples) with similar age ranges. Most of these studies reported decreased ReHo in middle and posterior cingulate, precuneus and medial prefrontal cortex, middle frontal gyrus (Shukla et al. 2010; Maximo et al. 2013; Di Martino et al. 2014; Dajani and Uddin 2016). Our results are similar to other works which showed increased ReHo in temporal pole, amygdala and posterior cerebellum (Paakki et al. 2010; Shukla et al. 2010). Such group differences between ASD and TD participants are present even when considering males and females separately.

In our analysis, we also consider changes in ReHo with respect to the RSN borders by computing the percentage of voxels with a significant z-score within a particular network. In this way, we assess the state of local connectivity within the nodes that belong to a particular RSN. Our analysis revealed that voxels within limbic and somatomotor networks had higher local connectivity in the ASD groups. This finding aligns with previous reports of somatosensory and limbic overactivation in participants with ASD in response to a tactile stimuli that was associated with sensory over-responsivity, a very common symptom of ASD that recently has being added to the diagnostic criteria specified in DSM-

5 (American Psychiatric Association 2013; Green et al. 2015). Speculatively, such overconnectivity at rest might predispose to the over-reactivity in the areas of somatomotor and limbic networks in response to sensory stimuli probably also interfering with large-scale network integration.

Our results also revealed that voxels belonging to default mode network were greatly involved in the group differences with lower ReHo in both ASD groups. Previously, the majority of connectivity studies were focusing on investigating RSN connectivity alterations based on long-range connections (Cerliani et al. 2015; Hull, Jacokes, et al. 2017; Nunes et al. 2018). There is mounting evidence reporting alterations in default mode network in ASD and the majority of these studies report underconnectivity in ASD (Weng et al. 2010; von dem Hagen et al. 2013; Padmanabhan et al. 2017). To our knowledge there was just one study focusing on sex differences and default mode network connectivity in ASD, which confirmed that underconnectivity in the default mode network is present in both males and females with ASD (Ypma et al. 2016). Such decrease in long-range connectivity and local connectivity within the default mode network suggests that the pathological changes in this network affects multiple scales of neuronal communication.

It has been well recognized that efficient information processing in the brain requires a balance between local and long-range connectivity (Bullmore and Sporns 2009). Although there is some evidence that the local connectivity might affect whole-brain dynamics (Deco et al. 2014), the understanding of the association between local and long-range connectivity is far from complete. Speculatively, both local and long-range underconnectivity might be interlinked, e.g. the local area being not able to activate and deactivate synchronously as a result of weaker input from other nodes of the network due to long-range underconnectivity. Or the other way around, the long-range communication being weakened by the inability to generate strong output signals due to the lack of local synchronization. Alternatively, there is evidence from brain stimulation studies that local underconnectivity might coexist with long-range overconnectivity, as the result of a compensation effect (Davis et al. 2017). It is clear that more research is needed to unravel the link between local and distributed connectivity alterations and their causality. This would aid our interpretation of atypicalities observed in ASD brain and provide insights on neural origin of such atypicalities.

Another significant LV obtained from the four-group comparison appeared to show sex differences in ReHo with males (both ASD and TD) in general having higher ReHo than females with and without ASD. More specifically males tended to have higher ReHo in dorsal attention, default mode and somatomotor network whereas females had higher ReHo within ventral attention network. The latter finding is in line with a recent study on sex differences in ReHo showing higher ReHo in females within the lateral attention network. They also reported higher ReHo in visual network in males (Xu et al. 2015). Another study showed higher ReHo in females mostly in middle frontal gyrus and precuneus of the left hemisphere while males exhibited higher ReHo mostly in the right hemisphere including middle frontal and precentral gyrus. (Wang et al. 2012). There is an evidence that higher ReHo can be reflective of higher functional segregation and modularity (Jiang and Zuo 2016). Considering this, our results are in line with another study focused on normal sex difference using a large sample of typical males and females reported higher modularity in males compared to females (Ingalhalikar et al. 2014).

3.5.3. Associations between local connectivity and ASD severity

The association between ReHo and ASD symptomatology, although being non-significant on the whole brain scale, revealed significant links on the network level. Overall, association with ASD symptomatology was also more extensive in females with ASD compared to males. Specifically, females with ASD demonstrated positive associations between increased ReHo in the limbic network and severity of ASD symptoms measured by three ADOS scales (communication, reciprocal social interaction, stereotyped behaviours and restricted interests). Higher scores in all five SRS sub-scales were correlated with higher ReHo within the ventral attention network. In contrast, males with ASD had poorer ratings on cognition (indexed by the SRS) correlated positively with higher ReHo within the somatomotor network. In the TD groups, while no correlations were found for TD males and SRS scores, TD females showed significant associations between SRS scores and local connectivity within somatomotor, limbic, and ventral attention networks.

The fact that local connectivity within somatomotor and limbic networks was related to ASD symptomatology is of particular interest, since higher ReHo there was featured in the group differences between ASD and TD groups. Worth mentioning in this regard, that numerous behavioral studies report differences in behavioral phenotypes between males and females with ASD. It has been shown that females with ASD,

compared to males, tend to have more 'internalizing' emotion-related difficulties such as social anxiety and in general, studies on anxiety often report the alterations in various parts of the limbic system (Hartley and Sikora 2009; May et al. 2014; Brooks and Stein 2015). In contrast, males with ASD typically display more repetitive and stereotyped behaviours that some models link to alterations in the motor system (Van Wijngaarden-Cremers et al. 2014; Kim et al. 2016). Future studies are needed to directly investigate such potential links between local connectivity within somatomotor and limbic networks and behavior to aid to our understanding of neural correlates of different behavioural phenotypes in males and females with ASD.

3.5.4. Associations between local connectivity and age and differences in developmental trajectories between groups

In the final part of our study, we show that ReHo is significantly associated with age in males and females with ASD as well as in TD groups. However, the age-related changes of ReHo are distinct in these groups. In particular, we demonstrate altered developmental trajectories for ReHo in females with ASD which are different from other groups. These findings add to the growing body of evidence supporting the developmental approach to investigating of atypical brain function and structure in ASD (Vakorin et al. 2017; Henry et al. 2018; Kozhemiako, Vakorin, et al. 2019; Nunes et al. 2019). Based on the structural findings, deviation from the typical trajectory of brain development most likely occurs at the very early life stages. The early overgrowth hypothesis suggests that brain volume enlargement, although not present at birth, becomes detectable by the end of the first year of life (Mosconi et al. 2006). The rate of change in brain volume and surface area in infants reliably predicted if they would develop ASD in the future (Hazlett et al. 2017). Such promising findings based on structural data motivate investigations of developmental changes in brain function as well.

The results of the current study indicate that females with ASD had the most distinct developmental pattern of ReHo as evidenced by the contrast explaining more than 40% of the variance in the data. Those differences in age-related changes involved the majority of RSN revealing a complex pattern of stronger positive correlation in visual network, weaker negative correlation in limbic and ventral attention network and stronger negative correlation in fronto-parietal control and default mode network. In contrast, males with ASD were different from other groups mostly due to stronger negative correlations in

somatomotor network. Another recent study investigated age and sex effect in functional integration and segregation in individuals with ASD (Henry et al. 2018). They also found that age-related changes in modularity involving the somatomotor network were contributing the most to observed group differences between ASD and TD groups. In another study employing developmental approach with interhemispheric connectivity, results indicated that both males and females with ASD were following typical male developmental trajectories (Kozhemiako, Vakorin, et al. 2019). However, previous studies as well as the current study were estimating age-related changes using cross-sectional datasets with large age ranges. Despite this limitation, they suggest that developmental approach can provide an important insight into ASD primary and secondary atypicalities. Other developmental approaches that include longitudinal datasets with dense sampling should be created to facilitate investigation of ASD neurodevelopment.

3.5.5. Limitations of the study

Overall, the findings of this study for the first time demonstrate that alterations in local connectivity and its developmental trajectories previously shown in males with ASD are equally (or even more robustly) present in ASD females. Importantly, ReHo alterations were also related to the symptom severity in ASD with females showing stronger and more robust correlations with multiple sub-scales of the SRS and ADOS. The findings of age-related changes of ReHo provide a valuable insight into the differences between ASD and TD groups, proving to be sensitive to capture sex-specific alterations in developmental trajectories of local brain connectivity in ASD and TD groups. The evidence of females with ASD having the most distinct developmental trajectories compared to other groups complement our overall finding of more robust alterations in females with ASD, indicating that being a female matters when ASD-related alterations of local connectivity are assessed. Although our findings are also at least partially concordant with the EMB hypothesis, these new insights from local connectivity and its developmental trajectories is most consistent with the view that being female offers neuroprotective benefits in the context of ASD.

3.5.6. Conclusion

Overall, the findings of this study for the first time demonstrate that alterations in local connectivity and its developmental trajectories previously shown in males with ASD

are equally (or even more robustly) present in ASD females. We also demonstrate how alterations in local connectivity are expressed within borders of RSNs, which provides strong context for the interpretation of these alterations in the context of established long-range connectivity disruptions in participants with ASD. Importantly, ReHo alterations were also related to the symptom severity in ASD with females showing stronger and more robust correlations with multiple sub-scales of the SRS and ADOS. The findings of age-related changes of ReHo provide a valuable insight into the differences between ASD and TD groups, proving to be sensitive to capture sex-specific alterations in developmental trajectories of local brain connectivity in ASD and TD groups.

Chapter 4.

Sex differences in brain connectivity and male vulnerability in very preterm children

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4.1. Abstract

Evidence indicates better cognitive and behavioural outcomes for females born very preterm (≤ 32 weeks gestation) compared to males, but the neurophysiology underlying this apparent resiliency of the female brain remains poorly understood. Here we test the hypothesis that very preterm males express more pronounced connectivity alterations as a reflection of higher male vulnerability. Resting state MEG recordings, neonatal and psychometric data were collected from 100 children at age 8 years: very preterm boys (n=27), very preterm girls (n=34), full-term boys (n=15) and full-term girls (n=24). Neuromagnetic source dynamics were reconstructed from 76 cortical brain regions. Functional connectivity was estimated using interregional phase-synchronization. We performed a series of multivariate analyses to test for differences across groups as well as to explore relationships between deviations in functional connectivity and psychometric scores and neonatal factors for very preterm children. Very preterm boys displayed significantly higher ($p<0.001$) absolute deviation from average connectivity of same-sex full-term group, compared to very preterm girls vs full-term girls. In the connectivity comparison between very preterm and full-term groups separately for boys and girls, significant group differences ($p<0.05$) were observed for boys, but not girls. Sex differences in connectivity ($p<0.01$) were observed in very preterm children but not in full-term groups. Our findings indicate that very preterm boys have greater alterations in resting neurophysiological network communication than girls. Such uneven brain communication disruption in very preterm boys and girls suggests that stronger connectivity alterations might contribute to male vulnerability in long-term behavioural and cognitive outcome.

4.2. Introduction

Preterm birth (≤ 37 weeks gestation) is the greatest cause of neonatal mortality and morbidity and, in case of survival, leads to long-term negative health consequences (Lawn et al. 2005; Moster et al. 2008). Mounting evidence suggests strong male disadvantage in the preterm population including increased preterm birth incidence, lower survival rates and worse long-term outcomes (Whitfield et al. 1997; Hindmarsh et al. 2000; Ingemarsson 2003; Hintz et al. 2006b; Rose et al. 2009; Peacock et al. 2012; Månsson et al. 2015; Schindler et al. 2017). Even in the absence of major pathologies and developmental impairments, preterm boys tend to experience more behavioral and cognitive difficulties, in particular: executive control (Urben et al. 2017a); language and speech problems (Wolke et al. 2008); language and cognitive ability (Wood et al. 2005; Skiöld et al. 2014; Young, Morgan, Powell, et al. 2016).

A number of neuroimaging studies aimed to clarify the underlying mechanism of male disadvantage, investigating sex differences in brain structure in the preterm population. The most consistent finding was a larger intracranial volume in preterm boys compared to girls (Vasileiadis et al. 2009; Liu et al. 2011; Skiöld et al. 2014; Kersbergen et al. 2016; Ball et al. 2017; Urben et al. 2017b; Benavides et al. 2018; Thompson et al. 2018). However, bigger brain volume in boys is also observed in the full-term population and was not shown to be related to preterm birth or long-term outcome. It has also been shown that preterm girls have more cortical folding despite smaller brain volume, suggesting more “compact” brain development compared to preterm boys (Vasileiadis et al. 2009). The proportional cerebral white matter volume has been shown to be larger in preterm boys, whereas proportional gray matter volume was larger in preterm girls (Benavides et al. 2018). Diffusion Tensor Imaging (DTI) studies also reported sex differences in the preterm population, which includes lower regional and mean fractional anisotropy (FA) and higher medium diffusivity in boys indicating less organized white matter microstructure (Constable et al. 2008; Liu et al. 2011; Thompson et al. 2018). Similarly, other studies have reported male sex to be associated with reduced corpus callosum microstructural growth trajectory during first 6 month of life as well as being a risk factor for diffuse white matter injury (Barnett et al. 2017; Teli et al. 2018). White matter features have also been shown to have different associations with long-term outcome in

boys and girls (van Kooij et al. 2011). Importantly, mean FA was shown to increase in preterm girls but not in boys in response to erythropoietin treatment indicating sex-specific medical therapy effectiveness in preterm population (Phillips et al. 2017). Such evidence of white matter alterations suggest that exploration of brain connectivity is a promising direction towards understanding of unequal consequences of preterm birth in males and females.

In support of the view that neurophysiological connectivity may be associated with developmental outcomes in very preterm children, we recently found that extremely preterm (≤ 28 weeks gestation) and very preterm children (28-32 weeks gestation) had pronounced alterations in connectivity which were associated with both adverse neonatal experience and poorer behavioral and cognitive performance at school age (Kozhemiako, Nunes, et al. 2019). These findings, combined with the long-established evidence of males being more affected by preterm birth that leads to poorer long-term outcome, led us to hypothesize that preterm males are characterized by more pronounced interregional connectivity alterations than preterm females. Additionally, we predicted that such larger deviations from the full-term typical connectivity would be associated with adverse neonatal experience and poorer long-term outcome in both preterm males and females. We also investigated sex differences in connectivity in full-term individuals and how typical sex differences are affected by very preterm birth.

4.3. Methods

4.3.1. Participants

Resting state MEG data were recorded from 100 children at age of eight. Participants were divided into four groups: preterm boys (24-32 weeks GA, n = 27), preterm girls (24-32 weeks GA, n = 34), full-term boys (40 weeks GA, n = 15) and full-term girls (40 weeks GA, n = 24). Children with major brain injury (periventricular leukomalacia or grade III-IV intraventricular hemorrhage on neonatal cranial ultrasound) or sensory or cognitive impairments were excluded. Clinical, demographic and psychometric data are presented in Table 4.1 Characteristics of the participants. The recruitment of the

participants was conducted within a prospective longitudinal study investigating effects of neonatal pain-related stress on neurodevelopment of very preterm children, e.g. (Grunau et al. 2007, 2009). This study was approved by the Clinical Research Ethics board of the University of British Columbia and the Research Ethics Board of the Children's & Women's Health Centre of BC. Written informed consent was obtained for every participant and their parent(s).

Table 4.1 Characteristics of the participants

Characteristics	Preterm		Full-term	
	Boys	Girls	Boys	Girls
Number of subjects	27	34	15	24
Age, years	7.8 (0.39)	7.7 (0.39)	8.0 (0.91)	8.0 (1.11)
MRI scans	16	25	5	8
Head circumference at age 8 years	51.5 (2.38)	51.7 (1.79)	52.8 (1.64)	52.3 (1.96)
GA at birth, weeks	29.7 (2.29) ***	29.5 (2.45) ***	39.6 (1.21)	40.1 (0.68)
Birth Weight, g	1382.9 (501.46) ***	1286.9 (370.42) ***	3496.4 (732.20)	3415.8 (300.91)
Number of subjects small for GA	2	2	0	0
Number of Skin-Breaking Procedures	103.6 (82.94)	94.6 (73.40)	n/a	n/a
Morphine Dosage	0.9 (1.67)	0.9 (3.70)	n/a	n/a
Days on Mechanical Ventilation	8.7 (13.29)	10.18 (18.02)	n/a	n/a
Early illness severity (SNAP-II)	13.8 (10.08)	10.3 (10.71)	n/a	n/a
Verbal Comprehension Index (WISC-IV)	100.9 (18.33)	98.2 (11.26) **	110.1 (14.87)	107.9 (12.15)
Perceptual Reasoning Index (WISC-IV)	101.0 (18.79) *	100.7 (12.86) **	113.6 (14.71)	112.8 (12.69)
Working Memory Index (WISC-IV)	95.2 (11.92)	99.7 (12.18)	102.1 (12.16)	102.3 (10.92)
Processing Speed Index (WISC-IV)	92.0 (10.47)	96.9 (14.64) *	98.4 (14.46)	108.5 (16.01)
Full-scale IQ (WISC-IV)	97.0 (16.40) *	98.7 (12.38) **	108.7 (13.67)	110.6 (12.40)
Internalizing Behavior (CBCL)	51.4 (12.49)	50.6 (9.23)	51.9 (10.96)	48.4 (12.49)
Externalizing Behavior (CBCL)	49.0 (11.60)	46.1 (9.23)	48.6 (10.83)	46.3 (10.52)
Behavioral Regulation Index (BRIEF)	54.6 (13.07)	49.2 (10.58)	50.6 (8.84)	50.0 (12.27)
Metacognition Index (BRIEF)	56.5 (11.99)	52.4 (14.52)	51.6 (6.01)	48.0 (12.41)
Visual Motor Integration (BEERY)	93.6 (7.66) *	94.5 (10.04) **	100.7 (15.47)	103.4 (10.36)
Visual Perception (BEERY)	101.8 (15.45) *	102.2 (15.40) **	114.4 (16.33)	113.5 (14.99)
Motor Coordination (BEERY)	90.9 (9.61)	92.5 (10.18) *	95.2 (11.60)	99.2 (10.90)

* [p < 0.05], ** [p < 0.01], *** [p < 0.001] group difference (preterm - full-term the same sex). Note that the statistics are reported in the terms of group mean (standard deviation), unless the number of subjects or scans is reported.

GA – gestational age, WISC-IV – Wechsler Intelligence Scale for Children, CBCL – Child Behaviour Checklist, BRIEF – Behavior Rating Inventory of Executive Function; BEERY – Beery-Buktenica Developmental Test of Visual-Motor Integration – 5th Ed.

4.3.2. Data collection

Resting state MEG data were recorded for two minutes in a magnetically shielded room using a 151 channel MEG system (CTF systems; Coquitlam, Canada). During the recording participants were supine and were instructed to fixate on a smiling face at the center of a screen. A research assistant accompanied participants in the magnetically shielded room to ensure that the children were following the instructions. Three fiducial coils were placed at the nasion and the left and right preauricular locations to enable continuous head position tracking. Data were collected at the sampling rate of 1200 Hz and stored for future analysis. The shape of each participant's head surface was also digitized using a Polhemus FASTRACK digitizer.

T1-weighted volumetric MRI images (1.5 Tesla) were also collected, but only for 54 children (the information on MRI availability for each group is provided in the Table 1). Additionally, 19 children who participated in this study had MRI, but no MEG data. In total we had a pool of 73 T1-weighted MRI images. Each of those participants who did not have the T1 MRI, was best-matched with one of the T1 MRI images from this pool with the goal to create a head model (a detailed description is provided below in the Data Analysis subsection).

4.3.3. Psychometric assessment

Cognitive assessment was performed following MEG data collection by a psychometrician who was blind to the participant's group status, and a parent completed questionnaires in a separate room. The assessment targeted the specific domains which frequently are reported to be altered in very preterm children. Those comprised verbal and nonverbal intelligence, visual-motor abilities, internalizing and externalizing behavior, and executive functions (Bhutta et al. 2002; Grunau et al. 2004; Aarnoudse-Moens et al. 2009; Loe et al. 2011; Ranger et al. 2014). Psychometric tests included the Wechsler Intelligence Scale for Children (WISC-IV) (Wechsler 2003) and Beery-Buktenica Developmental Test of Visual-Motor Integration – 5th Ed (Beery et al. 2004), and questionnaires included the

Child Behavior Checklist (Achenbach and Rescorla 2001), the Behavior Rating Inventory of Executive Function (BRIEF) (Gioia et al. 2000).

4.3.4. Neonatal data collection

During neonatal intensive care of the very preterm participants, daily chart reviews were conducted by an experienced research nurse to collect clinical information (for details see Grunau et al., 2009). In the present study we used GA, number of skin-breaking procedures, cumulative morphine dose adjusted for daily weight, days on mechanical ventilation and early illness severity (SNAP-II).

4.3.5. MEG analysis

Dipolar source solutions were calculated for three fiducial coils 30 times/s resulting in a continuous record of head position in the dewar for all participants individually. MEG data were down-sampled to 600 Hz. 60 Hz line noise was eliminated by applying a 2Hz-wide notch filter. For each participant, 15 segments of data were selected such that the deviations of the position of any fiducial coil from its median position were within 5 mm for any direction. The segments were 4 s long and did not overlap. Due to excessive movement in 4 subjects only 11-13 segments were available (1 preterm girl, 2 preterm boys, 1 full-term girl), which were still included in the analysis.

The following preprocessing steps were used to estimate comprised a reconstruction of neuromagnetic source activity using a beamformer. A head model was created for each subject using a single shell method as implemented in FieldTrip toolbox (Oostenveld et al. 2011). For those participants who did not have a T1 MRI image recorded, the pool of 73 child MRIs was searched for the best matched MRI for participant, as implemented in our previous study (Kozhemiako, Nunes, et al. 2019). Specifically, to choose the best match we calculated the mean distance between each Polhemus point and the closest point on the skull surface which was derived from segmented MRI image using Fieldtrip toolbox. Subsequently, the MRI scans with the smallest agregated distance between skull surface points and Polhemus points were selected for each participant. To ensure that the average distance was not different between our groups, we performed six two-sampled t-tests performed on a pair-wise basis. The results are reported in the

Results section. MEG and MRI were then co-registered using fiducial and head shape information.

To estimate neuromagnetic activity originating from the cerebral cortex all 76 cortical regions of interests (ROIs) were selected from the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al. 2002). Each brain region was represented by one seed computed as the center of mass, under an extra condition that the center is within the given region. MEG data were band-pass filtered from 1 to 150 Hz to remove low-frequency drifts and ultra-high frequencies. Then, for each pair of brain regions, we applied a Multiple Constrained Minimum Variance (MCMV) beamformer to estimate neuromagnetic activity at two selected locations (Moiseev et al. 2011). The main advantage of such approach is that it computes a unique inverse solution for each source pair nulling the contribution of one source onto another and alleviating a signal leakage (Moiseev et al. 2011).

4.3.6. Connectivity and statistical analysis

Functional connectivity is often quantified in terms of coordinated phases of neural dynamics reconstructed at spatially distinctive locations (Vakorin and Doesburg 2016). In our case, for each seed location, a complex wavelet decomposition was applied for each segment to estimate phase dynamics for each epoch at 50 frequency points equally spaced on a logarithmic scale from 4 to 50 Hz (Flandrin et al. 1996). More specifically, to estimate MEG functional connectivity, we calculated the Phase Locking Value (PLV) (Lachaux et al. 1999), a measure which quantifies stability of phase differences across time, for each pair of reconstructed neuromagnetic dynamics at each frequency point. PLV values were subsequently averaged across epochs for each source pair.

Partial Least Square (PLS) analysis was applied to test the significance of group differences in inter-regional connectivity and its association with neonatal and long-term behavioral and cognitive outcome in our participants. PLS is a multivariate technique, based on the singular value decomposition to decompose the entire data matrix (all the subjects within the groups by all the features) into latent variables (LVs) which explain the variance in the data, similar to Principal Component Analysis (Lobaugh et al. 2001; McIntosh and Lobaugh 2004). Each LV has three components: (i) a vector, which can be interpreted as a group contrast; (ii) a scalar value, which is ultimately related to explained variance; and (iii) a vector of saliences, representing a contribution of each feature (a combination of frequency and source pairing) to the group contrast. Two types of PLS

analysis were employed in the present study: mean-centered PLS and behavioral PLS. Both versions of PLS analysis comprise a global test for significance of overall group contrast in connectivity (in mean-centered PLS) or correlations between connectivity and neonatal or psychometric data (in behavioral PLS). The global test is based on random permutations of subjects across groups, resulting in one p-value for a group contrast. Additionally, there are series of feature-specific (local) tests based on bootstrapping subjects within the groups, performed with the goal to estimate the contribution of individual MEG features (PLV for unique combinations of frequencies and source pairs, in our case) to the overall group contrast or correlations. The resulting measures from the bootstrap resampling can be interpreted as z-scores. The positive or negative z-scores of 2.5 or -2.5 approximately corresponds to the 95% confidence interval (McIntosh and Lobaugh 2004). In this study, the number of permutations and the number of bootstrap resamplings were equal to 5000.

In the Results section, each significant LV obtained from the mean-centered PLS indicates a contrast representing overall group differences, a corresponding p-value from the global test, and a set of z-scores, each expressing the robustness of contribution of a given source pair at a specific frequency to the overall group contrast. To assess the overall directionality of the group differences we compute the sample skewness (Sk) the sample median (Md) for each z-score distribution. For the behavioral PLS p-values and overall correlations between connectivity alterations and neonatal or psychometric scores are presented. Statistical differences in neonatal and psychometric scores were investigated by series of pair-wise two-tailed t-tests with subsequent correction for multiple comparisons using Bonferroni method.

4.3.7. Testing the hypothesis of male disadvantage

To test our main hypothesis that very preterm boys are characterized by more pronounced connectivity alteration from same-sex full-term group than girls, the magnitude of connectivity alterations in preterm boys and girls were compared. For each subject from the preterm groups, the magnitude of connectivity alterations was defined for each unique combination of source pairing and frequency point as the absolute connectivity deviation from the same-sex full-term connectivity averaged across the full-term boys or girls. Accordingly, the average connectivity for each connection and frequency bin was computed for full-term boys and girls separately. Then for each

individual male from the preterm group and for each MEG feature, the mean connectivity values of the full-term male group were subtracted which resulted in connectivity deviation matrix for preterm boys. Similar procedures were conducted for preterm girls by subtracting the average connectivity values of the full-term female group. Subsequently, a mean-centered PLS analysis was performed to compare the absolute connectivity deviations from full-term group between preterm boys and girls. Additionally, two separate mean-centered PLS analyses were used to compare connectivity between preterm and full-term participants for boys and girls separately.

To test if the deviations from the full-term averaged connectivity in preterm groups are related to adverse neonatal experience and behavioral and cognitive abilities at school age, four behavioral PLS analyses were run. Thus, we investigated correlations, separately for preterm girls and boys, between the absolute deviations in functional connectivity, and two clusters of 'behavioral' data: (i) neonatal factors and (ii) behavioral and cognitive scores collected on the same day as MEG acquisition.

4.3.8. Analysis of sex differences in connectivity

To investigate sex differences in connectivity between the control groups, as well as how these differences are affected by very preterm birth, two mean-centered PLS analyses were performed comparing the phase locking values between boys and girls in the full-term and preterm groups, separately. Additionally, similarities in general sex differences pattern in full-term and very preterm children were assessed by comparing the female/male connectivity ratio between the preterm and the full-term group. The mean of connectivity across subjects was computed for each group resulting in one estimate of averaged connectivity for each MEG feature, specifically, for every connection at each frequency, per group. These values were used to calculate the female to male ratio by dividing the females' average connectivity estimate by averaged connectivity of males from the same group for each MEG feature. Two distributions of the ratio values (for full-term and preterm groups) were thus obtained for each unique combination of source paring and frequency: ratio value > 1 reflects higher connectivity in females, whereas ratio value < 1 implies higher connectivity in males. To quantify the size effects of differences between two distributions Cohen's d was calculated. To assess spatial and temporal variations in the effect size of differences in female/male connectivity ratio Cohen's d was

computed across source pairings within lobes (frontal, parietal, temporal, occipital and limbic) within the canonical bands (theta [4-8 Hz], alpha [8-13 Hz], beta [13-35 Hz] and gamma [35-50 Hz]), resulting in 16 distributions, in total, separately for preterm and full-term cohorts. To check the significance of female/male ratio 20 random subsamples of 10 participants for all four groups were generated. Then, for each subsample female/male connectivity ratio for preterm and full-term groups were computed separately, resulting in two matrices (subsamples x connections x frequency bins). Mean-centered PLS analysis was performed comparing female/male connectivity ratio between the full-term and preterm groups. It was performed similar to how the group differences in connectivity (regional synchrony) were explored, with two distinctions: (i) instead of phase-locking value (PLV), our variable of interest was the female/male ratio in PLV; and (ii) instead of subjects, our observations were sub-samples.

4.4. Results

4.4.1. Demographic, neonatal and behavioural characteristics of the cohort

The results of statistical comparison between groups in clinical scores collected during neonatal period and psychometric scores assessed at school age are provided in the Table 4.1. The only significant differences in neonatal characteristics which survived after Bonferroni correction were lower GA and weight at birth for preterm boys vs full-term boys, and preterm girls vs full-term girls.

4.4.2. Best-matched MRI selection

On average, the distance between the Polhemus digitization and reconstructed MRI-based head surface was slightly smaller in the MRI-matched participants compared to those with native MRI (2.1±0.28 mm and 1.9±0.34 mm, respectively). These differences, however, were not statistically significant across the four groups, according to the six two-sampled t-tests performed on a pair-wise basis.

4.4.3. Preterm boys demonstrate more pronounced connectivity alterations

Using mean-centered PLS analysis deviations in PLV from same-sex full-term groups in preterm boys and girls were compared. This analysis revealed significantly ($p < 0.001$) larger deviations in functional connectivity for the preterm boys with respect to the preterm girls, as illustrated on Figure 4.1, a, b. The overall distribution of all the z-scores for all connections and frequencies is shown in Figure 4.1, c wherein the largest negative (z-score < -2.5) and positive (z-scores > 2.5) tails are marked in blue and red colour, respectively. This distribution was skewed towards positive z-scores: the sample skewness Sk = -0.23, and the sample median Md = 0.39. In Figure 4.1, d represents the distribution of the tails, and c across frequencies. Specifically, for each frequency point, we calculated the number of connections associated with z-scores, which were either higher than 2.5 or lower than -2.5 (the most robust effects). Such measures represent the strength of positive (deviations are higher for preterm boys) and negative (deviations are higher for preterm girls) effects. On average, preterm boys had larger deviations in connectivity from full-term boys across all the frequencies with peaks around theta (4-8 Hz) and beta (13-25 Hz) bands, as compared to preterm girls' deviations from full-term girls' connectivity.

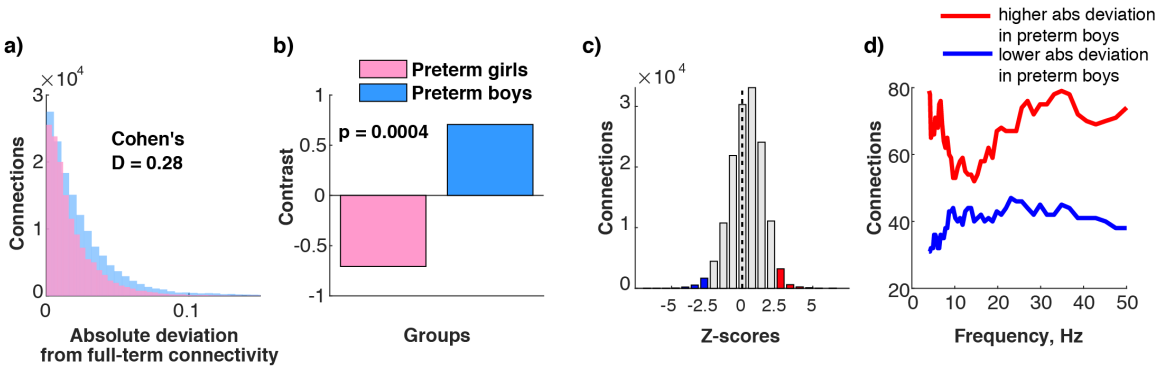

Figure 4.1 More pronounced connectivity alterations in preterm boys than girls

a) distribution of absolute connectivity deviation from full-term same-sex groups in preterm males and females; b) PLS contrast reflecting significant group difference in absolute connectivity deviation from full-term groups; c) distribution of z-scores; d) distribution of z-scores across different frequencies.

Another PLS analysis performed separately for boys and girls to investigate differences in connectivity between preterm and full-term groups, revealed significant differences only for the boys ($p = 0.03$; Figure 4.2, a). Comparisons of the preterm and

full-term girls in terms of their inter-regional connectivity did not reach statistical significance (p = 0.13; Figure 4.2, f). On average, preterm boys had significantly higher connectivity than full-term boys of the same sex as illustrated in the group contrast (Figure 4.2, a). Figure 4.2, b displays the corresponding overall distribution of z-scores, marking the two tails in red and blue in the same way as described above. The sample skewness of this distribution was close to zero (Sk = -0.003) but the median of 0.50 suggested that the distribution was skewed towards positive z-scores. Note that given the contrast in Figure 4.2, a, positive z-scores reflect increases in connectivity in the preterm boys compared to the full-term boys. Negative z-scores in this case are associated with lower connectivity in the preterm boys. To compare the strength of these two effects and to identify the temporal patterns for increases and decreases in connectivity, we calculated the number of connections with z-score > 2.5 and negative connections with z-score <-2.5 for each frequency point (Figure 4.2, c). On average, increased connectivity in the preterm boys dominated over decreased connectivity. The effects associated with increased connectivity in preterm boys were mostly located in lower frequencies including higher theta (6-8 Hz) and alpha (8-13 Hz). The spatial localizations of increased connectivity were scattered across the brain, mostly involving long-range connections between frontal areas and the rest of the brain (Figure 4.2, d, e).

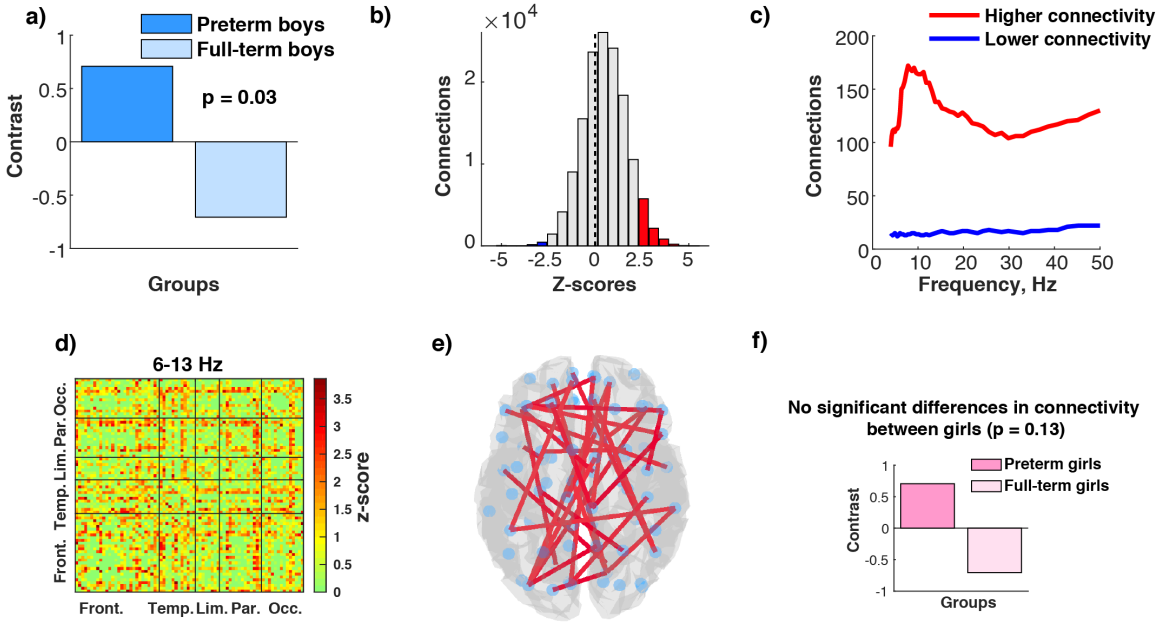

Figure 4.2 Connectivity differences between preterm and full-term groups
a) PLS contrast illustrating connectivity difference between preterm boys and full-term boys; b) distribution of z-scores associated with PLS contrast between preterm boys and full-term boys; c) distribution of z-scores across different frequencies that indicates on which

frequencies the connectivity differences between preterm and full-term boys were the most pronounced; d) connectivity matrix reflecting spatial distribution of connections with positive z-scores for 5.5-13 Hz; e) one percent connections with highest z-scores for the same frequency range in the brain space demonstrating higher connectivity in preterm boys compared to full-term boys; f) PLS contrast between preterm girls and full-term girls.

4.4.4. Association with neonatal factors and long term behavioural and cognitive outcomes

Behavioral PLS analysis of associations between deviations in connectivity from the full-term same sex group and adverse neonatal experience revealed significant overall associations ($p = 0.024$) for preterm girls (Figure 4.3, I). The z-score distribution was almost symmetrical, with skewness of - 0.03, and positive median of 0.10 (Figure 4.3, b). The distribution across frequencies illustrates that for most of the frequency bins there were more positive connections with z-score > 2.5 than negative connections with z-score < -2.5 indicating that in general higher deviation from average full-term connectivity in preterm girls was negatively correlated with gestational age and positively correlated with number of skin-breaking procedures, total dose of morphine, duration of mechanical ventilation and early illness severity (Figure 4.3, I a). In preterm boys we found similar pattern of association with neonatal factors, however, it was significant only at 90% confidence interval ($p = 0.098$) (Figure 4.3, II). The z-score distribution was negatively skewed ($Sk = -0.32$) with positive median ($Md = 0.20$) indicating more connections with positive z-score and suggesting that, similarly to girls, the higher absolute deviation from averaged full-term connectivity was positively associated with adverse neonatal experience and lower GA.

When investigating the relationships between the deviations in connectivity and the long-term behavioral and cognitive outcomes we did not find significant relationships for either of the preterm groups. However, in preterm boys we again registered a ‘trend’ towards an association ($p = 0.087$). Slightly negative skewness ($Sk = -0.13$) of z-score distribution and positive median ($Md = 0.4$) indicated that the majority of connections had positive z-scores. Thus, on average higher absolute deviation from full-term group in preterm boys was positively associated with Metacognition and Behavior Regulation Indices (BRIEF), Externalizing and Internalizing Behavior (CBCL) and had a negative relationship with visual-motor integration (BEERY) and the Perceptual Reasoning Index

(WISC-IV). Note that for BRIEF and CBCL scores, higher score indicates more difficulties in corresponding behavioral or cognitive domains.

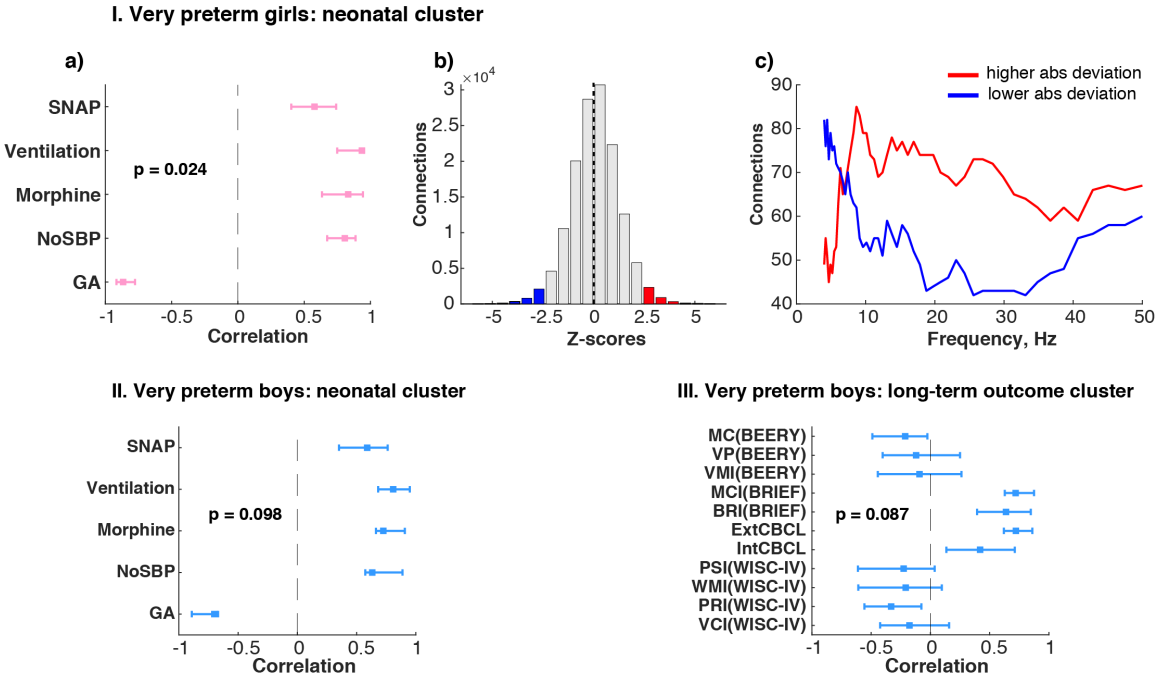

Figure 4.3 Associations between absolute connectivity deviation and adverse neonatal experience and long-term behavioral and cognitive outcome

The graphs I, II and III illustrate the results from three separate behavioral PLS analyses investigating correlations between the absolute deviation in connectivity from the same-sex full-term group I. in preterm girls and neonatal factors: I, a - PLS correlation coefficients are shown as a tick mark with whiskers representing bootstrap upper and lower boundary for correlation coefficient for each neonatal factor; I, b – overall z-score distribution; I, c – z-score distribution across frequencies. II. correlations between connectivity deviations in preterm boys and neonatal factors; III. correlations between connectivity deviations in preterm boys and behavioral and cognitive outcome at school-age. Neonatal cluster: GA – gestational age, NoSBP – number of skin-breaking procedures, Morphine – cumulative morphine dose with dosing adjusted for weight, Ventilation – days on mechanical ventilation and SNAP – early illness severity. Long-term outcome cluster: VC(WISC-IV) – verbal comprehension, PRI(WISC-IV) – perceptual reasoning index, WMI(WISC-IV) – working memory index, PSI(WISC-IV) – processing speed index from the Wechsler Intelligence Scale for Children; IntCBCL – internalizing index, ExtCBCL – internalizing index from the Child Behavior Checklist; BRI(BRIEF) – behavioral regulation index, MC(BRIEF) – metacognition index from the Behavior Rating Inventory of Executive Function; VMI(BEERY) – visual-motor integration, VP(BEERY) – visual perception from the Beery-Buktenica Developmental Test of Visual-Motor Integration – 5th Ed.

4.4.5. Sex differences in connectivity within full-term and preterm groups

To investigate whether sex differences in connectivity are altered in preterm children, we ran two mean-centered PLS analyses: preterm boys vs preterm girls and full-term boys vs full-term girls. While we did not find significant sex differences in the full-term group (Figure 4.4, f; p = 0.28), the comparison within preterm participants showed significant differences in inter-regional connectivity between boys and girls (p = 0.002, Figure 4.4, a). The distribution of z-scores with sample skewness of -0.21 and a median of 0.46 indicated that the majority of connections had positive z-scores (Figure 4.4, b). That indicates overall increased connectivity in boys compared to girls. In particular, such over-connectivity was present in the low theta (4-6 Hz), alpha (8-13 Hz) and low beta (13-20 Hz) bands (Figure 4.4, c). The spatial localization of the connectivity differences around 8-20 Hz were evident across the brain and involved mostly frontal and parietal connections (Figure 4.4, d, e).

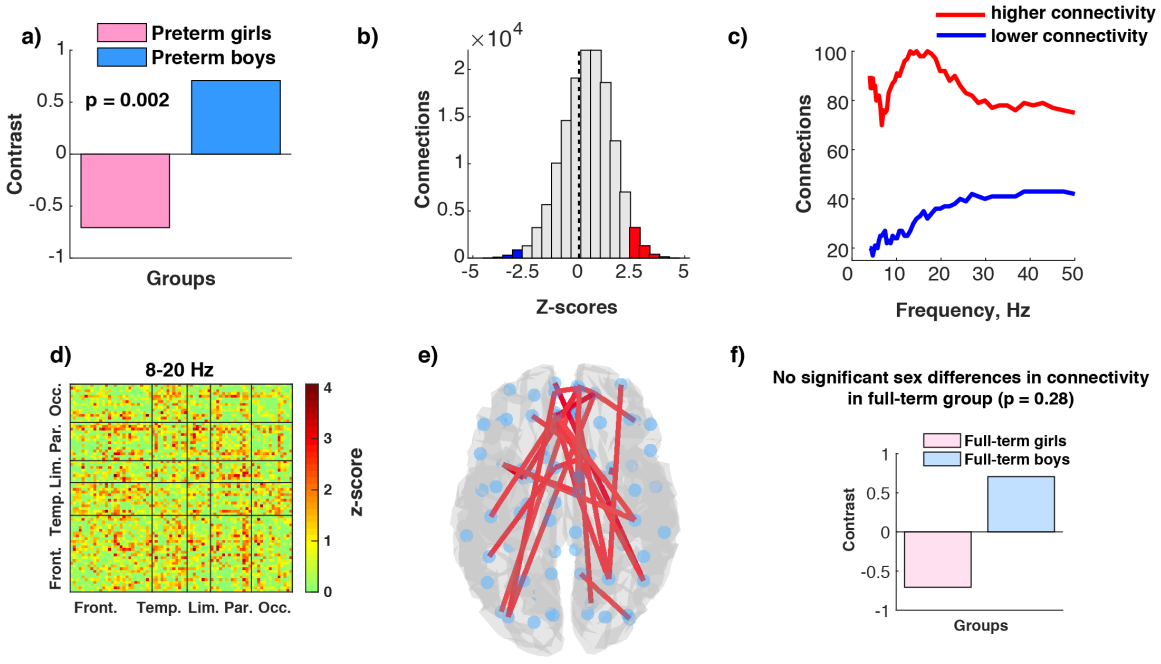

Figure 4.4 Sex differences in connectivity within full-term and preterm groups

a) PLS contrast demonstrating significant sex differences in connectivity in preterm groups; b) z-scores distribution associated with significant contrast between preterm boys and girls; c) z-scores distribution across frequency bins that illustrates which frequencies contributed the most to the revealed differences between preterm boys and girls; d) spatial distribution of positive z-scores in 8-20 Hz frequency range; e) connections with the highest z-score (top one

percent) in the brain space reflecting higher connectivity in preterm boys compared to preterm girls; f) PLS contrast between full-term girls and full-term boys.

To further investigate the revealed sex differences in preterm children, we compared the average female/male connectivity ratio in preterm and full-term children. Specifically, we were interested whether preterm children display exaggerated sex differences, which are present in full-term group to a much smaller extent. Thus, we compared the distributions of female/male connectivity ratios between preterm and full-term groups (Figure 4.5). The ratio values > 1 mean higher connectivity in girls and ratio values < 1 mean higher connectivity in boys. As illustrated in Figure 4.5, on average full-term girls tended to have higher connectivity than boys, whereas the preterm children showed the opposite trend of higher connectivity in boys compared to girls. The largest differences between female/male ratio distribution in preterm and full-term children as measured by Cohen's d, were observed in alpha frequency in parietal, limbic and temporal areas. Additionally, we run mean-centered PLS analysis using randomly generated subsamples and confirmed that there was a significant difference ($p < 0.001$) in female/male connectivity ratio between full-term and preterm groups.

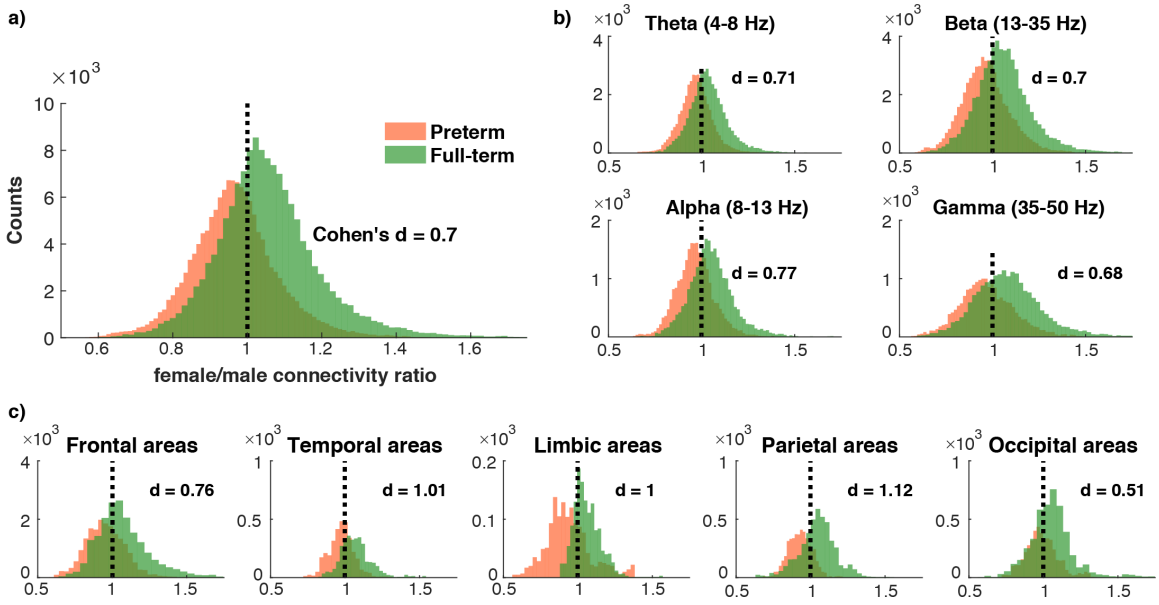

Figure 4.5 Female/male connectivity ratio distributions in preterm and full-term groups

a) averaged female/male connectivity ratio across all connections and frequencies; b) averaged female/male connectivity ratio across canonical frequency bands; c) averaged female/male connectivity ratio across brain areas.

4.5. Discussion

The present study investigated the neurophysiological bases of male vulnerability to adverse effects of very preterm birth resulting in poorer behavioral and cognitive outcomes later in life. We tested the hypothesis that very preterm boys would express more pronounced alterations in interregional brain connectivity compared to very preterm girls. We found greater absolute deviation from the full-term connectivity pattern in preterm boys compared to preterm girls and significant group differences in connectivity between preterm and full-term boys, but not girls. These findings indicate that connectivity alterations, as apparent in preterm boys, were not present or at least not to the same extent in preterm girls further supporting our hypothesis. Similarly, in volumetric MRI studies, differences between preterm and full-term boys have been previously reported at school age, with no differences among girls. For example, lower gray matter volumes in prefrontal cortex, basal ganglia, temporal lobe and lower white matter volumes in corpus callosum, corticospinal tract, prefrontal cortex have been reported in preterm boys compared to full-term (Kesler et al. 2008). However, those results were shown not to be correlated with neonatal variables or cognitive outcome. Another study reported reduced white matter volume in preterm boys but not in girls, however, lower white matter volume was significantly associated with birth weight in both boys and girls (Reiss et al. 2004). These findings are in line with white matter dysmaturation frequently reported in the preterm population (Fischi-Gomez et al. 2016; Karolis et al. 2016; Batalle et al. 2017). It was hypothesized that in the condition of white matter scarcity there is a tendency to prioritize rich-club connections (maintain communication between 'rich-club' nodes in distributed brain networks) over local connections that sometimes even leads to stronger rich-club architecture in preterms (Karolis et al. 2016). Thus, increased long-range connectivity involving frontal connection in low frequencies reported in this study between very preterm and full-term boys might reflect such prioritization strategy in very preterm brain. In contrast Kontis et al. (2009), in their study on adults born very preterm found that preterm females had increased mean diffusivity in corpus callosum compared to full-term females which also correlated with lower Performance IQ, but neither differences in white matter microstructure nor its association with cognitive outcome were found for males (Kontis et al. 2009). However, due to the lack of demographic details separately for very preterm males and females, it is unknown if there were differences in sample size, age,

GA, or cognitive outcome for males and females, which could possibly explain such unusual results.

Alternatively, the resting state overconnectivity might be an indicator of abnormal cortical maturation in very preterm boys. At school age, the brain is still in the process of tuning the connections to increase overall efficiency of information transduction and processing (Benasich and Ribary 2018). Such age-related brain reorganization is a complex dynamic process that involves axons growing and retracting with new synapses being formed while others being eliminated by pruning (Spear 2013). It has been shown in animal models that during preadolescence dendritic ramification in prefrontal cortex follows a sex-specific pattern which gets disrupted in males exposed to prenatal stress, whereas females remain unaffected (Markham et al. 2013). Nevertheless, current understanding of the premature birth long-term implications is far from complete and future investigations of the neurophysiological mechanisms of altered connectivity in children born very preterm will be of great importance.

Our analysis of associations between absolute deviation from full-term connectivity and adverse neonatal experience and long-term outcomes revealed a strong link between connectivity alterations and neonatal events in preterm girls, whereas no such correlation was observed with cognitive and behavioral performance at school age. Preterm boys, in turn, demonstrated a 'trend' towards significant association between connectivity deviation and both neonatal factors and long-term outcomes. These results suggest that connectivity alterations in girls might be only partially impacted by adverse neonatal events but do not relate to later behavioral and cognitive abilities whereas connectivity alteration in boys seem to be indicative of both, although only at the 90% confidence interval.

In the present study, we showed that significant sex differences in connectivity are present between preterm boys and girls, but not in full-term children. Interestingly, the spatial and temporal pattern of connectivity differences between preterm girls and preterm boys were similar to the differences between preterm boys and full-term boys. Potentially, this might indicate that pathological connectivity alterations in preterm boys were differentiating them from all other groups. In a study on ERP oddball task responses in children at age 6 years, there were opposite results of significant sex differences in the full-term group showing larger amplitude of P3 component in full-term boys, and no sex difference in the extremely preterm group (Lavoie et al. 1998). However, similar to our results, the discrepancy between sex differences in full-term but not in the extremely

preterm group was due to atypical preterm male brain response. Extremely preterm boys had diminished amplitude of P3, while extremely preterm girls displayed amplitudes typical to full-term girls. This motivated us to compare the overall pattern of full-term and preterm female/male connectivity ratio that distinctly showed that sex differences pattern in preterm children does not resemble the full-term. We confirmed that sex differences observed between preterm boys and preterm girls do not represent magnified full-term sex differences, but rather appear to reflect unequal alteration in brain connectivity in this vulnerable population.

4.5.1. Conclusion

This study provides the first evidence of more pronounced alterations of interregional neurophysiological connectivity in very preterm boys than in girls that potentially might reflect neurophysiological basis of male disadvantage in very preterm children. The investigation of sex differences in expression of preterm birth consequences on brain function is of great importance, to delineate underlying neurophysiological mechanisms which are responsible for the long term behavioral and cognitive alterations related to prematurity. Moreover, understanding sex-specific mechanisms is important for development of targeted therapeutic strategies.

4.5.2. Limitations

The data collection did not include EOG or ECG electrodes, limiting capacity for accurate monitoring of eye movements, given that children born very preterm tend to display high anxiety in novel situations and poor attention span, therefore might ask to end the session. The length of resting state recording was only three minutes, which was further reduced to 60 seconds after discarding the segments of data with head motion exceeding 5 mm in any direction.

For some participants, the structural MRI was not acquired so the best match of other T1 scans was used for the headmodel creation. Although for the MEG data analysis the best match approach was applied, the absence of native MRI scans for some children limited our investigation in terms of volumetric properties of gray matter and white matter and their association with higher connectivity in very preterm boys. Despite these limitations inherent to studying children born very preterm at age 7-8 years, our findings

provide novel information on neurophysiological function in very preterm boys that will inform future studies in this field.

Chapter 5.

Discussion

In this thesis, I investigated sex-specific alterations in brain connectivity in participants with ASD and very preterm children and how those alterations are related to male vulnerability previously reported in these clinical populations. Multiple hypotheses were suggested in attempt to explain male disadvantage in ASD (please see Figure 1.2) and I discuss our findings in context of supporting or advocating against these hypotheses.

5.1. Does sex matter in connectivity alterations in ASD?

The profound male bias in ASD cases encourages sex differences investigation in individuals with ASD but, at the same time, such disproportion greatly complicates the research process. Due to the considerably lower number of females with ASD diagnosis it is hard to acquire a decent sample size for a neuroimaging study. However, a relatively recent release of the large multicenter database ABIDEI&II containing more than 2200 structural and resting state functional MRI scans created a unique opportunity to investigate differences between males and females with ASD using the largest sample size up to date (Di Martino et al. 2014).

In Chapter 2 and Chapter 3 I used ABIDEI&II resting state fMRI data to investigate sex differences in interhemispheric and local connectivity. Importantly, those were the first studies that examined interhemispheric and local connectivity in ASD populations having females with ASD as a separate group in the study design. Such advantage allowed us to directly test the hypothesis of distinct connectivity alterations in males and females with ASD.

As reported in the Chapter 2, examination of the interhemispheric connectivity between groups revealed no differences. While contradictory to previously reported decreased interhemispheric connectivity in participants with ASD (Anderson et al. 2010; Di Martino et al. 2014), our results are in line with the recent study that also failed to find ASD-related alterations in homotopic connectivity (Hahamy et al. 2015). Local connectivity comparison described in Chapter 3, however, revealed differences between ASD groups and TD groups as well as sex differences between male and female groups. The most

robust local connectivity alterations in ASD groups were detected within the boundaries of the default mode, limbic and somatomotor network. Reports of altered long-range connectivity between the hubs of those networks, and the default mode network in particular, are very common in ASD (Padmanabhan et al. 2017) suggesting that disruption in brain communication happens on multiple scales in this clinical population. Importantly, the pattern of alterations from typical connectivity was similar in females and males with ASD. However, the differences between females with ASD and typical females were more significant than the differences between male groups, as demonstrated in the pairwise comparison. Such results suggest that if there are sex-specific connectivity alterations related to ASD pathology, they are rather quantitative than qualitative ruling out the GI hypothesis. To remind the reader, the GI hypothesis assumes qualitative differences in ASD-related alterations in males and females (Bejerot et al. 2012). It suggests that alterations in males with ASD would be shifted towards the female-like pattern, whereas females with ASD would have more male-like pattern of connectivity. Although such patterns were reported in whole brain connectivity study (Alaerts et al. 2016), the GI hypothesis was not supported by our local connectivity analysis results. In contrast, the findings of more robust alterations in females with ASD relatively to males are in line with the EMB theory that predicts that there should be more similarities between participants with ASD and typical males as compared to typical females (Baron-Cohen 2002). Analogously, the FPE hypothesis would predict similar or more pronounced alterations in females given that they have developed ASD despite the protective features of female sex. Such explanation, though, assumes that the cumulative load of etiological factors was greater in females compared to males in our ASD cohort. Testing this hypothesis further would need additional genetic, endocrinological and clinical information that unfortunately was not available for our sample.

The imperfections in the diagnostic tools and procedures could also potentially explain more robust alterations in local connectivity in females with ASD. It has been shown that to be diagnosed with ASD, girls should present more severe behavioural impairments than boys (Dworzynski et al. 2012). Assuming that same applies to our cohort, there could be potential differences in the severity of ASD between males and females which are not captured by tools measuring ASD symptoms. Stronger alterations in local connectivity and its developmental trajectories in females could reflect those differences in severity of ASD manifestation. Some of our results, however, appear to challenge this explanation. We analysed how the alterations in local connectivity were

linked to ASD symptoms severity. Interestingly, while such association was subtle in ASD males, females with ASD displayed significant correlation between ASD symptoms severity and local connectivity alterations in multiple scores and resting state networks. Such results might suggest that scores measuring ASD severity (ADOS and SRS were used in this study) were able to capture ASD related symptomatology in females in a sufficient manner.

5.2. Sex-specific developmental trajectories

Growing body of connectivity studies in ASD with different age of cohorts and considerable amount of inconsistencies between their results led to a suggestion that connectivity alterations were not stationary across lifespan. Developmental approach was proposed to better characterize ASD-specific alterations (Uddin et al. 2013). Reports of distinct developmental trajectories in males and females further promoted the developmental approach as a promising way to capture sex-specific alterations in ASD (Giedd and Rapoport 2010; Raznahan et al. 2011; Nguyen et al. 2013). Our developmental trajectories investigation confirmed the validity of such approach in studying sex-differences in ASD cohort in local and interhemispheric connectivity.

While no differences between groups were observed in interhemispheric connectivity in Chapter 2, its age-related changes where different between groups. Importantly, these differences were mostly expressed through similarity between males and females with ASD and typical males in contrast to typical females. In other words, males and females with ASD were following the typical male developmental trajectory supporting the EMB theory. In addition, the more distinct developmental trajectories of males and females with ASD were from the typical female trajectories the higher severity of ASD symptoms they had providing additional arguments in favour of the EMB theory. Congruently, similar pattern of differences in developmental trajectories was shown in modularity of resting state connectivity (Henry et al. 2018).

The analysis of age-related trajectories of local connectivity revealed group differences as well. Unlike the results obtained investigating interhemispheric connectivity, we found that the most of the variance of the data was explained by distinct trajectories of local connectivity in female with ASD compared to other groups and typical females in particular. Such distinctions involved the majority of resting state networks and extended

the previous conclusion based on connectivity findings of more pronounced alterations in females with ASD compared to males with ASD. Such results of increased alterations in females who were diagnosed with ASD suggest the suitability the hypotheses which assume FPE and rely on assumption that females need to have an increased load of risk factors to manifest ASD. Alternatively, the EMB theory stipulates there should be more similarities between participants with ASD and typical males as compared to typical females and that is somewhat congruent to our findings. However, we did not find similarity between ASD-related alterations and typical male local connectivity trajectories to support EMB theory entirely. The imperfections in ASD diagnostic procedures cannot be excluded as a potential explanation of observed higher alterations in developmental trajectories in females with ASD, similarly to the connectivity results.

5.3. More pronounced alterations in connectivity in very preterm males compare to very preterm females

Using very preterm children as another clinical child population with male sex disadvantage, I further investigated the connectivity alterations in Chapter 4. Choosing the initial hypothesis regarding the results I excluded the GI hypothesis due to the lack of evidence supporting it in our findings on ASD cohort and insufficient theoretical framework for explaining the male bias. The relevance of the EMB theory for preterm children was questionable because it is very specifically tailored to explain ASD symptoms in the context of typical sex differences in systematizing and empathizing. At the same time, the hypothesis of FPE seem to be more inclusive and capable to explain male vulnerability not only in ASD but in other conditions that involve early developmental disturbances. In addition, its reliance on differential outcome in males and females based on the number of predisposing events or factors could be tested. In very preterm cohort such variables as gestational age, number of skin-braking procedures, daily dose of morphine-based medication, total ventilation duration and early illness severity scores were available. Speculatively, they could be considered as a proxy to the risk factors that are associated with probability of developing the long-term behavioural and cognitive complication (Anand and Scalzo 2000; Ranger and Grunau 2014; Ranger et al. 2014; Johnco et al. 2016). There are also reports of adverse neonatal factors produce different long-term effects in males and females even in the absence of quantitate differences due to sex-specific pain sensitivity, medication efficiency, etc. It has been shown that most of these

factors affect boys to the greater extent than girls possibly due to some protective features of early female development being congruent with the FPE hypothesis (Hindmarsh et al. 2000; Johnson 2007; Tyson et al. 2008; Torrance et al. 2010; Binet et al. 2012). Importantly, the measures of adverse neonatal experience, which were available for the cohort investigated in Chapter 4, were not quantitively different between very preterm boys and girls. Thus, based on the FPE hypothesis, very preterm boys would display more pronounce alterations, including connectivity alterations, compared to girls.

The inter-regional connectivity results revealed significantly larger absolute deviation in connectivity from the typical pattern in very preterm boys compared to girls. Also, in the direct pairwise comparison we found significant differences between very preterm boys and full-term boys but not in the girl groups. Speculatively, if the load of risk factors and events is similar between males and females, males tend to be affected more confirming our initial hypothesis. The findings in local connectivity in ASD cohort, apart from the FPE hypothesis, could be explained by the flaws in ASD diagnostic tools when assessing females. Such explanation seems to be quite unlikely in very preterm population. The cognitive and behavioural impairments in preterm children were measured by tools which were created based on balance samples in terms of sex. Therefore, the FPE theory was to be able to explain better sex-specific connectivity alterations in both cohorts.

The results of correlation analysis, however, were quite unexpected in the light of higher alterations in connectivity in very preterm boys. Significant association between absolute deviation connectivity from the typical pattern and adverse neonatal experience was found only in very preterm females. In preterm boys, connectivity alterations were correlated with both adverse neonatal factors and long-term behavioural and cognitive outcome but only significant at 90% confidence interval. Such infirm correlations in very preterm boys could be explained by lower statistical power due to smaller sample size of the boys' cohort compared to girls. To support this idea, when similar analysis was performed using whole cohort, it demonstrated robust correlation between connectivity and adverse neonatal experience and long-term behavioural and cognitive outcome in very preterm children (Kozhemiako, Nunes, et al. 2019).

5.4. Large-scale brain connectivity contributes to understanding male vulnerability

Investigation of large-scale brain connectivity has a potential to facilitate our understanding of sex bias in neuropsychiatric and neurodevelopmental conditions. We found increase in local connectivity in limbic and somatomotor brain areas in both ASD males and females. Such results could indicate an amplified segregation of functional areas and excessive local information processing in the absence of regulatory feedback and similar findings were reported by other studies (Keown et al. 2013; Maximo et al. 2014). It was proposed that such local overconnectivity in ASD could be related to altered excitation/inhibition balance mostly due to impaired GABA-related inhibition (Casanova et al. 2003). It has been shown that GABA concentration measured by MR spectrography was negatively correlated with strength of functional connectivity in somatomotor network (Stagg et al. 2014). In addition, multiple studies have shown decreased concentration of GABA in ASD participants (Ajram et al. 2017). Interestingly, according to an animal study there are also indication of decreased number of the GABAa (gamma-aminobutyric acid A type receptor) receptors following preterm birth and their link to behavioral alterations in males but not females (Shaw et al. 2016). Going back to our results in very preterm children cohort, we also found increased inter-regional connectivity in very preterm boys compared to full-term boys while no differences was observed between girls.

Importantly, there is extensive evidence of GABA-inhibitory signaling maturation being dependent on estrogen (McCarthy 2008). In particular, the explicit effect of GABAa receptor activation is determined by the transmembrane chloride gradient that steadily changes soon after birth. In adults the intracellular concentration of chloride is lower than extracellular levels, thus opening of GABAa associated ionophore leads to chloride influx. Since chloride is a negatively charged ion its influx hyperpolarizes membrane leading to an inhibitory effect. However, in fetuses and neonates the transcellular chloride concentration is opposite (less chloride outside the cell and more inside). Thus, when the GABAa receptors are activated it results in chloride exiting the cell which depolarizes its membrane resulting in excitatory effect. It has been shown that estrogen enhances and extends the depolarizing action of GABA by keeping the chloride intracellular concentrations high (Nuñez et al. 2005). During the neonatal period males have higher levels of estrogen compared to females that results in longer period of depolarizing GABAa effect in males and faster shift to hyperpolarizing GABA-signalling in females.

Such pattern of sex differences in GABA-inhibitory circuits was reported in substantia nigra in rats (Galanopoulou 2005).The immaturity of GABA interneurons inhibitory effect was hypothesized to be the primary reason of spontaneous activity transients (SATs – intermittent slow potentials with large amplitude) extensively present in neonatal EEG (Vanhatalo and Kaila 2006b). A recent study aimed to assess the level of EEG signal maturity partially based on the number of spontaneous activity transients in female and male infants born very preterm over seven timepoints during the first four weeks after birth. They found that at each assessed timepoint males had on average more SATs compared to females indicating less mature brain activation pattern in males compare to females during first weeks of life. Such maturational delay in boys compared to girls could be responsible for higher adversity of developmental disruptions in males potentially leading to long-lasting alterations in brain coordinated activity. Although this phenomenon still has to be extensively investigated and replicated, sex differences in GABA-signalling maturation could potentially explain the male vulnerability at early age which is observed in very preterm children and ASD population.

Apart from increased local communication in ASD groups we also found decreased local connectivity within default mode network in males and females with ASD. Such underconnectivity pattern does not seem to be directly linked to GABA-signaling alterations due to directionality of connectivity differences and inconsistent reports of relation between GABA concentration and connectivity strength in default mode network (Kapogiannis et al. 2013; Stagg et al. 2014). Despite this, underconnectivity in default mode network seem to be one of the most replicable findings in ASD population (Padmanabhan et al. 2017). There are also reports of reduced default mode network connectivity related to preterm birth (Damaraju et al. 2010; Wisnowski et al. 2013). Moreover, there is evidence that alterations in default mode network connectivity or activation pattern of its main hubs has sex-specific character (Holt et al. 2014; Kirkovski et al. 2016; Ypma et al. 2016). Thus, investigation of why default mode connectivity alterations are expressed differently in males and females with ASD and what are potential neurophysiological mechanisms behind such sex differences would be crucial for unravelling the causes of the male bias in ASD.

Biological sex differences in the brain originate from differences in sex hormones and differential gene expression of sex-linked genes as well as some autosomal genes modulated by hormonal exposure. The hormonal regulation is very time specific and has

multiple critical timepoints over the lifespan causing organizational and activational physiological changes including those in the brain (McCarthy 2008). It has been shown that male and female mice had different developmental trajectories of glia gene expression with males having slower age-related expression increase (Hanamsagar et al. 2017). Such age-dependent hormonally and/or genetically induced distinctions could be potentially be reflected in sex differences in developmental trajectories in brain structure and function. Multiple studies confirm distinct growth trajectories in multiple brain regions between girls and boys (Giedd and Rapoport 2010; Raznahan et al. 2011). We obtained very promising results studying developmental changes in interhemispheric and local connectivity. Such approach revealed both sex- and ASD-specific deviations in how connectivity changes over age. Similar findings of distinct age-related modularity trajectories between males and females with ASD (Henry et al. 2018). Perhaps, due to existence of typical sex-specific pattern in maturational trajectories it is easier to capture sex differences caused by early disruption in brain development using developmental approach supporting its high potential in investigating male vulnerability.

5.5. Significance and future directions

Despite arising awareness about how important is to understand the neurophysiological basis underling male vulnerability and functional connectivity as very promising candidate for it, the sex differences in brain connectivity in clinical child populations remained underexplored. Present work provides evidences indicating the presence of sex-specific alterations in local and interhemispheric connectivity and/or their developmental trajectories in ASD and very preterm children. While interpreting our results we found some indications pointing at the relevance of the FPE hypothesis for both populations and its capability to explain most of our results. However, the EMB theory and the imperfections of the diagnostic process in ASD could also explain some of our findings in participants with ASD.

Future studies should aim to establish large neuroimaging datasets which would include females in the numbers sufficient to investigate them separately from males. Based on our results, the developmental approach is very promising in investigating sex-specific alterations in this clinical populations. Hence, longitudinal settings would be very

important to further clarification of how connectivity changes over time in individuals with ASD and very preterm children, especially covering the early life period. Most of our findings supported the FPE hypothesis, however, to confirm it further more information about participants is critical. Extensive behavioural and cognitive assessment scores, clinical and family medical history, sociocultural background, genomic samples, and endocrinological information combined with neuroimaging data would be necessary to pinpoint the impact of environment, genetic predisposition and hormonal exposure and diagnostic strategies on the etiology of male disadvantage or female protective effect in early life.

5.6. Conclusion

Investigation of connectivity within and between brain regions and/or its age-related changes has proven to be able to capture sex differences in clinical child populations which are impacted by male vulnerability. While only local connectivity exhibited ASD-related alterations, developmental trajectories were informative of both sex- and ASD- specific alterations. Overall, females with ASD were expressing more pronounced alterations from typical females compared to differences between males with ASD and typical males. In contrast, in the very preterm children cohort it was the opposite. Very preterm boys had more distinctive connectivity from typical connectivity patterns than very preterm girls. Such results feet very well to the hypotheses which rely on the assumption that females need to be confronted by more harmful events and biological factors to exhibit pathological disruptions of normal development. When the cumulative effect of predisposing factors (like in the case of very preterm children) does not differ between males and females, males show more alterations in connectivity than females. In contrast, whenever such factors overweight the effect of female protective features resulting in pathological outcome (females diagnosed with ASD), the connectivity disruptions are the same or even more robust in females.

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Appendix A.

Supplementary tables describing ASD cohort

Table A1 ABIDE I and II acquisition sites and participants IDs

Acquisition site ID			Typical Development (TD)		Autistic Spectrum Disorder (ASD)	
			Males	Females	Males	Females
ABIDEI	Kennedy Krieger Institute	N = 27	9	6	8	4
		ID	50773, 50775, 50781, 50785, 50787, 50814, 50817, 50819, 50822	50778, 50780, 50784, 50790, 50812, 50820	50793, 50797, 50799, 50800, 50802, 50804, 50823, 50825	50792, 50795, 50796, 50798
	University of Leuven: (Sample 2)	N = 18	6	5	4	3
		ID	50725, 50727, 50731, 50733, 50738, 50740	50722, 50723, 50730, 50735, 50736	50746, 50747, 50748, 50750	50743, 50744, 50749
	NYU Langone Medical Center	N = 36	8	11	8	9
		ID	51075, 51083, 51087, 51096, 51100, 51109, 51113, 51147	51038, 51040, 51042, 51045, 51047, 51049, 51051, 51053, 51055, 51057, 51059	50967, 50972, 50977, 50998, 51007, 51008, 51016, 51026	50952, 50954, 50955, 50956, 50957, 50958, 50959, 50961, 50962
	Olin, Institute of Living at Hartford Hospital	N = 11	3	2	3	3
		ID	50102, 50104, 50111	50113, 50114	50118, 50120, 50136	50119, 50125, 50127
	University of Pittsburgh School of Medicine	N = 16	4	4	4	4
		ID	50033, 50043, 50045, 50047	50036, 50038, 50049, 50059	50006, 50015, 50027, 50028	50005, 50023, 50029, 50057
	Stanford University	N = 16	4	4	4	4
		ID	51182, 51185, 51187, 51194	51180, 51184, 51188, 51192	51163, 51169, 51172, 51178	51162, 51164, 51167, 51170
	University of California Los Angeles	N = 18	5	4	4	5
		ID	51253, 51269, 51274, 51278, 51281	51264, 51267, 51279, 51282	51204, 51214, 51220, 51224	51207, 51215, 51219, 51226, 51230
	University of Miami	N = 41	10	11	12	8
		ID	50335, 50344, 50349, 50350, 50355, 50360, 50366, 50371, 50372, 50381	50343, 50348, 50353, 50354, 50356, 50357, 50361, 50369, 50374, 50375, 50379	50272, 50273, 50274, 50275, 50277, 50279, 50280, 50281, 50282, 50283, 50286, 50288	50276, 50278, 50284, 50285, 50300, 50302, 50319, 50321
	YALE	N = 35	9	8	10	8
		ID	50552, 50554, 50559, 50561, 50564, 50570, 50573, 50575, 50578	50555, 50557, 50558, 50563, 50565, 50569, 50572, 50576	50601, 50602, 50605, 50606, 50608, 50609, 50610, 50611, 50616, 50622	50604, 50615, 50619, 50620, 50620, 50621, 50623, 50624, 50627
AB	Erasmus University	N = 21	5	5	6	5

IDEII	Medical Center Rotterdam	ID	29898, 29903, 29895, 29906, 29915	29892, 29894, 29896, 29899, 29917	29869, 29870, 29873, 29868, 29872, 29885	29867, 29871, 29875, 29882, 29889
	Georgetown University	N = 36 ID	8 28781, 28792, 28794, 28798, 28806, 28828, 28837, 28845	11 28751, 28753, 28757, 28759, 28769, 28772, 28775, 28783, 28801, 28826, 28829	9 28800, 28808, 28815, 28819, 28821, 28825, 28832, 28838, 28843	8 28756, 28758, 28779, 28780, 28805, 28807, 28834, 28839
	Institut Pasteur and Robert Debre Hospital	N = 32 ID	8 29580, 29583, 29589, 29594, 29599, 29608, 29614, 29617	6 29590, 29596, 29601, 29607, 29620, 29630	10 29587, 29588, 29606, 29609, 29612, 29615, 29619, 29626, 29627, 29629	8 29584, 29591, 29603, 29610, 29613, 29623, 29624, 29628
	Kennedy Krieger Institute	N = 52 ID	15 29311, 29313, 29316, 29317, 29319, 29320, 29321, 29323, 29324, 29325, 29328, 29345, 29340, 29341, 29346	11 29298, 29301, 29309, 29315, 29334, 29337, 29366, 29371, 29439, 29442, 29466	12 29275, 29281, 29286, 29290, 29291, 29292, 29385, 29394, 29400, 29401, 29413, 29456	14 29276, 29278, 29279, 29285, 29287, 29289, 29293, 29344, 29375, 29409, 29411, 29412, 29416, 29477
	Oregon Health and Science University	N = 28 ID	6 28951, 28956, 28961, 28969, 28974, 29002	8 28945, 28950, 28971, 28977, 28994, 29004, 30154, 30167	7 28924, 28931, 28937, 28941, 28958, 28967, 28987	7 28934, 28942, 28962, 28968, 28988, 28997, 30156
	Olin Neuropsychiatry Research Center	N = 19 ID	5 28701, 28705, 28715, 28726, 28733	5 28699, 28706, 28714, 28719, 28724	6 28677, 28681, 28686, 28688, 28696, 28697	3 28678, 28680, 28693
	San Diego State University	N = 16 ID	4 28854, 28867, 28870, 28891	2 28880, 28882	3 28874, 28879, 28901	7 28869, 28871, 28873, 28876, 28885, 28903, 28907
	University of California Davis	N = 18 ID	5 30015, 30020, 30023, 30024, 30029	4 29997, 29998, 30006, 30008	5 30010, 30011, 30014, 30016, 30019	4 29999, 30001, 30021, 30026
Total number per group			**114**	**107**	**115**	**104**

Table A2 Numbers of participants across ABIDE I&II acquisition sites that have been included to the analysis

Acquisition site ID		**Typical Development (TD)**		**Autistic Spectrum Disorder (ASD)**		**Total number per site**
		Males	**Females**	**Males**	**Females**	
ABIDE I	Kennedy Krieger Institute	8	5	7	4	24
	University of Leuven: (Sample 2)	5	4	4	3	16
	NYU Langone Medical Center	8	8	8	8	32

	Olin, Institute of Living at Hartford Hospital	3	2	3	3	11
	University of Pittsburgh School of Medicine	4	4	4	4	16
	Stanford University	4	4	4	4	16
	University of California Los Angeles	5	3	4	5	17
	University of Miami	9	9	9	8	35
	YALE	8	8	9	7	32
ABIDE II	Erasmus University Medical Center Rotterdam	5	5	5	4	19
	Georgetown University	8	11	7	6	32
	Institut Pasteur and Robert Debre Hospital	6	3	9	5	23
	Kennedy Krieger Institute	12	9	11	12	44
	Oregon Health and Science University	6	7	7	7	27
	Olin Neuropsychiatry Research Center	5	5	4	2	16
	San Diego State University	4	2	3	7	16
	University of California Davis	5	4	5	3	17
Total number per group		**105**	**93**	**103**	**92**	

Appendix B.

Developmental trajectories differences using quadratic and cubic function to model age-related changes in ReHo.

Here we investigate age-related effects using quadratic and cubic model. We used exactly the same data and procedure of generating the sub-samples to perform this analysis with two distinctions. First, in order to preserve quadratic and cubic effect of age we added them as components of the linear model at the stage of regressing out center-specific variability. Then, we generated the subsamples and fitted quadratic and cubic model to estimate curvature and aberrance coefficients respectively for each subsample for each voxel. Using PLS analysis, we compared curvature and aberrance coefficients between groups.

PLS analysis of age-related changes in ReHo using quadratic function revealed three significant latent variables (LVs). Overall, these results were quite similar to the results presented in the main manuscript obtained using linear model. The group contrast associated with the first LV expressing the most variance in the data matched closely to the LV #2 in the manuscript. Basically, it illustrates differences between males with ASD and other groups (Fig.B1A) which were the most pronounced in somatosensory network. The second LV in this analysis shared similarities with original LV #1 in the manuscript separating females with ASD from other groups (in particular TD females). Differences in developmental trajectories were distributed across multiple networks including visual, dorsal attention, ventral attention and default mode network. The last significant LV was showing the differences between TD males and both females groups which were the most pronounced in limbic network. Overall, the comparison of developmental trajectories where age effect is modeled using quadratic function is rather congruent with the analysis obtained with applying linear model and described in the manuscript.

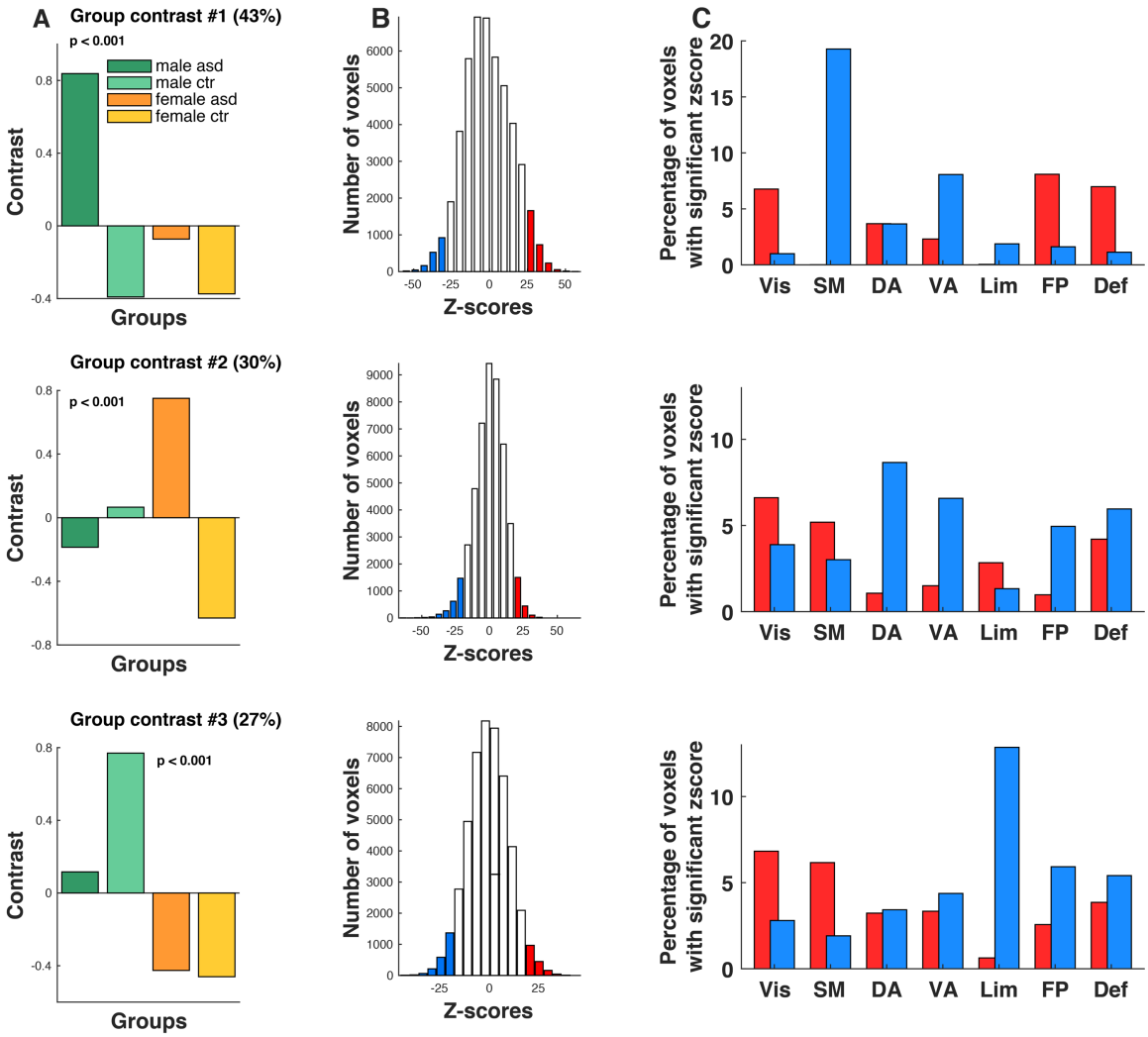

Fig. B1 Differences in developmental trajectories in ReHo assessed with a quadratic model: the first row is LV #1 representing 43% of variance in the data that separates males with ASD from the other three groups; the second row represents LV #2 that expresses 30% of variance in the data and revealed different developmental trajectories in ASD females as compared to other groups; the third row illustrates LV #3 that represents 27% of variance in the data and shows differences in developmental trajectories between TD males and other three groups (in particular females). Column A – group contrasts showing group differences; column B – distribution of Z-scores with tails representing 5% of voxels with highest (in red) and lowest (in blue) z-scores; column C – a bar graph illustrating percentage of voxels that belong to the 5% tails for each RSN.

Similarly to the analyses using linear and quadratic model, the results of comparing the developmental trajectories of ReHo where age effect is modeled using cubic function also revealed three significant LVs (Fig. B2). The group contrast associated with the first LV separated groups by diagnosis analogously to the differences we observed when comparing the ReHo per se. Revealed differences were mostly driven by females and

located in visual network. The second LV demonstrated differences between male and female groups that was again similar to the LV#2 group differences in ReHo. The resting state networks involved in these differences were somatosensory and ventral attention networks. The third LV juxtaposed TD males and ASD females to ASD males and TD females and apparently was mostly driven by contrast between males.

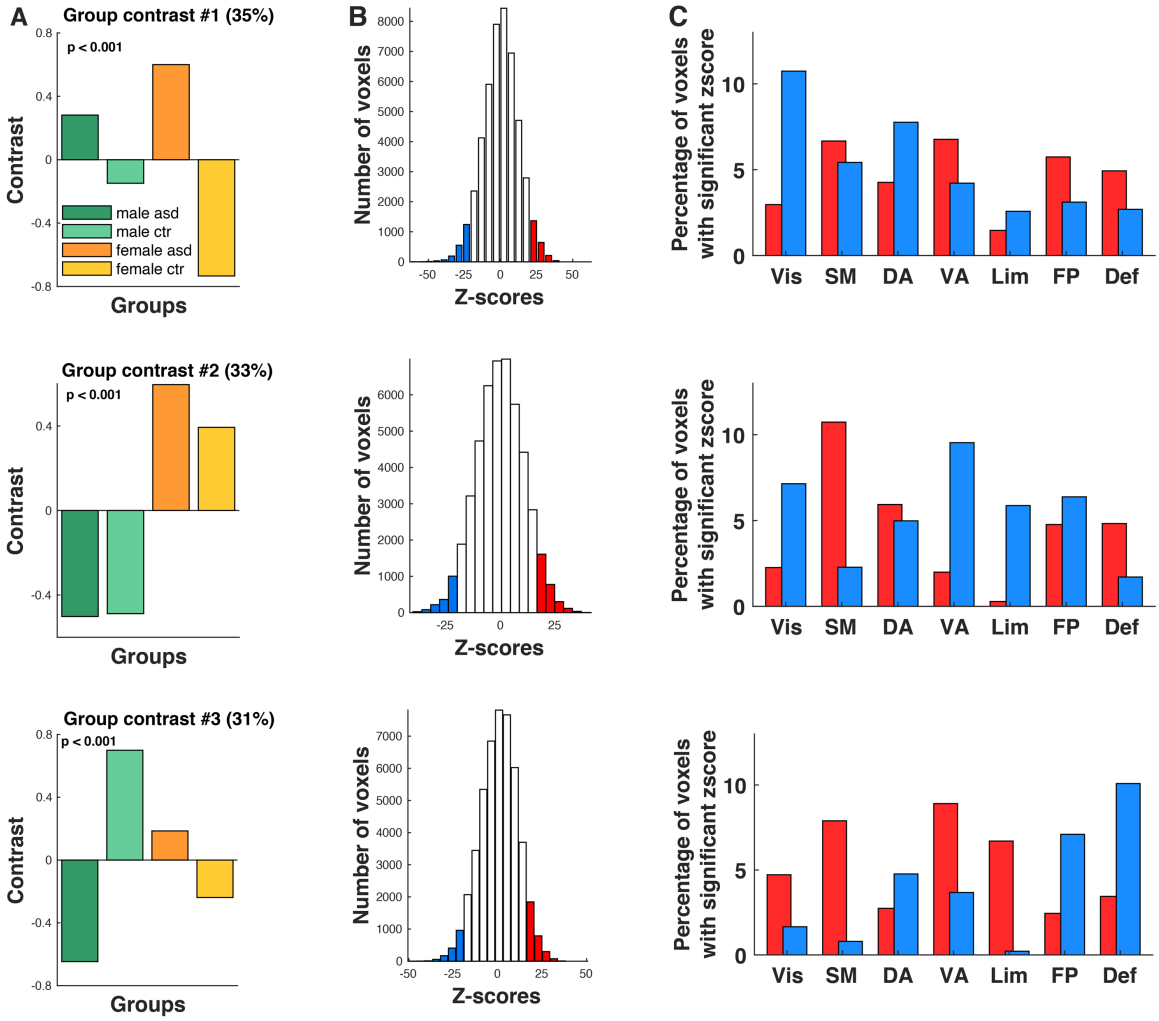

Fig. B2 Differences in developmental trajectories in ReHo assessed with a cubic model: the first row is LV #1 representing 35% of variance in the data that separates ASD groups from the other TD groups; the second row represents LV #2 that expresses 33% of variance in the data and revealed different developmental trajectories in females as compared to male groups; the third row illustrates LV #3 that represents 31% of variance in the data and shows differences in developmental trajectories mostly driven by TD males and ASD males. Column A – group contrasts showing group differences; column B – distribution of Z-scores with tails representing 5% of voxels with highest (in red) and lowest (in blue) z-scores; column C – a bar graph illustrating percentage of voxels that belong to the 5% tails for each RSN.