KOÇ UNIVERSITY

GRADUATE SCHOOL OF SOCIAL SCIENCES & HUMANITIES

UNDERSTANDING AUTISM:

BIOLOGICAL BASIS, COGNITION, AND BEHAVIOR

BY

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A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Psychology

in the Graduate School of Social Sciences and Humanities

of

Koç University

June 2018

KOÇ UNIVERSITY

GRADUATE SCHOOL OF SOCIAL SCIENCES & HUMANITIES

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This dissertation contains no material which has been accepted for any award or any other degree or diploma in any university or other institution. It is affirmed by the candidate that, to the best of her knowledge, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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THESIS ABSTRACT

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with increasingly rising prevalence, where as of 2018, 1 in 59 children is reported to have ASD. ASD has no known cause or treatment, or a broad theory that accounts for all its behavioral and physiological manifestations. Thus, comprehensive investigations across multiple domains are important for understanding ASD, which is the focus of the present thesis that explores the biological basis, cognitive signature, and behavioral problems in individuals with ASD. Firstly, this thesis investigated the two risk factors of gut dysbiosis and inflammation in ASD together and revealed potential common mechanisms of action for these two risk factors, which are their effects on neurodevelopment, ASD gene expression, and gut and brain barrier integrity. This then segued into suggesting probiotics as a potential treatment or prevention that can target all these areas that are possibly influenced by ASD risk factors to result in the ASD phenotype. Secondly, the unique cognitive style of individuals with ASD was investigated empirically with a thorough design that explored error-monitoring in two cognitive domains of decision-making and timing. Results revealed a circumscribed deficit in error-monitoring in the presence of similar task performance with typically developing (TD) controls. Thirdly, the relationships between parent psychological problems and child behavior problems were investigated in families with children with ASD and TD children. It was found that parent obsessive-compulsive characteristics predicted child ASD symptoms. Overall, this thesis contributes to understanding ASD by offering novel findings about its biological basis, cognitive signature, and behavioral inheritance profile, which can guide investigations into more comprehensive models that may one day be able to explain this complex disorder.

Keywords: autism spectrum disorder, biology, physiology, cognition, error-monitoring, behavior, parent-child.

TEZ ÖZETİ

Otizm spektrum bozukluğu (OSB), yaygınlığı giderek artan nörogelişimsel bir hastalıktır, öyle ki 2018 senesinde 59 çocuktan 1'inin OSB tanılı olduğu bildirilmiştir. OSB'nin bilinen bir nedeni veya tedavisi ve tüm davranışsal ve fizyolojik belirtilerini açıklayabilen bir teorisi yoktur. Bu bağlamda, çoklu alanlardaki kapsamlı araştırmalar OSB'yi anlamak için önem taşımaktadır. Bu konu, mevcut tezin odak noktasıdır ve bu amaç doğrultusunda OSB'nin biyolojik temeli, bilişsel imzası ve anne-çocuk arasında ilişki gösteren psikolojik ve davranışsal özellikleri araştırılmıştır. İlk olarak, OSB için iki önemli risk faktörü olan bağırsak bakterilerindeki bozulmuş denge ve inflamasyon birlikte incelemekte ve bu iki risk faktörünün ortak etki mekanizmaları olarak sinirgelişim, OSB genlerinin ekspresyonu ve bağırsak ile beyin bariyeri bütünlüğü üzerindeki etkileri önerilmektedir. Daha sonra, OSB fenotipinin ortaya çıkmasında rol oynayan OSB risk faktörlerinden etkilenebilecek bu alanları hedefleyecek potansiyel bir tedavi veya önleme yöntemi olarak probiyotikler önerilmektedir. İkinci olarak, OSB olan bireylerin kendilerine has düşünme tarzları, iki farklı bilişsel alanda (karar verme ve zamanlama) hataların fark edilmesi becerisi üzerinden incelenmiştir. OSB olan çocuklar, normal gelişim gösteren çocuklar ile benzer görev performansı ile uyaran algılama ve zamanlama becerisi göstermiş, fakat OSB olan çocuklarda hata izleme becerisine özgü bir eksiklik ortaya çıkarılmıştır. Üçüncü olarak, ebeveynlerin psikolojik problemleri ile çocuk davranış sorunları arasındaki ilişkiler araştırılmıştır. Ebeveyn obsesif kompulsif özelliklerinin çocuk OSB belirtilerini yordadığı bulunmuştur. Genel olarak bu tez, OSB'nin biyolojik temelleri, bilişsel imzası ve davranışsal kalıtım profili hakkında yeni bulgular sunarak OSB'nin anlaşılmasına katkıda bulunmakta olup, OSB'yi açıklayabilecek daha kapsamlı modellere rehberlik etme niteliğindedir.

Anahtar Kelimeler: otizm spektrum bozukluğu, biyoloji, fizyoloji, biliş, hata izleme, davranış, ebeveyn-çocuk.

DEDICATION

To my dearly beloved professor and role model Prof. Çiğdem Kağıtçıbaşı,

who is an exquisite exception to the case of great people seldom being good people, who continues to and always will inspire me to become the best academician and person I can be.

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CHAPTER I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social interaction deficits, and repetitive and restrictive behaviors and interests. It has no known single cause, and has an increasingly rising prevalence, with the most current estimates revealing a 15% increase from the estimate of the previous two years, i.e. from 1 in 68 to 1 in 59 individuals having ASD (Baio et al., 2018). By combining the three related domains of biology, cognition, and behavior, this thesis aims to contribute to filling the empirical and theoretical gaps in our understanding of ASD. This attempt that has both theoretical and practical implications can help achieve a more complete characterization of this disorder based on these multiple complementary bases and inform future models of ASD etiology accounting for multiple areas of deficit. Specifically, this thesis investigates the common mechanisms of action of different physiological pathways influencing ASD neurology, broad thinking styles in ASD that are hitherto unexplained by a single model, and relations between parent psychological problems and child behaviors in families with individuals with ASD. Such an integrative approach enables taking a comprehensive perspective to understanding ASD and bringing together multiple areas of differences in individuals with ASD under one broad investigation.

Gastrointestinal problems have been prevalently reported in individuals with ASD for years. With the global frenzy in the past couple of years on the gut-brain axis in various physiological and neurological disorders, the gastrointestinal investigations in ASD moved from the symptom domain to the microbial domain by exploring gut microbial differences in ASD. With increasing evidence for the bidirectional effects of gut and brain on each other, such investigations hold value both for explaining the development of ASD and for potential interventions targeting the gut dysbiosis. Additionally, ASD has been associated with abnormal immunity or inflammation, where maternal immune activation is considered a risk factor for ASD and aberrant immune profiles are believed to persist across development in ASD. The first chapter in this thesis combines these two physiological risk factors of gut microbiota and immune

activation/inflammation and investigates potential common mechanisms that enable their influence on ASD neurology. Such a comprehensive outlook is likely to inform holistic understanding of the physiological differences in ASD and point to potential treatments that may target these potential common mechanisms. Such targeting may make these treatments successful in subgroups of ASD with different comorbidities or etiological profiles, and may pave the way for discovering broadly applicable treatments that can benefit as many subgroups of individuals with ASD as possible. They may also be used as preventative measures in mothers at risk for giving birth to children with ASD, with their potential to counteract multiple risk factors during development. Thus, exploring how two different physiological risk factors for ASD may have common mechanisms of effect on ASD neurology is important both for advancing the theoretical understanding of ASD and suggesting potential preventative and treatment methods.

Understanding the cognitive correlates of ASD has also been a hot topic, which is predicted to help unravel the neurological underpinnings of deficits observed in ASD. In their analysis of cognitive explanations of ASD, Frith (1996) overviewed three hypotheses to explain the different impairments in ASD; a) theory of mind deficit for social communication impairments, b) executive function deficit for perseveration and rigidity, and c) weak central coherence for uneven pattern of intellectual abilities in individuals with ASD, who may have a distinctive cognitive style. Frith concludes by noting that identifying the multiple cognitive deficits in ASD can help learn more about the underlying brain abnormalities in ASD. By looking at various cognitive abilities of individuals with ASD together as part of the same study design, this study made a comprehensive attempt at understanding the proposed distinctive cognitive style of individuals with ASD. In ASD, most cognitive studies remain isolated in their specific domains and broader investigations into the cognitive style in ASD that span multiple domains are lacking. Cognitive domains that especially warrant attention are perceptual decision-making, interval timing, and awareness of errors (i.e., error/performance monitoring), because they are all important components that underlie smooth social interactions, an area that is impaired in

individuals with ASD. Combining these different cognitive processing domains will help specify the nature of dysfunctions in cognitive processing in ASD and help make sense of sometimes contrasting findings that come from these different domains when they are investigated separately from each other. Additionally, understanding how error-monitoring works across different cognitive domains in individuals with ASD can give valuable information about one of the mechanisms underlying their repetitive and stereotypic behaviors, which are not corrected even when inappropriate possibly due to both their tendency to perseverate and inability to generate novel responses (Lopez et al., 2005). Lastly, understanding others' mental states and inferring intentions from their behaviors are impaired in individuals with ASD and this mind-reading ability is suggested to be related to metacognition. Thus, if the awareness of one's own errors and behaviors is indeed related to understanding intentions behind others' behaviors, then error-monitoring can also be a cognitive mechanism underlying mind-reading difficulties in ASD. So, for its importance in smooth social interactions, correcting inappropriate behaviors, and potentially helping understand others' intentions, exploring error-monitoring in ASD with a comprehensive and controlled design is a valuable research endeavor that is taken up in the second chapter of this thesis.

Finally, ASD is characterized by dysfunctional behaviors in communication and social interaction, and by repetitive behaviors. Though they mainly serve as the prerequisites for an ASD diagnosis, investigations into ASD behaviors can also inform domains other than diagnosis in ASD. ASD is accepted to have a strong hereditary component. The majority of hereditary studies in ASD has focused on twins, however inheritance studies between parent and child traits can also be informative regarding the hereditary components of ASD. For this reason, addressing which parent psychological problems relate with which child behavior problems in ASD differently than the neurotypical population can be informative in understanding hereditary relationships in ASD. Yet, such an investigation has not yet been made and this gap in the literature was addressed with the third chapter of this thesis. It explored the relations between

parent psychological symptoms and child problem behaviors in both families with ASD and families with typically developing children, and also between parent psychological symptoms and ASD symptoms in families with children with ASD. Such a fresh approach to hereditary studies in ASD can guide and assist investigations into the behavioral genetics of ASD. These findings will show which parent psychological problems predict which ASD child symptoms to what degree in this disorder that spans a spectrum, with different symptoms manifested and levels of symptom severity observed in each individual with ASD.

Overall, this thesis has looked at where we are in understanding ASD in today's world, what the recent advances are, and how we can take them one step further in the domains of neurodevelopment, cognition, and behavior to expand our knowledge on this neurodevelopmental disorder that still evades etiological and genetic explanations.

CHAPTER II

GUT MICROBIOTA, INFLAMMATION, AND PROBIOTICS ON NEURAL DEVELOPMENT IN ASD

Doenyas, C. (2018). Gut microbiota, inflammation, and probiotics on neural development in autism spectrum disorder. *Neuroscience, 374,* 271-286. doi:10.1016/j.neuroscience.2018.01.060

Gut Microbiota, Inflammation, and Probiotics on Neural Development in Autism Spectrum Disorder

Abstract

Recent evidence implicates immune alterations and gut microbiota dysbiosis in at least some subpopulations of individuals with autism spectrum disorder (ASD). Immune and gut alterations in ASD have mostly been studied separately, and the reviews and theoretical models up to now have mainly considered the immune system as one of the routes for gut-brain communication. We take a different perspective and consider possible common mechanisms of action for the gut microbiota and inflammation on the neural basis of ASD. We propose these to be their effects on ASD-susceptibility genes, neurodevelopment, and intestinal and blood-brain barrier integrity. We then use these common mechanisms to offer pathways for potentially beneficial effects of early-life probiotics on the neural development in ASD. This new perspective yields a conceptual framework for creating effective preventions for mothers at risk of giving birth to children with ASD. Such a framework may also inform effective interventions targeting these common mechanisms of action, which may be shared in many ASD cases regardless of their different etiological profiles. Probiotics may be one example of such preventions and interventions. Finally, the common mechanisms offered by this perspective can be useful in the search of comprehensive theories that can account for the complete neurobiological and behavioral symptoms of ASD.

Keywords: autism spectrum disorder, gut microbiota, immunity, inflammation, neurodevelopment, probiotics.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by difficulties in social interaction and communication, and repetitive behaviors and interests (American Psychiatric Association, 2013). There is a strong genetic component in ASD (Chahrour et al., 2016) and ASD is one of the most heritable neuropsychiatric disorders. Yet, no specific gene for ASD has been identified, known single gene mutations can only explain less than 1% of all ASD cases, and the concordance between monozygotic twins is not 100%, implicating environmental factors and complex gene-gene and gene-environment interactions in the etiology of ASD (Korkmaz, 2013a). Substantial increases in the prevalence of ASD (from 1 in 500 in 1992 to 1 in 110 in 2007 and 1 in 68 currently in the US) has taken place in too short of a time frame for genetic changes to occur at a population level. Such prevalence data also points to environmental factors, which are now believed to have a much greater role in ASD than previously assumed (Estes & McAllister, 2015).

Recently emerging evidence points to a combination of gut microbiota changes, intestinal permeability, and inappropriate immune responses in individuals genetically predisposed to ASD (Coury et al., 2012). Children with ASD experience significantly more gastrointestinal (GI) symptoms than children without ASD (McElhanon et al., 2014), and their GI symptoms correlate strongly with their ASD severity (Adams et al., 2011). Additionally, of four decades of publications on the physiological and metabolic abnormalities in ASD, 95% show an association between ASD and immune dysregulation/inflammation (Rossignol and Frye, 2012a). Yet, these two lines have mostly remained segregated in research. GI comorbidities in ASD have mainly been investigated in relation to ASD severity and other behavioral manifestations such as anxiety and sensory over-responsivity (Adams et al., 2011; Mazurek et al., 2013). Conversely, inflammation studies operated under a more biological perspective, focusing mostly on neuroinflammation (Pardo et al., 2005; El-Ansary &Al-Ayadhi, 2014) and immune mediators

in peripheral tissues (Estes & McAllister, 2015), without much interest in connecting inflammatory findings with behavioral ASD manifestations. Such a divide hinders comprehensive attempts at understanding biological underpinnings of ASD. In the reviews and theoretical models published thus far, the immune system and its products have mostly been considered as one of the ways for gut-brain communication to take place (e.g., Collins et al., 2012; Cryan & Dinan, 2012; Fung et al., 2017). The present discourse takes a different perspective and investigates potential common principles of influence for the gut microbiota and inflammation on the neural basis of ASD. Such a perspective seems to have been invited by de Magistris et al. (2014), who are the only researchers I have come across to refer to a "gut-brain-immune system" axis of communication. Yet, they have sufficed with only referring to this axis of communication without elaborating its details, which is a task taken up in the present paper.

When talking about the neural basis of ASD, it should be noted that although regions have been suggested to mediate clinical ASD phenotypes such as the frontotemporal lobe, amygdala, hippocampus, basal ganglia, and frontoparietal cortex, brain imaging studies fail to implicate a single brain region or system in all cases of ASD. This may in part be due to the clinical diversity, heterogeneous subgroups of ASD, and developmental changes in the brain (Ha et al., 2015). However, synaptic deficits and altered functional connectivity between brain regions with instances of both hypo- and hyper-connectivity (Belger et al., 2011) seem to be consistent findings for ASD. Synaptic protein defects are considered to result in changes in synaptic structure, function, and neuronal circuits, leading some researchers to conceptualize ASD as a synaptopathy (Won et al., 2013). Synapse integrity also is related to functional connectivity in the brain, as the functioning of synapses is important for proper neural connectivity (Belger et al., 2011). Thus, the focus of the present paper is on the common mechanisms whereby gut microbiota and inflammation can influence synaptic and connectivity

properties of neurons during development and how probiotics may be employed to counteract such effects during neurodevelopment in ASD.

Recent findings on the importance of the gut microbiota in neural development and behavior has created a "paradigm shift in neuroscience" (Mayer et al., 2014). With the latest investigations into the gut microbiota, GI symptoms have acquired biological correlates besides their usually considered behavioral implications. The gut microbiota is the complex and dynamic population of microorganisms in the GI tract, which influences the host (humans) during homeostasis and disease (Thursby & Juge, 2017). Now, through the gut microbiota, GI symptoms can be considered on a common molecular ground with the immune/inflammatory abnormalities. Such an approach facilitates the investigation of potential common mechanisms via which the gut and the immune abnormalities can influence the neurology of ASD. Searching for common, overlapping, or interacting pathways between the gut microbiota and inflammation in ASD is useful in several ways. This search may pave the way for more informed interventions that target multiple pathways to ameliorate as many symptoms as possible from the heterogeneous behavioral constellation of the autism spectrum. Additionally, if such common mechanisms do in fact operate, then it may be possible that certain genes or epigenetic modifications influencing these common mechanisms also exist. Unraveling such genetic or epigenetic correlates may then contribute to understanding the specificities of the gene-environment interactions in the pathogenesis of ASD. Finally, these common mechanisms can serve to inform probiotic mechanisms of action. Such an endeavor is important as the recent interest in the therapeutic roles of probiotics and their use to ameliorate ASD symptoms is accompanied by a lack of knowledge about their mechanisms of effect.

Gut Microbiota and Inflammation in ASD

Gut microbiota refers to the complex gut microbial community that has a symbiotic relationship with their host (Bäckhed et al., 2004; Collins et al., 2012). Whereas the gut microbiome refers to the collective genomes of this microbial community (Bäckhed et al., 2005), the gut microbiota comprises the microbes and the metabolites they produce. Though its composition shows high diversity even among healthy individuals (The Human Microbiome Project Consortium, 2012) the gut microbiota is mainly dominated by organisms belonging to the *Firmicutes* and *Bacteroidetes* phyla (Eckburg et al., 2005). A "human intestinal microbiota phylogenetic core" shared by at least 50% of the sampled individuals has been noted, which includes members of the *Faecalibacterium, Ruminococcus, Eubacterium, Dorea, Bacteroides, Alistipes, and Bifidobacterium* genera (Tap et al., 2009). The gut microbiota follows an overlapping course of development with the intestinal barrier (Kelly et al., 2015). Intestinal or gut barrier is a dynamic physical barrier controlled by tight junctions, which are complex protein structures that regulate intestinal barrier permeability (Ulluwishewa et al., 2011). Disruption of the gut barrier is associated with the transit of luminal contents into the bloodstream, which activates the immune response and induces an inflammatory state (Viggiano et al., 2015), and with increased permeability and various GI disorders (Wang et al., 2015). Thus, intestinal barrier integrity is implicated in both immune/inflammatory responses and GI symptoms.

One of the factors influencing gut barrier integrity is the gut microbiota, which regulates intestinal barrier function by changing the expression and distribution of tight junction proteins (Ulluwishewa et al., 2011). The gut microbiota is also involved in many important biological and metabolic functions, from the synthesis and metabolism of nutrients, hormones, and vitamins to the clearance of drugs and toxins; and from the supply of energy from dietary sources otherwise unavailable to the host to the modulation of brain activity and behavior via the gut-brain axis

(Louis, 2012; Mangiola et al., 2016; Sharon et al., 2016). In recent years, the microbiota-gut-brain axis has become a focus of considerable investigation for its role in the generation of ASD behaviors (Li & Zhou, 2016). This has come about especially as a result of animal findings implicating gut microbiota in ASD-related behaviors. Germ-free mice show a lack of the normal preference for spending time with another mouse over spending time in an empty chamber, and a lack of the normal increase in time spent exploring a novel over a familiar mouse (Desbonnet et al., 2014). They also have differential gene expression associated with neuronal structure and function in the amygdala, a brain region important for emotions, anxiety, and social behaviors (Stilling et al., 2015).

For the gut microbiota of individuals with ASD, findings about decreased and increased prevalence of specific strains vary from study to study and no clear trend for a gut microbiota profile of ASD has emerged yet. There are some consistent findings of increase in certain strains such as the toxin-producing *Clostridia*, yet ASD has been linked to both increases and decreases other strains such as *Bacteroidetes* and bacterial products such as short-chain fatty acids in different studies, which may be due to methodological differences and heterogeneity inherent in ASD, and these findings may be confounded by higher antibiotic usage and different diets/repetitive dietary choices of individuals with ASD (Cryan & Dinan, 2012; Louis, 2012; Mangiola et al., 2016; Vuong & Hsiao, 2017). The researchers on the ongoing quest for deciphering the gut microbiota alterations should keep in mind the wide heterogeneity even in the microbiota of healthy individuals and the heterogeneity of co-morbid conditions in ASD and given its nature as a “spectrum”, the differential levels of symptoms in individuals collectively diagnosed with ASD. The recent surge of interest in any research involving the gut-brain axis may have resulted in the elusion from attention of this diversity, as well as the following proposition that has not been yet made or investigated. Inflammation or other intestinal or

metabolic abnormalities may break down the tolerance of the body to normal gut flora, as was shown to be the case for individuals with inflammatory bowel disease (Macpherson et al., 1996).

Inflammatory and immunological insults during prenatal years or early in life have been implicated in ASD. Epidemiological findings show associations between a diagnosis of ASD in the offspring and maternal viral infection in the first trimester, maternal bacterial infection in the second trimester (Atladóttir et al., 2010), and maternal fever in the second trimester, with risk rising dose dependently with exposure to three or more fever episodes after 12 weeks of gestation (Hornig et al., 2017). This association was specific to maternal fever and did not hold for influenza experienced during pregnancy, where fever-associated ASD risk was reduced in mothers who took antipyretic medications compared to those who did not (Zerbo et al., 2013). In addition, the different chemokine profiles of newborns who are later diagnosed with ASD compared to those who did not suggests early life inflammation in children with ASD (Zerbo et al., 2014), which may result from either prenatal or early postnatal immune insults. Increasing evidence points to an important role for immune dysregulation in ASD, including findings of ongoing immune dysregulation/ongoing inflammation, infection, fetal reactive antibodies, autoimmunity, altered immune cell function, and elevated pro-inflammatory cytokine profiles in the blood and the brain of individuals with ASD (Onore et al., 2012; Estes & McAllister, 2015). Recently, astrocytes derived from individuals with ASD have been shown to have higher levels of proinflammatory cytokines, and were found to be physiologically impaired compared to control-derived neurons. When ASD neurons were combined with control astrocytes, ASD neuronal morphology and synaptogenesis improved, but when control-derived neurons were combined with ASD-derived astrocytes, the control neurons displayed the ASD neuronal phenotype (Russo et al., 2017). These findings suggest that inflammation in the astrocytes of individuals with ASD may influence neuron and synapse development, and contribute to the pathogenesis of ASD in at least come cases. Additionally, the neuroglial and

innate neuroimmune system activation in the brains of individuals with ASD has been suggested to contribute to the diversity of ASD phenotypes (Pardo et al., 2005).

Colonization with a diverse microbiota in early years of life is crucial for the proper development and regulation of the immune system (Slattery et al., 2016). The gut microbiota stimulates both specific and nonspecific immunity in the first years of life, and though this low-grade inflammation resulting from the gut microbiota's continuous immune stimulation is generally considered beneficial, it could be harmful for children at risk for ASD (Madore et al., 2016). A major portion of the immune system, estimated around 80%, is located in and around the intestinal mucosa and gut microbiota play an important role in the maturation and modulation of the immune system (Critchfield et al., 2011), where specific strains such as *Bifidobacterium* and *Lactobacillus* produce anti-inflammatory cytokines and others such as *Clostridium* and *Ruminococcus* produce pro-inflammatory cytokines (Heberling et al., 2013). The relationship between the gut microbiota and the immune system is bidirectional (Vuong & Hsiao, 2017), and in mouse models of ASD, immune activations, such as maternal immune activation, result in gut microbiota alterations and deficits in sociability, communication, and repetitive behaviors (Hsiao et al., 2013).

Critical Look at Gut Microbiota and Inflammation Findings in ASD

There are three points that are important to consider when interpreting the immune-gut findings in ASD. First, immune and gut microbiota abnormalities are not specific to ASD and are also present in obesity, allergies, autoimmune disorders, irritable bowel syndrome (IBS), inflammatory bowel disease, dementia, mood disorders, and schizophrenia (Campbell-McBride, 2008; Mangiola et al., 2016; Young et al., 2016). Second, GI problems and inflammation are not found in all studies or for all participants with ASD. Though children with ASD experience significantly more GI symptoms than children without

ASD (Ibrahim et al., 2009; McElhanon et al., 2014) and their unaffected siblings (Wang et al., 2011b), the reported prevalence of GI symptoms range from 9% to 91% in individuals with ASD (Coury et al., 2012), suggesting that though common, they are not ubiquitously present in everyone with ASD. Children with ASD and gut microbiota alterations are suggested to constitute a subgroup of ASD children with GI symptoms, for whom gut dysbiosis may play a role in ASD etiology (Slattery et al., 2016). Similarly, given the significant within- and between-subject variability of serum immune profiles in individuals with ASD, Young et al. (2016) suggest that the observed differences in inflammation may indicate potential immunological dysregulation processes as contributors to ASD pathology for certain subgroups of individuals with ASD. Therefore, GI/gut microbiota and immunological abnormalities may characterize certain ASD subgroups and may be involved in ASD etiology for those individuals, but may not be common ASD correlates.

Third, there are two methodological issues that may undermine obtained results or underlie discrepant findings. Fecal matter has around 50% viable cells and may more likely show cells excreted from the body and not the true bacterial composition within the gut (Heberling et al., 2013), yet most studies analyze fecal samples and extrapolate about the gut microbiota compositions from the concentrations in the fecal matter, which may be one reason for the disagreement on the specific gut bacterial composition in individuals with ASD between studies using intestinal biopsies and fecal analyses. Similarly, a recent study found that within-subject stability of measured metabolites across time was low, suggesting that single measurements may not be reliable indicators of immune mediator concentrations (Pardo et al., 2017). Gut microbiota compositions can also be affected by comparable daily influences, and measurements at different time points may yield different microbial compositions in the same individuals. It is known that food influences the composition of the gut microbiota (Li & Zhou, 2016), and being exposed to or restricted certain foods for a period of time before the testing may influence the gut microbiota

composition measured at the time of testing, again questioning the reliability of one-time measurements. Thus, time of measurement may be a factor contributing to some of the inconsistent findings in the literature.

When considering the rising excitement and accumulating findings in the field about the GI, gut microbiota, and immunological abnormalities in ASD as offering potential explanations and treatments for ASD, it should be kept in mind that they are neither ASD-specific or ASD-ubiquitous, and that when integrating evidence across different studies, important methodological considerations should not be overlooked.

Though gut microbiota may also be involved in GI symptoms in other disorders such as the IBS, the focus of the present paper is on whether gut dysbiosis during brain development is related to the pathophysiology of ASD, and how such dysbiosis and immune alterations/inflammation during brain development, i.e. approximately until age 2, can have an effect on ASD pathogenesis.

Present Measurements Do Not Preclude Earlier Effects

Another important point in interpreting inconsistent findings about gut dysbiosis, inflammation, or other biological indications such as a leaky gut in ASD relates to the timing of effects. Measurements made in childhood, adolescence, or adulthood do not preclude the possibility that these physiological disruptions were present during a developmentally critical early period, exerted their effect on neurodevelopment, but did not continue to be present in later years. Dalton et al. (2014) note that though their study did not find support for a persistent leaky gut in children with ASD, this does not rule out the possibility that there was transient permeability of the gut at an earlier age. Pardo et al. (2005) interpret their findings to suggest innate rather than adaptive neuroimmune responses to be associated with immune abnormalities

in ASD, but they also point out that they cannot exclude the possibility that specific cellular or humoral immune reactions may be occurring at early stages of brain development during prenatal or postnatal periods. Additionally, though in some cases maternal immune activation (MIA) may lead to permanent immune dysregulation as evidenced by abnormal immune profiles of adult offspring of these mothers (Hsiao et al., 2012), MIA may also exert its effects via other mechanisms than triggering an immune response in the offspring. One example comes from a study in rats. MIA was found to influence oxidative stress and alter gene expression profiles including the expression of ASD-associated genes without triggering an immune response in the fetus. These offspring later developed to become socially deficient juveniles and young adults. Such an effect that occurred without triggering an immune response may be due to the transfer of maternal cytokines to the fetus from serum, amniotic fluid, or placenta (Oskvig et al., 2012). This finding suggests that maternal immune activation may still influence the offspring's neural development even if studies fail to find an increased inflammatory profile in the offspring.

Thus, immunity, gut microbiota, or gut barrier integrity changes early in life may influence ASD neurology. Such a possibility is not excluded by measurements later in life failing to find altered immune or gut profiles in individuals with ASD and is particularly relevant to the present discussion investigating the common mechanisms of influence of gut dysbiosis and immune abnormalities on the neurology of ASD.

Common Principles of Influence

Although gut microbiota alterations and immune abnormalities may not be present in all individuals with ASD, they are increasingly implicated in ASD symptoms in at least some subgroups with ASD. Moreover, it is possible that they may be present in early development but

not continue to do so in all individuals with ASD, and exert their most consequential effects in this sensitive period. Thus, further investigations into the mechanisms of action of gut microbiota and immune alterations implicated in ASD are warranted. To offer a new perspective to the relations between the gut microbiota and immune alterations in ASD beyond the traditionally assumed role of immunity as one of the multiple pathways mediating the gut-brain connection, we outline their possible common mechanisms of influence on the neural profile of ASD.

Gene expression in ASD and ASD susceptibility genes. The first common mechanism of influence of gut microbiota and inflammation on the neural basis of ASD comes from gene expression evidence and ASD susceptibility genes. Though the specific role played by genetics in ASD etiology is still not clear, more than 100 ASD-susceptibility genes have been identified (Ansel et al., 2016), with 206 genes found as prime ASD susceptibility candidates (Carter & Blizard, 2016). ASD-susceptibility genes are related to early brain development, synapse formation, brain connectivity, inflammation, and immune or microglia markers (Madore et al., 2016). These multiple ASD susceptibility genes are influenced not only by their interactions with other genes, but also by environmental factors (Kazlauskas et al., 2015).

The specific influence of the environment on ASD-susceptibility genes can be seen with the recent finding that many environmental compounds implicated in ASD such as pesticides, heavy metals, industrial, agrochemical, and household pollutants or drugs,
and neurotransmitters and hormones such as serotonin, dopamine, and noradrenaline selectively target multiple ASD-susceptibility genes, with the authors suggesting that the rise in ASD incidence may be chemically driven in a gene-dependent manner (Carter & Blizard, 2016). Many of these ASD-susceptibility genes are enriched and localized in barriers such as the blood–brain,

skin, or intestinal barrier, and thus are likely to influence the absorption, metabolism, or physiological effects of environmental or endogenous toxicants (Carter & Blizard, 2016).

In gene expression studies listing main affected pathways in individuals with ASD, those most commonly implicated are the cell cycle/cell death, neurogenesis, GI disease, and immune function pathways (Ansel et al., 2016). Both post-mortem brain tissue gene expression studies and peripheral blood gene expression studies reveal differential expression of genes related to the immune system function in samples with ASD (Ansel et al., 2016). Moreover, the three top biological functions associated with the unique gene expression profile of children with ASD and GI complaints are inflammatory disease, endocrine system development and function, and digestive system development and function (Ansel et al., 2016), suggesting connections between inflammation and digestive system problems in individuals with ASD experiencing GI symptoms. These findings imply that there may be altered expression patterns of immune function and GI system genes in individuals with ASD in different degrees and combinations, which may lead to the heterogeneity of GI and inflammatory co-morbidities observed across individuals with ASD.

There is some evidence that gut microbiota products and inflammatory cytokines can influence gene expression directly. Though not evidenced in ASD or ASD-susceptibility genes yet, microbial metabolites have been shown to influence the epigenetics for cancer risk, which refers to the heritable changes in gene expression (Hullar & Fu, 2014) and pro-inflammatory cytokines affect gene expression of diabetes-associated autoantigens (Steinbrenner et al., 2002). These findings from other disorders suggest that gut microbiota metabolites and inflammatory cytokines may also have such an effect on the expression of ASD-susceptibility genes, which if shown could inform the specifics of gene-environment interactions in ASD.

The gut microbiota and immune pathways may lie at the intersection between genes and the environment. The gut microbiota has the potential to connect genetic and environmental influences given that its composition depends on both genetic background and is shaped by environmental factors (Vuong & Hsiao, 2017). Similarly, many different environmental factors contributing to ASD have been proposed to converge on immune response alterations during prenatal or early postnatal development (Estes & McAllister, 2015). Early brain inflammation is a well-recognized risk factor for ASD where ASD pathogenesis is linked to neuroinflammatory events in the developing brain, which are influenced by environmental factors including maternal immune activation and gut microbiota (Madore et al., 2016). Thus, environmental toxins or endogenous agents including inflammatory cytokines and gut microbiota products may specifically target ASD susceptibility genes during pre- or early post-natal development, which may in turn influence the expression of genes involved in the development and functioning of GI and immune systems, thereby instigating continued dysfunction in these systems which are shown to be differentially regulated in ASD. It is likely that the type, level, duration, and combination of environmental toxin and endogenous agent exposures may be some of the factors leading to the high heterogeneity observed in ASD cases.

Effects on neurodevelopment. The above findings of immune function and GI disease being two of the four most commonly implicated pathways in ASD gene expression studies (Ansel et al., 2016) segue into the next possible common mechanism whereby gut microbiota and inflammation can influence the neural basis of ASD, which is their role in neural development. Both the gut microbiota and inflammatory processes are implicated in neurodevelopmental processes, albeit to different degrees and in different processes.

Most of the evidence on the effects of gut microbiota and inflammation on neurodevelopment comes from animal findings. For the former, germ-free mouse models are used, which enable a direct assessment of the roles played by microbiota on different aspects of physiology via comparisons between these mice, whose postnatal colonization of GI tract is prevented, and conventionally colonized mice (Cryan & Dinan, 2012). For the latter, maternal immune activation (MIA) mouse model is used where generic inflammatory agents are administered to the mother that model factors common to several infections and are independent of the pathogen's nature, and their effects on offspring neural development is observed, where offspring show the three core symptoms of ASD (impaired communication, decreased social interaction, and repetitive/stereotyped behavior) (Kazlauskas et al., 2015).

Studies on germ-free mice revealed that basic neurodevelopmental processes are modulated by gut microbiota colonization, including neurogenesis, neuronal differentiation and survival, myelination, formation and integrity of BBB, development and maturation of microglia, expression of neurotrophins (signals for neuron survival), neurotransmitters and their receptors, apoptosis, and synaptic pruning (Sharon et al., 2016). Given that ASD has been associated with alterations in neural development, synaptic connections, microglial activations, and BBB integrity, disruptions or abnormalities in the modulation of these functions by the gut microbiota are likely to result in these neural characteristics of ASD. One mechanism whereby the gut microbiota may modulate neurodevelopment and behavior is via the effect of metabolic products on the CNS. Though no agreement has yet emerged on which microbial strains and metabolites are the most important for ASD, there are various findings and related theories that suggest an involvement of certain dysregulated gut microbiota and their metabolites in ASD symptoms. Given the findings of increased *Clostridia* species in stools of children with ASD compared to controls (Finegold et al., 2002), Finegold (2008) theorized *Clostridia* spores that are resistant to antimicrobial agents and germicides may explain the relapse of individuals with ASD

after drug treatment, and the unexplained increase in the incidence of ASD in recent years and in the same family, which would have important therapeutic implications. In a later study, Finegold and colleagues (2010) found *Desulfovibrio* to be significantly increased in stools of children with ASD compared to controls, upon which Finegold (2011) hypothesized about the importance of *Desulfovibrio* for ASD, again with implications for treatment and prevention. Though it is possible that these strains may not be solely responsible for the etiology or unexplained recent increase in ASD, they nonetheless may have a role in ASD, as their levels correlate with severity of ASD symptoms (Tomova et al., 2015).

Metabolic products of the gut microbiota, short-chain fatty acids (SCFAs), have also been implicated in ASD, with propionic acid (PPA) being the one receiving the most attention. PPA is produced by the ASD-associated bacterial strains of *Clostridia* and *Desulfovibrio* (MacFabe, 2015) and can cross both the gut barrier and the BBB and enter the CNS, where it can influence neurotransmitter release, inhibit gap junctions, stimulate secretion of pro-inflammatory cytokines (Kazlauskas et al., 2015) and modulate gene expression (Nankova et al., 2014). Intraventricular PPA infusions in mice resulted in social impairments, repetitive behaviors, increased oxidative stress, and activated microglia that indicate neuroinflammation, which suggest a role for PPA in changing brain and behavior in accordance with ASD symptoms (MacFabe et al., 2007; Shultz et al., 2008). In one study, SCFAs, especially concentrations of acetic, propionic, and butyric acid, were observed to be significantly higher in fecal samples of children with ASD compared to controls (Wang et al., 2012). In another study, SCFAs were observed to be lower, but PPA was observed to be higher, in fecal samples of children with ASD compared to controls (De Angelis et al., 2013). In yet another study, SCFAs including propionate was found to be lower in children with ASD compared to controls (Adams et al., 2011). It is important to note here the specification made by Adams et al. (2011) that stool analysis cannot determine the reason for changed amounts of SCFAs excreted, and if findings of

decreased SCFAs in the stool is due to increased absorption potentially due to increased gut permeability, this would result in more SCFAs in the blood and exacerbated ASD symptoms. Thus, not only measuring the levels of metabolites but understanding the reasons underlying their changed levels is important for making sense of such findings, similar to the conclusion of Kelly et al. (2015)that microbiota research needs to go beyond compositional assessments to understanding potential mechanisms via which gut dysbiosis contributes to disease pathophysiology. Though there are both reports of increased and decreased SCFAs in ASD, the significance of these differences regardless of their direction suggest a disruption in fermentation processes in ASD that may influence ASD symptoms and neurology, where PPA and other SCFA products of the gut microbiota can influence gene expression, synaptic plasticity, metabolic and immune pathways, and result in the neurological profiles reported in ASD (Slattery et al., 2016). Although there are controversies about the gut microbial profile in ASD and the most important metabolites in the etiology or symptomatology of ASD, the metabolic products of the dysbiotic gut of individuals with ASD may influence neural development or communication via both their effects on the brain and in combination with the immune system.

The neurodevelopment and behavior of offspring is influenced by the variations in the maternal microbial populations as well as the maternal immune activation during pregnancy, which modulates offspring microbiota, physiology, neurodevelopment, and behavior (Sharon et al., 2016). Amniotic fluids of children with ASD were shown to have significantly higher levels of inflammatory cytokines (Abdallah et al., 2013), and cohort study findings indicate that mothers of children with ASD are four times more likely to have brain-reactive antibodies than other women of child-bearing age, providing robust evidence for an increase in brain-reactive antibodies in mothers of children with ASD (Brimberg et al., 2013). These anti-fetal brain autoantibodies can cross the placenta during gestation and can bind to the fetal brain proteins, whereby they can interfere with important neurodevelopmental mechanisms and these maternal

anti-brain autoantibodies have been identified as one risk factor for developing ASD (Fox-Edmiston & Van de Water, 2015). Cytokines and chemokines not only coordinate inflammatory responses but also mediate normal, ongoing communication between non-immune cells including the CNS, like major histocompatibility complex family members that are involved in cell-mediated immunity and that also play an important role in activity-dependent brain development and plasticity besides their immunoregulatory functions (Young et al., 2016). Similarly, TGF-β1 is an anti-inflammatory cytokine that is also involved in brain development and glial function in addition to its role in controlling immune responses. The levels of this cytokine are altered in post-mortem tissues of individuals with ASD, implicating TGF-β1 in the abnormal neural development leading to ASD neural basis and behaviors (Kazlauskas et al., 2015). Likewise, microglia that are the resident immune cells of the CNS do not only contribute to inflammation but also to neurodevelopment, where deficits in microglial activity during brain development undermines mature synapse formation, which can lead to increased immature synapses and to possibly cognitive and ASD-like behavioral impairments (Madore et al., 2016). The microglia activation observed in brains of individuals with ASD can result in focal brain inflammation and damage normal synaptic connectivity (Patel et al., 2016). So, microglia that are commonly known for their immune functions can modulate developmental brain wiring via their effects on synaptic pruning and formation of mature synapses, and contribute to the neural profiles seen in ASD. Thus, gut dysbiosis and immune dysregulation can have adverse effects on neurodevelopment and can potentially result in the assumed neural basis of ASD.

Leaky barriers. Another common principle of effect of gut microbiota and inflammation on ASD neurology may be via intestinal and BBB integrity. Evidence for a leaky gut in ASD comes from findings of increased intestinal permeability in children with ASD and their relatives compared to typically developing controls (D'Eufemia et al., 1996; de Magistris et al., 2010);

significantly higher plasma levels of the intestinal permeability-modulating protein zonulin in children with ASD compared to typically developing controls, where levels of zonulin positively correlate with the severity of ASD symptoms (Esnafoglu et al., 2017); and decreased abundance of the mucin-degrading bacterium *Akkermansia muciniphila* pointing to a thinner mucus barrier than controls (Wang et al., 2011a). There are also studies failing to support increased intestinal permeability in ASD, yet some methodological concerns undermine the validity of their findings. Kushak et al. (2016) observed increased intestinal permeability in children with ASD that did not reach statistical significance, yet both their ASD and control group comprised of children suspected of having GI disorders who had clinical indications for an endoscopy or colonoscopy, suggesting that a leaky gut may also have been present in their "control" group suffering from GI symptoms, thus preventing the trend of increased intestinal permeability in children with ASD from reaching statistical significance. Another study with a questionable control group is by Dalton et al. (2014) who did not find statistically significant group differences in small intestine permeability of children with ASD and special education needs (SEN), a broad group comprising children not meeting the criteria for ASD and having a variety of other diagnoses including ADHD, intellectual disability, cerebral palsy, and language disorders. Because the control group also includes children with atypical instead of typical development, findings of this study can as readily be interpreted as indicating leaky gut in other disorders besides ASD, which is corroborated by evidence for intestinal permeability in stress-related psychiatric disorders (Kelly et al., 2015). Given that compared to controls, 75% of the ASD sample show reduced expression of intestinal barrier-forming tight junction components and 66% of the ASD sample have increased pore-forming claudins (Fiorentino et al., 2016), the current evidence suggests increased intestinal permeability in at least some subgroups of individuals with ASD compared to typically developing individuals. Alternatively, as suggested earlier, intestinal permeability may be present at a developmentally critical early period, and this permeability may persist only in some subgroups but not in all individuals with ASD across life.

The association between intestinal inflammation and permeability has been known for some time (Ramage et al., 1988) and is present in other conditions such as Crohn's disease, an inflammatory disorder of the intestine with unknown causes (Hollander et al., 1986; Teahon et al., 1991), suggesting a role for inflammation in intestinal permeability. Children with ASD who have GI symptoms show an increased pro-inflammatory cytokine and a decreased regulatory profile compared to typically developing children (Ashwood et al., 2004), and exhibit a unique pattern of blood and mucosal cytokines and mucosal lymphocyte density, indicative of significant immune dysregulation (Torrente et al., 2002; Ashwood & Wakefield, 2006). Studies investigating intestinal inflammation in individuals with ASD mostly use samples being tested for GI problems due to ethical constraints of performing invasive operations on asymptomatic children (Ashwood et al., 2003), an exception being de Magistris et al. (2010) who found no correlation between presence of GI symptoms and abnormal intestinal permeability values in children with ASD. Thus, it is likely that the inflammation present in the intestines of children with ASD may relate to intestinal permeability in these individuals.

Animal findings indicate that the composition of the gut microbiota not only shapes the intestinal mucous barrier and its permeability (Jakobsson et al., 2015), but also influences the integrity of the structurally similar blood–brain barrier (BBB), where the gut microbiota affects the prenatal development BBB and its permeability later in life (Braniste et al., 2014). Similarly, exposure to prolonged inflammation in early development results in increased BBB permeability in rats (Stolp et al., 2005), indicating a role for inflammation in BBB as well as the intestinal barrier. Modifying the gut microbiota in a mouse model of obesity decreased the expression of inflammatory markers and improved gut barrier functions (Cani et al., 2009), pointing to the interactive effects of inflammation and gut microbiota on intestinal permeability. Increased prevalence of *Clostridia* and decreased prevalence of *Bifidobacteria* observed in some

individuals with ASD have been proposed to lead to an abundance of pro-inflammatory cytokines, decreasing the ability to resolve inflammation after a threat, and resulting in prolonged gut permeability and possibly tissue damage, which also exacerbates gut permeability (Heberling et al., 2013). This proposed pathway, partial support for which comes from the finding that toxins produced by *Clostridia* increase human intestinal epithelial cells by augmenting tight junction permeability (Hecht et al., 1988), is in line with our proposition that intestinal and blood–brain barrier effects may be one of the common mechanisms of actions for the gut microbiota and inflammation on ASD neurology.

The increased gut permeability in ASD can enable the leakage of microbial products with inflammatory properties including opioid peptides derived from the digestion of dietary products such as gluten and casein and lipopolysaccharide (LPS) that is a bacterial cell wall component with strong pro-inflammatory properties, which can instigate immune reactions that can then influence the brain, given the altered regulation of BBB genes in ASD. Such a mechanism has been biologically demonstrated with amyloid beta peptides that cross a defective BBB and interact with neurons but not glial cells in the brain (Clifford et al., 2007). Opioid peptides have been proposed to cross the BBB and produce ASD symptoms that resemble the actions of opioids on the brain (Panksepp, 1979; Whiteley & Shattock, 2002). LPS activates liver cells to produce TNF-α that induces pro-inflammatory cytokine production in the liver, serum, and the brain, leads to brain microglial activation, and results in a progressive loss of dopamine neurons (Qin et al., 2007). This finding, along with the presence of increased levels of TNF-α secreting cells in intestines with inflammation (Breese et al., 1994), suggests one potential pathway for peripheral inflammation to influence neuroinflammation and neural profiles in ASD, where increased levels of pro-inflammatory cytokines either resulting from intestinal inflammation or systemic inflammation may be interfering with microglial activation and neuronal development. If peripheral inflammation does indeed increase brain TNF-α levels (Qin et al., 2007), then it may

also influence BBB integrity, since the TNF-α levels in the brain modulate BBB permeability (Kim et al., 1992). However, there may be another pathway of influence that is independent of brain TNF-α levels. Peripheral TNF-α has been shown to mediate the communication between the peripheral immune system and the CNS in a mouse model of Alzheimer's disease (AD) where systemic and local brain inflammation are implicated, and to be able to affect the brain via regulating peripheral inflammation, independent of brain TNF-α levels (Paouri et al., 2017). Pardo et al. (2017) found no differences in TNF-α levels between the serums of ASD and control subjects, but Jyonouchi et al. (2001) found individuals with ASD to produce higher levels of TNF-α compared to controls, suggesting excessive innate immune response patterns in certain children with ASD which may not be present in all individuals with ASD. Thus, the AD mechanism of peripheral TNF-α inducing changes in the brain may not be directly applicable to all individuals with ASD but be present in certain subgroups with elevated immune profiles, or as previously noted, may be active in a developmentally sensitive earlier period to exert its effects on the neurology of ASD, but may not be persistently active in later years. Additionally, there may be a broader mechanism of peripheral monocytes regulating microglial responses in ASD via other inflammatory molecules or gut microbiota products.

Gut permeability is thus implicated as a possible common principle whereby gut microbiota and inflammation can act on ASD neurology. ASD-susceptibility genes targeted by environmental and endogenous toxicants are abundant in the barriers of the body, including not only the intestinal barrier but also the BBB (Carter & Blizard, 2016), and genetic markers of autism susceptibility include genes encoding neuronal cell-adhesion molecules (Korkmaz, 2013a). ASD subjects show altered expression of genes associated with BBB integrity and function, which is coupled with increased neuroinflammation and is specific to ASD as opposed to schizophrenia, another neurological disorder where immunity abnormalities are implicated (Fiorentino et al., 2016). Since this differential BBB integrity is associated with

neuroinflammation and the gut microbiota are implicated in the formation and integrity of the BBB (Braniste et al., 2014), another common mechanism of action for gut microbiota and inflammation on ASD neurology may be their effects on BBB or ASD-susceptibility genes encoding barrier proteins, which can enable the passage into the brain of environmental toxins or endogenous metabolites/hormones that can target ASD genes and result in the neural profile of ASD.

Limits to gut and immune effects on ASD neurology. The gut microbiota and inflammation provide important real-life information to the nervous system about the environment and influence its development (Sharon et al., 2016), and such information controls certain developmental processes as neurogenesis, barrier function, and immunity. However, although specific neural pathways evolved to respond to the cues of the gut microbiota, other pathways are unaffected by it and are influenced only by genetic or other environmental cues (Sharon et al., 2016). Several neuronal circuits may be responsible for ASD behaviors that have different sensitivities to the effects of gut microbiota (Kazlauskas et al., 2015). Moreover, the existence of studies failing to show atypical inflammatory activity in ASD and neuroinflammation being a common finding in other neurological disorders question the specificity of inflammation as a mechanism contributing to the emergence of ASD and whether it is a causal or reactive process (Young et al., 2016), and a similar argument can be made for the gut microbiota.

Since symptoms result from two opposing processes of pathological functioning and compensatory strategies aimed at reducing this dysfunction (Korkmaz, 2013b), a possible compensatory role should also be considered when interpreting abnormal gut microbiota and inflammation findings. Fiorentino et al. (2016) found increased levels of some BBB tight junction

proteins in individuals with ASD, and interpreted this as either a compensatory mechanism to repair the compromised BBB or as the inability of mutated BBB proteins to be integrated into the barrier leading to a sustained compensatory gene expression and accumulation of this protein. In a parallel vein, Pardo et al. (2017) propose that their findings that do not support active inflammation in ASD may indicate that the previous observations of brain cytokine and chemokine increases may actually reflect homeostatic non-inflammatory processes in response to CNS dysfunction. Under this light, the knowledge of autoantibodies having a neuroprotective role following tissue damage (Wills et al., 2007) may offer a compensatory explanation to the elevated levels of autoantibodies found in some individuals with ASD. So, higher levels of inflammation should not always be attributed a pathological role but should also be considered as potential reactions or compensatory mechanisms against some other dysfunction in ASD.

Although these two factors can contribute to the assumed neural basis of ASD, future investigations need to clarify how they specifically lead to ASD neurology/symptomatology as opposed to other neurological disorders with gut dysbiosis and inflammation. While the gut microbiota and inflammation can provide important cues and shape certain aspects of neurodevelopment, it would not be reasonable to purport them as the sole determinants of the neural basis of ASD.

Probiotics and ASD

Probiotics refer to products that contain live microorganisms, which have beneficial and desirable effects on humans and animals when provided in adequate amounts (FAO/WHO, 2001). Probiotics were shown to be effective treatments for certain childhood disorders associated with GI symptoms and inflammation, such as necrotizing enterocolitis and infantile colic (Lin et al., 2008; Xu et al., 2015). A role for probiotics has been suggested for children with ASD as well, as preliminary findings from mice and human studies provide some evidence that probiotics may

improve the gut microbial, gastrointestinal, and behavioral abnormalities in children with ASD (Navarro et al., 2016; Slattery et al., 2016).

Assumed Mechanisms of Probiotic Action

The testing of probiotics for ASD is still in its infancy and much remain unknown about their effects and mechanisms of action. The only notable publication listing potential mechanisms of actions via which probiotics can help individuals with ASD is the review by Critchfield et al. (2011), which is summarized here. Firstly, given the similarities of GI symptoms between patients with IBS and those with ASD, and the recent successes in treating IBS with probiotics, one mechanism of action of probiotics may be improving GI symptoms such as bloating, bowel movement difficulty, and abdominal pain. Secondly, *Clostridium* species are elevated in children with ASD and their high levels are associated with GI problems, and treatment with a drug targeting *Clostridium difficile* improved behavior and communication in children with ASD during treatment period but not after it was discontinued, implicating correction of gut microbiota balance as another mechanism of action of probiotics. Thirdly, the increased intestinal permeability in ASD may be ameliorated by probiotics, which are able to stabilize the mucosal barrier by reducing bacterial overgrowth, synthesizing antioxidants, increasing mucin expression, and stimulating mucous immunity, and short-term exposure to probiotics has been shown to enhance epithelial tight junction proteins in humans. Lastly, given the multiple findings of aberrant immune activation in a subset of individuals with ASD, and that a major part of the immune system is concentrated in/around the intestinal mucosa, that the gut microbiota play an important role in the maturation and the regulation of the immune system, another mechanism of action of probiotics may be on immune system. Probiotics can modulate the immune system in a species- and strain-specific manner, but these anti-inflammatory effects of probiotic strains are mainly observed in vitro and authors note that their effects on

systemic cytokines and interactions with other gut microbiota remains to be determined (Critchfield et al., 2011).

Some of these proposed mechanisms are corroborated by the seminal study on the effect of probiotics on ASD symptoms and physiology by Hsiao et al. (2013)on a MIA mouse model. This study showed that offspring of immune-activated mothers show increased gut permeability, abnormal intestinal cytokine levels, and gut dysbiosis driven by changes in *Clostridia* and *Bacteroidia*, which show that in addition to the behavioral and neuropathological ASD features, this mouse model shows inflammatory, microbial, and gut permeability profiles similar to those reported in subsets of individuals with ASD. Treatment with the probiotic *Bacteroides fragilis* corrected intestinal permeability in MIA offspring and restored the increases in the pro-inflammatory cytokine IL-6 in the colon, but not other cytokines, revealing a specificity for IL-6. Moreover, probiotic treatment significantly restored the relative abundance of 6 out of 67 bacterial species units that discriminated MIA from control offspring, suggesting that *B. fragilis* partly ameliorates the gut dysbiosis associated with the MIA mouse model of ASD. Finally, this probiotic improved communicative, repetitive, sensorimotor, and anxiety-like behavioral abnormalities of MIA offspring, but it did not affect their deficits in sociability and social preference, leading the authors to suggest that social behaviors may be governed by a different circuity and *B. fragilis* may only modulate specific circuits when improving ASD-related behavioral impairments. They found that the effect of *B. fragilis* on ASD behaviors was also observed in a treatment with *Bacteroides thetaiotaomicron*, but not when treated with *Enterococcus faecalis*. Thus, this study shows that probiotic treatment can help ASD symptoms by reducing inflammation, improving the gut permeability, restoring microbial imbalances, and ameliorating non-social ASD symptoms. However, the mechanisms whereby this probiotic improves behavior have not been speculated on by the authors and remain to be discovered.

Potential Links between Probiotics and Neurodevelopment

Since the probiotic research on ASD is still nascent, there is no evidence or propositions of how the mechanisms of effect of probiotics may relate to neurodevelopment. Using the possible common mechanisms of action for gut microbiota and inflammation on ASD neurology discussed above, we can make certain postulations (Figure 1).

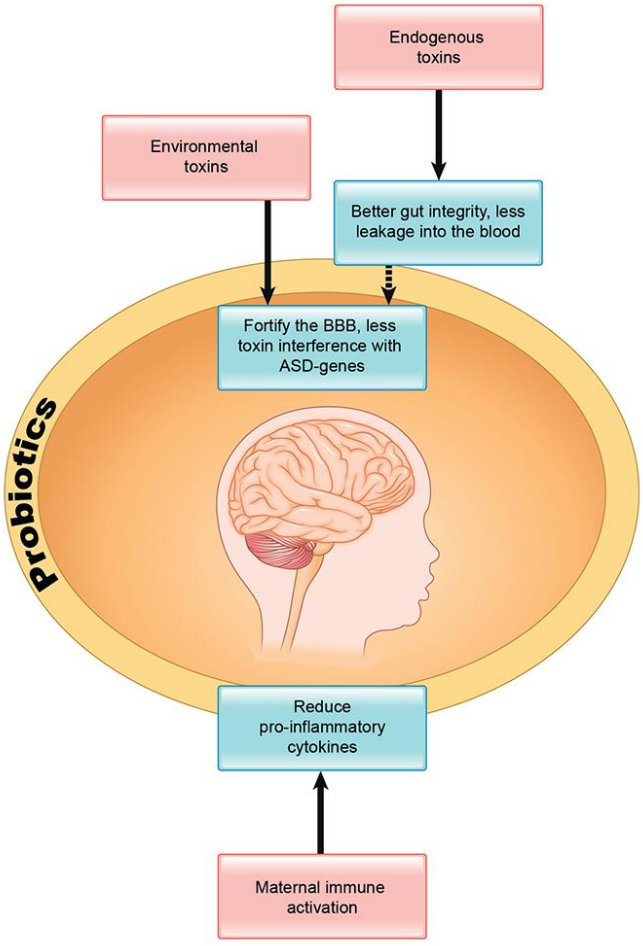

Figure 1. Potential mechanisms of action for probiotics on neurodevelopment in ASD. Probiotics may protect neurodevelopment by fortifying the blood–brain barrier, which would prevent both endogenous and environmental toxins from reaching the developing brain; by improving gut integrity to provide an additional barrier for endogenous toxins before reaching the brain; and by reducing maternally or fetally derived pro-inflammatory cytokines that may otherwise target ASD-genes and neurodevelopment in the developing brain.

First, Hsiao et al. (2013) found that *B. fragilis* improved the increase in the pro-inflammatory cytokine IL-6 in the colon. Systemic inflammation can influence the brain, one mechanism for which was discussed above for the case for peripheral TNF-α modulating microglial responses in the brain. Thus, if the mothers of children with ASD experience infections during critical periods of pregnancy, this systemic inflammation can influence the neural development of their children, but if probiotics such as *B. fragilis* and others discovered to influence pro-inflammatory cytokines other than IL-6 are administered during these maternal inflammatory periods, they could prevent brain inflammations and, if MIA models of ASD are accurate representations of etiological processes in at least certain subgroups of ASD, also prevent ASD symptomatology. In a similar vein, Young et al. (2016) have suggested that maternal immune activation can result in increased pro-inflammatory cytokine levels and decreased anti-inflammatory cytokine levels in the placenta and amniotic fluid, and if the actions of these pro-inflammatory cytokines are blocked during maternal infection, development of ASD-like behavior may be prevented in offspring. Whereas they allude to interventions blocking the effects of inflammatory cytokines during maternal infection, we propose using probiotics to normalize the aberrant immune responses in the prenatal or early postnatal period for children at risk for having ASD, which is corroborated by recent findings showing probiotic administration to correct the metabolomic disruption and reduce multiorgan inflammation in mice (He et al., 2017).

Second, gut microbiota has important roles in neurodevelopment from neurogenesis to myelination, from synaptic pruning to neurotransmitter and receptor generation. If similar to Hsiao et al. (2013)'s findings, probiotics can restore the gut dysbiosis that may be due to genetic reasons or other reasons unidentified thus far in individuals with ASD during gestation, then they can prevent the harmful effects an imbalanced gut microbiota can have on neural development. Third, Hsiao et al. (2013) found that *B. fragilis* treatment improved MIA-

related changes in the expression of certain claudins, which are tight junction proteins. This study looked at changes in the gut, but it may be possible that probiotics may have similar effects on tight junction proteins of the BBB since Fiorentino et al. (2016) found both altered expression of genes associated with BBB function and integrity, and increased pore forming and decreased barrier forming tight junction component expressions in guts of ASD individuals, which is also supported by findings that gut microbiota plays an important role in the formation and maintenance of BBB. Thus, the environmental toxicants that are shown by Carter and Blizard (2016) to selectively target ASD-susceptibility genes may be kept out of the brain if the BBB is fortified with probiotic administration, which would prevent the targeting of ASD-susceptibility genes and inhibit the initiation of a cascade of genetic expression events that may result in the neural profile of ASD.

Support for this proposed pathway of action comes from the finding that early postnatal probiotic treatment decreases ASD risk. Pärtty et al. (2015) showed that from children randomly assigned to probiotic or placebo groups during the first six months of life, none in the probiotic and 17% in the placebo group was diagnosed with ASD or ADHD. The authors note that the effect of probiotic administration on CNS is likely not via alterations in the microbiota composition, since no single microbiota composition, or the difference thereof, was observed in children with or without neuropsychiatric disorders and probiotics had no significant effect on microbiota composition (Pärtty et al., 2015). As suggested presently, probiotics may be exerting their effects on the CNS via other mechanisms such as reducing inflammation or fortifying barriers, or they may correct the imbalance in the activity of the gut microbiota without changing its composition, by potentially correcting the over-production of harmful and under-production of beneficial gut bacterial products.

In our model, we did not include the gene expression mechanism of action that we postulated for gut microbiota and inflammation effects on ASD neurology for probiotics because of the lack of evidence for such a relationship so far. Findings from animals that probiotic administration systematically influences global gene expression in cows including immunity and homeostasis genes (Adjei-Fremah et al., 2017) and leads to higher expression of intestinal mucin genes in chicks (Aliakbarpour et al., 2012), and from human bacteria transplanted mice that probiotics change the expression of gut microbiome-encoded enzymes involved in metabolic pathways such as carbohydrate metabolism (McNulty et al., 2011) suggest that future investigations can show a potential role for probiotics in gene expression. The present evidence in humans only indicates effects on the expression of intestinal genes, and such a function would be encompassed by the probiotic effects on gut barrier integrity included in the model, so an additional genetic expression path was not added until future evidence shows whether probiotics can also influence gene expression in the brain and thereby affect ASD neurology. Such a discovery would be promising for preventive and restorative treatments for neurological disorders such as ASD, if their complete mechanisms of actions on gene expression can be delineated and no harmful effects are detected on the expression of other vital genes.

Probiotics Over Fecal Microbiota Transplantation for ASD

Given the gut microbiota abnormalities and GI symptoms in ASD, and the effectiveness of fecal microbiota transplantation (FMT) in treating *Clostridium difficile* infection, this procedure of delivering the fecal microbiota of a healthy individual to one with gut dysbiosis has been suggested for use in individuals with ASD (Li et al., 2017). In 18 children with ASD, the modified FMT procedure microbiota transfer therapy, which includes an initial antibiotic treatment, bowel cleansing, and extended fecal microbiota transplant, resulted in 80% reduction in GI symptoms and significant improvements in ASD symptoms (Kang et al.,

2017). However, following this administration, the abundance of *Desulfovibrio* increased in the gut of children with ASD, which has been previously implicated in causing or exacerbating ASD symptoms (Finegold et al., 2010; Finegold, 2011), which suggests that indiscriminately administering the gut microbiota of healthy individuals to those with ASD may lead to the increase of some bacteria that may in fact be harmful to individuals with ASD. Moreover, FMT has been shown to have some side effects that may exacerbate the GI symptoms experienced by many individuals with ASD, where after infusion of donor feces, 94% of patients had diarrhea, 31% had cramping, and 19% had belching, and in follow-up, 19% had constipation (Van Nood et al., 2013), which undermine the suitability of FMT for individuals with ASD. In a recent consensus conference on the use of FMT in clinical practice, no evidence-based recommendation emerged to use FMT in clinical practice but that its use should be limited to research purposes (Cammarota et al., 2017) and Navarro et al. (2016) state that they do not believe that this treatment will have a role in the treatment of GI symptoms of individuals with ASD. Thus, given its questionable effects on gut microbiota strains implicated in ASD, its side effects that are likely to exacerbate GI symptoms in ASD, and concerns about its safety currently preclude FMT from being considered as a feasible intervention for individuals with ASD, at least until these issues are satisfactorily addressed.

We have proposed earlier that it is possible that intestinal, inflammatory or metabolic abnormalities in ASD may break down the body's tolerance to normal gut flora similar to the case in other disorders (Macpherson et al., 1996). If this proposition if valid, then simply administering healthy gut flora without targeting the physiological mechanisms that are leading to intolerance to normal gut flora may not be the optimal solution. Probiotics have the potential to address the intestinal, inflammatory or metabolic dysfunctions that may be resulting in this intolerance, and to decrease the commonly experienced GI problems in ASD. Improving GI symptoms has been associated with improvements in ASD symptoms (Horvath et al., 1998). Yet,

though GI symptom severity correlated with ASD symptom severity, levels of *Desulfovibrio* and *Clostridia* were positively associated with ASD symptom severity but not with GI symptoms, suggesting that these strains may be involved in ASD but not in GI symptoms (Tomova et al., 2015), and that the gut microbiota may have effects on ASD neurology independent of its effect on GI symptoms. Thus, the mechanism of action for improvements in GI decreasing ASD symptoms that explains the association between GI and brain function in individuals with ASD is more likely to be the restoration of intestinal permeability or normal excretion of gut products, enabling the gastrointestinal tract to eliminate compounds harmful to the CNS (Horvath et al., 1998) and not a direct effect of reducing GI symptoms on ASD neurology. This line of evidence again points to probiotics, which can improve the physiological and metabolic abnormalities in the gut, as a more effective treatment option than the FMT procedure of simply administering normal gut microbiota, which may serve to decrease GI symptoms but lack the beneficial agents contained in the probiotics or increase levels of bacteria associated with ASD symptom severity such as *Desulfovibrio* (Kang et al., 2017).

Thus, probiotics emerge as a safer alternative than FMT for modulating the gut microbiota of individuals with ASD that is devoid of the side effects of FMT and is likely to have multiple beneficial effects for the various physiological dysfunctions observed in ASD.

Gaps in the Literature

There seem to be five main gaps in the literature on the gut microbiota and inflammation effects on ASD. First, the molecular mechanisms for the remote effects of the gut microbiota on brain physiology are still not known, and future investigations are called for to find specific microbial factors, immune functions, and microbiota-immune pathways that are involved in modulating brain function and behavior (Fung et al., 2017). Though the present discourse aimed

to propose some common mechanisms of effect for gut microbiota and inflammation on ASD neurology, the literature lacks any concrete knowledge about how such distant processes can influence brain physiology. Second, the biological mechanisms of action of probiotics are not established and are only speculated upon using the existing knowledge about the roles played by probiotics in normal physiology, and thus how probiotics lead to behavioral restorations in ASD animal models are still unknown. Third, although it is known that probiotics implement strain-specific effects, knowledge about the molecular, microbial, immunological, neurological, and behavioral consequences of different probiotics on the host is lacking. For instance, *B. fragilis* affects communication, stereotypic, anxiety-like, and sensorimotor abnormalities but not sociability and social preference deficits (Hsiao et al., 2013), whereas *Lactobacillus reuteri* corrects the social deficits induced by maternal high-fat diets (Buffington et al., 2016). Understanding which behavioral improvements are induced by different strains of probiotics and their different pathways of effect on neurology and behavior can help elucidate the neural pathways open to modification in ASD, which can provide important therapeutic information. The fourth gap is the lack of practices or measures of identification of ASD subgroups who exhibit GI symptoms and immune abnormalities that can benefit from treatments directed at these conditions (e.g., probiotics aimed at restoring the gut epithelium and normalizing the pro-inflammatory profiles). And the fifth gap is the lack of definitive knowledge of whether these abnormalities are accompanied by other more dominant (e.g. more systemic or metabolic) dysfunctions that may override any treatment directed at the gut microbiota or inflammation, which could undermine any treatment created with the aim of ameliorating the GI and inflammatory atypicalities in ASD. For instance, both inflammation/immune dysfunction (Rossignol and Frye, 2014) and higher prevalence of GI problems (Rossignol and Frye, 2012b) have been associated with oxidative stress and mitochondrial dysfunction in ASD, which may implicate stronger and broader mechanisms of action on ASD neuropathology and symptomatology than inflammatory or GI problems.

Conclusion

In this review, we parted from the previous models considering immunity as one of the pathways in the gut-brain axis and took a different perspective investigating potential common mechanisms of the gut microbiota and inflammation on neural profiles in ASD. We proposed that both may lead to their effects on ASD neurology via targeting ASD-susceptibility gene expression, influencing neurodevelopment, and the intestinal and blood–brain barrier. We showed how these mechanisms can also shed light on possible mechanisms of actions of probiotics on neurodevelopment in ASD, which can elucidate if they may be used pre-emptively to prevent the effects of maternal inflammation and prenatally induced gut dysbiosis in resulting in ASD neurology.

There are many unknowns in ASD research, the most fundamental being the enigma of its etiology. Many different treatments have been proposed and tested for individuals with ASD, without much knowledge of their mechanisms of effect. If pathways whereby probiotics improve ASD behaviors can be elucidated, treatments that provide the most benefits with least challenges to administer for parents and their children with ASD can be formulated. For instance, dietary interventions are very popular and reported effective by some studies, yet they are very restrictive and difficult to implement. These diets try to eliminate any substance that may create inflammation, allergies, or other reactions in the body. Yet, the reason that dietary products are leading to these symptoms in the first place may be the leaky gut and the lack of healthy microbiota that digest and absorb them appropriately (Campbell-McBride, 2008). So, instead of restricting most of the foods in a regular diet, if the body's potential to digest and contain these products within the gut is fortified via probiotics that increase the colonization of healthy bacteria and improve the gut barrier, then these dietary products may not be allergenic, and other endogenous toxins such as LPS from bacterial walls can either be decreased by correcting

the gut dysbiosis and decreasing LPS-producing bacteria, or preventing LPS from passing into the blood.

Recent evidence suggests that an immune signaling pathway may regulate social behavior (Bordon, 2016), and *L. reuteri* correcting the social deficits resulting from maternal high-fat diets (Buffington et al., 2016) implicates gut microbiota in the modulation of social behaviors, but the mechanisms of action of either are unclear. Elucidating the pathways between the gut microbiota, immunity, and the social behavior changes induced by them can help formulate more informed biological treatments for the social deficits in ASD. If social behaviors can be found to be in fact modulated by gut microbiota or immune pathways regardless of their initial etiology, such treatments may correct social behaviors independent of the specific etiology or combination of different instigating factors (e.g., maternal immune activation, genetics, toxin exposure) in different ASD cases, and can help broader ASD populations, overcoming the need to identify specific subgroups and opening the way to restorative treatments for most individuals with ASD.

CHAPTER III

ERROR-MONITORING IN ASD

Error Monitoring in Decision-Making and Timing is Disrupted in Autism Spectrum Disorder

Abstract

Individuals with autism spectrum disorder (ASD) have difficulties in social interactions. The smooth navigation of social interactions is supported by the abilities of perceptual decision-making, timing, and error-monitoring, which enable one to appropriately understand and react to the other individual in communicative settings. This study constitutes a comprehensive exploration of decision-making and interval timing in ASD as well as the first investigation of error-monitoring abilities of individuals with ASD regarding their first-order performance in the corresponding domains. We found that children with ASD fared similar to typically developing (TD) children in their first-order task performance in two-alternative forced choice perceptual decision-making task and temporal reproduction task. Yet, they had a deficit in error-monitoring in both tasks where their accuracy did not predict their confidence ratings, which was the case for the TD group. The difference between ASD and TD groups was limited to error-monitoring performance. The first-order performance in the primary tasks and secondary tasks (signal detection and free finger tapping tasks) did not differ between the two groups. This study attests to a circumscribed impairment in performance monitoring in individuals with ASD, which may partially underlie their social interaction problems. This difficulty in cognitively evaluating one's own performance may also relate to theory of mind deficits reported for individuals with ASD, where they struggle in understanding the mental states and intentions of others.

Keywords: autism spectrum disorder, error-monitoring, metacognition, decision-making, time perception.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and communication, and repetitive behaviors (American Psychological Association, 2013). Three cognitive domains that have important implications for the smooth execution of social interactions seem to be decision-making, interval timing, and error-monitoring. Specifically, one needs to be able to decide when to take turns during the conversation given visual (e.g., facial expressions) and auditory cues (e.g., pauses) from the other person so that they can react properly (Barraclough et al., 2005); to produce their response after an appropriate amount of time during communication and pace their speech understandably (Lambrechts, Falter-Wagner, & Wassenhove, 2018); and to notice their errors in these perceptual and temporal judgments in order to correct them in their next turn in conversation or social settings (Sokhadze et al., 2010). Such performance monitoring is an important component of metacognition (Fleming & Dolan, 2012; Garofalo & Lester, 1985). Metacognition in decision-making refers to both error-monitoring and confidence, and characterizes the ability of individuals to be aware of their mistakes in the absence of explicit feedback and give confidence ratings in line with their objective performance (Yeung & Summerfield, 2012). Importantly, recent work has shown that typically developing (TD) individuals are not only aware of the errors they commit but also their magnitude and direction in paradigms that enable such quantitative characterizations (Akdoğan & Balcı, 2017).

In the present study, we combined the three domains of decision-making, timing, and error-monitoring, which have remained as segregated research topics in ASD up until now, in order to take a more comprehensive approach to understanding cognitive processing in ASD. Our design encompasses both perceptual decision-making and timing, and investigates performance monitoring of children with ASD in these two domains. This way, we aimed to test whether potentially different metacognitive abilities of individuals with ASD generalize to multiple

cognitive domains. The integrative empirical approach adopted in this study can be helpful in understanding which of these cognitive skills needed for the successful communication and possibly in other functions are impaired in individuals with ASD.

Perceptual Judgments in ASD

Processing of visual information has been a topic of interest in the ASD population, where individuals with ASD have been repeatedly observed to focus on details, starting from Kanner (1943)'s original description of children with ASD including their repetitiousness and insistence on sameness. Yet, more refined experiments show that the deficits observed in individuals with ASD in stimulus processing can be better explained by stimulus complexity rather than local-global processing, as individuals with ASD were superior in identifying the orientation of simple gratings but impaired in identifying the orientation of complex gratings, which has been attributed to a problem in neuro-integrative function (Bertone et al., 2005). Similarly, although a difference between ASD and control participants was found in random dot motion (RDM) discrimination, this deficit was shown to be better explained by IQ (Koldewyn, Whitney, & Rivera, 2010). This study attests to the importance of having a homogeneous group of participants with ASD, which was a major consideration in the present study.

Along these lines, a recent study showed that although looking only at behavioral data would suggest reduced perceptual sensitivity in individuals with ASD (based on slower response times - see also Bogte et al., 2007), the computational decision theoretic approach to performance that can separate perceptual sensitivity from other components of decision-making did not support this conclusion (Pirrone et al., 2017). Specifically, Pirrone et al. (2017) showed that slower response times of participants with ASD resulted from their more cautious decision-threshold setting and longer delays in signal-detection/motor response rather than weaker perceptual integration. These results point to the importance of theoretic approaches to behavioral

data for delineating the components of observed differences, which was achieved by the application of ex-Gaussian fits in the current study.

Interval Timing in ASD

Timing deficits in ASD have been reported by parents who see their children as having a qualitatively poor sense of time (Allman, DeLeon, & Wearden, 2011). Yet, there is experimental evidence showing both similar performance to controls by individuals with ASD (Falter et al., 2012; Jones, Lambrechts, & Gaigg, 2017; Wallace & Happe, 2008) and impaired performance (Allman et al., 2011; Szelag et al., 2004). One potential confound resulting in such different results may be task instruction complexity, which was intended to be minimized in the present study. This possibility aligns with Russell et al. (1999)′s demonstration that individuals with ASD are not in fact impaired in executive function tasks when the task contains no arbitrary or novel rules. These authors suggest that individuals with ASD struggle with executive tasks primarily because they are less likely to encode the rules verbally.

Thus, there is still relatively little evidence on temporal abilities in individuals with ASD, which are contradictory in nature (Lambrechts et al., 2018). A recent study with adults with ASD found them to be relatively unimpaired in temporal bisection with visual stimuli, and call for future work with children with ASD (Jones et al., 2017), which is taken on in the present paper by investigating temporal reproduction and free finger tapping of children with ASD using visual stimuli.

Error-Monitoring in ASD

In individuals with ASD, differences in error-related behaviors have been noted compared to those of neurotypical individuals. For instance, Brosnan et al. (2016) probed math performance

monitoring, asking if participants thought they got the answer to a math problem right or wrong, and found that individuals with ASD were more likely than TD participants to think that they got an incorrect answer correct. In another related domain, some studies have investigated metamemory. Metamemory, the knowledge of and ability to monitor own memory processes, was found to be impaired in children and adults with ASD (Cooper et al., 2016; Grainger et al., 2014; Grainger et al., 2016). Beyond performance monitoring, Sokhadze et al. (2010) investigated brain signals corresponding to error related processing; error-related negativity (the neural activation occurring 50-100ms after committing an error, which reflects initial automatic error detection processes) and positivity (neural activation occurring 200-500ms after committing an error, which reflects conscious error recognition and comprehension). The differences they observed in brain signals of individuals with ASD from TD controls suggested insensitivity in the detection and monitoring of errors in ASD. On the other hand, more recently, Hüpen et al. (2016) reviewed the existing evidence in individuals with ASD on error-related negativity. They concluded that a relation between ASD symptoms and ERN is inconclusive and noted that ASD symptomatology per se may not be directly related to ERN amplitude.

Post-error behavioral adjustment is a process presumably related to error processing, and individuals with ASD have been reported not to correct their errors after learning the outcomes of their actions (Russell & Jarrold, 1998). Additionally, they have been observed not to show the post-error slowing that characterizes the response of TD individuals after an error in one study (Bogte et al., 2007), but to show similar post-error slowing with controls in another (South et al., 2010), and yet even to show post-error acceleration in another where TD group exhibited post-error slowing (Sokhadze et al., 2010). Given these contradictory findings, there is a need for more specific measurements that take into consideration both post-error behavioral adjustments and explicit awareness to bring some clarity to the topic of error monitoring in individuals with ASD, which is taken up in the present study.

Present Study

Moving toward a more comprehensive understanding of the cognitive abilities of individuals with ASD and toward finer distinctions in such abilities and impairments, we investigated performance and error awareness in two different tasks in individuals with ASD and TD controls. We compared children with ASD and TD children in the two-alternative forced choice (2AFC) RDM discrimination task and temporal reproduction task. In the former task, responses were given in a binary manner where the judgment regarding the direction of motion is either correct or not, and in the latter task, responses were parametric where there was a numerical range of proximity of reproduction times to the target duration.

First, we used the RDM discrimination task with the addition of confidence questions after each choice, inquiring how confident the participant is of the correctness of their decision. In relation to this 2AFC task, we also assessed if the potential slower responses of children with ASD could be due to their slowness in signal-detection and/or motor response by testing participants in a separate signal detection task.

Second, we applied a paradigm that has lately shown neurotypical young adults to be aware of the direction and magnitude of their timing errors (Akdoğan & Balcı, 2017). We tested if high-functioning children with ASD were aware of the direction and magnitude of their timing errors. This task for the first time addresses the temporal error monitoring ability of individuals with ASD (and children in general) and test the generalizability of a potential error monitoring disruption (see 2AFC task above). In order to assess the potentially different temporal information processing of individuals with ASD, we also utilized an independent timing task (self-paced finger tapping) that required participants to keep a stable rhythm at a pace comfortable to them. This task was chosen as it has been recently argued to be more sensitive to

clock-related deficits or age-related differences in temporal information processing (e.g., see Turgeon et al., 2012; 2016 for this argument in aging; Paraskevoudi, Balcı & Vatakis, 2018).

Based on previous findings (e.g., De Jonge et al., 2007, Wallace & Happe, 2008 but see Pirrone et al., 2017), we did not expect significant differences between the perceptual decision-making and timing performances of participants in two groups. In line with the ERN, metamemory, and math performance monitoring findings suggesting differences in error awareness brain signals and impaired metamemory and math error awareness in individuals with ASD (Cooper et al., 2016; Grainger et al., 2014; Grainger et al., 2016; Sokhadze et al., 2010), we expected disrupted error awareness in children with ASD in visual and temporal tasks. However, given the contradictory findings on post-error behaviors in individuals with ASD (Bogte et al., 2007; Sokhadze et al., 2010; South et al., 2010), we did not have specific predictions regarding post-error behavioral adjustments.

Method

Sample

Power analyses (alpha = 0.05, power = 0.80, 1:1 ratio) from previous studies that have found significant differences between individuals with ASD and control groups yield the following sample size requirements for each group: 4 and 7 from the different durations in Allman et al. (2011); 7 from Pirrone et al. (2017), and 5 and 7 from first-order and second-order stimuli, respectively from Bertone et al. (2005). Thus, given that these studies suggest 4, 5, or 7 participants in each group to detect a difference, we decided to use 8 participants in each group that are age and gender-matched and matched for socio-economic backgrounds.

Participants were 8 children with ASD (9-17 years old, *M* = 14, 1 female) and 8 TD children (12-17 years old, *M* = 14, 1 female). An inducement of 20 Turkish Liras (~$5) worth of bookstore gift card was provided for their participation. For participants with ASD, the inclusion criteria included being aged between 9 and 17, having a diagnosis of ASD and being high functioning. Exclusion criteria included having a co-morbid diagnosis of OCD or moderate/severe ADHD and medication use. One participant with ASD was on an allergy medication that he took the previous night and was also using a supplement containing Omega 3 that he last took the day before testing. Two participants with ASD used medications (i.e., methylphenidate) for mild ADHD-related symptoms, which they last took 36 and 48 hours prior to testing, providing enough time for their clearance from the blood system. Another participant with ASD was using daily insulin injections for diabetes mellitus Type I, which were not stopped for testing. For ASD participants, 13 participants were recruited that satisfied these criteria. The testing of two was terminated due to inability to sit down and concentrate on the tasks, one said he did not want to continue with the experiment after completing the second task, and two participants did not understand or follow the instructions. Their data were excluded from the dataset prior to running any analyses.

For control participants, the inclusion criteria included being aged between 9 and 17, an absence of any psychiatric diagnosis, having similar age and socio-economic status with participants with ASD, can be other patients visiting the hospital or patient relatives. Exclusion criteria were psychiatric diagnosis and being referred to the hospital for gastrointestinal symptoms (see Drossman et al., 1999). Two of the control group participants were on medications; one used hypertension medication and took it the afternoon before testing and another used a painkiller for headaches but did not take one in the 24 hours prior to testing.

ASD symptoms were evaluated by both a clinician and parents. A child psychiatrist completed the Childhood Autism Rating Scale (CARS) and Clinical Global Impression (CGI) scale, and the parents filled the Aberrant Behavior Checklist (ABC). All participants with ASD ranked above the cutoff score for an ASD diagnosis, and their CARS scores ranged between 30 and 39.5, which correspond mostly to mild-moderate autism. Their CGI score that represents a global rating of illness severity were either 2 or 3 in all cases, which correspond to borderline-to-mild illness in terms of ASD severity. Their ABC scores as rated by their parents ranged between 9-47 (maximum possible score of 174), which suggests that they display certain disruptive behaviors related to ASD symptoms, but these are in a moderate level, corroborating their diagnosis of mild-moderate autism and high functioning levels. The verbal behaviors of participants with ASD were comparable to their TD peers and they all went to regular schools, showing similar academic abilities to TD peers and independence in their daily living skills.

Tasks

Figure 1 illustrates the general conceptual relations that underlie the choice of the tasks utilized in this study.

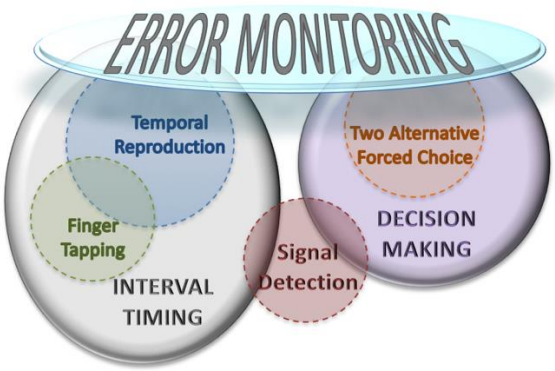

Figure 1. Illustration of the general conceptual relations between different cognitive domains and tasks used. Error monitoring is depicted as a meta-cognitive ability that encompasses interval timing and decision-making performances.

Random dot motion discrimination task. Participants saw moving dots on the screen, where a sub-group of dots moved coherently (12% coherence) either to the left or right while the others moved randomly. Participants were asked to decide which way the majority of the dots were moving. They responded by pressing the 'D' key on a mechanical keyboard with their left index finger for left and the 'K' key with their right index finger for right. Then, they were asked how sure they are of their response, and responded by pressing the 'Q' key for "not sure," the 'W' key for "moderately sure", and the 'E' key for "very sure". The response to stimulus interval (RSI) for this task was randomly sampled from a uniform distribution ranging between 1.5-2 seconds. Participants played 10 practice trials where feedback was given regarding their accuracy, followed by 15 minutes of the actual task with no feedback.

Signal detection task. Participants were asked to respond as soon as they saw the dot motion stimulus appear on the screen. Participants were clearly instructed to respond as soon as the stimuli that they were already familiar with appeared on the screen, without judging the direction of motion. For 45 seconds, they responded by hitting the 'K' key with their right index finger, and for the next 45 seconds, they responded by hitting the 'D' key with their left index finger. The RSI was randomly sampled from a uniform distribution between 1.5-2 seconds.

Temporal reproduction task. Participants saw a turquoise square on the screen with a black background. They were asked to watch the stimulus as long as it stayed on the screen and then reproduce the duration for which it was on screen without counting or using other chronometric strategies. Based on a review of previous studies that used various durations with children with ASD (Allman & Falter, 2015), the target duration was determined as 2.2 seconds. In order to reproduce the experienced duration, participants pressed the spacebar and then

released it when they believed the target duration had elapsed. Then, they were asked how sure they were of their reproduction by pressing the 'Q' key for "not sure," the 'W' key for "moderately sure", and the 'E' key for "very sure". Finally, participants were asked if they over-reproduced or under-reproduced the target duration in each trial, which they indicated by pressing the 'V' key for a reproduction shorter, and 'N' key for a reproduction longer than the target.

Participants had 10 practice trials. In the first five practice trials, they learned how to press the spacebar to reproduce the target duration and that the box disappeared when they stopped pressing the spacebar. In the last five practice trials, they saw the whole task and learned that they will be asked to retrospectively assess their reproductions. Upon finishing the practice trials, they performed the task for 20 minutes, with a break offered after the first 10 minutes.

Self-paced finger tapping task. Participants were asked to tap the spacebar repeatedly at a comfortable rate while keeping their inter-tap interval constant for a duration of 90 seconds.

Procedure

Written consent was obtained from the parents and verbal consent from the children for their participation. Parents completed the initial screening form to ensure that their children met the inclusion criteria. All tasks were performed in front of an iMac located in a quiet testing room in Koç University Hospital. The brightness of stimuli and the room were set to accommodate sensory hypersensitivities of individuals with ASD. First, a short finger-tapping task was completed, followed by the RDM discrimination task. Children were given the opportunity to take a before continuing to the next task. A short signal detection task ensued, followed by the temporal reproduction task. The entire study took around 45 minutes. The experimenter explained the tasks slowly, and instructed the children by pointing to the screen and guiding their hands towards the keyboard during the practice trials, which is a widely used Applied Behavior

Analysis-based procedure in ASD education called graduated guidance, and specifically manual prompting (Cooper, Heron, & Heward, 2007).

Data Analysis

Data were analyzed at two levels; first-order task performances and error monitoring in a subgroup of these tasks (RDM discrimination and temporal reproduction). Whenever we could not reject the null hypothesis based on the conventional frequentist tests, we conducted Bayesian analyses and reported BF_{01} values to indicate how much more likely were the data under the null vs. alternative hypothesis. Tenets of signal detection theory (SDT) was applied to the decision outputs of RDM discrimination and temporal reproduction tasks.

Random dot motion discrimination. The units of analysis were accuracy, response times (RT), and inverse efficiency (RT/Accuracy). For the computation of d', the sensitivity index in signal detection theory, seeing a left motion was arbitrarily treated as the 'signal'. Thus, the computed d' corresponded to participants' ability to respond left when the dots were moving leftward, and respond right when the dots were moving rightward ([z(Hit)-z(FA)]). Criterion (-[z(Hit)+z(FA)]/√2) scores were also computed to estimate the response bias. Then, a regression was conducted to see whether performance (accuracy) predicted confidence ratings of participants. Finally, post-error behavioral adjustment was addressed via post-error slowing based on the approach suggested by Dutilh et al. (2012).

Temporal reproduction. The units of analysis were mean reproduction time, coefficient of variation (CV: (SD of temporal reproductions)/ (mean of temporal reproductions), z-score transformed reproduction times, and probability of saying short given negative z-scores (underreproduction) and long given positive z-scores (overreproduction). Estimates of CV

indicate relative variability as the ratio of standard deviation to the mean and indicates a standardized measure of dispersion of data points. A regression analysis was performed to test if performance (distance from the mean reproduction) predicted confidence ratings of participants. We also devised a measure of post-error behavioral timing adjustments; the post-error behavior was treated as the comparison of the temporal reproductions prior to and after reporting an under-reproduction as well as the temporal reproductions prior to and after reporting an over-reproduction. These difference scores were compared between ASD and TD groups.

Secondary Tasks. In the finger tapping task, the mean, SD and CV of inter-tap intervals were compared whereas in the signal detection task response times were compared.

Results

Our data analysis is grouped under three main titles: comparison of first-order task performance in the two primary tasks (i.e., RDM discrimination and temporal reproduction), comparison of error-monitoring performance in these two tasks, and the comparison of performance on the two secondary tasks (i.e., finger tapping and signal detection).

Primary Decision Making and Timing Tasks

First Order Performance

Random dot motion discrimination. The accuracy of children with ASD and TD children were .66 and .70, respectively ($t(14) = .44$, $p = .67$). The Bayesian analysis of the same data showed that the data are more likely under the null hypothesis than the alternative hypothesis ($BF_{01} = 2.19$). The mean RTs of two groups were 7632ms and 11026ms for ASD and TD groups, respectively, which did not significantly differ from each other ($t(14) = 0.99$, $p = .338$, $BF_{01} =$

1.68). The median RTs, which are more robust to outliers, also did not differ significantly between groups ($t(14) = 1.22$, $p = .243$, $M_{TD} = 9.94$, $M_{ASD} = 6.21$, $BF_{01} = 1.43$). There were also no significant differences between the inverse efficiency scores of two groups computed either using mean RTs ($t(14) = 1.02$, $p = .33$, $M_{TD} = 19.50$, $M_{ASD} = 12.04$; $BF_{01} = 1.65$) or median RTs ($t(14) = 1.18$, $p = .26$, $M_{TD} = 17.63$, $M_{ASD} = 9.85$; $BF_{01} = 1.48$).

We also compared the d' as the sensitivity index between the two groups. An independent samples t-test did not reveal a difference in the d' scores ($t(14) = .472$, $p = .64$, $M_{TD} = 1.03$, $M_{ASD} = .77$; $BF_{01} = 2.17$) or the response bias (criterion) of two groups ($t(14) = -.69$, $p = .50$, $M_{TD} = .04$, $M_{ASD} = .19$, $BF_{01} = 1.99$).

Finally, we characterized the RT distributions with exponentially modified Gaussian distributions to decompose them into their constituents (Matzke & Eagenmakers, 2009). The chi-square statistics indicated satisfactory fits of all participants' data to Ex-Gaussian (all ps > .05; Figure 2). None of the Ex-Gaussian parameters differed between the two groups (μ: $t(14) = 1.77$, $p = .10$, $BF_{01} = .87$; σ: $t(14) = 1.43$, $p = .17$; $BF_{01} = 1.20$; τ: $t(14) = -.25$, $p = .81$, $BF_{01} = 2.29$). Overall, these results show that TD participants and participants with ASD did not differ in their first-order decision-making performance.

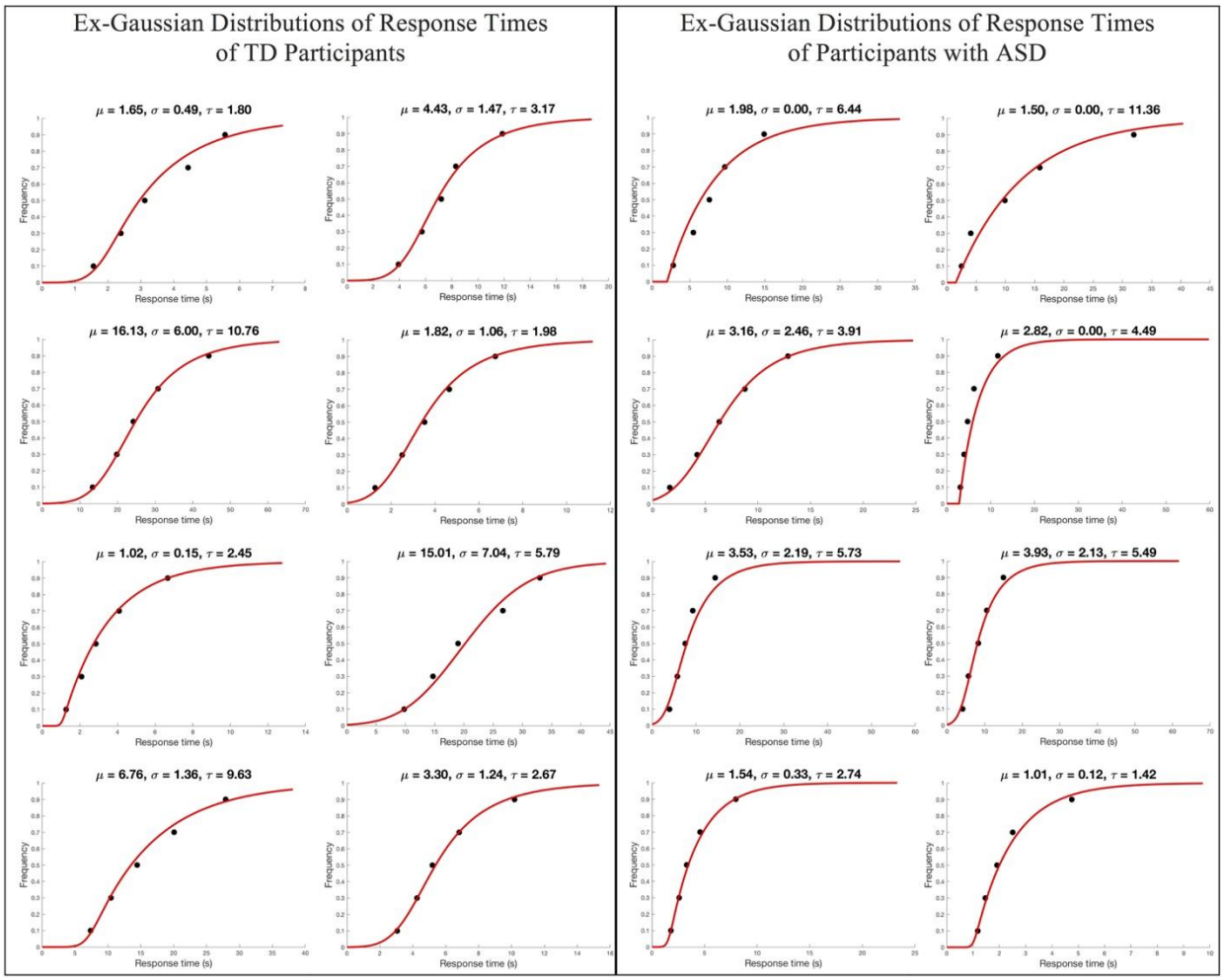

Figure 2. Ex-Gaussian fits of the response times in the dot motion discrimination task with the relevant μ, σ, and τ values of TD participants (left) and participants with ASD (right). Data and Ex-Gaussian fits are depicted as cumulative density functions.

Temporal reproduction. The mean reproduction times were 1991 ms and 1986 ms for the ASD and TD groups, respectively ($t(14) = -.01$, $p = .99$; $BF_{01} = 2.34$). The comparison of the CV between groups did not reveal significant differences, either ($t(14) = -2.10$, $p = .054$, $M_{TD} = 0.24$, $M_{ASD} = 0.42$, $BF_{01} = .60$). These results show that the primary timing performance of ASD and TD children are comparable.

Error Monitoring

Random dot motion discrimination. In order to capture the relationship between the objective performance and its subjective rating, we regressed the participants' average confidence ratings on their accuracy separately for TD and ASD groups (Figure 3). Accuracy of TD participants significantly predicted their confidence scores (b = .76, $t(7)$ = 2.87, p = .029), and explained a significant proportion of variance in their confidence ratings (R^2 = .51, $F(1,7)$ = 8.21, p = .029), supporting the awareness of the TD group of their decision accuracy. For the ASD group, the accuracy was not a significant predictor of their confidence scores (b = .10, $t(7)$ = .13, p = .90; R^2 = -.16, $F(1,7)$ = .02, p = .90). These results suggest that unlike TD children, children with ASD were not aware of their performance accuracy in the RDM discrimination task.

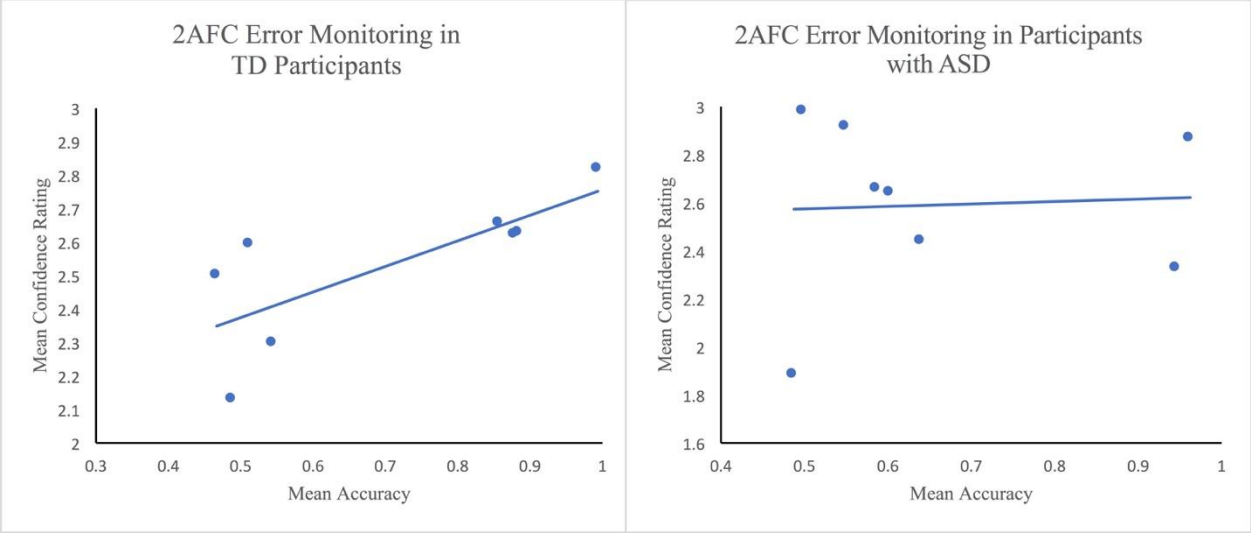

Figure 3. Graphs depicting the regression of confidence ratings on performance (accuracy) in dot motion discrimination task for TD participants (left) and participants with ASD (right).

Temporal reproduction. For the temporal reproduction task, we had three confidence rating levels (not sure, moderately sure, very sure), and two duration rating levels (longer, shorter), thus resulting in six possible response combinations. We first z-score transformed the reproduction times of each participant and then regressed six confidence rating pairs on this

reproduction performance. Confidence rating pairs containing less than 5 data points were excluded from further analyses (Figure 4). The regression for the TD group showed a significant regression of the confidence rating pairs on the objective timing errors (b = 1.83, $t(23)$ = 4.04, p = .001; R^2 = .42, $F(1,23)$ = 16.34, p = .001 - the same results held even without excluding rating pairs with less than 5 data points) pointing to their awareness of timing performance accuracy in this task. For children with ASD, we did not find a significant regression of the confidence rating pairs on the objective timing errors, (b = 1.15, $t(23)$ = 1.38, p = .18; R^2 = .04, $F(1,23)$ = 1.90, p = .18). Consistent with the error monitoring performance observed in the 2AFC task, these findings suggested that unlike TD children, children with ASD are not aware of their performance accuracy in the temporal reproduction task.

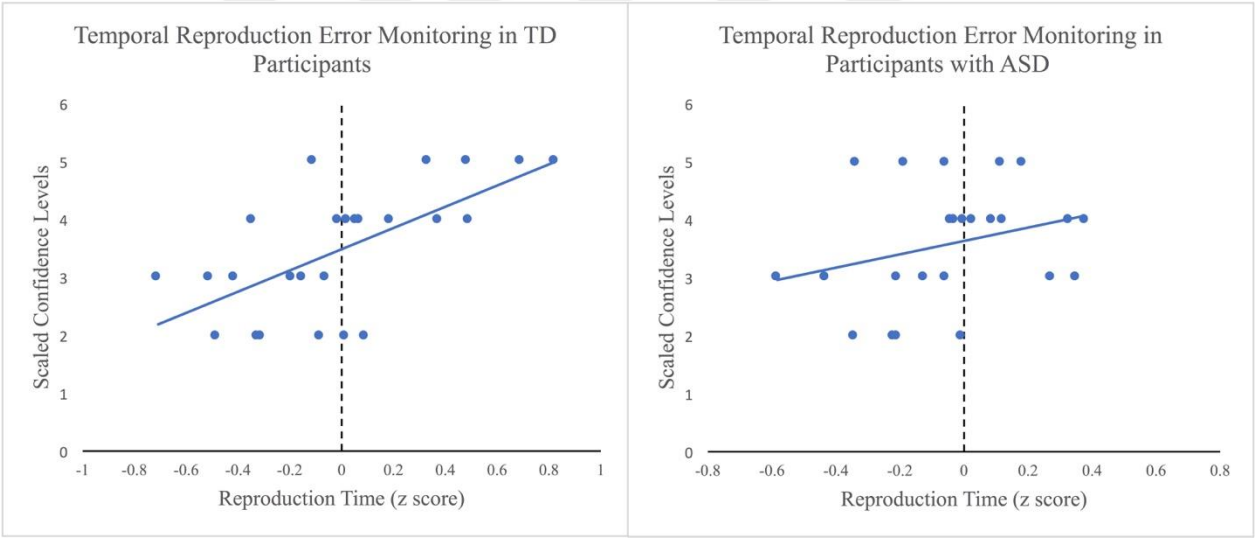

Figure 4. Graphs depicting the regression of scaled confidence levels on performance (temporal reproduction) for TD participants (left) and participants with ASD (right). Scaled confidence levels were coded as combinations of confidence rating and reported underreproduction or overreproduction (1: not sure + under; 2: moderately sure + under; 3: very sure + under; 4: very sure + over; 5: moderately sure + over; 6: not sure + over).

Post-Error Behavioral Adjustments

An analysis controlling for spurious differences in RTs that may be due to boredom or other factors (Dutilh et al., 2012) revealed that the groups did not differ in their post-error slowing ($t(14) = -1.38$, $p = .19$, $M_{TD} = .10$, $M_{ASD} = 2.26$, $BF_{01} = 1.25$). One participant in the ASD group and one participant in the control group were outliers as they committed less than three errors. Same results held after removing these participants from the analysis ($t(14) = 1.10$, $p = .15$, $M_{TD} = -.02$, $M_{ASD} = .86$, $BF_{01} = 1.52$).

For the temporal reproduction task, differences in reproduction times after participants indicated over-reproduction or under-reproduction were investigated. For each group, the difference between their mean reproduction time in the trials after the ones for which they have stated under-reproduction and their mean reproduction time in the trials after the ones for which they have stated over-reproduction was computed. Comparing these differences between the two groups yielded non-significant results ($t(14) = 1.24$, $p = .24$, $M_{TD} = .04$, $M_{ASD} = -.12$, $BF_{01} = 1.41$).

These results suggest that participants with ASD display similar post-error behavioral adjustments to TD participants in the decision-making and timing tasks used in this study.

Secondary Tasks: Signal Detection and Timing

We assessed the early perceptual/motor processing ability and timing performance of our participants with two additional tasks.

Signal detection. Data were averaged across the two blocks. Independent samples t-test revealed no significant differences between the mean RTs ($t(14) = -1.04$, $p = 0.32$, $M_{TD} = 0.39$, $M_{ASD} = 0.56$, $BF_{01} = 1.63$), standard deviations of the RTs ($t(14) = -.85$, $p = 0.41$, $M_{TD} = 0.13$,

M_{ASD}= 0.28, BF_{01}= 1.84), or their CV ($t(14) = -0.49$, $p = 0.63$, $M_{TD} = 0.27$, M_{ASD}= 0.33, BF_{01}= 2.15), suggesting similar early perceptual processing and motor responding in two groups.

Finger tapping. The average intertap intervals ($t(14) = 1.52$, $p = 0.15$, $M_{TD} = 0.62$, $M_{ASD} = 0.52$, BF_{01}= 1.11), standard deviations of intertap intervals ($t(14) = -.07$, $p = 0.95$, $M_{TD} = 0.22$, M_{ASD}= 0.22, BF_{01}= 2.34), and their CV ($t(14) = -.57$, $p = 0.58$, $M_{TD} = 0.36$, M_{ASD}= 0.45, BF_{01}= 2.10) did not significantly differ between TD participants and participants with ASD, suggesting that the groups have similar internal rhythms and endogenous timing variability.

Discussion

Metacognition, the ability to monitor one's performance, is especially valuable for individuals with ASD, who experience social difficulties and would benefit highly from noticing their social faux pas and correcting them. Perceptual decision-making and interval timing are two cognitive domains with particular significance for the social difficulties in ASD, where inferring meaning from visual input and arranging the timing of verbal and non-verbal responses in communications are crucial for conducting smooth social interactions (e.g., Barraclough et al., 2005; Lambrechts et al., 2018; Sokhadze et al., 2010). For these reasons, we tested the metacognitive abilities in children with ASD and TD children in both perceptual decision-making and timing tasks. Our comprehensive empirical approach included the analysis of first-order task performances as well as error monitoring in tasks probing these cognitive domains with different forms of responding (binary vs. parametric). Previous studies have investigated metamemory (Cooper et al., 2016; Grainger et al., 2014; Grainger et al., 2016) or math performance monitoring (Brosnan et al., 2016) in individuals with ASD and found them to display impaired metamemory and a greater likelihood of stating their inaccurate responses to be accurate. Yet, none of these tasks investigated error monitoring in low level processing such as perceptual decision-making or

time perception. Moreover, none of these previous studies used multiple tasks with different response structures (binary and parametric) and those that probe different cognitive abilities.

The analyses of first-order task performances showed that individuals with ASD performed similar to the TD group in both RDM discrimination and temporal reproduction tasks (but there was a trend for a difference in their coefficient of variations, with higher intraindividual variation in the reproductions of children with ASD). The lack of differences in these primary tasks was coupled with and corroborated by comparable performances between the groups also in the free-finger tapping and signal detection tasks (secondary tasks). These results suggest that children with ASD perform as well as the TD children in these basic cognitive tasks. Yet, what was different between the two groups was in the metacognitive domain.

We found that children with ASD have a circumscribed deficit in performance monitoring. In neither the perceptual decision making nor the interval timing task and unlike the TD children, did the confidence ratings of children with ASD match their objective performance. This is in line with Sokhadze et al. (2010)'s brain response findings that suggest individuals with ASD to be less sensitive to and less aware of their errors than TD individuals, as well as with behavioral findings (Grainger et al., 2016; Brosnan et al., 2016). Probing metamemory from what participants remember of a video about kangaroos, Grainger et al. (2016) found that although both individuals with ASD and TD individuals were able to use the judgment of confidence scale appropriately, where both TD participants and participants with ASD gave higher confidence ratings for easy questions compared to impossible questions, there were significant differences between groups in the confidence ratings for correct and incorrect answers, which are in line with our findings. Similarly, Brosnan et al. (2016) found similar math performance but different awareness of errors in individuals with ASD, which also match our findings of similar first-order task performance but differential error monitoring abilities in individuals with ASD.

Given that Brosnan et al. (2016) found individuals with ASD to be more likely than the TD group to erroneously think they got an incorrect question correct and that individuals with ASD self-reported superior metamemory abilities than TD participants (Grainger et al., 2014), we questioned whether this finding was due to children with ASD consistently giving higher confidence ratings, which was not the case ($t(14) = .40$, $p = 0.69$, $M_{TD} = 2.53$, $M_{ASD} = 2.59$, $BF_{01} = 2.21$; $t(14) = -1.14$, $p = 0.28$, $M_{TD} = 2.57$, $M_{ASD} = 2.74$, $BF_{01} = 1.53$ for confidences in RDM discrimination and temporal reproduction tasks, respectively). The average frequencies of the three levels of confidences were indeed similar across groups. Thus, in the absence of such a bias, it can be concluded that individuals with ASD are impaired in their metacognitive abilities.

Metacognition also has implications for an area that has been shown to be significantly impaired in ASD, which is understanding others' mental states. A relationship between monitoring one's own cognitive processes and understanding others' minds have been proposed (Carruthers, 2009; Misailidi, 2010). Similarly, both reality monitoring and metamemory (Cooper et al., 2016) and thinking about people and social relations, or social cognition (Saxe & Young, 2013) have been suggested to be underlain by the medial prefrontal cortex (mPFC). High-functioning adults with ASD were shown to be impaired in both mindreading and metamemory abilities compared to TD adults (Grainger et al., 2014). Future investigations into cognitive and neural commonalities between metacognitive and mentalizing impairments in ASD can pave the way for a more comprehensive understanding of the social, and other behavioral and cognitive deficits in ASD.

As ASD is mainly characterized by deficits in social interaction, the impairments observed in biological motion in children with ASD are expected. Though individuals with ASD performed equivalently to controls when grouping line elements into a global figure, they were significantly impaired in perceiving the human activity in the biological motion task (Blake et al.,

2003). So, we deliberately made the choice of not using biological human motion in our tasks in order to avoid the confounding variable of social information processing deficits in individuals with ASD. Most studies probing cognitive aspects in ASD either use adult participants, or when they use children/adolescents, they do not probe as many different domains/parameters as we did in the present design. Because our study design intended to probe multiple different cognitive aspects of individuals with ASD, we had multiple tasks that required a certain level of instruction understanding and following, and of focus and attention. For this reason, our sample of children with ASD was restricted to high functioning individuals without a comorbid diagnosis of OCD or severe ADHD, and with no medication use or use of medications that could be discontinued with clinician guidance prior to testing. Since there is a particularly high prevalence of comorbid ADHD and also OCD in ASD cases and children with ASD are usually on some type of medication, our criteria were highly restrictive. Additionally, from those who satisfied these already stringent criteria, the data of five participants had to be discarded prior to any analysis, as either they could not focus on the task or they did not understand the instructions and what the task required. These exclusions were made to control for confounding effects that could have affected even the first-order performances. Our small sample size can be a limitation, although it was nonetheless shown to be enough based on the power analyses and yielded significant differences in metacognition. This limitation can be overcome in future studies if more participants satisfying the inclusion criteria can be recruited, as not to confound the findings with other co-morbidity or medication issues. Future studies with larger sample sizes may reveal a difference between ASD and TD children in the coefficient of variation that indexes relative timing variability. Additionally, although we matched the mean age of our participants, the ASD group ranged between 9-17 and TD group between 12-17 in age, which can be more closely matched in future studies as well.

There seems to be improvements in metacognitive abilities across development. Metacognitive abilities were shown to improve significantly between ages 11-17 and stay stable in adulthood (19-41 years), leading the researchers to conclude a prolonged developmental trajectory for awareness of one's performance during adolescence (Weil et al., 2013). The present results in combination with previous findings suggest that this trajectory may spread over a more extended period in individuals with ASD, or may remain impaired across the lifespan, which is supported by findings of impaired metamemory and reality monitoring in adults with ASD (Cooper et al., 2016). Relatedly, Rinne and Mazzocco (2014) investigated calibration in mental arithmetic, which is the alignment of accuracy and confidence of judgments. They found that calibration continued to develop through grades 5 to 8, even when arithmetic accuracy neared ceiling. Thus, it seems that metacognitive abilities follow a more extended developmental trajectory than accuracy or ability, and may take even longer to develop or remain impaired across lifetime in individuals with ASD.

On the other hand, adequate task performance in the absence of metacognitive access to performance in individuals with ASD may suggest their use of cognitive strategies other than error-monitoring to accomplish task performance. This brings to mind the savant abilities seen in certain individuals with ASD, such as knowing to which day of the week any given date in any year corresponds. It is still not known how they are able to accomplish such difficult feats, but it is possible that they are using a cognitive strategy for it, of which we are not yet aware, and a similar cognitive strategy may be utilized by individuals with ASD in task performance that is different from error-monitoring. For instance, these savant calendrical calculators did not differ from TD controls in their general short and long-term memory so that a general mnemonic advantage was not the explanation for this skill, and the authors suggest that the cognitive processing style characteristic of ASD may have played a role in acquiring this date calculation savant ability (Heavey, Pring, & Hermelin, 1999). Such investigations can help move one step

further in understanding the unique cognitive styles of individuals with ASD, and hopefully one day enable the revelation of the mysteries behind how the ASD mind works.

CHAPTER IV

RELATIONS BETWEEN MOTHER PSYCHOLOGICAL SYMPTOMS AND CHILD BEHAVIORAL PROBLEMS IN ASD AND TYPICAL DEVELOPMENT

What runs in the family?

Relations between parent-child characteristics in autism and typical development

Abstract

Autism spectrum disorder (ASD) is widely accepted to have a genetic component, but there are still many unknowns regarding the genetics of ASD and a complete genetic profile for ASD is yet to be found. Though the literature has mainly turned to twin studies for information in this domain, trait inheritance studies between parents and children are also highly valuable in guiding such genetic investigations. The present study was the first to compare the relationships between child behavior problems and mother psychological symptoms in a group of children with ASD and their parents (n = 64) and a control group with typically developing children and their parents (n = 53). Results show that whereas there exist multiple relations between child behavior problems and mother psychological symptoms in the ASD group, such relationships are much less prominent in the control group. This comparison was followed by a more detailed investigation focusing on ASD behavioral difficulty domains (e.g., social withdrawal, stereotypic behaviors). In the ASD group, beyond demographic variables, mother obsessive-compulsiveness significantly predicted child stereotypic behaviors and inappropriate speech, which includes repetitive speech and repeating words or phrases. Mother depression also significantly predicted inappropriate speech in children with ASD beyond demographic variables. Our findings point to OCD-related genes as potential targets of investigation in the quest to unravel the genetic profile of ASD.

Keywords: Autism spectrum disorder, parent-child, parent symptoms, child behaviors, inheritance, family, obsessive-compulsive

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder presenting with deficits in social interaction and communication, and with repetitive behaviors and restrictive interests (American Psychiatric Association, 2013). Though twin studies indicate a strong genetic component in ASD and the estimated ASD heritability is around 50%, the common variants of the genetic composition of ASD are still unknown (Huguet et al., 2016). Additionally, strong evidence was recently provided for the contribution to ASD of epistasis or gene-gene interaction (Mitra et al., 2017), suggesting that unraveling the genetic makeup of ASD may be more complicated than previously believed; not being limited to single genes but also including their interactions. Though there is genetic heterogeneity in ASD, the associated genes seem to converge on certain molecular pathways such as protein synthesis and degradation, chromatin remodeling, and intracellular signaling, which are suggested to be related to changes in social and cognitive behaviors (Chahrour et al., 2016). So, genetic studies seem to imply that the commonly influenced pathways in ASD may translate to behavioral differences. Such an implication points to inheritance studies as valuable contributions to inform this field, alongside the more popular twin studies. With this purpose, the present study looked at the relationships between parent and child traits in ASD and compared these relationships to those in families with neurotypical development. We believe that such a study can assist genetic studies by providing behavioral and symptomatological inheritance information in ASD as different from neurotypical families, which can highlight potential areas of focus for investigating the commonalities in the genetics of ASD.

The relatives of individuals with ASD are repeatedly reported to exhibit certain language, personality, and social-behavioral characteristics that resemble ASD symptoms but expressed in a milder form (Losh et al., 2009). Such broad autism phenotype (BAP) characteristics have

previously been investigated with the hopes to provide a complementary approach to finding the genes related with ASD, and individuals with ASD and their parents with BAP were found to differ from controls and parents without BAP in measures of social cognition, but not in measures of executive function and central coherence (Losh et al., 2009). Though such social cognition deficits are expected given the social nature of impairments in ASD, we believe that looking at other, more extensive clinical symptoms will be informative in revealing specific behavior or symptom groups that are more likely to be inherited in ASD than in neurotypical development. Insistence on sameness in children with ASD was shown to be positively correlated with obsessive-compulsive behaviors in parents (Abramson et al., 2005). Obsessive-compulsive disorder (OCD) and ASD are both highly heritable disorders that share genetic risk factors. the authors describe a combined genome-wide association study (GWAS) of ASD and OCD. This combined genome-wide association study (GWAS) of ASD and OCD revealed a significant polygenic component of ASD, predicting 0.11% of the phenotypic variance in an independent OCD data set (Gua et al., 2017). More broadly, children of parents with a neurodevelopmental or a neuropsychiatric disorder were found to display more restricted and repetitive behaviors than children of parents without such disorders (Evans et al., 2017).

Though these few studies investigated the inheritance of symptoms in families with and without ASD, they have looked at predictable domains for ASD that are restricted to a single area, such as social cognition, repetitive behaviors, and insistence on sameness. Taking these studies one step further, we set out to look at a wider range of symptoms and behaviors in parents and children and their relationships, and how these relationships differ in families with children with ASD and typically developing children.

The present study measured child behaviors and characteristics comprehensively using Child Behavior Checklist that informs about withdrawal; somatic complaints; and

anxious/depressed, delinquent, and aggressive behavior of children, for both children with ASD and typically developing children. We measured parent psychological problems and symptoms using Symptom Checklist 90 in parents of both groups. In addition, we used Aberrant Behavior Checklist for children with ASD to get a clearer understanding of how different behaviors associated with ASD relate to psychological symptoms of their parents. Given the previous findings, we expected obsessive-compulsive behaviors of parents to relate to stereotypic behaviors in children with ASD, but given the lack of evidence otherwise, we approached the relations between other parent-child characteristics in an exploratory manner.

Method

Participants

Participants comprised 64 children with a clinical ASD diagnosis and their mothers, and 53 typically developing children and their mothers (Table 1). Exclusion criteria included having a diagnosis of a genetic, neurological, metabolic, respiratory or chronic infectious disorder, having a history of severe head injury or organic brain damage, and using pharmacological agents during the previous month. All parents had at least an elementary school education (Table 1). Upon approval from the Ethical Committee of Medical Faculty of Istanbul University, informed consent was obtained from all children and parents.

Table 1

Participant information for ASD group and TD group

	ASD Group (*n* = 64)				**TD Group (*n* = 53)**			
Demographics	*M / n*	*SD / %*	Min	Max	*M / n*	*SD / %*	Min	Max
Child age	11.66	3.82	6	18	11.75	.85	10	13
Mother age	38.25	6.27	25	52	40.08	4.59	31	48
Mother education[a]	2.95	1.12	2	5	2	5	3.11	1.20
Number of children in family	2.14	1.01	1	5	1.91	.56	1	3
Child gender	53 male	82.8%			40 male	75.5%		
Mother employment status	50 unemp.	78.1%			24 unemp.	45.3%		
Consanguineous marriage	14 cases	21.9%			-			
C-section births	29 cases	46%			32 cases	60.4%		
Problematic pregnancy period	21 cases	33.3%			4 cases	7.5%		

[a]Mother education was scored as follows: 1- no education, 2- elementary school graduate, 3- middle school graduate, 4- high school graduate, 5- university graduate

Measures

For all children. Demographic form filled by mothers indicated child age, parent age, and parent education. Childhood Behavior Checklist (CBCL) was used to measure children's behavioral and emotional problems, which are categorized into withdrawn, delinquent, aggressive behaviors; social, attention, thought problems; somatic complaints, and being anxious/depressed. The Turkish standardized version (Erol et al., 1995) of this scale, which was originally created by Achenbach (1991), was used in this Turkish sample.

For children with ASD only. Aberrant Behavior Checklist (ABC) is a list scored by mothers that measures irritability, social withdrawal, stereotypic behaviors, hyperactivity/noncompliance, and inappropriate speech in children with ASD. This scale was created by Aman and colleagues (1987) and its Turkish version that has been approved for validity and reliability by Karabekiroglu and Aman (2009) was used in this sample.

Childhood Autism Rating Scale (CARS) is a behavior observation scale used by trained observers to rate the symptoms of children with ASD to yield a total score for ASD severity. The original scale (Schopler & Reichler, 1971)'s Turkish version was used in this sample as a diagnostic tool, which was approved for validity and reliability (Sucuoğlu et al., 1996).

For all parents. Symptom Checklist 90 (SCL-90) is a self-report scale measuring mother psychological symptoms under the categories of somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, phobic anxiety, hostility, paranoid ideation, and psychoticism (Derogatis et al., 1973). Higher scores in this scale indicate higher problems in these domains, for instance having higher scores in interpersonal sensitivity does not suggest better interpersonal sensitivity but more problems in that area. The Turkish version of this scale that shows acceptable validity and reliability for the Turkish population (Güleç et al., 2012) was used.

Analysis Plan

First, Pearson correlations between parent psychological symptoms and child behaviors and characteristics were investigated and compared for two groups. Second, Pearson correlations between ABC scores of children with ASD and psychological symptoms were examined. The significantly correlated variables were then put into a hierarchical regression analysis to see if these predictions held over and above socio-demographic variables.

Results

The results revealed significantly higher CBCL and SCL scores for the ASD group compared to the control group ($t(108) = 6.49$, $p < .001$ and $t(115) = 7.33$, $p < .001$, respectively). The mean CBCL score in the ASD group was 62.84 and the mean score was 48.96 in the control group. The mean SCL score was 11.65 in the ASD mothers group and it was 4.58 in the control mothers group. In both measures, higher scores indicate more problems. The CARS scores of participants with ASD ranged between 24.00 and 52.50, with $M = 36.97$, indicating that all participants in the ASD group were on the spectrum, ranging from mildly to heavily autistic.

Table 2

Correlations between mother psychological symptoms and child behavioral problems in ASD group

Child Behavior Problems / Mother Symptoms	Withdrawn	Somatic complaints	Anxious/ depressed	Social problems	Thought problems	Attention problems	Delinquent behavior	Aggressive behavior
Somatization	.23	.26	*.42***	.19	.12	.15	.29*	*.42***
Anxiety	.19	.18	.29*	.23	.20	.14	.25	.32*
Obsessive-compulsive	.39**	.27*	**.48*****	*.44****	.27*	.35**	.32*	**.48*****
Depression	.33*	.27*	*.43***	.38**	.30*	.34*	.41**	**.50*****
Interpersonal sensitivity	.26	.18	.33*	.29*	.28*	.26	.23	.33*
Psychoticism	.26	.33*	.31*	.26	.28*	.20	.25	.28*
Paranoid ideation	.28*	.32*	.36**	.31*	.34**	.25	.23	.40**
Hostility	.32*	.27*	.41**	.30*	.21	.16	.28*	.31*
Phobic anxiety	.28*	.19	.30*	.22	.22	.15	.17	.22

*$p < .05$, **$p < .01$, ***$p < .001$.

Note: Bolded values indicate significance at the .05 level, and italicized values indicate marginal significance at the .10 level after Holm-Bonferroni correction.

Relations between mother-child characteristics

When the relations between mother symptoms and child behaviors were investigated, for the ASD group (Table 2), a lot of correlations emerged. Yet, when Holm-Bonferroni correction was applied, only the relations between mother depression and child aggressive behaviors, mother obsession and child anxious-depressed behaviors, and mother obsession and child aggressive behaviors remained significant in the ASD group.

Table 3

Correlations between mother psychological symptoms and child behavioral problems in TD group

Child Behavior Problems / Mother Symptoms	Withdrawn	Somatic complaints	Anxious/ depressed	Social problems	Thought problems	Attention problems	Delinquent behavior	Aggressive behavior
Somatization	.12	.18	.10	.26	.15	.17	.22	.09
Anxiety	.14	.13	.14	.11	.13	.12	.38**	.16
Obsessive-compulsive	.09	.04	.19	.17	.16	.29*	.37**	.13
Depression	.11	.04	.16	.16	.15	.22	.30*	.11
Interpersonal sensitivity	.02	.01	.06	.07	.17	.12	.24	.07
Psychoticism	.13	.16	.19	.27	.20	.24	**.47*** **	.23
Paranoid ideation	.02	-.04	.05	.01	.06	.06	.34*	.12
Hostility	.23	.17	.12	.22	.17	.17	.30*	.22
Phobic anxiety	-.06	-.00	-.02	.05	.19	.10	.18	-.06

*$p < .05$, **$p < .01$, ***$p < .001$.

Note: Bolded values indicate significance at the .05 level after Holm-Bonferroni correction.

When these relations were examined for the typically developing group, the correlations in Table 3 were obtained. After Holm-Bonferroni correction, the only significant correlation was between mother psychoticism and child delinquent behavior.

These analyses showed that mother psychological problems were associated with child behavior problems to a greater degree in the ASD group compared to the control group, and both mother psychological problems and child behavior problems were higher in the ASD group compared to the control group.

Relations between mother characteristics and ASD behaviors

For children with ASD, their total aberrant behavior score was significantly and positively correlated with only their mothers' obsessive-compulsiveness out of all parent psychological symptoms (Table 4). From ABC's subscales, the significant correlations were between mother obsessiveness and child stereotypic behavior and inappropriate speech, and mother depression and child inappropriate speech (Table 4).

Table 4

Correlations between mother psychological symptoms and aberrant behaviors in ASD group

Child Aberrant Behaviors / Mother Symptoms	Irritability, agitation, crying	Lethargy and social withdrawal	Stereotypic behavior	Hyperactivity, noncompliance	Inappropriate speech	Total Aberrant Behavior Score
Somatization	.00	-.03	.06	-.04	.08	.00
Anxiety	.10	-.02	.07	.04	.13	.06
Obsessive-compulsive	.22	.23	.26*	.11	.26*	.27*
Depression	.18	.21	.17	.12	.27*	.23
Interpersonal sensitivity	.13	.08	.04	.06	.16	.11
Psychoticism	.08	.01	.12	-.04	.14	.07
Paranoid ideation	.07	.11	.07	.04	.22	.11
Hostility	.10	.08	.12	.14	.13	.12
Phobic anxiety	.11	.08	.14	.01	.22	.13

*$p < .05$.

When put into a hierarchical linear regression, mothers' obsessive-compulsiveness continued to predict children's aberrant behaviors beyond child age, mother age, and mother education ($\beta = .27$, $p < .05$). It explained an additional 7% of variance in child aberrant behaviors beyond these variables ($\Delta R^2 = .067$, $p < .05$). Mother obsessiveness continued to predict child stereotypic behaviors ($\beta = .26$, $\Delta R^2 = .062$, $p < .05$) and inappropriate speech ($\beta = .26$, $\Delta R^2 = .064$, $p < .05$) beyond these demographic variables. Mother depression also continued to predict inappropriate speech beyond these variables ($\beta = .28$, $\Delta R^2 = .076$, $p < .05$).

Discussion

This study for the first time looked at the relationship between different child behavior problems and parent psychological problems in both children with ASD and their parents, and typically developing children and their parents. Children with ASD displayed significantly more

problem behaviors than typically developing children, and mothers of children with ASD had significantly more psychological symptoms than mothers of typically developing children. Mothers' symptoms were associated to a greater extent with the behavioral problems in ASD group compared to control group. When a child with ASD displays aggressive or anxious-depressed behaviors besides the core ASD symptoms, these are associated with higher depressive or obsessive-compulsive problems in mothers. In the typically developing group, only significant relation was between mother psychoticism and child delinquency, and no relations between obsessive-compulsive problems in mothers and child behavior problems emerged.

In the ASD group, when ASD core symptoms were investigated beyond the broader child behavior problems, mothers' obsessive-compulsiveness predicted children's aberrant ASD-related behaviors. This prediction held beyond demographic variables as child age, mother, and mother education. Mothers' obsessive-compulsiveness especially predicted stereotypic behaviors and inappropriate speech, where the latter was also significantly predicted by parent depression. Findings are in support of our prediction that parent obsessive-compulsiveness would be related to children's stereotypic behaviors. Stereotypic behaviors were characterized in this checklist with items such as "repetitive hand, body or hear movements", "waves, shakes extremities repeatedly", "rocks body back and forth", and "stereotyped, repetitive movements." (Aman et al., 1987). It makes sense that such repetitive actions would be related to obsessive-compulsiveness of mothers, which are measured with items as "having to check and double-check what you do" and "having to repeat the same actions such as touching, counting, washing" (Derogatis et al., 1973). This study adds to the previous finding that obsessive-compulsive traits in parents are associated with insistence on sameness in children with ASD (Abramson et al., 2005) by showing that obsessive-compulsive traits in parents not only predict insistence on sameness, but also stereotypic behaviors and inappropriate speech in children with ASD.

Inappropriate speech in children with ASD was significantly predicted beyond demographic variables by mother obsessive-compulsiveness and parent depression. Inappropriate speech was characterized with the following items: “talks excessively”, “talks to self loudly”, “repeats a word or phrase over and over”, and “repetitive speech” (Aman et al., 1987). It makes sense that obsessive-compulsive traits in mothers would predict the latter two items, which suggest repetition or stereotypy and resemble in nature the items for stereotypic behaviors listed above, which were also significantly predicted by mother obsessive-compulsiveness. Mother depression may be more related to the former two items in this list of talking excessively and talking to self loudly. Since this was a cross-sectional study, data cannot be interpreted in a causal direction. Either mothers who are depressed may have no patience or energy to correct the excessive or loud talking of their children, or children’s excessive or loud talking may precipitate mother depression. The directionality of this prediction can be clarified with future longitudinal studies. Alternatively, depressed mothers may also be more likely to report more problems with their children. So, future studies using objective clinician evaluations as well as studies reaching both mothers and fathers to look at the behavioral similarities between children and both parents will be valuable extensions of the present results.

Interestingly, whereas obsessive-compulsiveness in mothers of children with ASD correlated with withdrawn behaviors, somatic complaints, anxious-depressed behaviors, social problems, thought problems, attention problems, delinquent behaviors, and aggressive behaviors in their children with ASD significantly and positively, mothers’ obsessive-compulsiveness only correlated with attention problems and delinquent behaviors in the typically developing group. These findings suggest that obsessive-compulsiveness in parents by itself may not be a leading cause for the social problems in children with ASD, but may be a risk factor that comes into play when in combination with multiple other risk factors implicated in ASD.

Obsessive-compulsiveness in parents may be a risk factor for ASD, as it seems to be the parental trait most strongly correlated with child ASD symptoms. Therefore, investigating genes commonly implicated in OCD and ASD can help narrow the search for the genetic fingerprint for these disorders and point to specific gene groups that can help accelerate investigations on the genetics of ASD.

CHAPTER V

GENERAL DISCUSSION

GENERAL DISCUSSION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with no clear etiology or treatment. Thus, efforts from multiple domains targeted to understand this disorder more comprehensively are truly valuable. This was the aim of the present thesis, which investigated the biological, cognitive, and behavioral correlates of ASD in the light of most recent evidence and developments.

Firstly, the effects of gut microbiota and inflammation on ASD neurology were reviewed. ASD has been separately associated with inflammation/immune dysregulation and gut dysbiosis. Previously, the immune system has been considered a route in gut-brain communication, but its effects on ASD neurology have not been previously investigated in detail, which was done as part of this thesis study. A review of the literature and especially the physiological effects of the gut microbiota and inflammation/immune system separately enabled me to find three possible common mechanisms of action for inflammation and gut microbiota on the neural profile of ASD. These mechanisms of action are their influence on ASD-susceptibility genes, neurodevelopment, and integrity of intestinal and blood-brain barriers. There are around 100-200 genes associated with ASD and a recent study showed them to be specifically targeted by ASD risk factors. Such a mechanism can constitute a link in the gene-environment interaction through epigenetic mechanisms that is believed to underlie ASD. Secondly, studies on animals show the gut microbiota to have important effects on the healthy development of neurons, their myelination, and their connections. Inflammation is reported in astrocytes in the ASD brain, and when inflamed astrocytes are placed together with regular neurons, they influence them in such a way as to result in behavioral ASD symptoms in the organism. Thirdly, the gut microbiota is involved in the development and maintenance of the gut and the blood-brain barrier, and inflammation is associated with disrupted integrity of the gut and the blood-brain barrier. As these three mechanisms are open to influence from both the gut microbiota and inflammation, I suggested them to be the common mechanisms of effect of these two risk factors on ASD

neurology. Moreover, they can also be the mechanisms through which probiotics may exert a protective role on neurodevelopment in ASD. Specifically, probiotics can improve gut barrier integrity, which would keep endogenous toxins restricted in the gut and away from the developing brain. Probiotics can also strengthen the blood-brain barrier and thus prevent endogenous and environmental toxins from disrupting neurodevelopment. Finally, with their anti-inflammatory properties, probiotics can reduce maternally or fetally derived pro-inflammatory cytokines that would target ASD-genes and thus influence neurodevelopment. This is an important contribution as the mechanisms of action of potential beneficial probiotic effects are still unknown. Different subgroups of individuals with ASD may have been affected by different risk factors. So, discovering potential common mechanisms of action of different risk factors on ASD neurology is valuable, as it can inform potential treatments and interventions targeting these mechanisms and be effective for a wide population of individuals with ASD.

Secondly, our empirical study on children with ASD delineated their specific cognitive signature. Children with ASD did not have any deficits in perceptual decision-making and timing, but they had a specific impairment in their awareness of their performance in these domains. Thus, we showed a domain-general metacognitive impairment in individuals with ASD in the presence of conserved first-order task performance (i.e., timing and choice behaviors). This finding is important because being aware of one's own actions and errors (metacognition) and understanding others' actions and attributing intentions to them (mentalizing) may be related, and thus such circumscribed error-monitoring deficiencies in ASD can also enlighten what underlies their mentalizing impairments that are widely reported behaviorally. Additionally, since children with ASD are impaired in error-monitoring, they may be using another strategy for their successful task performance. Understanding of these different strategies can pave the way to discovering what underlies savant abilities in individuals with ASD, which are exceptionally difficult cognitive feats performed relatively effortlessly by some individuals with ASD. This finding of impaired error-monitoring in different domains in individuals with ASD can also pave

the way for creating new interventions that can target this domain and have a broad-ranging influence on multiple cognitive and behavioral areas of impairment. Importantly, if the error-monitoring ability can be improved with such interventions, it can improve online social communications of individuals with ASD by enabling immediate error correction in social interactions for individuals with ASD. Finally, if monitoring one's own behaviors and decisions is indeed related to understanding the intentions behind others' behaviors and the mental states underlying their decisions, then such interventions can ultimately improve Theory of Mind abilities of individuals with ASD, a deficit in which is a hallmark of this population, commonly referred to as "mindblindness" in ASD (Baron-Cohen, 1995).

Thirdly, as the genetic blueprint of ASD is still unknown, hereditary studies provide valuable information in the way to understanding the etiology of ASD. Until recently, hereditary studies in ASD mostly focused on twins. We instead looked at relationships between parent-child psychological problems in families with children with ASD and typically developing children. Parents' obsessive-compulsive traits emerged as a significant predictor of child behavioral problems and ASD symptoms in the ASD group, but not in the typically developing group. This finding suggests a potential common genetic basis that may be shared between ASD and OCD, which can guide genetic investigations of these disorders. OCD is characterized by repetitive behaviors, which is also a diagnostic criterion for ASD. Both disorders also involve rigidity, where both individuals with ASD and OCD stick to their behavioral patterns even when they are not adaptive. Beyond the overlapping behavioral repetitiveness and rigidity, the hereditary relations between OCD and ASD suggest that investigating these two disorders together and continuing the search for their commonalities in terms of genetics, etiology, and disrupted physiological and cognitive mechanisms can be informative in understanding more about both disorders.

Overall, understanding ASD has remained an important yet ever challenging task ever since Kanner first described ASD in 1943 (Kanner, 1943). Though certain advances have been

definitely made in our understanding of ASD, there is still much more to be learned and discovered. Future comprehensive attempts such as the present thesis that investigates multiple aspects of ASD can contribute to endeavors of unraveling the etiology and complete physiology of ASD, which can then reveal effective treatments and potential preventions for this increasingly prevalent neurodevelopmental disorder.

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