# NETWORK COHERENCE IN AUTISM SPECTRUM DISORDER: A MULTIMODAL NEUROIMAGING STUDY OF FUNCTIONAL CONNECTIVITY AND SPECTROSCOPY MRI

---

A Dissertation

presented to

the Faculty of the Graduate School

at the University of Missouri

---

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

JOHN P. HEGARTY II, M.A.

Dr. David Q. Beversdorf, Dissertation Supervisor

JULY 2015

The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

NETWORK COHERENCE IN AUTISM SPECTRUM DISORDER:
A MULTIMODAL NEUROIMAGING STUDY OF FUNCTIONAL CONNECTIVITY AND SPECTROSCOPY MRI

presented by John Hegarty,

a candidate for the degree of doctor of philosophy

and hereby certify that, in their opinion, it is worthy of acceptance.

David Q. Beversdorf, M.D.

Shawn E. Christ, Ph.D.

Jeffrey D. Johnson, Ph.D.

Dennis K. Miller, Ph.D.

Gene J. Blatt, Ph.D.

# ACKNOWLEDGEMENTS

First and foremost, I would like to thank the participants and their families for their involvement in this research. Without their contributions none of this would be possible. I would also like to thank Dr. Beversdorf and the many students in the Cognitive Neuroscience Lab for facilitating the development of this project and support throughout the process. These experiments required collaboration across a multidisciplinary team and could only have been accomplished with their assistance and dedication. Finally, I would like to thank the faculty and research staff at the Thompson Center for Autism and Neurodevelopmental Disorders and Department of Psychological Sciences Brain Imaging Center. They provided the tools and expertise necessary to complete this research.

# TABLE OF CONTENTS

# LIST OF FIGURES

Figure Page

# LIST OF TABLES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ABC | Aberrant Behavior Checklist |
| ADHD | Attention Deficit Hyperactivity Disorder |
| ADI | Autism Diagnostic Interview |
| aINS | Anterior Insula |
| aIPL | Anterior Inferior Parietal Lobule |
| aMPFC | Anterior Medial Prefrontal Cortex |
| ANCOVA | Analysis of Covariance |
| ANOVA | Analysis of Variance |
| ASD | Autism Spectrum Disorder |
| aTL | Anterior Temporal Lobe |
| BAI | Beck Anxiety Inventory |
| BOLD | Blood Oxygen Level Dependent Response |
| BPM | Beats Per Minute |
| C | Mean Clustering Coefficient |
| CNS | Central Nervous System |
| Cre | Creatine |
| Cr+PCr | Creatine+Phosphocreatine |
| CSF | Cerebrospinal Fluid |
| daCC | Dorsal Anterior Cingulate Cortex |
| DAN | Dorsal Attention Network |
| DLPFC | Dorsolateral Prefrontal Cortex |
| DMN | Default Mode Network |
| dMPFC | Dorsal Medial Prefrontal Cortex |
| DSM III | Diagnostic Statistical Manual 3rd Edition |
| DSM IV | Diagnostic and Statistical Manual 4th Edition |
| DSM 5 | Diagnostic and Statistical Manual 5th Edition |
| E/I | Excitatory/Inhibitory Balance |
| Eglobal | Global Efficiency |
| Elocal | Local Efficiency |
| FC | Functional Connectivity |
| fcMRI | Functional Connectivity Magnetic Resonance Imaging |
| FDR | False Discovery Rate |
| FEF | Frontal Eye Fields |
| fMRI | Functional Magnetic Resonance Imaging |
| FPC | Frontoparietal Control Network |
| FSIQ | Full Scale Intelligence Quotient |
| FSL | FMRIB Software Library |
| FXS | Fragile X Syndrome |
| GABA | gamma-aminobutyric acid |
| GAD | Glutamic Acid Decarboxylase |
| Glx | Glutamate+Glutamine |

| | |
|---|---|
| GSOM | General Social Outcomes Measure |
| GWAS | Genome-wide Association Study |
| HF | Hippocampal Formation |
| IFG | Inferior Frontal Gyrus |
| iPCS | Inferior Precentral Sulcus |
| IQ | Intelligence Quotient |
| k | Cost |
| L | Characteristic Path Length |
| LTC | Lateral Temporal Cortex |
| M | Mean |
| MANOVA | Multivariate Analysis of Variance |
| MFGBA6 | Middle Frontal Gyrus BA6 |
| MFGBA9 | Middle Frontal Gyrus BA9 |
| mg | milligrams |
| MR | Magnetic Resonance |
| MRS | Magnetic Resonance Spectroscopy |
| msPFC | Medial Superior Prefrontal Cortex |
| MT | Middle Temporal Motion Complex |
| MTL | Medial Temporal Lobe |
| NAA | N-acetylaspartate |
| PCC | Posterior Cingulate Cortex |
| PDD | Pervasive Developmental Disorder |
| PDD-NOS | PDD- Not Otherwise Specified |
| PHC | Parahippocampal Cortex |
| pIPL | Posterior Inferior Parietal Lobule |
| PIQ | Performance Intelligence Quotient |
| PNS | Peripheral Nervous System |
| PPM | Parts Per Million |
| rlPFC | Rostrolateral Prefrontal Cortex |
| ROI | Region of Interest |
| RsP | Retrosplenial Cortex |
| SFG | Superior Frontal Gyrus |
| SOG | Superior Occipital Gyrus |
| SPL | Superior Parietal Lobule |
| SRS | Social Responsiveness Scale |
| STS | Superior Temporal Sulcus |
| TempP | Temporal Pole |
| TLC | Test of Language Competence |
| TPJ | Temporal Parietal Junction |
| TS | Tuberous Sclerosis |
| VIQ | Verbal Intelligence Quotient |
| vMPFC | Ventral Medial Prefrontal Cortex |

Network Coherence in Autism Spectrum Disorder:
A Multimodal Neuroimaging Study of Functional Connectivity and Spectroscopy MRI

John Hegarty

Dr. David Q. Beversdorf, Dissertation Supervisor

Abstract

The underlying neuropathology and effects on neuronal activity in individuals with ASD are still being elucidated as well as their impact on intervention and treatment outcomes. Frontal, temporal, parietal and cerebellar pathways exhibit disrupted structural and functional connectivity in individuals with ASD and we sought to investigate the potential clinical utility of altered network coherence. *Beta*-adrenergic antagonism improved information processing in a subset of individuals with ASD and improved performance was related to pharmacologically-mediated alterations in functional connectivity in the fronto-parietal control network. These findings support the potential utility of *beta*-adrenergic antagonists for some patients with ASD and the clinical significance of alterations in network coherence. There are also additional considerations for functional connectivity investigations in ASD. The cerebellum is interconnected via feedback loops to the neocortex and thus has some modulatory influences on cortical and subcortical neuronal circuits. The cerebellum is consistently implicated in the neuropathology of ASD but has been largely ignored in investigations of functional network coherence. Functional connectivity between the cerebellum and neocortex was anticorrelated in a subset of individuals with ASD. These individuals exhibited reduced glutamate levels in the cerebellum and diminished interpretive linguistic abilities, suggesting a potential mechanism underlying altered cerebrocerebellar connectivity in some individuals with ASD as well a cognitive outcome of alterations in cerebrocerebellar network coherence.

# CHAPTER 1: INTRODUCTION

## Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a behaviorally defined disorder characterized by impairments in social communication and the presentation of stereotyped interests and repetitive behaviors early in life.[1] Dr. Eugen Bleuler first coined the term autism in 1908 to refer to patients with schizophrenia who exhibited a tendency to withdraw within one self (drawing from the ancient Greek term "autós" meaning "self").[2] The first formal description of early infantile autism was not until 1943 by Dr. Leo Kanner.[3] Dr. Kanner presented a case series of children displaying symptoms differing from previous cases of childhood schizophrenia and suggested a new unique syndrome. These patients exhibited a preference for aloneness from the beginning of life and responded atypically during interactions with others, such as no adjustment in posture when being held or an anxious response to interaction. Many patients also displayed aversion to loud noises or moving objects and exhibited an extreme preference for sameness with activities. Language development was delayed and their utterances were often governed by rigidity, such as preference for identical order. Language was not often used for communication but to exhibit object or wrote memory, perhaps for a self-serving interest or to appease a caretaker's request for repetition. Dr. Hans Asperger also described a class of patients as autistic psychopaths in 1944 that largely resembled Dr. Kanner's patients[4]; however these patients did not exhibit language delays.

Autism did not begin to receive widespread attention as a distinct disorder until Bernard Rimland published the book *Infantile Autism: The Syndrome and Its Implications for a Neural Theory of Behavior* in 1964.[5] An early prevalence study in 1966 estimated that approximately 4.5

in every 10,000 children exhibited evidence of the syndrome.[6] Following greater clinical recognition, infantile autism was added to the third edition of the Diagnostic and Statistical Manual of Mental Disorders in 1980 (DSM III).[7] Autism was now formally separated from childhood schizophrenia. Children presenting with widespread distortions in the development of multiple psychological functions, such as gross and sustained impairment in social relationships paired with excessive anxiety, constricted affect, resistance to change, abnormal speech or motor movements, and sensitivity to sensory stimuli, were diagnosed with a pervasive developmental disorder (PDD), which included infantile autism. Infantile autism was characterized by onset before 30 months of age with a pervasive lack of responsiveness to other people, gross deficits in language development, peculiar speech patterns, bizarre responses to various aspects of the environment (e.g. resistance to change or peculiar interest or attachment to objects), and importantly an absence of delusions, hallucinations, loosening of associations, and incoherence associated with schizophrenia. Revisions to the DSM III shifted autism classification from symptom description to distinctive areas of functioning in which a minimum number of concrete and observable abnormalities had to be met.[8] By 1992 approximately 19 in every 10,000 children in the United States met diagnostic criteria for infantile autism.

Pervasive developmental disorders, including autism, were further segregated and redefined in the DSM-IV.[9] Autistic Disorder required a minimum of six criteria that must be met with at least two symptoms in qualitative impairment in social interaction, one impairment in communication, and one presentation of restricted, repetitive, and stereotyped patterns of behavior, interests, and activities. Delays or abnormal functioning in social interaction, language for social communication or symbolic or imaginative play were also required and symptom onset had to occur before 3 years of age. Furthermore, these associated disturbances could not be

accounted for by other disorders such as Rett's syndrome (a genetic disorder), or childhood disintegrative disorder, which was characterized by normal development until at least the third or fourth year of life but often not occurring until after approximately 10 years followed by a severe loss of social, communication, or other skills. Asperger Disorder was also added following the incorporation of Dr. Asperger's original work into research published in English journals in 1981[10] and translation of his original German work into English in the 1991 book *Autism and Asperger Syndrome*.[11] Asperger disorder was characterized by impairment in social interaction and the presentation of restricted repetitive and stereotyped patterns of behavior or interests; however, there could be no clinically significant delay in language or cognition. The classification of Asperger disorder accounted for patients presenting with social interaction symptoms associated with autistic disorder that appeared to otherwise have normal cognitive development. By 2000 approximately 67 in every 10,000 children in the United States met diagnostic criteria for autistic or Asperger disorder.[12]

The new DSM 5 edition released in 2013 has further altered diagnostic criteria and collapses Asperger, PDD-NOS, and autistic disorder into a single autism spectrum disorder (ASD) category.[1] ASD is now defined by persistent deficits in social communication and social interaction across multiple contexts with a minimum of two manifestations of restricted, repetitive patterns of behavior or interests presenting early in development. The DSM 5 also classifies ASD without a requirement for language delay, which allows inclusion of individuals whom previously would have received an Asperger diagnosis. Overall, ASD is a behaviorally-defined disorder defined by deficits in social interaction and communication with the presentation of circumscribed interests and behaviors. Current estimates suggest approximately 147 in every 10,000 children meet diagnostic criteria for ASD[13]; however new estimates of

disorder prevalence with DSM 5 criteria have yet to be obtained from a population sample.

ASD is diagnosed based on the presentation of symptoms across different cognitive and behavioral domains. Symptom presentation and severity within domains is highly variable, ranging from mild to severe and may or may not involve the onset of cognitive impairments, approximately 70% of cases exhibit such impairments.[14] ASD is also highly comorbid with other disorders, compounding diagnostic boundaries, with approximately 70% of patients diagnosed with ASD meeting criteria for at least one other psychiatric disorder.[15,16] The underlying biological mechanisms causing the ASD phenotype have been very difficult to identify due to the heterogeneity across individuals; however twin studies of concordance rates of ASD in monozygotic and dizygotic pairs support a high rate of genetic influences in the disorder. Concordance rates of diagnosis are between 36-91% for monozygotic pairs and closer to 0% for most dizygotic pairs but ranging up to 21% in some estimates.[17-20] Considering a broader dimensional definition of ASD-related abnormalities, concordance in monozygotic pairs still remains much higher than dizygotic pairs, 77-92% vs. 10-31%.[20,21] ASD appears to be highly heritable but is not entirely genetic in origin.

Genetic contributions to ASD-related outcomes are largely multigenic as only 6-15% of ASD cases express a known associated monogenic mutation.[17,22,23] Genomic sequencing and genome-wide association studies (GWAS) have identified genomic variant regions and loci mutations associated with ASD, but many of these genetic regions are not distinct to ASD and show some overlap in familial and genetic susceptibility to other psychiatric disorders,[24] further confounding the identification of ASD-specific mechanisms. The majority of genetic contributions to ASD etiology are rare variants and common allelic variation and can be organized into eight general classes[25]: 1) genes regulating activity-dependent alterations to

molecular pathways, such as TSC1&2,[26,27] 2) genes associated with translation and protein stability, such as fMRI1,[28,29] 3) genes involved with neuronal development and synapse formation, such as neurexin/neuroligins,[30-32] 4) genes affecting production and signaling of neurotransmitters, such as the serotonin transporter,[33,34] 5) genes regulating intracellular ion concentrations,[35] 6) genes affecting protein metabolism, such as phenylalanine hydroxylase,[36,37] 7) genes associated with genomic expression, such as MeCP2[38,39] and 8) genes of unknown function, such as AHI1.[40,41] Overall, genetic susceptibility appears to play a major role in the development of ASD-related symptoms and the majority of these genetic contributions involve genetic regions associated with neuronal development and signaling mechanisms.

The core symptoms of ASD were originally thought to be emergent from a common underlying mechanism but recent research has suggested ASD may more accurately reflect a dimensional disorder originating from difficulty and impairments at the extreme end of a continuum across independent domains.[42] For example, ASD-related traits exhibit significant heritability but express low levels of covariation,[43] even when examining those with the most severe impairments.[44] Furthermore, genetic influences associated with individual traits further supports fractionation of autism-related impairments because genetic modeling indicates that one half to two thirds of the genes associated with variation in social symptoms of ASD are not associated with nonsocial symptoms.[43,45] Considering variation of ASD-related symptoms in the normal population, the prevalence of non-overlapping genetic influences for individual cognitive and behavioral outcomes, and largely multigenic susceptibility associated with diagnosis; ASD does not appear to be due to a single underlying genetic cause. Multifactorial genetic influences may independently contribute to specific behavioral outcomes in ASD; therefore, much attention has been given to identifying neurodevelopmental trajectories and neuropathological outcomes

associated with the disorder. Unfortunately the identification of ASD-specific neuropathology and/or biomarkers has proven difficult and is only beginning to be elucidated.

Investigations of cellular structure in individuals with ASD implicate widespread microstructural neuronal abnormalities. In general, cellular abnormalities associated with ASD include altered neuronal orientation, irregular laminar patterns, and altered neuronal density, increased or decreased depending on the region being investigated.[46] For example, individuals with ASD exhibit reduced neuronal size and increased cellular density in the limbic system[47]; whereas investigations of the cerebellum report a decreased number of Purkinje cells.[46-50] Abnormalities in cerebellar circuits are one of the most consistently reported neuropathological perturbations in individuals with ASD; however more recent investigations have also noted abnormal minicolumn structure in the frontal and temporal lobes, specifically more numerous, smaller, less compact columnar organization.[51] Microstructural abnormalities in neuronal circuit structure in the frontal, temporal, and cerebellar cortices are implicated in the presentation of the ASD phenotype; however a major limitation in the investigation of neuropathological outcomes in ASD is the reliance on post-mortem brain samples. Post-mortem investigations typically have limited sample sizes, due to lack of tissue availability. They also generally rely on samples from older individuals whom died many years after neuropathological perturbations have occurred, which may obscure neuropathological outcomes due to treatment-related and compensatory mechanisms. To address these limitations recent investigations have utilized non-invasive *in vivo* neuroimaging techniques.

Neuroimaging techniques such as magnetic resonance imaging (MRI) allow the assessment of younger (living) individuals, larger samples, and most importantly the longitudinal assessment of neuropathological and behavioral outcomes associated with ASD. Structural MRI

techniques, which allow differentiation between white matter, grey matter, and cerebrospinal fluid, have identified morphological differences in the brain related to ASD. Abnormal brain volume has been consistently reported in younger individuals with ASD, with those diagnosed typically exhibiting significantly larger brains even after adjustment for body mass, IQ, and intra-cranial volume.[52-56] Cerebral development throughout adolescence to adulthood is also implicated such that following initial hyperplasia in individuals with ASD, patients typically exhibit significantly reduced cerebral growth rates compared to unaffected individuals, such that brain volumes are comparable to unaffected individuals by adulthood.[52,53] The reported cerebral enlargement and altered growth trajectories in ASD do not appear to be globally mediated as regionally specific abnormalities have been reported that may underlie whole-brain volume differences. The most consistently implicated cortical regions include those comprising the frontal, temporal, parietal,[57-59] and cerebellar cortices[53,54,57,60] with the occipital lobe remaining largely unaffected.[61] Furthermore, there have also been selective alterations in volumes such as decreased hippocampal volumes[62,63] but increased amygdala volumes[56,57,64] within the medial temporal lobe, suggesting regional specificity of alterations to neuronal circuits. Although some inconsistencies exist across studies such as discrepant reports of frontal cortex[59] and amygdala volumes,[62] the pattern of prefrontal, tempo-parietal, limbic, and cerebellum perturbations are generally supported within the available literature.[61] These macrostructural investigations are consistent with the previously reported microstructural findings of a distributed network of abnormalities in ASD involving the frontal, temporal, and cerebellar cortices and cumulatively this evidence suggests altered organization of neuronal networks in ASD. Due to the widespread neuropathological abnormalities in ASD, investigations of neuropathological abnormalities have largely shifted to understanding network-level effects on network organization. Neuronal cells

are regionally organized into distinct neuronal clusters[65] with short-range projections within clusters and long-range projections between different regions of the brain. Neuronal projections are organized into white matter tracts connecting regions in the brain. The widespread cellular abnormalities in individuals with ASD across different regions in the brain and network organization of neuronal clusters suggests that white matter tracts connecting these regions may also be affected.

Diffusion tensor imaging (DTI) is an MRI technique that allows assessment of white matter tracts in the brain. DTI measures water diffusion based on molecular interactions with the surrounding environment, such as biological tissue, providing a means of characterizing white matter tracts in the brain. Two of the primary outcome variables associated with DTI analysis are fractional anisotropy (FA) and mean diffusivity (MD).[66] An FA value reflects the directional variation in molecular diffusion, such that smaller values reflect generally spherical isotropic diffusion and larger values reflect highly directional diffusion along a specific axis. FA is sensitive to microstructural differences in white matter, thus providing a measure of white matter integrity. A MD value represents the average molecular motion independent of directionality and provides an inverse measure of tissue density.

Typical patterns of healthy white matter tracts in the brain would present with high FA values and low MD values. Whole-brain analysis of individuals with ASD have consistently reported reduced FA[67-69] and increased MD[67,70] compared to unaffected individuals, suggesting microstructural white matter abnormalities. Some studies have reported discrepant findings such as generally increased FA in individuals with ASD[71,72] but across the available literature reduced FA and increased MD appears to be associated with ASD.[73] Perturbations in specific white matter tracts appear to be driving these effects. The corpus callosum is the primary whiter matter

bundle connecting the contralateral hemispheres and typically exhibits reduced FA[74-77] and increased MD[67,75,76] in individuals with ASD, suggesting decreased interhemispheric connectivity. The superior longitudinal fasciculus is the primary ipsilateral/within-heimsphere white matter bundle and connects the prefrontal, parietal, posterior temporal, and occipital lobes. The arcuate fasciculus is a bundle comprising a subsection of the superior longitudinal fasciculus connecting the inferior parietal cortex/caudal temporal cortex to the inferior frontal cortex and most notably connects language regions such as Broca's and Wernicke's areas. The superior longitudinal fasciculus and arcuate fasciculus exhibit more heterogeneous findings in ASD such that some report bilaterally decreased FA,[77-79] whereas others report only right hemisphere differences,[80,81] left hemisphere differences,[69] or no differences in individuals with ASD compared to unaffected individuals.[82] These seemingly discrepant findings on the integrity of white matter pathways connecting language-related regions in ASD may be partially due to the heterogeneity within the ASD population at the behavioral level, which is especially relevant when comparing individuals with ASD and Asperger disorder whom do not present with the same delays in language. Overall, global inter-hemispheric white matter integrity in ASD seems to be reduced with more heterogeneous effects in intrahemispheric white matter integrity, depending on the individuals and white matter tracts being assessed. These findings suggest abnormal neuronal circuitry in ASD, consistent with previous neuropathological reports from post-mortem investigations, and further suggests altered structural connectivity between regions in the brain as an underlying neuropathology in ASD.

Altered structural connectivity in the brain may affect functional utilization of distinct neuronal clusters during cognitive processing causing atypical cognitive and behavioral outcomes, such as those seen in ASD. The association between structural abnormalities and

functional utilization in the brain appears to be highly dependent on network-level connections and not solely due to direct connections between regions,[83] suggesting alterations in structural connectivity affect coherence of functional networks in the brain. Optimized network coherence in the brain is believed to rely on dense local connections between neighboring neuronal clusters with additional long-range connections between distant clusters, which minimizes the metabolic cost of information processing[84] while still allowing efficient information transfer between spatially distributed systems.[85] Thus, atypical white matter integrity in individuals with ASD may alter neuronal network activation and organization in the brain and modify information processing.

Neuronal activation in the brain can be assessed non-invasively with functional magnetic resonance imaging (fMRI). fMRI measures a correlate of neuronal activation, the blood oxygen level dependent (BOLD) response,[86] and fluctuations in the BOLD response can be correlated between regions in the brain, termed functional connectivity (FC),[87] allowing an assessment of functional network coherence. Investigations into the development of functional networks in the brain have suggested network maturation exhibits a general segregation of anatomical neighbors with concurrent integration of more distant brain regions into functional networks.[88] Functional network organization seems to be affected in ASD with initial investigations reporting general global hypoconnectivity between neuronal clusters.[51,89-96] These disturbances are present across a widespread network within the brain as affected regions comprise the frontal, temporal, and parietal lobes, consistent with previous structural neuropathological abnormalities in these cortices and further demonstrating functional outcomes of these perturbations. However, additional research into functional connectivity patterns in ASD has found more complex outcomes in functional network organization and utilization than just global hypoconnectivity.

Additional investigations have also reported local hyperconnectivity of neighboring regions.[89,94] Although these findings may seem somewhat contradictory, these patterns of functional network abnormalities suggest a shift in developmental maturation of functional network coherence in ASD that warrants further investigation.[97]

Previous studies have utilized both resting state and task-based fMRI designs to assess functional connectivity in ASD. Resting state fMRI measures neuronal activation in the absence of a cognitive processing task whereas task-based fMRI measures activation during the performance of a cognitive processing task. Resting-state connectivity focuses on intrinsic functional connectivity in the brain, which reflects spontaneous synchronous low-frequency fluctuations between different regions. The default mode network (DMN) is of particular interest in the study of resting state fMRI because the DMN is the primary network activated during passive states[98] and dissociates from other networks during cognitive processing.[98,99] Investigations of resting state functional connectivity of the DMN in ASD generally report global patterns of hypoconnectivity,[96,100-104] consistent with previous underconnectivity theories of ASD. However, increased connectivity within the DMN network[105] and between the DMN and visual and motor networks has also been reported.[106] Additional discrepancies also exist from investigations of task-based functional connectivity in ASD. Task-based fMRI primarily focuses on activation-based functional connectivity in the brain or intrinsic functional connectivity patterns during a specific cognitive processing task. An initial investigation of sentence comprehension in individuals with ASD reported reduced connectivity between language regions[107], illustrating altered functional connectivity in regions underlying a specific cognitive domain affected in the disorder and suggesting hypoconnectivity in ASD. Further investigations of functional connectivity in individuals with ASD during cognitive processing in

other domains have also exhibited alterations in functional connectivity of networks related to symptom outcomes. Individuals with ASD exhibit reduced connectivity in networks underlying executive functioning,[93,108] language processing,[95,109] working memory,[110,111] mental imagery,[112] theory of mind,[113] stimulus inhibition,[91] visuomotor processing,[114,115] and motor control.[116] These findings are also consistent with theories of general underconnectivity across cognitive domains implicated in the core characteristics of ASD; however higher functional connectivity in ASD has also been reported in networks associated with language and imitation,[117] memory, [118] emotional processing, [119] and visuomotor processing. [120] Connectivity was higher between regions within the frontal [117] and temporal lobes [119] and more spatially extensive across cortico-thalamic [120] and bilateral networks. [118] Abnormal functional connectivity in individuals with ASD may be more accurately reflected by hypoconnectivity of more distant network regions and perhaps hyperconnectivity of anatomically neighboring regions. Additionally, altered functional connectivity within networks coupled with more extensive networks can be conceptualized into a neural systems framework to suggest reduced functional network integration and less network segregation during neuronal network development. [121] Overall, functional connectivity measures provide a powerful tool for assessing domain-specific networks in the brain and implicate abnormal functional organization and utilization of neuronal networks in ASD; however additional research is necessary to develop more definitive theories of functional connectivity patterns in ASD.

Investigations of functional connectivity in ASD have provided important information regarding the association between specific cognitive and behavioral symptoms of the disorder and network level processing in the brain; however due to the heterogeneity across patients with ASD and methodological differences across studies,[122] these techniques are not currently reliable

as diagnostic markers. These techniques may be more applicable as patient stratification and treatment markers providing a means of identifying subjects expressing abnormal function within specific networks or allowing the ability to track treatment-related changes in network coherence. Within-subject designs can account for more patient heterogeneity when assessing treatment outcomes as well as account for some methodological concerns because patients and processing techniques remain constant across time points. Additionally, focusing on treatment-related changes in functional connectivity in ASD will help researchers determine the clinical utility of these techniques. If differences in functional connectivity are clinically relevant to the study of ASD then treatment paradigms that improve symptom outcomes of the disorder should also modulate connectivity patterns in patients benefiting from treatment. Therefore, we propose that individuals with ASD will express abnormal functional connectivity in networks underlying cognitive processing domains associated with the disorder and that treatment paradigms that benefit core symptoms of the disorder should modulate functional connectivity within these networks. *Beta*-adrenergic antagonism, such as that resulting from the administration of propranolol, may benefit individuals with ASD by reducing aggressiveness and improving language and communication abilities.[123] Propranolol has been shown to alter functional connectivity in language regions in individuals with ASD[124]; however these propranolol-meditated benefits may also be associated with changes in coherence of other functional networks.

There are also additional considerations for functional connectivity investigations in ASD. Functional connectivity research in ASD has primarily investigated the relationship between cortical and subcortical networks with cognitive and behavioral domains associated with symptom outcomes of the disorder; however the cerebellum has largely been ignored in these

investigations. As previously reported, the cerebellum consistently expresses neuropathological outcomes associated with ASD. The cerebellum is interconnected via feedback loops to the neocortex and thus has some regulatory control of cortical and subcortical neuronal circuits. Although traditionally thought to be exclusively involved with motor control, imaging and lesion studies have implicated the cerebellum in higher-order cognitive domains as well. Patients with cerebellar lesions or atrophy exhibit deficits in general intelligence, verbal learning,[125,126] language, executive functioning[127,128] memory,[129] visuospatial planning,[126,130] and modulation of affect.[128] Neuroimaging studies have also reported that the cerebellum is activated during language processing,[131-135] visuospatial processing,[135-137] emotional processing,[135,138,139] working memory,[135,140,141] and executive functioning.[142,143] The cerebellum is believed to play an important role in cognitive functions such as attention, language, working memory, and sensory integration in addition to motor control. Due to the multiple efferent/afferent projections between the cerebellum and motor control and cognitive regions of the brain, and the consistent reports of cerebellar perturbations in ASD; cerebellar alterations may underlie some of the core aspects of ASD symptomatology. We propose that cerebellar abnormalities may influence functional networks underlying cognitive processing domains in individuals with ASD and that these influences are related to symptom presentation of the disorder. Furthermore, altered functional network coherence in ASD may be due to an altered balance of excitation to inhibition (E/I) in the brain,[144,145] which is largely defined by glutamate and GABA signaling. Reduced Purkinje cell output in individuals with ASD may alter the E/I balance in the cerebellum causing abnormal functional relationships with cortical and subcortical networks during cognitive processing.

**Summary**

ASD is a behaviorally defined neurodevelopmental disorder characterized by impairments in social communication and the presentation of stereotyped interests and repetitive behaviors early in life. Symptom presentation and severity are highly variable, and current estimates suggest approximately 1 in 68 children in the United States meet diagnostic criteria for ASD. Genetic susceptibility appears to play a major role in the development of ASD-related symptoms and the majority of these genetic contributions involve genes associated with neuronal development and signaling mechanisms. There have been no reports of gross brain abnormalities associated with the disorder; however, investigations of underlying neuropathology have implicated widespread microstructural and macrostructural abnormalities across the frontal, temporal, parietal, and cerebellar cortices, suggesting abnormal circuit formation within local neuronal clusters and altered long range projections between clusters. Functional MRI techniques have been utilized to assess the functional outcomes of these abnormalities, and individuals with ASD exhibit general patterns of reduced functional network integration and less functional segregation between networks. Overall, genetic susceptibility for perturbed neuronal development appears to affect neuronal migration and network organization in the brain causing abnormal functional utilization of networks underlying specific cognitive and behavioral processing domains associated with the disorder.

The proposed investigations outlined in this manuscript will apply MRI techniques to assess functional connectivity in individuals with ASD and unaffected individuals to assess the relationship between perturbations of functional networks with behavioral and cognitive outcomes. FMRI and connectivity analyses will be applied to a group of participants following *beta*-adrenergic antagonism in order to assess alterations in functional connectivity in response to

treatment. FMRI and additional MRI techniques that can assess glutamate and GABA levels will also be applied to a separate group of participants with the goal of measuring cerebellar influences on network dynamics in the brain. The proposed research will allow an assessment of the potential clinical utility of measurements of functional connectivity in individuals with ASD as well as allow a better understanding of the neuropathological effects of cerebellar abnormalities on functional neocortical networks.

# CHAPTER 2:

# PHARMACOLOGICAL MODULATION OF NETWORK COHERENCE

## *Beta*-adrenergic antagonism and ASD

Current pharmacological treatments for ASD affect multiple mechanistic pathways, are primarily directed at managing secondary manifestations,[146] and may cause substantial side effects in patients. The underlying neuropathology and effects on neuronal activity in ASD are still being elucidated, which makes mechanistically driven pharmacological intervention difficult. The only currently FDA-approved medications for ASD are risperidone, aripiprazole, and antipsychotics. Risperidone is an atypical antipsychotic that acts as a dopamine and serotonin antagonist as well as a partial *alpha*-adrenergic antagonist and histamine inverse agonist. Aripiprazole is also an atypical antipsychotic but acts as a partial dopamine and serotonin agonist at some receptor subtypes and a dopamine and serotonin antagonist at other receptor subtypes, with antagonist effects on *beta*- and *alpha*- adrenergic and histamine receptors. Antipsychotics have widespread effects in the central nervous system making the exact mechanisms of action on individual outcomes largely unknown. For patients with ASD, antipsychotics are primarily prescribed to treat aberrant behaviors, generally reducing aggression, self-injurious behaviors, and rapid mood changes[147,148]; however, antipsychotics do not affect core symptoms of the disorder. Additionally, antipsychotics can have moderate to severe side effects such as weight gain, diabetes mellitus, dyslipidemia, myocarditis, anxiety, insomnia, gastrointenstinal dysfunction, and even cardiac death in elderly individuals.[149,150] Patients with ASD also express higher rates of anxiety,[151] insomnia,[152] and gastrointestinal dysfunction[153] and these issues could be exacerbated with antipsychotic medications.

Pharmacological agents affecting distinct mechanistic pathways have been investigated in the treatment of ASD but there is currently no pharmacological intervention for the core symptoms of the disorder. Selective serotonin reuptake inhibitors (SSRI) have exhibited beneficial effects in individuals with ASD such as reduced aggression, anxiety, affective reactions, and repetitive thoughts and behavior. Some patients even exhibited improved language and social behaviors; however, these effects varied across small open-label trials and case series.[154,155] Double-blind, placebo-controlled studies primarily assessed repetitive thoughts and behaviors and reported some improvement,[156,157] but these effects were not replicated in a large-scale placebo controlled trial.[158] The most robust effect of SSRIs in individuals with ASD was reduced anxiety. SSRIs may also be able to reduce repetitive thoughts and behaviors in some individuals with ASD but these effects were inconsistent within larger placebo-controlled trials.

Glutamatergic and GABAergic agents have also been investigated and exhibited beneficial effects in patients with ASD for hyperactivity, irritability, and inappropriate speech as well as some reported effects on socialization; however these effects also varied across open-label trials and case series[159,160] A double-blind, placebo-controlled study found that memantine ( an NMDA receptor antagonist) decreased irritability, stereotypic behaviors, and hyperactivity but this was in the context of being used as an adjunctive treatment to risperidone[161]; whereas a double-blind, placebo controlled study of lamotrigine (an antiepileptic drug that is thought to inhibit glutamate release) reported no treatment benefits in individuals with ASD.[162] A double-blind, placebo controlled trial of bumetanide (a diuretic that reinforces GABAergic inhibition) reduced global ASD symptom severity, which was mostly driven by diminished restricted interests and stereotypical behaviors.[163] Large-scale placebo controlled trials have either failed to replicate these results[164] or are still ongoing. The most robust effects from

glutamatergic/GABAergic agents were reduced irritability and hyperactivity in individuals with ASD.[161]

Oxytocin is a neuropeptide that mediates complex social behaviors such as attachment and social recognition.[165] Oxytocin exhibits beneficial effects in social cognition[166] and affect in individuals with ASD[167] as well as a potential reduction in repetitive behaviors in small-scale, double-blind, placebo-controlled trials.[168] However, these effects also vary across trials with reports of no clinical efficacy for some patients with ASD,[169] and large-scale trials are necessary to better assess the effects of this type of intervention.

In summary, pharmacological intervention in ASD can benefit certain symptom presentations in some individuals but there is currently no pharmacological treatment across the core symptoms of the disorder. Antipsychotic medications can reduce aberrant behaviors but are often accompanied by side effects. Serotonergic medications can reduce anxiety-related symptoms and perhaps some repetitive thoughts and behaviors. Glutamatergic/GABAergic medications can reduce hyperactivity and irritability. However, the latter two types of treatments do not consistently mitigate core social and language impairments. Oxytocin may reduce social impairments but not those in the communication and language domains. There is currently no pharmacological intervention for the core symptoms of ASD, especially for language and communication domains.

**Noradrenergic system in ASD**

A pharmacological intervention with clinical efficacy for the core symptoms of ASD has yet to be discovered but there are additional mechanisms of action that warrant further investigation. Norepinephrine (NE), or noradrenaline, is a monoamine and the primary neurotransmitter released by the sympathetic nervous system, which initiates the stress response.

The noradrenergic system may be affected in individuals with ASD. Individuals with ASD exhibit heightened sympathetic nervous system arousal[170-172] and stress reactivity[173,174] compared to unaffected individuals. Peripheral levels of NE and stress hormones released following NE signaling may be upregulated in individuals with ASD[176,177]; [175,176] however some investigations have reported no differences in NE compared to controls and suggested previous results may have been due to a heightened stress reaction to sample collection.[177] ASD is also commonly accompanied by comorbid diagnoses such as ADHD[15] and anxiety[151] and secondary symptoms such as autonomic nervous system dysfunction,[172] which also implicate the noradrenergic system. Dysfunction of the noradrenergic system impacts cognitive and affective processing and has been implicated in disorders such as attention deficit hyperactivity disorder (ADHD),[178] anxiety, and post-traumatic stress disorder.[179] The noradrenergic system may also be related to hypersensitivities often reported in patients with ASD such that top-down attentional processes, which are modulated by the NE, are thought to underlie some aspects of hypersensitivity to environmental stimuli.[180] Although a definitive noradrenergic mechanism has not been established in ASD, these associated disturbances suggest that modulation of the noradrenergic system may provide clinical benefit for some individuals.

Pharmacological interventions directly affecting the noradrenergic system can target *alpha*- or *beta*- adrenergic receptors. Double-blind, placebo-controlled studies of clonidine and guanfacine, primarily *alpha* 2-adrenergic agonists which inhibit NE release, have reported diminished irritability and hyperactivity[181,182] as well as modest social and affective improvements in individuals with ASD.[183] However, drowsiness was often reported following treatment and language and communication domains were unaffected. An initial open trial of *beta*-adrenergic antagonists in individuals with ASD reported diminished aggressiveness along

with some improvement in speech and socialization abilities,[123] suggesting potential beneficial effects in some of the core domains. Most importantly, *beta*-adrenergic antagonism improved domains not currently addressed by other pharmacological interventions. The *beta*-adrenergic antagonist propranolol has been further investigated in pilot trials of individuals with ASD. Propranolol is a lipophilic non-selective *beta*-adrenergic antagonist. In a series of double-blind, placebo-controlled, single-dose studies, individuals with ASD exhibited significantly lower response latencies during verbal problem solving (i.e. anagrams),[184] increased performance for semantic fluency (i.e. number of items generated in response to a categorical cue),[185] and decreased error rates on a working memory task[186] following propranolol administration compared to placebo. Additionally, individuals with ASD exhibited improved social reciprocity.[187] *Beta*-adrenergic antagonism may benefit individuals with ASD by reducing aggressiveness and potentially improving speech, language, associative processing, and working memory abilities; however large-scale clinical trials are necessary to determine the clinical efficacy of this type of intervention across the ASD population.

*Beta*-adrenergic antagonism in the peripheral nervous system (PNS) leads to lower blood pressure and heart rate.[188] In the central nervous system (CNS), it affects initiation and maintenance of functional network coherence,[189] ( i.e. coordinated functional utilization of distinct neuronal clusters) which is modulated by noradrenergic activity.[190] NE release is mediated by the locus coeruleus (LC); which is located in the rostral pons. *Alpha* 1- and *beta*-adrenergic receptor binding in the central nervous system typically hyperpolarizes cells causing neuronal activation following NE release from the LC.[191,192] The locus coeruleus-norepinephrine (LC-NE) system initiates and maintains neuronal states appropriate for the acquisition of sensory information and modulates processing of salient information via sensory, memory, attentional,

and motor processes.[192] The LC has projections throughout the neocortex and considering NE effects on cognitive processing has particularly salient connections to the basal forebrain and medial temporal lobe.[193] During typical cognitive processing states, the LC-NE responds phasically to task-relevant stimuli primarily from prefrontal cortex regulation of attentional networks.[194] Arousal causes the LC-NE to shift to tonic firing, which is associated with distractibility and hypervigilance. This shift impairs attentional control in favor of re-orientation to environmental stimuli[194,195] and alters activity patterns within networks underlying cognitive processing.[196] Therefore, changes in LC-NE output may modulate networks underlying cognitive processing tasks in favor of networks that prompt environmental adaptation. For example, cognitive flexibility (not including set-shifting) represents the ability to inhibit a dominant response and examine remote alternatives that may produce an optimal solution. The ability to examine these remote alternatives is theorized to be dependent on flexible network access in the brain, which may be due to alterations in signal-to-noise with and between neuronal networks. [197,198] Upregulation of the LC-NE via stress induction or pharmacological modulation decreases performance on cognitive flexibility tasks whereas *beta*-adrenergic antagonism improves performance,[199,200] and these effects are especially robust for individuals experiencing difficulty with the problem.[201] Thus, states of hyperarousal or instances of LC-NE dysfunction may limit access to networks underlying certain information processing domains in favor of greater adaptation to the environment, which affects cognitive processing.

*Beta*-adrenergic antagonism appears to mitigate some of the effects of increased LC-NE output on the brain. An fMRI investigation found that exposure to an acute stressor (aimed at upregulating the LC-NE) increased activation and functional connectivity between regions associated with stimulus salience and sensory and attentional re-orienting, and increased

connectivity within these networks was significantly associated with response to stress.[202] Propranolol administration significantly reduced this increase in connectivity,[202] suggesting that *beta*-adrenergic antagonism can reduce the upregulation of salience and re-orienting networks during instances of increased LC-NE activation. Additionally, an fMRI investigation of individuals with ASD reported that *beta*-adrenergic antagonism increased functional connectivity in regions involved with associative and language processing.[124] Functional connectivity of regions activated during phonological associative processing (i.e. rhyming) was significantly higher during propranolol administration compared to nadolol, a hydrophilic *beta*-adrenergic antagonist that cannot pass through the blood brain barrier and thereby controlling for general cardiovascular effects.[124] *Beta*-adrenergic antagonism can increase network access in domain relevant networks in individuals with ASD during cognitive processing, suggesting a link between the previously outlined behavioral benefits of propranolol in individuals with ASD and coherence of network underlying cognitive processing. Our goal with this investigation is to further examine *beta*-adrenergic effects on functional networks in the brain in individuals with ASD and compare these to unaffected individuals. Specific aims include:

**Aim 1:** Utilize resting-state fMRI to assess baseline changes in network coherence in individuals with and without ASD during *beta*-adrenergic antagonism. This investigation will allow us to determine if *beta*-adrenergic antagonism affects network coherence and if any of the changes are specific to ASD.

**Aim 2:** Utilize task-based fMRI to assess changes in network coherence during cognitive processing in individuals with and without ASD during *beta*-adrenergic antagonism and assess how these changes relate to language-based associative processing

performance. This investigation will allow us to determine if changes in network coherence are associated with cognitive and behavioral improvements in individuals with ASD.

## RESTING STATE fMRI

Resting state fMRI assesses the BOLD response while individuals have their eyes closed or are passively viewing a basic fixation cross, i.e. with a lack of stimulus or specific cognitive processing task. Resting state fMRI allows an assessment of intrinsic functional connectivity when correlations in the BOLD response between regions of interest are calculated. The default mode network (DMN) is of particular interest in the study of resting state fMRI because the DMN is the primary network activated during passive states[98] and dissociates from other networks during cognitive processing tasks.[98,99] Activation of the DMN in the absence of an overt cognitive processing task does not simply reflect an idle brain state. The DMN is involved with internal mentation such as constructing self-relevant decisions, mental imagery, and future-oriented thought.[99] Due to a role in internal mentation, the DMN has received considerable attention in its contribution to dysfunction in psychiatric disorders,[203] such as ASD. The DMN is relevant in the study of network coherence in ASD because the regions comprising the DMN are involved with social cognition and Theory of Mind,[204,205] which are affected in the disorder.[206]

Investigations of resting state functional connectivity of the DMN in ASD generally report global patterns of hypoconnectivity,[96,100-106] consistent with previous underconnectivity theories of ASD. However, increased connectivity within the DMN network[105] and between the DMN and visual and motor networks has also been reported.[106] The DMN is comprised of multiple subnetworks including a midline core, a dorsal medial prefrontal cortex (dMPFC) subnetwork, and a medial temporal lobe (MTL) subnetwork.[207] The MTL subnetwork is preferentially activated during self-referential prospective thought and mental imagery, and the dMPFC is preferentially activated during self- and other- mental state inference, suggesting a role in social cognition. The midline core is activated during internal mentation associated with

both the MTL and dMPFC subnetworks as well as evaluation of personal significance.[207] Hyperconnectivity within the DMN in individuals with ASD was reported specifically within the MTL subnetwork and between regions across DMN subnetworks,[106] suggesting less segregation between subnetworks of the DMN and a potential bias towards MTL-based processing. Overall, DMN coherence appears to be affected in ASD with general hypoconnectivity across the DMN but potentially hyperconnectivity with MTL regions and less segregation between the DMN and other functional networks in the brain.

Previous research into pharmacological modulation of network coherence in ASD has shown that *beta*-adrenergic antagonism can increase functional connectivity during associative processing[124] in regions associated with lexical, semantic, and associative networks.[199] Although a definitive noradrenergic mechanism has not been established, individuals with ASD exhibit heightened sympathetic nervous system arousal,[170-172] stress reactivity,[173,174] and peripheral levels of NE and stress hormones,[175,176] suggesting that modulation of the noradrenergic system may provide some clinical benefit. *Beta*-adrenergic antagonist trials in individuals with ASD have reported diminished aggressiveness, [123] verbal problem solving,[184] semantic fluency,[185] working memory,[186] and social reciprocity.[187] The widespread effects of *beta*-adrenergic antagonism on cognitive processing in individuals with ASD indicate that networks underlying other cognitive domains may also be altered following pharmacological intervention. Given that the DMN is involved in social cognition, network coherence of the DMN is affected in ASD, and *beta*-adrenergic antagonism modulates FC, we proposed that *beta*-adrenergic antagonism could modulate network coherence of the DMN in individuals with ASD.

The purpose of this study was to assess the effects of *beta*-adrenergic antagonism on resting-state network coherence in ASD. ASD and matched controls were administered propranolol, a CNS and PNS *beta*-adrenergic antagonist, nadolol, a PNS only *beta*-adrenergic antagonist, and placebo across three separate within-subject conditions. Nadolol served as a control for the PNS effects of *beta*-adrenergic antagonism because it does not cross the blood-brain barrier yet yields identical peripheral physiological effects as propranolol. To assess the PNS effects of *beta*-adrenergic antagonism, heart rate and blood pressure were measured. To assess the CNS effects of *beta*-adrenergic antagonism, resting-state fMRI data were acquired. Functional network coherence of cortical and subcortical networks was assessed within drug conditions and between ASD and control groups, with particular emphasis on the DMN due to the reported alterations in network coherence in individuals with ASD and its purported role in social cognition. Graph theory techniques were utilized because graph theory provides a data-driven assessment of network coherence that allows evaluation of different aspects of network topology.[208]

Based on previous reports of hypoconnectivity of the DMN, we hypothesized that individuals with ASD would exhibit significantly lower functional connectivity in the entire DMN and dMPFC subnetwork at baseline; however, the MTL subnetwork may exhibit hyperconnectivity due to increased connectivity between MTL and midline core regions. Following propranolol administration, we hypothesized that individuals with ASD would exhibit significantly higher functional connectivity in the DMN compared to nadolol and placebo, due to diminished LC-NE effects, and that these changes would be greater in individuals with ASD than controls. We also hypothesized that individuals with the lowest functional connectivity estimates at baseline would show the largest changes in functional connectivity following propranolol

administration because LC-NE activation modulates network access and *beta*-adrenergic antagonism may mitigate these effects in individuals with ASD and allow greater functional integration of the DMN.

## Methods

### PARTICIPANTS

Fifteen individuals with ASD, confirmed from clinical report and the Autism Diagnostic Interview-Revised,[209] with a full-scale IQ (FSIQ) of at least 80 and aged between 15 and 35 years were recruited from the University of Missouri Thompson Center for Autism and Neurodevelopmental Disorders. Fifteen gender, age, FSIQ and handedness matched controls without any previous major medical or psychiatric diagnoses were recruited from the surrounding community. All participants were interviewed by a physician ensuring drug administration safety and that enrollment criteria were met. IQ was estimated with the Wechsler Abbreviated Scale of Intelligence.[210] Demographic information (e.g. ethnicity, years of education, and socio-economic status) were collected with questionnaires. All participants were consented in accordance with the University of Missouri Health Sciences Institutional Review Board.

### ENROLLMENT CRITERIA

Inclusion criteria included: 1) an ASD diagnosis confirmed from clinical report and the Autism Diagnostic Interview-Revised[209] and no previous major medical or psychiatric diagnoses, 2) between 15 and 35 years old, and 3) a minimum low average IQ of 80. Exclusion criteria included: 1) a history of hypersensitivity or adverse reactions to *beta*-adrenergic antagonists, 2) diabetes, 3) a current diagnosis of reactive airway or pulmonary disease, 4) thyroid disease, 5) bradyarrhythmia, 6) narrow angle glaucoma, 5) schizophrenia, 6) major depression, 7) bipolar

disorder, 8) non-ASD related learning disability, 9) previous major head trauma, or 10) pregnancy.

Control participants were also interviewed by a clinician experienced in neurodevelopmental disorders to confirm no current or previous history of neurodevelopmental diagnoses. Participant medications were also screened. Control participants were not enrolled if currently or regularly taking any psychoactive medications. ASD participants prescribed medication commonly used to treat ADHD, including but not limited to Ritalin, Adderall, Clonidine, Focalin, Methylin, Concerta, or Strattera, were advised that these medications could not be used within 24 hours of participation. Before altering medication regimes, participants were required to consult with a personal physician and obtain written verification of the ability to safely withhold medication.

**DRUG ADMINISTRATION**

Participants attended three sessions counterbalanced for drug order separated by at least 24 hours in which propranolol, nadolol, or placebo were administered orally in a blinded manner. A dose of 40 mg propranolol was administered because this dose was previously shown to be sufficient to benefit cognitive flexibility for those struggling to complete difficult problems.[201] A dose of 50 mg nadolol was administered because it does not cross the blood-brain barrier and yields identical heart rate and blood pressure changes as 40 mg propranolol.[211] Drug administration was followed by wait times for peak effects: 60, 90 or 120 minutes for propranolol, placebo and nadolol, respectively.

Heart rate, blood pressure, and self-report anxiety, as assessed by the Beck Anxiety Inventory (BAI),[212] were measured before drug administration, at peak effects, and following testing. Difference scores between baseline and each subsequent time point were computed for

further analysis. Researchers were blinded during data processing to participant diagnostic group and treatment condition.

**MRI ACQUISITION**

Following the proper wait time for peak drug effects, MRI was carried out at the Brain Imaging Center of the University of Missouri, Department of Psychological Sciences utilizing a Siemens 3T Trio scanner (Siemens, Malvern, PA). Structural T1-weighted images were acquired for anatomical localization (TR=1920 ms, TE=2.9 ms, Flip Angle=9 degrees, 1 $mm^3$ resolution) and functional T2*-weighted images were acquired for BOLD activation (TR=2200 ms, TE=30 ms, Flip Angle=90 degrees, 35 ACPC-aligned slices at 4 $mm^3$) during 5 minutes of passive rest in which the participant viewed a blank screen with a cross-hair fixation point.

**MRI PREPROCESSING**

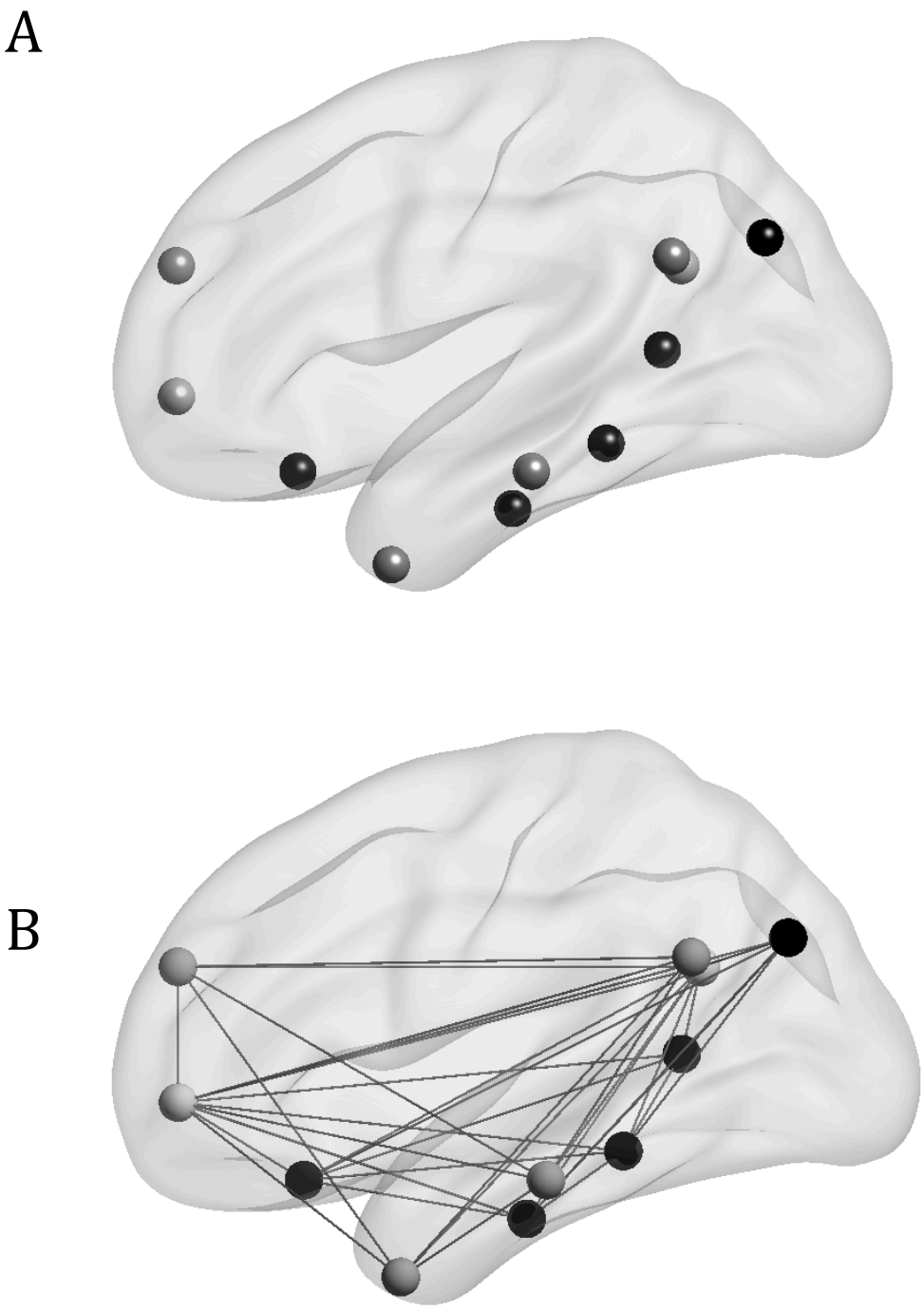
Preprocessing of fMRI data consisted of slice timing correction, rigid body realignment, intensity normalization, brain extraction and registration to the structural T1-weighted image with the FMRIB Software Library (FSL).[213,214] To account for spurious fluctuations in the BOLD signal,[215] translation and rotation parameters (x, y, z, pitch, roll, and yaw) from realignment were combined with average BOLD signals from the whole brain, ventricles and white matter and their temporal derivatives and regressed out of the timeseries data with the REST toolkit.[216] Temporal band-pass filtering ($0.01 < f < 0.08$ Hz) was also applied to reduce the effects of low-frequency drift and high-frequency noise and focus the analysis on intrinsic fluctuations.[217] FMRI data were then conservatively motion corrected as motion can substantially influence functional connectivity analyses.[218,219] BOLD acquisitions were scrubbed for excess motion and signal intensity using in-house MATLAB programs (The Mathworks, Inc., Natick, MA). Any acquisitions that exceeded 2 standard deviations from the within-subject within-run mean for any

translation, rotation, or intensity parameter or exceeded motion of more than 2 mm in any direction were removed.

Structural T1-weighted images were registered to the MNI atlas[220,221] and inverse matrices were generated for each subject for each session. Standard space regions of interest (ROIs) from the Automated Anatomical Labeling method [222] were converted to each participant's native space and timeseries were averaged from all voxels within each ROI. The AAL template includes 45 ROIs from the cerebrum and 9 from the cerebellum in each hemisphere and an additional 8 ROIs from the cerebellum along the vermal midline, totaling 116 separate brain regions. Regions comprising the DMN, as defined by Andrews-Hanna, et al.,[223] were localized within AAL ROIs and included: the midline core, consisting of the anterior medial prefrontal cortex (aMPFC) and posterior cingulate cortex (PCC), (2) the dMPFC subnetwork, consisting of the dorsal medial prefrontal cortex (dMPFC), temporal parietal junction (TPJ), lateral temporal cortex (LTC) and temporal pole (TempP), and (3) the MTL subnetwork, consisting of the ventral medial prefrontal cortex (vMPFC), posterior inferior parietal lobule (pIPL), retrosplenial cortex (RsP), parahippocampal cortex (PHC) and hippocampal formation (HF), Figure 1. The midline core regions were included in both the dMPFC and MTL subnetworks for further analyses due the robust connectivity between the midline core and each DMN subnetwork.

To account for covariance and allow for assessment of unique functional connectivity, partial correlation matrices containing all possible ROI pairs were generated for each participant and each condition. Fischer's r-to-z transformations were then applied to standardize the data, and self- and negative correlations were set to zero. Negative correlations were removed from

**Figure 1 Default Mode Network structure.** (A) Regions comprising the default mode network (DMN), as defined by Andrews-Hanna et al. [223]. The midline core (light grey) and dMPFC (dark grey) and MTL (black) subnetworks are displayed. (B) Connections within subnetworks of the DMN are displayed for the left hemisphere. Images were generated with BrainNet Viewer [224].



further analysis because false negative correlations may be introduced by removal of the whole brain signal during preprocessing.[225,226]

**NETWORK COHERENCE ANALYSIS**

Network coherence analyses were conducted utilizing data-driven graph theory techniques to assess properties of the whole-brain and sub- networks. A graph is a mathematical representation of a network and consists of a set of nodes, ROIs, and a set of edges, connections between nodes. Graph theory has been applied previously to assess networks in the brain and allows comparison between normal and pathological states.[227] Most importantly, graph theory allows a comprehensive assessment of different aspects of network coherence expanding on previous functional connectivity investigations between specific neuronal clusters.[208] Graph metrics, Table 1, were calculated for the whole-brain and DMN using the Brain Connectivity Toolbox.[228] Clustering coefficient measures the likelihood of connections between regions connected to a common region, and local efficiency measures the average efficiency of information exchange of clusters within the network, representing local processing. Characteristic path length measures the average shortest number connections between regions and global efficiency measures the efficiency of information exchange across the network, representing global processing. Therefore these additional measures provide assessment of global vs. local processing within networks of interest. Abnormal functional connectivity in individuals with ASD typically presents patterns of global hypoconnectivity but some hyperconnectivity of anatomically neighboring regions.[94] We hypothesized that individuals with ASD would exhibit increased local processing and decreased global processing at baseline and that propranolol administration would increase global processing within the DMN.

**Table 1 Definitions of graph metrics.** Description of graph metrics computed to assess network coherence.

| Graph Metrics | Analysis Level and Interpretation |
|---|---|
| **Functional Connectivity** | ***(FC)*** The average weight of connections |
| **Clustering Coefficient** | ***(C)*** The fraction of a node's neighbors, connected by edges, that are also neighbors |
| **Characteristic Path Length** | ***(L)*** The average shortest number of edges, connections, between nodes |
| **Global Efficiency** | ***(Eglobal)*** Efficiency of information exchange across the network |
| **Local Efficiency** | ***(Elocal)*** The average efficiency of information exchange in clusters within the network |

Standardized partial correlation matrices were first analyzed at a whole-brain or global level, and graph metrics were generated to characterize network coherence across all regions within the brain and those comprising the bilateral DMN. Before graph metrics were calculated the matrices were thresholded to control for sparsity of the network (i.e. number of connections)

because graph theory techniques are most applicable to sparse graphs and differences in sparsity between groups and drug conditions may obscure the interpretation of differences across metrics.[229] For example if an individual exhibited a greater number of low magnitude connections between regions of interest simply due to individual differences in network topology but expressed the same high magnitude connections representing underlying functional network structure, a difference in clustering may be due to the low magnitude connections in the network and not represent meaningful differences in network coherence. Therefore, global matrices were thresholded based on cost (*k;* the number of actual connections in the network) at the minimum cost value across all participants, $k$=0.49. We chose this value because it standardized sparsity across participants while still allowing the greatest number of potential connections of interest to be represented in our analyses. Additionally, individual standardized thresholds have not yet been identified that provide meaningful cut-offs within this network approach. We limited our threshold to a single value to control for potential Type 1 error within our analyses. Relevant subnetwork graph metrics were also generated for lateralized dMPFC and MTL subnetworks to determine if lateralization or domain-specific differences existed across groups and drug conditions. Unthresholded weighted matrices were used to calculate graph metrics at the DMN subnetwork level because we were assessing small networks with *a priori* structure, which did not exhibit enough connections for graph metric calculations when thresholded. Clustering coefficients and characteristic path lengths were not examined because these metrics would be largely redundant with measures of global and local efficiency at this level.

**STATISTICAL ANALYSES**

One ASD participant was unable to complete the study due to an adverse reaction to the imaging environment and one additional ASD participant was removed following motion

correction. These subjects' matched controls were subsequently removed and statistical analyses were conducted on 13 individuals with ASD and 13 matched controls. Analyses mainly consisted of a (multivariate) analysis of variance (M)ANOVA approach with an additional $\chi^2$ (chi-squared) for categorical variables. Analyses included 1) a one-way ANOVA between groups for continuous demographic variables (age, IQ, and years of education) and a $\chi^2$ (chi-squared) for categorical demographic variables (gender, ethnicity, handedness, and family income), 2) a 2 X 3 repeated measures MANOVA between diagnostic groups and within drug conditions for difference from baseline to peak effect and ending time points for cardiovascular and anxiety measures (heart rate, blood pressure (systolic and diastolic), and BAI), 3) a set of two (one for the whole brain and one for the bilateral DMN) 2 X 3 repeated measures MANOVAs between groups and within drug conditions for global level graph metrics (*C, L, Eglobal, Elocal,* FC), and 4) a set of four (the right and left lateralized dMPFC and MTL subnetworks) 2 X 3 repeated measures MANOVAs between groups and within drug conditions for DMN level graph metrics (*Eglobal*, *Elocal*, FC). One-sample t-tests were also utilized to assess if the change in cardiovascular measures from baseline to peak effect were significant within each drug condition.

Analyses were carried out with IBM SPSS Statistics Software.[230] Due to the small sample size and pilot nature of this investigation, correction for multiple comparisons was only completed across drug conditions, which may increase Type I error but will allow hypothesis generation for future investigations. Follow-up paired samples t-tests or independent samples t-tests were conducted when significance was indicated at the univariate level, and correction for multiple comparisons was completed across drug conditions by controlling for the false discovery rate (FDR).[231] Significance following FDR correction is indicated (*).

**Table 2 Demographic and diagnostic information for participants undergoing *beta*-adrenergic antagonism.** Data represents average scores +/- standard deviation or number of subjects within each categorical group. Categorical groups include males and females (M/F), white, black, Hispanic and other (W/B/H/O), right and left (R/L), and most frequently reported income bracket (mode).

| | **ASD** | **CTRL** | **Statistics** | **p** |
|---|---|---|---|---|
| ***Demographics*** | (n=13) | (n=13) | | |
| Age (years) | 22.21 +/- 4.16 | 22.86 +/- 2.68 | F(1,24)=0.23 | 0.64 |
| Gender (M/F) | 11/2 | 11/2 | $\chi^{2223}$=0.00 | 1.00 |
| Race (W/B/H/O) | 10/1/1/1 | 10/1/0/2 | $\chi^2$(3)=1.33 | 0.74 |
| Handedness (R/L) | 10/3 | 11/2 | $\chi^{2223}$=0.25 | 0.62 |
| Education (years) | 13.46 +/- 2.85 | 15.23 +/- 2.45 | F(1,24)=2.88 | 0.10 |
| Family Income (mode) | $25,000-$34,999 | $50,000-$74,999 | $\chi^2$(5)=7.52 | 0.19 |
| ***Intelligence Quotients*** | | | | |
| VIQ | 105.31 +/- 13.71 | 114.08 +/- 8.50 | F(1,24)=3.84 | 0.06 |
| PIQ | 103.77 +/- 15.71 | 105.69 +/- 12.20 | F(1,24)=0.13 | 0.73 |
| FSIQ | 105.46 +/- 14.30 | 111.08 +/- 10.00 | F(1,24)=1.35 | 0.26 |
| ***Diagnostics (cutoff)*** | | | | |
| ADI Social [223] | 19.85 +/- 4.39 | - | | |
| ADI Communication (8) | 12.76 +/- 3.92 | - | | |
| ADI Non-verbal (7) | 7.33 +/- 2.90 | - | | |
| ADI Abnormality [223] | 2.92 +/- 0.86 | - | | |

# Results

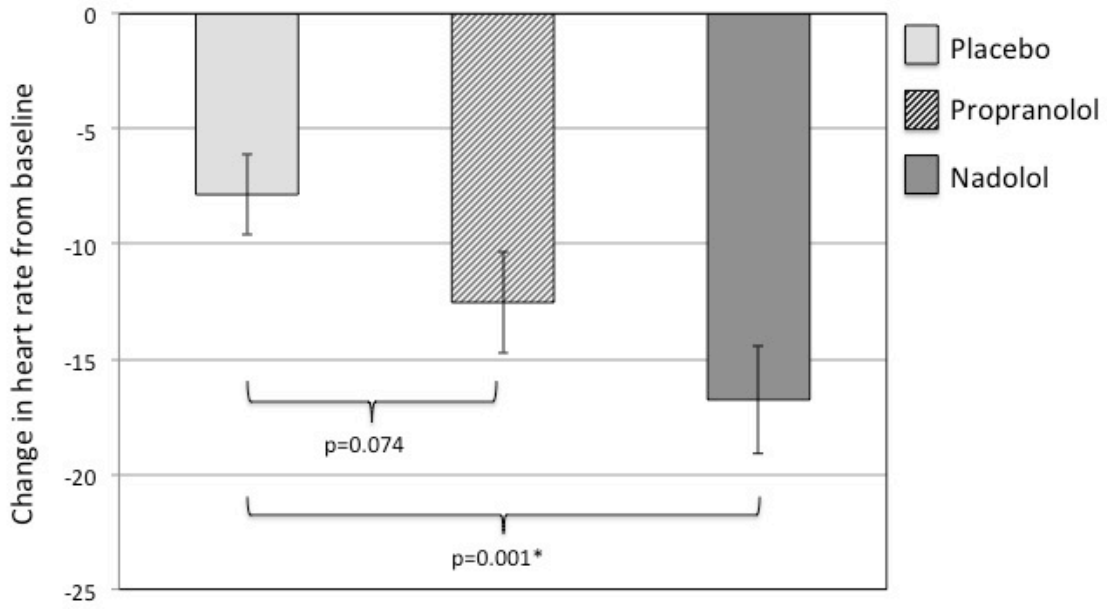
## PARTICIPANTS

The ASD and control groups did not significantly differ in age, IQ, or years of education, $p>0.05$, nor gender, ethnicity, handedness or family income, $p>0.05$, Table 2. VIQ approached significance, $p=0.06$, which was primarily due to matching participants based on FSIQ. One ASD participant's IQ was low average, which is difficult to match without introducing additional confounds. This participant was matched to a control participant within the average IQ range.

## CARDIOVASCULAR AND ANXIETY MEASURES

Cardiovascular and anxiety measure difference scores significantly changed within-subject across drug conditions, driven by heart rate changing from baseline to the peak drug effect time point, $F(2,48)=7.00$, $p=0.002$. Heart rate significantly decreased during placebo (M=-7.85 BPM), $t(25)=4.447$, $p<0.001$*, nadolol (M=-16.8 BPM), $t(25)=7.191$, $p<0.001$*, and propranolol administration (M=-12.54 BPM), $t(25)=5.724$, $p<0.001$, but decreased significantly more during nadolol compared to placebo, $t(25)=3.70$, $p=0.001$*, and exhibited a trend towards a greater reduction during propranolol compared to placebo, $t(25)=1.866$, $p=0.074$. Decreased heart rate during placebo was most likely due to the period of quiet rest used to control for wait time effects and the greater reduction following *beta*-adrenergic antagonism indicated a significant drug effect on the PNS across participants, Figure 2. Self-reported anxiety did not differ across drug conditions or diagnostic groups, $p>0.05$. The absence of drug-related changes in anxiety measures may be due to insufficient self-report sensitivity within the time domain of assessment, general lack of agreement between physiological and self-report measures of anxiety,[232] and difficulty individuals with ASD exhibit with emotional introspection.[233]

**Figure 2 *Beta*-adrenergic antagonism effects on heart rate.** Difference scores of heart rate in beats per minute (BPM) from baseline to peak drug effects are displayed for placebo (light grey), propranolol (striped), and nadolol (dark grey). Error bars represent standard error and significant differences are indicated (*).




**GRAPH METRICS**

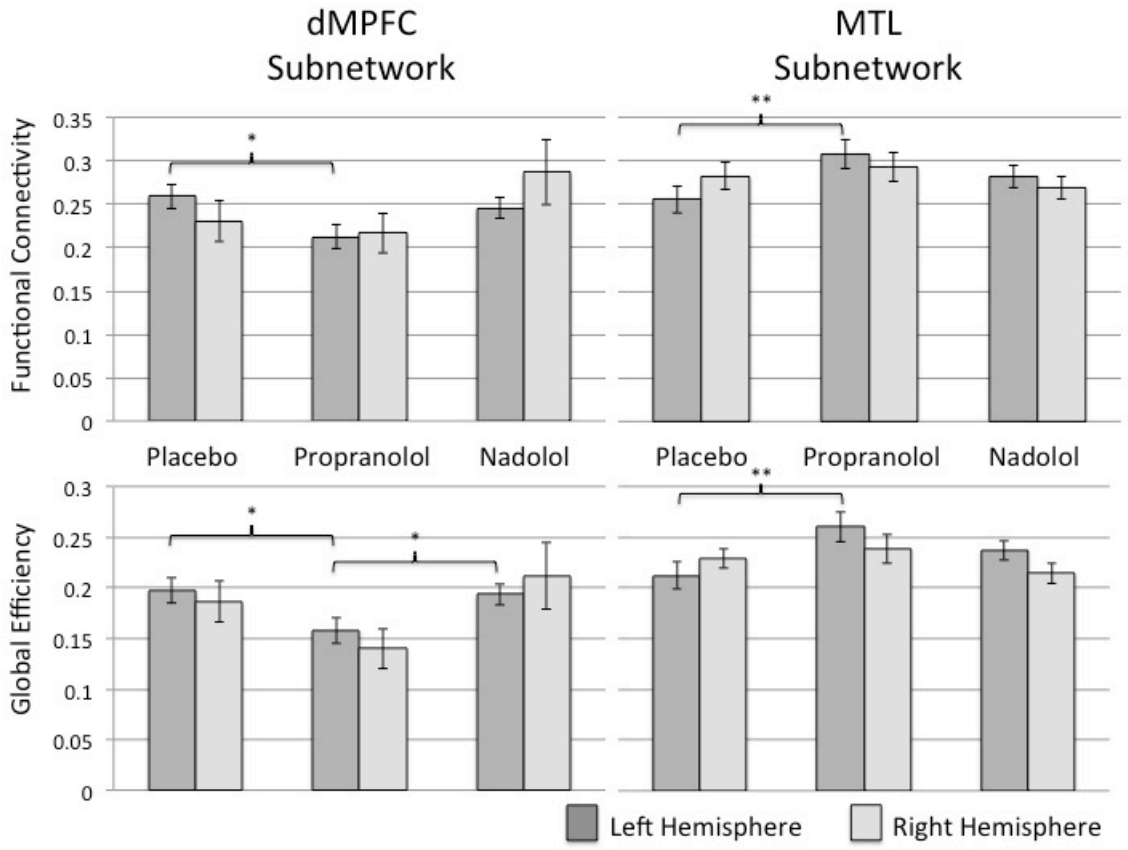
Whole-brain and bilateral DMN graph metrics did not significantly differ based on diagnostic group or drug condition nor was there a group by drug interaction observed, $p>0.05$.

**dMPFC Subnetwork**

For the left dMPFC, DMN graph metrics displayed a significant within-subject effect of drug at the omnibus level, $F(8,92)=2.252$, $p=0.030$, driven by *Eglobal* significantly changing within drug conditions, $F(2,48)=3.503$, $p=0.038$, Figure 3. During propranolol administration, *Eglobal* ($M=0.158$) significantly decreased compared to nadolol ($M=0.194$), $t(25)=2.203$,

p=0.037, and placebo (M=0.197), t(25)=2.297, p=0.030, but there was no change between placebo and nadolol, p>0.05. There was also a trend towards a difference in mean FC across

**Figure 3 *Beta*-adrenergic antagonism effects on the DMN during resting state.** Average functional connectivity and global efficiency of the dorsal medial prefrontal cortex (dMPFC) and medial temporal lobe (MTL) subnetworks are displayed for the left (dark grey) and right (light grey) hemisphere across placebo, propranolol, and nadolol conditions. Error bars represent standard error and significant differences are indicated with (**) and without (*) correction for multiple comparisons.




drug conditions, F(2,48)=3.005, p=0.059, Figure 3, with a significant decrease in FC during propranolol administration (M=0.213) compared to placebo (M=0.259), t(25)=2.499, p=0.019, but no difference compared to nadolol nor placebo compared to nadolol, p<0.05. Drug effects on network coherence in the dMPFC subnetwork did not significantly differ at the group-level

based on ASD diagnosis; however more individuals with ASD appeared to exhibit a change in network coherence following *beta*-adrenergic antagonism compared to controls with a subset of these individuals expressing higher connectivity at baseline exhibiting a greater response to drug, Figure 4. There were no significant changes in DMN graph metrics for the right dMPFC subnetwork across drug conditions or diagnostic groups, $p>0.05$, which appeared to be due to greater variability in network coherence in right hemisphere regions.

**Figure 4 Effects of propranolol on the DMN dMPFC subnetwork during resting state.** Functional connectivity is displayed across the (A) ASD and (B) control groups following the administration of placebo (dark grey) and propranolol (light grey). Colored lines indicate individual participants FC across drug conditions. Error bars represent standard error.

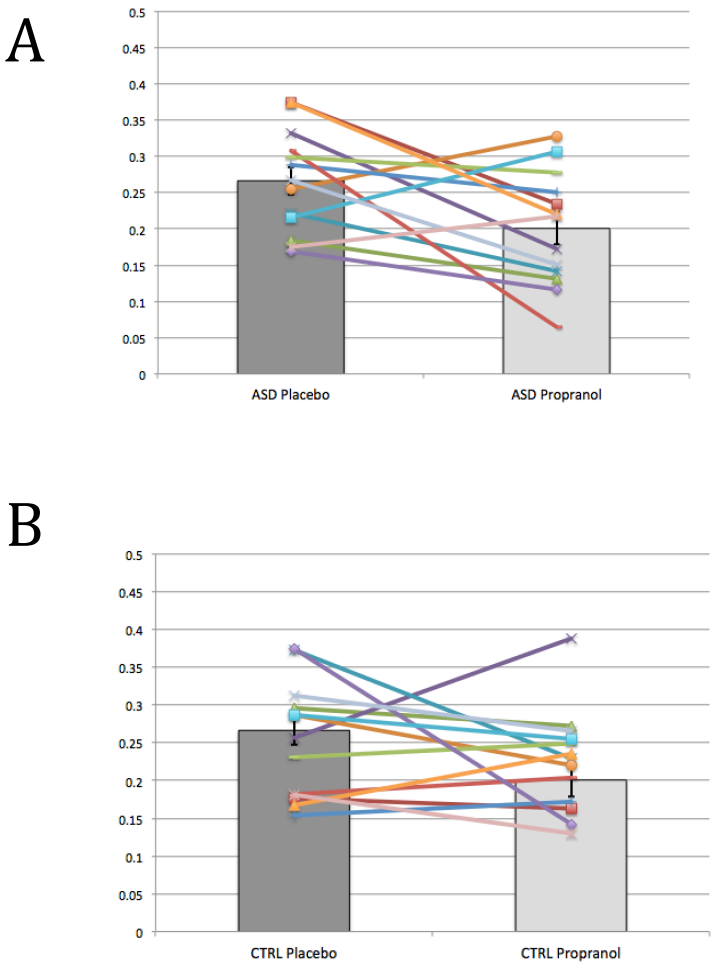
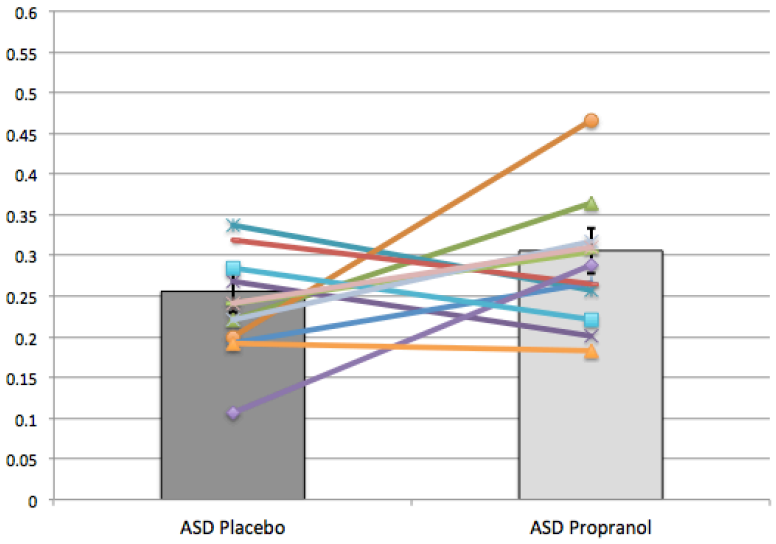
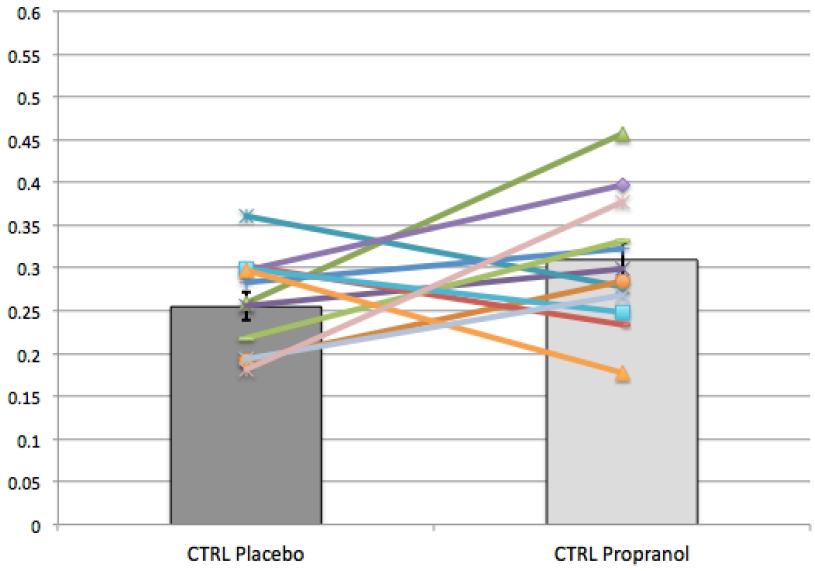


**Figure 5 Effects of propranolol on the DMN MTL subnetwork during resting state.** Functional connectivity is displayed across the (A) ASD and (B) control groups following the administration of placebo (dark grey) and propranolol (light grey). Colored lines indicate individual participants FC across drug conditions. Error bars represent standard error.

A




B



**MTL Subnetwork**

For the left MTL, there were no significant differences at the omnibus level, $p>0.05$; however there was a significant change in *Eglobal* across drug conditions at the univariate level, $F(2,48)=3.251$, $p=0.047$, Figure 3. During propranolol administration, *Eglobal* (M=0.260) significantly increased compared to placebo (M=0.212), $t(25)=2.696$, $p=0.012$*, but not nadolol (M=0.237), $p>0.05$, nor placebo compared to nadolol, $p<0.05$. There was also a trend towards a drug effect on FC, $F(2,48)=2.604$, $p=0.084$, with a significant increase in FC during propranolol administration (M=0.308) compared to placebo (M=0.255), $t(25)=2.561$, $p=0.017$, but not nadolol (M=0.282), $p>0.05$, nor placebo compared to nadolol, $p<0.05$ , Figure 3. Drug effects on network coherence in the MTL subnetwork did not significantly differ based ASD diagnosis with rather homogenous effects across groups, Figure 5. There were no significant changes in DMN graph metrics for the right MTL subnetwork across drug conditions or diagnostic groups, $p>0.05$.

## Discussion

*Beta*-adrenergic antagonism effects on network coherence in ASD were previously examined during an associative processing task and propranolol administration increased functional connectivity in networks underlying this cognitive processing domain. [124] The purpose of this study was to extend the investigation of *beta*-adrenergic antagonism effects on network coherence in individuals with ASD to additional networks implicated in the disorder during resting-state fMRI and assess whether these alterations in network coherence were specific to ASD. Our focus was on the DMN because it is more active during rest and exhibits altered functional connectivity in individuals with ASD[96,100,234,235] that is related to symptom presentation.[102] Utilizing a data-driven graph theory approach to assess network coherence, we found that *beta*-adrenergic antagonism altered FC and network efficiency of the DMN across all

participants regardless of diagnostic group. Relative to placebo, propranolol, a lipophilic *beta*-adrenergic antagonist, led to decreased connectivity and global efficiency in the dMPFC subnetwork and increased connectivity and global efficiency in the MTL subnetwork. Network coherence during nadolol administration, a hydrophilic *beta*-adrenergic antagonist that cannot pass through the blood-brain barrier, did not significantly differ from placebo, suggesting the propranolol-mediated changes in network coherence were not exclusively due to PNS effects on the BOLD signal. Furthermore, the general lack of differences between propranolol and nadolol suggest alterations of network coherence in the DMN may not be exclusively due to CNS effects, as previously reported. Rather, *beta*-adrenergic agents that cross the blood-brain barrier may generally modulate intrinsic functional connectivity and network coherence in the brain as a result of combined CNS and PNS mechanisms. Overall, *beta*-adrenergic antagonism modulated DMN network coherence within all individuals, but may influence inherent ASD-related disturbances in DMN organization and activation.[96,100,234,235] This is especially relevant in the study of ASD because the DMN is involved with prospective thought, introspective thought and theory of mind,[207] which are affected in the disorder.[206,236,237]

Relevant to this, we hypothesized that individuals with ASD would exhibit hypoconnectivity within the DMN[96,100-104] but potentially hyperconnectivity within the MTL subnetwork[106] compared to unaffected individuals. Within our sample, we found no significant baseline differences in connectivity between DMN subnetworks, which may be due to the utilization of large anatomically-defined regions of interest. The use of anatomically-defined regions, in contrast to functionally-defined ROIs, allows standardized segmentation of the brain but these regions may be comprised of multiple functional subunits,[229] diluting the ability to assess domain-specific functional clusters. Although there were no baseline differences in

network coherence of the DMN, *beta*-adrenergic effects on network coherence in individuals with ASD were supported.

The LC-NE has efferent projections throughout the brain and is the sole source of norepinephrine in the prefrontal cortex and medial temporal lobe.[193] *Beta*-adrenergic antagonism altered network coherence in the dMPFC and MTL subnetworks of the DMN, which contain multiple prefrontal and medial temporal lobe regions, suggesting pharmacological modulation of LC-NE influences on network coherence. We hypothesized a general increase in functional network coherence following propranolol administration because altered LC-NE output modulates networks underlying cognitive processing tasks in favor of networks that prompt environmental adaptation,[194-196] and increased connectivity with cognitive processing networks was previously reported following propranolol administration in individuals with ASD. *Beta*-adrenergic antagonism decreased network coherence in the dMPFC subnetwork of the DMN but increased network coherence in the MTL subnetwork. *Beta*-adrenergic receptor binding in the central nervous system typically hyperpolarizes cells[191,192] modulating networks dynamics; therefore some reductions in network coherence following *beta*-adrenergic antagonism would not be unexpected. Selective network effects of *beta*-adrenergic antagonism are also relevant for individuals with ASD considering reports of decreased integration within networks and segregation between networks in ASD.[238]

The most robust effects of *beta*-adrenergic antagonism altering network coherence in the brain were found in the left dMPFC subnetwork of the DMN. Functional connectivity and global efficiency were significantly lower during propranolol administration compared to placebo, and global efficiency was also lower compared to nadolol, suggesting this alteration in network coherence was not solely due to general cardiovascular changes in response to drug. Network

coherence was also altered in the MTL subnetwork in response to propranolol, however these effects did not significantly differ from nadolol, suggesting potential contributions from cardiovascular influences. Changes in MTL network coherence were also more general across participants whereas a greater proportion of individuals with ASD exhibited propranolol-mediated effects in the dMPFC compared to unaffected individuals. Individuals with ASD are more prone to heightened sympathetic nervous system arousal[170-172] and stress reactivity[173,174] compared to unaffected individuals, which is associated with upregulation of the locus coeruleus-norepinephrine system.[175,176] Heightened baseline noradrenergic activity, at least in a subset of individuals, may have contributed to a greater number of individuals with ASD responding to *beta*-adrenergic antagonism. Furthermore, decreased connectivity in the dMPFC subnetwork appeared to be driven by a subset of individuals with higher connectivity at baseline expressing larger changes in network coherence in response to propranolol, especially individuals with ASD. *Beta*-adrenergic antagonism can modulate network coherence of the DMN and these alterations are particularly robust in a subset of individuals with ASD exhibiting higher levels of connectivity at baseline. Future investigations with larger more heterogeneous samples of individuals with ASD will help further stratify these patients in order to identify salient subgroups that may be more likely to respond to this type of treatment.

*Beta*-adrenergic antagonism was previously shown to benefit cognitive processing, especially in verbal domains. We found that *beta*-adrenergic antagonism modulates network coherence, and these alterations were localized primarily in the left hemisphere, which supports potential benefits to language and communication domains. Communication deficits are core symptoms of ASD, and previous reports have shown that language-related improvements following propranolol administration are associated with increased FC.[124] Our findings suggest

that the effect of propranolol on network coherence is more complex than increased functional connectivity and that *beta*-adrenergic antagonism may be able to up- or down- regulate specific subnetworks in the brain, such as the DMN and alter global network efficiency. Reduced DMN network coherence could support cognitive benefits because the DMN dissociates from task-related networks during task performance,[98] which may allow greater network integration and better segregation between networks during cognitive processing. Additionally, increased network access to MTL regions such as the hippocampus may further support clinical benefits of *beta*-adrenergic antagonism given the pervasive role of the hippocampus across cognitive processing domains. Pharmacological modulation of network coherence allowing more efficient information processing may underlie the aforementioned propranolol-mediated benefits to cognitive processing; however assessment of network coherence during cognitive processing tasks will be necessary to further elucidate the effects of *beta*-adrenergic mediated changes in network coherence on symptom outcomes in ASD.

**LIMITATIONS**

Due to the pilot nature of our investigation, correction for multiple comparisons was only applied across drug conditions and the potential for increased Type I error should be considered regarding interpretation of this work. The general lack of diagnostic group differences in baseline comparisons of the DMN in our sample may have been due to the utilization of anatomically defined regions of interest. Future investigations of network coherence following propranolol administration in ASD should examine functionally-defined regions of interest in order to better localize domain-specific neuronal clusters. The functional roles of the MTL subnetwork during self-referential prospective thought and the dMPFC during mental state inference suggests that differential effects of *beta*-adrenergic antagonism across subnetworks may modulate these

domains but we did not assess participant mentation during passive rest. Evaluation of DMN network coherence during directed internal mentation will be necessary to elucidate the effects of *beta*-adrenergic antagonism on specific domains. Following assessment of functional connectivity, graph theory analysis was applied to further examine the effects of beta-adrenergic antagonism on network coherence. Sparsity within these networks was not controlled and potentially contributed to the aforementioned differences in network efficiency. Altered functional connectivity following propranolol administration was still supported regardless of these consideration. Furthermore, our analysis of resting-state data supports the ability of *beta*-adrenergic antagonism to alter network coherence, which is associated with improved cognitive processing, but does not directly assess changes in network coherence related to cognitive benefits. Further research is necessary to assess the effects of propranolol on network coherence in task-related networks during cognitive processing in order to more directly examine the influence of pharmacologically mediated changes in network coherence on clinical outcomes in ASD.

## ASSOCIATIVE PROCESSING DURING TASK-BASED fMRI

*Beta*-adrenergic antagonism, such as the use of propranolol, provides cognitive and behavioral benefits to individuals with ASD. An initial open-trial in older adults with ASD found that patients initially displayed diminished aggressiveness followed by some improvements in speech and socialization,[239] supporting the potential to pharmacologically treat both the secondary and core symptoms of the disorder. Single dose trials have reported additional benefits in verbal problem solving,[184] semantic fluency,[185] working memory,[186] and social reciprocity.[187] Individuals without neurodevelopmental diagnoses also benefit from the use of propranolol during cognitive processing but these benefits are primarily during difficult tasks that the individual is struggling to complete, whereas propranolol can hinder performance on simpler tasks in these same individuals.[201] Individuals with ASD appear to benefit from propranolol even on simple tasks,[184] and these additional benefits may be due to inherent differences in network coherence of functional networks affecting cognitive processing.

Individuals with ASD exhibit altered functional network topology and utilization in the brain. Generally, functional networks exhibit hypoconnectivity of more distant network regions and perhaps hyperconnectivity of anatomically neighboring regions.[94] Conceptualized into a neural systems framework, these patterns suggest reduced functional network integration and less network segregation[121] causing abnormal functional utilization of networks underlying specific cognitive and behavioral processing domains associated with the disorder. *Beta*-adrenergic antagonism can alter network coherence of functional networks underlying cognitive processing by modulating the effects of the locus coeruleus-norepinephrine (LC-NE) system on its targets in the cerebral cortex. The LC-NE initiates and maintains neuronal states appropriate for the acquisition of sensory information[192] and increased LC-NE output diminishes attentional

control in favor of re-orientation to environmental stimuli,[194,195] which alters activity patterns within networks underlying cognitive processing.[196] *Beta*-adrenergic antagonism can modulate the LC-NE shifting of network access. In our previous investigation, propranolol significantly altered network coherence of the default mode network (DMN) in individuals with and without an ASD diagnosis during passive rest. These findings support the ability of *beta*-adrenergic antagonism to modulate functional network coherence in the brain, but did so in the absence of an overt cognitive processing task. *Beta*-adrenergic antagonism can also increase coherence within networks underlying specific cognitive domains in individuals with ASD, [124] suggesting a link between the previously outlined behavioral benefits of propranolol in individuals with ASD and coherence of networks underlying cognitive processing; however this investigation did not examine performance during cognitive processing, and did not include a control group comparison. Examining network coherence across additional networks relevant for cognitive processing between individuals with and without ASD during performance trials with and without the administration of *beta*-adrenergic agents will allow a better understanding of the effects of propranolol on performance in individuals with ASD and allow an assessment of how these effects are mediated by alterations in functional network coherence.

In this investigation, we examine a semantic fluency task in order to assess the effects of *beta*-adrenergic antagonism underlying verbal processing in individuals with ASD. During verbal processing, regions comprising the primary language network are preferentially activated; however functional networks in the brain must also work in concert to shift network access and optimize performance. The default mode network (DMN) subserves internally directed cognition, e.g. internal mentation during passive rest, whereas the dorsal attention network (DAN) subserves attention orienting during externally directed cognition, e.g. stimulus based

cognitive processing.[240,241] Additionally, the frontoparietal control network (FPC) dynamically couples with either the DMN or DAN during internally vs. externally directed cognition, respectively, integrating information between systems and processes[242]. During presentation of a verbal processing task, the DMN is primarily activated during rest blocks interspersed between stimulus presentation and task performance. Upon the presentation of a verbal stimulus, the FPC shifts network access from the DMN to the DAN to allow orientation to the stimulus and the language network then processes the information necessary to complete the task. Once the stimulus is removed the FPC shifts network access back to the DMN until the next stimulus presentation. Cortical and subcortical regions comprising the language network[95,112], DAN,[243] DMN,[96] and FPC[244,245] exhibit disrupted connectivity in ASD, suggesting diminished network integration. Individuals with ASD typically exhibit reduced connectivity within these networks compared to unaffected individuals, and network disruptions are thought to underlie the language deficits associated with ASD.[107,112,246] Given that network integration during cognitive processing typically optimizes performance, coherence of networks involved with language processing are affected in ASD, and *beta*-adrenergic antagonism affects network coherence, we propose that *beta*-adrenergic antagonism may modulate network coherence of language, attention, and control networks during verbal processing in individuals with ASD and these alterations in network coherence will be associated with performance.

The purpose of this study was to assess the effects of *beta*-adrenergic antagonism on network coherence in ASD during cognitive processing. Individuals with ASD and matched controls were administered propranolol, a CNS and PNS *beta*-adrenergic antagonist, nadolol, a PNS only *beta*-adrenergic antagonist, and placebo across three separate within-subject sessions. Nadolol served as a control for the PNS effects of *beta*-adrenergic antagonism because it does

not cross the blood-brain barrier yet yields identical peripheral physiological effects to propranolol. To assess the PNS effects of *beta*-adrenergic antagonism, heart rate and blood pressure were measured, and to assess the CNS effects of *beta*-adrenergic antagonism, task-based fMRI data during an associative processing task were acquired. Network coherence of language, DMN, DAN, and FPC networks were assessed within drug conditions and between ASD and control groups.

Based on previous reports of semantic fluency performance benefits selectivity in individuals with ASD,[124] we hypothesized that individuals with ASD would exhibit a greater increase in semantic fluency performance compared to controls. Additionally, hypoconnectivity of language networks has also been reported in individuals with ASD during cognitive processing[107] and *beta*-adrenergic antagonism significantly increased connectivity of language regions in a separate sample.[124] Therefore, we hypothesized that individuals with ASD would exhibit significantly lower functional connectivity in language regions at baseline compared to unaffected individuals and exhibit a larger increase in network coherence following propranolol administration. We also examined the aforementioned networks that may be affected by *beta*-adrenergic antagonism and are preferentially activated or deactivated during cognitive processing. We proposed that minimal differences would be found in the DMN due to down regulation of the DMN during cognitive processing.[241] Attentional and control networks were examined because the previously noted changes in network coherence following *beta*-adrenergic antagonism could have been due to alterations related to these aspects of cognitive processing. We hypothesized that additional baseline differences would be found in the DAN and FPC with potentially lower network coherence in individuals with ASD, as previously reported.[243,244] Furthermore, *beta*-adrenergic antagonism may increase functional connectivity in the FPC allowing better

integration within networks involved in cognitive processing demands due to reduced LC-NE effects downregulating the DAN and allowing the FPC to modulate internetwork communication between other cognitive processing regions.[247] LC-NE activation modulates network access and *beta*-adrenergic antagonism may mitigate these effects in individuals with ASD and allow greater functional network integration. We hypothesized that individuals with the lowest functional connectivity estimates at baseline would exhibit the largest performance benefits following propranolol administration due to greater network access.

## Methods

### PARTICIPANTS, ENROLLMENT CRITERIA, AND DRUG ADMINISTRATION

Participants, Table 2, enrollment criteria, and drug administration and effects, Figure 2, were previously described in the resting-state fMRI portion of this manuscript.

### MRI ACQUISITION

Following the acquisition of previously described structural and resting-state data, functional T2*-weighted images were acquired for BOLD activation (TR=2200 ms, TE=30 ms, Flip Angle=90 degrees, 35 ACPC-aligned slices at 4 mm3) during the completion of a semantic associative processing task.

### VERBAL PROCESSING TASKS

Verbal processing was assessed utilizing a fluency task. While in the MR scanner, each participant completed two independent versions of a semantic fluency task during each session. Semantic fluency stimuli consisted of the presentation of a verbal cue for 33 seconds (15 TRs). In response to the semantic cue, each participant was instructed to verbally name as many items as possible that belonged to the cue category, for example ANIMALS. An independent rater that was blind to diagnostic group and drug condition

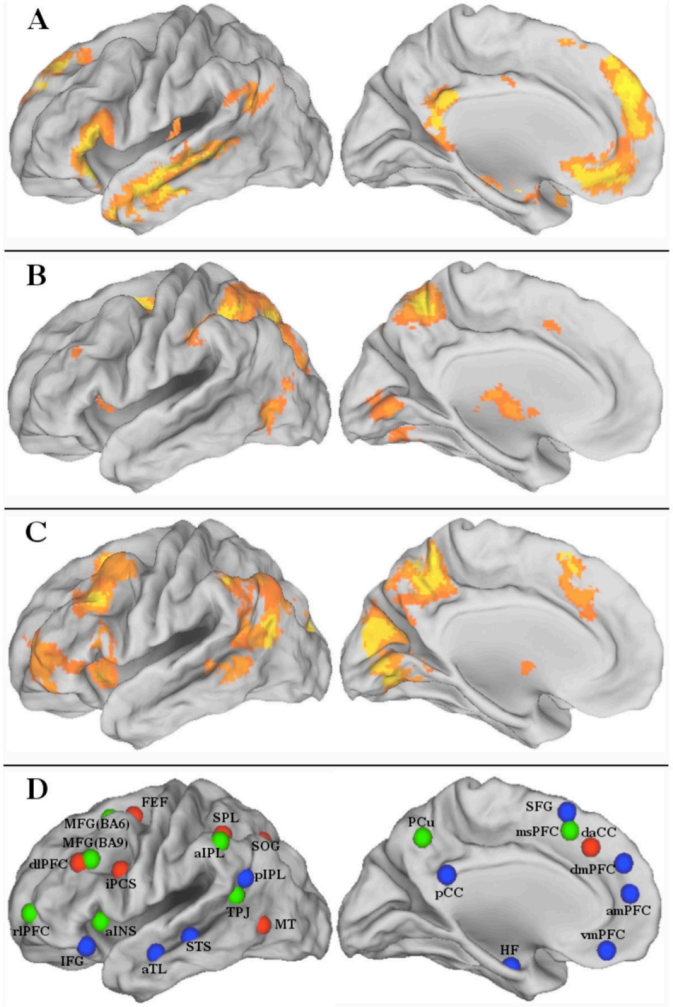
recorded each verbal response. Each cue and response block was preceded by 11 seconds (5 TRs) of passive rest in which a cross-hair fixation point was displayed. Fluency cue response was utilized as a means of assessing performance based on the quantity of words produced per cue.

**MRI PREPROCESSING**

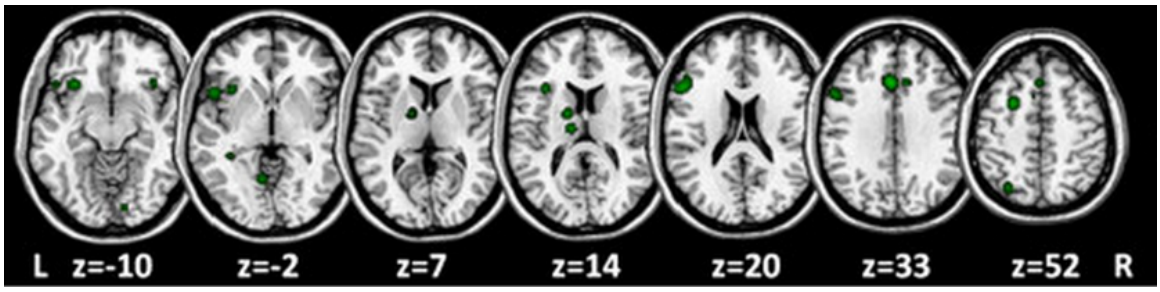
Preprocessing procedures were previously outlined. Additional considerations for task-based data were also applied. Initial analyses were conducted on timeseries with an additional regression of stimulus design in order to account for activation magnitude differences between groups and drug conditions and to focus the analyses on residual fluctuations in the BOLD response. Due to methodological concerns regarding preprocessing methods in task-based functional connectivity analyses in ASD,[122] we also examined the BOLD response only during task blocks. A combination of task regression analysis and analysis of only stimulus blocks should allow us to identify the most robust diagnostic group and drug condition differences in network coherence.

Structural T1-weighted images were registered to the MNI atlas[220,221] and inverse matrices were generated for each subject for each session. 5 mm spherical standard space regions of interest from were determined *a priori* for a language-based network that is preferentially activated during semantic fluency, hereafter referred to as the semantic association network (SAN),[248] and the DMN, DAN, and FPC as described in Spreng et al,(2013),[247] which were previously defined by significant and reliable task-based activation during multiple cognitive processing tasks across multiple independent samples, Figure 6. ROIs were converted to each participant's native space and timeseries were averaged from all voxels within each ROI. The SAN included the left anterior cingulate cortex (aCC),

**Figure 6 DMN, DAN, FPC, and SAN functional networks.** Left hemisphere lateral and medial surfaces for task-based localization of regions comprising the (A) default mode, (B) dorsal attention, and (C) frontoparietal control networks. (D) ROIs from the default (dark blue), dorsal attention (red) and frontoparietal control [23] networks. (E) Task-based localization of regions comprising the semantic association network. Images reproduced from Spreng et al. 2013 and Wagner et al. 2014.




**E**



the left inferior frontal gyrus BA45 (IFG45), the left superior/middle frontal gyrus (SMFG), the left orbitofrontal cortex (OFC), the left inferior frontal gyrus BA47 (IFG47), and the superior parietal cortex (SPC). The DMN included the left anterior medial prefrontal cortex (amPFC), right and left anterior temporal lobe (aTL), left dorsal medial prefrontal cortex (dmPFC), right and left hippocampal formation (HF), right and left inferior frontal gyrus (IFG), left posterior cingulate cortex (pCC), right and left posterior inferior parietal lobule (pIPL), right and left superior frontal gyrus (SFG), right and left superior temporal sulcus (STS), and left ventral medial prefrontal cortex (vmPFC). The DAN included the right and left frontal eye fields (FEF), the right and left inferior precentral sulcus (iPCS), the right and left middle temporal motion complex (MT), the right and left superior occipital gyrus (SOG), the left and right superior parietal lobule (SPL), the left and right dorsolateral prefrontal cortex (dlPFC), and the right dorsal anterior cingulate cortex (daCC). The FPC included the right and left anterior inferior parietal lobule (aIPL), the right and left anterior insula (aINS), the left medial superior prefrontal cortex (msPFC), the right and left middle frontal gyrus BA6 (MFGBA6), the right and left middle frontal gyrus BA9 (MFGBA9), and the right and left rostrolateral prefrontal cortex (rlPFC). All regions included in this analysis were non-overlapping. To account for covariance and allow for assessment of unique functional connectivity, partial correlation matrices containing all possible ROI pairs within each network were generated for each participant. Fischer's r-to-z transformations were then applied to standardize the data, and self- and negative correlations were set to zero.

**NETWORK COHERENCE ANALYSIS**

Network coherence analyses were conducted utilizing graph theory techniques to assess properties within each network and were previously described in the resting-state portion of this

manuscript. Standardized partial correlation matrices were analyzed across all possible ROI pairs within the SAN, DMN, DAN, and FPC networks separately. Before graph metrics were calculated the matrices were thresholded to control for sparsity of the network, number of connections, because graph theory techniques are most applicable to sparse graphs and differences in sparsity between groups and drug conditions may obscure the interpretation of differences across metrics,[229] as has been previously described. Therefore, matrices were thresholded based on cost (*k*). To account for native topology within our dataset, the matrices were thresholded at the minimum cost value across all participants, $k$=0.45. Thresholded weighted matrices were used to calculate weighted graph metrics. Metrics of interest included: (1) mean functional connectivity (FC), (2) mean clustering coefficient (*C*), (3) characteristic path length (*L*), (4) global efficiency (*Eglobal*), and (5) mean local efficiency (*Elocal*), which were previously described, Table 1. Due to the small number of ROIs within the SAN, there was insufficient sparsity to calculate some graph metrics at the 0.45 cost level and graph metrics were analyzed with unthresholded matrices for examination of network properties. Functional connectivity was still evaluated at k=0.45 threshold for comparison of the most robust connections across networks of interest. Differences in sparsity will affect graph metric calculations and this confound should be taken into account with interpretation of graph metrics of the SAN.

**STATISTICAL ANALYSES**

One ASD participant was unable to complete the study due to an adverse reaction to the imaging environment and one additional ASD participant was removed following motion correction. These subjects' matched controls were subsequently removed and statistical analyses were conducted on 13 individuals with ASD and 13 matched controls. Analyses consisted of a

(multivariate) analysis of variance (M)ANOVA approach. Analyses included: 1) a 2 X 3 repeated measures MANOVA between diagnostic groups and within drug conditions for maximum number of words produced between the two assessments of fluency and 2) a 2 X 3 repeated measures MANOVA between groups and within drug conditions separately for graph metrics from the SAN, DMN, DAN, and FPC networks (*C, L, Eglobal, Elocal,* FC). Graph metric analyses were also assessed controlling for the effects of task performance to determine whether changes in network coherence were related to performance differences across drug conditions.

Analyses were carried out with IBM SPSS Statistics Software.[230] Due to the small sample size and pilot nature of this investigation, correction for multiple comparisons was only completed across drug conditions, which may increase Type I error but will allow hypothesis generation for future investigations. The additional analyses assessing differences related to performance and focusing only on tasks blocks should further allow us to identify the most robust diagnostic group and drug-related changes. Follow-up paired samples t-tests or independent samples t-tests were conducted when significance was indicated at the univariate level and corrected for multiple comparisons across drug conditions by controlling for the false discovery rate (FDR).[231] Significance following FDR correction is indicated (*).

## Results

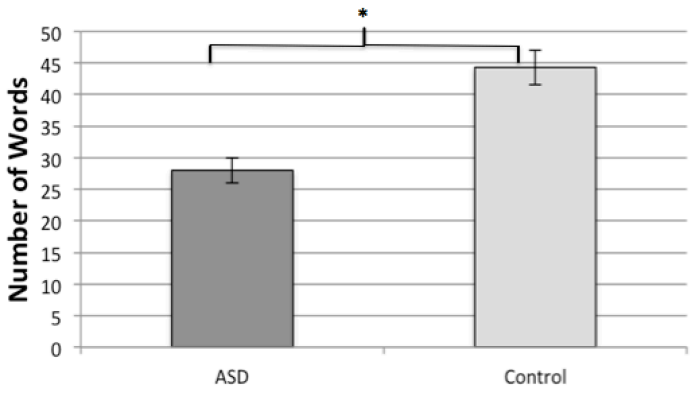
### SEMANTIC FLUENCY

There was a significant diagnostic group difference in the maximum number of words participants were able to produce across trials, $F(1,24)=25.667$, $p<0.001$. The control group produced significantly more words (M=44.30, SD=14.17) compared to the ASD group (M=28.00, SD=10.50), during placebo $t(24)=4.32$, $p<0.001$*, propranolol $t(24)=5.026$, $p<0.001$*, and

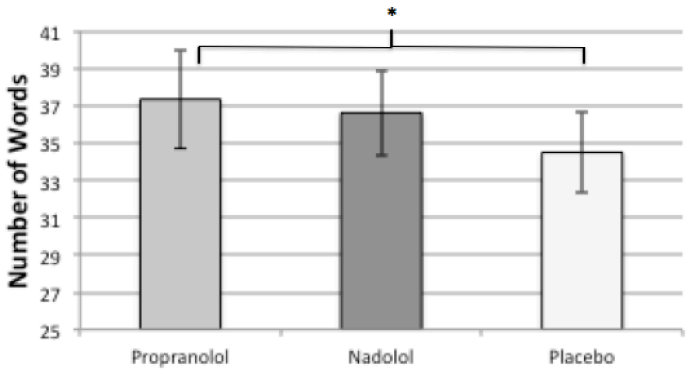
nadolol conditions t(24)=4.58,p<0.001*. Semantic fluency scores also exhibited a trend across drug conditions, F(2,48)=2.99, p=0.059. The number of words generated during semantic fluency was significantly higher during propranolol administration (M=37.35, SD=13.18) compared to placebo (M=34.50, SD=10.90), t(25)=2.155, p=0.042, but not compared to nadolol (M=36.62, SD=11.5), t(25)=0.617, p>0.05, nor nadolol compared to placebo, t(25)=1.822, p=0.081, Figure 7. There was no significant drug by group interaction, F(2,48)=1.81, p=0.175.

**Figure 7 *Beta*-adrenergic antagonism effects on semantic fluency performance.** Number of words produced during the semantic fluency task are displayed across (A) ASD (dark grey) and control (light grey) groups, and (B) drug conditions including propranolol (light grey), nadolol (dark grey), and placebo (white). Error bars represent standard error and significant differences are indicated (*).

A

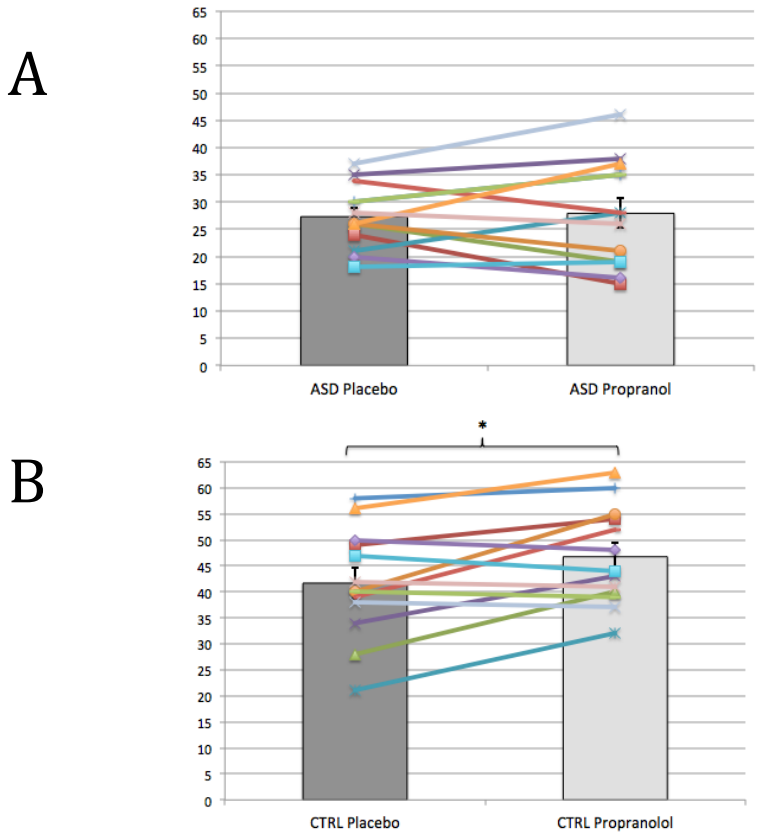



B



Although no drug by group interaction was found, we examined group differences in drug-related changes within diagnostic groups based on our *a priori* hypothesis of individuals with ASD exhibiting a performance response to *beta*-adrenergic antagonism. At the group level, the effect of propranolol on semantic fluency appeared to be primarily due to control individuals exhibiting more responses during propranolol (M=46.77, SD=9.41) compared to placebo (M=41.69, SD=10.50), t(12)=2.839, p=0.015*, and not individuals with ASD (M=27.92, SD=9.71, M=27.31, SD=5.86), p>0.05, Figure 8.

**Figure 8 *Beta*-adrenergic antagonism effects on semantic fluency performance across diagnostic groups.** The number of words produced during the semantic fluency task are displayed across the (A) ASD and (B) control groups following the administration of placebo (dark grey) and propranolol (light grey). Colored lines indicate individual participants responses across drug conditions. Error bars represent standard error and significant differences at the group level are indicated (*).



Controls exhibited a general trend such that individuals exhibited either an increase in the number of words produced or very minimal change across sessions; whereas individuals with ASD exhibited a general trend towards either an increase in the number of words produced or a decrease in the number of words produced. To test these potential response differences, we performed a median split on the data based on behavioral response to propranolol and compared performance effects across individuals within each diagnostic group, hereafter referred to as responders and non-responders. The ASD non-responder group (M=-5.50, SD=2.43, 95% CI [-8.05, -2.95]) generated significantly less words during propranolol relative to placebo compared to the control non-responder group (M=-1.00, SD=1.67, 95% CI [-2.76, 0.76]), $t^{223}$=3.737, p=0.004*, which most likely contributed to the non-significant group level drug effect within individuals with ASD. Cardiovascular response to drug, as assessed by change in heart rate, did not appear to underlie performance differences between responders (M=-13.36, SD=8.86) and non-responders (M=-11.58, SD=13.74), t(24)=0.397, p>0.05, nor between non-responders with ASD (M=-10.67, SD=14.92) and controls (M=-12.50, SD=13.81), t(10)=0.221, p>0.05. Thus, differences in performance effects may have been related to more central mechanisms such as network coherence. Responders and non-responders were also compared within diagnostic groups in our analysis of network coherence to investigate these effects.

**GRAPH METRICS**

One ASD participant and one control participant exhibited average functional connectivity estimates across all possible ROI pairs that were greater than 3 standard deviations from the group mean and thus classified as outliers. These participants were removed from subsequent analyses of network coherence and all additional comparisons were completed on groups of 12 ASD participants and 12 controls. Metrics of interest included: (1) mean functional

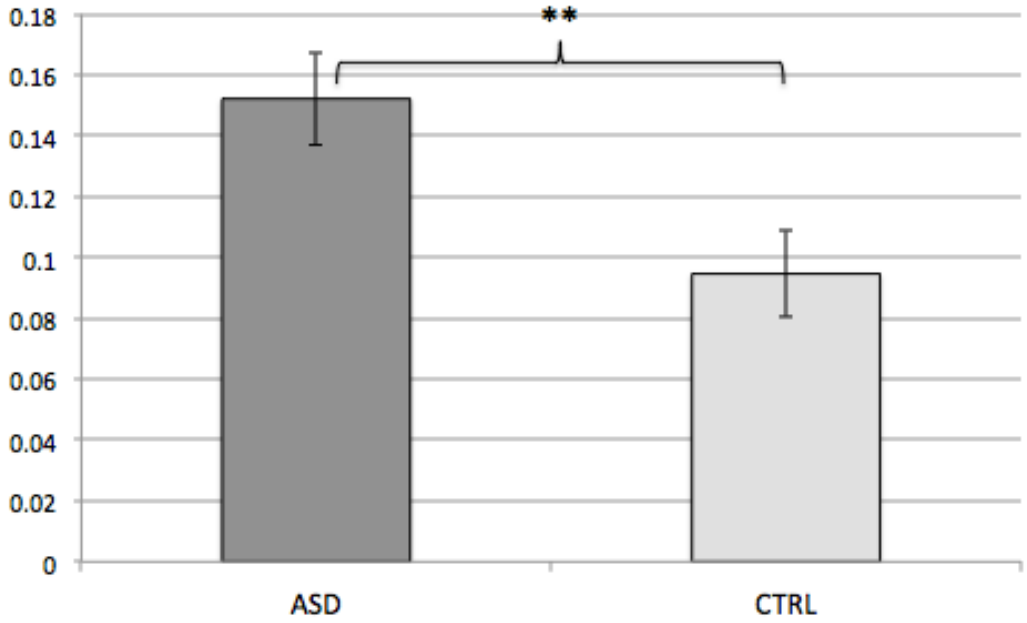
connectivity (FC), (2) mean clustering coefficient (*C*), (3) characteristic path length (*L*), (4) global efficiency (*Eglobal*), and (5) mean local efficiency (*Elocal*), Table 1. Clustering coefficient is a measure of the likelihood of connections between regions connected to a common region, and local efficiency is a measure of the average efficiency of information exchange of clusters within the network. Clustering and local efficiency represent the local processing level. Characteristic path length is a measure of the average shortest number of connections between regions and global efficiency is a measure of the efficiency of information exchange across the network. Path length and global efficiency represent the global processing level.

Initial analyses were completed on whole session timeseries regressing out stimulus design. These effects were then examined controlling for performance. If between groups or within drug conditions differences that were indicated that were not accounted for by performance, these effects were then examined focusing only on the BOLD response during task blocks. A combination of task regression analysis and analysis of only stimulus blocks should allow us to identify the most robust diagnostic group and drug condition differences in network coherence, and examining these differences in a hierarchical manner will help reduce the potential Type I error rate within our analyses.

**Semantic Association Network**

There was a significant diagnostic group difference in local efficiency, $F(1,22)=6.166$, $p=0.021$, which exhibited a trend when controlling for task performance, $F(1,21)=3.450$, $p=0.077$. Baseline diagnostic groups differences in the SAN were not based on performance effects. *Elocal* was significantly higher in the ASD group (M=0.152, SD=0.053) compared to the control group (M=0.095, SD=0.049) at baseline, $t(22)=2.780$, $p=0.011$*, Figure 9, which exhibited a trend when only examining task blocks, $t(22)=1.966$, $p=0.062$. Baseline diagnostic group

**Figure 9 Baseline differences in the SAN during task-based fMRI.** Local efficiency at baseline is displayed between individuals with ASD (dark grey) and controls, CTRL, (light grey) for the semantic association network. Error bars represent standard error and significant differences are indicated (*) as well if these differences were supported when only examining task blocks (**).

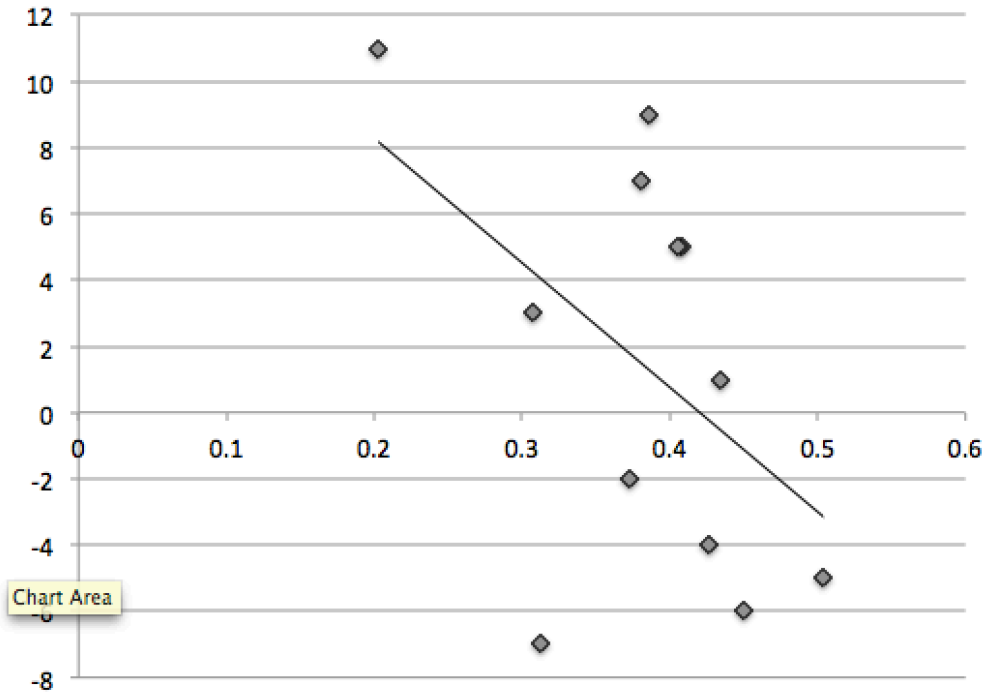



differences in the SAN were not solely due to activation magnitude effects. There was no difference between groups following propranolol administration (M=0.172, SD=0.054, M=0.152, SD=0.043) or nadolol administration (M=0.133, SD=0.057, M=0.116, SD=0.071). Individuals with ASD exhibited a greater reliance on local processing in the SAN during associative processing.

There was a significant effect of drug on *Elocal*, F(2,44)=3.717, p=0.032, and *C*, F(2,44)=3.673, p=0.033, but no drug by group interaction, p>0.05; however, drug effects on the SAN were no longer significant when controlling for task performance, p>0.05. Due to our *a priori* hypothesis regarding *beta*-adrenergic effects on network coherence in the SAN and the aforementioned performance effects during the semantic fluency task, we also examined drug

effects on network coherence in responders and non-responders. Examining responders and non-responders regardless of diagnostic group, there were no group by responder interactions for local efficiency or clustering, $p>0.05$. There were also no significant differences comparing only individuals with ASD who were responders and non-responders, $p>0.05$. There were no drug effects on baseline differences in local processing in the SAN during semantic fluency.

**Figure 10 Baseline functional connectivity of the SAN in individuals with ASD.** Baseline functional connectivity of the SAN is displayed for individuals with ASD in relation to number of responses during propranolol administration compared to placebo.



In the SAN, baseline FC regardless of diagnostic group exhibited no association with change in performance in response to propranolol; however the ASD group exhibited a moderate negative association, r=-0.481, p=0.113, Figure 10, whereas the control group exhibited a weak positive association, r=0.181, p=0.574. Examining only timepoints from stimulus blocks, there was a trend towards a negative association between baseline FC and change in performance in response to propranolol regardless of diagnostic group, r=-0.362, p=0.083, which was due to negative associations in both the ASD, r=-0.383, p=0.197, and control group, r=-0.270, p=0.422. There is a trend towards an association between baseline FC in the SAN and performance response to propranolol, which appears to be more robust in individuals with ASD; however this relationship appears to be due to a relatively small number of subjects.

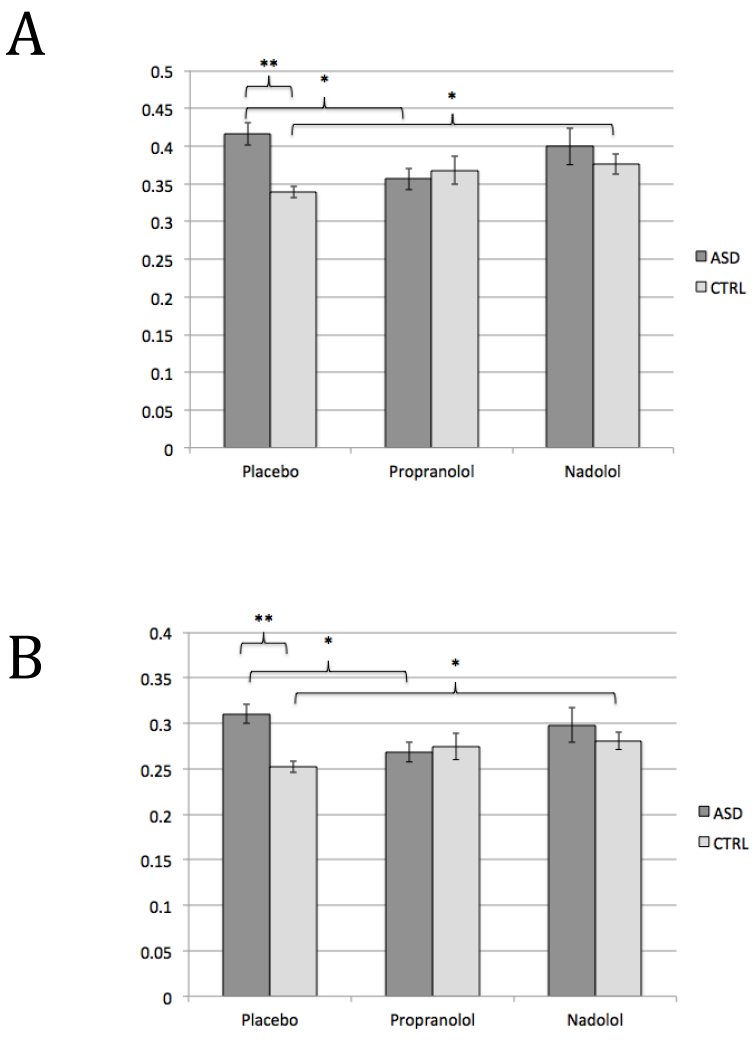
Individuals with ASD exhibited greater reliance on local processing in the SAN at baseline, but there were no direct effects of *beta*-adrenergic antagonism on network coherence in the SAN. Individuals with ASD who benefited from propranolol administration during task performance exhibited moderately lower FC at baseline compared to those who did not benefit from propranolol; however these effects were heterogeneous across responders and nonresponders. A larger sample size will be necessary to determine the ability of baseline FC in language and association regions to predict performance response.

**Frontoparietal Control Network**

There was a significant diagnostic group difference in functional connectivity, $F(1,22)=4.318$, $p=0.019$, due to higher FC in the ASD group ($M=0.42$, $SD=0.05$) compared to the control group ($M=0.34$, $SD=0.03$), at baseline, $t(22)=4.515$, $p<0.001$*, and a significant diagnostic group difference in global efficiency, $F(1,22)=3.962$, $p=0.026$, due to higher *Eglobal* in the ASD group ($M=0.31$, $SD=0.04$) compared to the control group ($M=0.25$, $SD=0.02$),

t(22)=4.749, p<0.001*, at baseline, Figure 11. Baseline differences between groups remained significant when controlling for performance and when only examining task blocks. Individuals with ASD exhibited altered network coherence at baseline compared to controls such that there was greater global processing within the FPC.

**Figure 11 *Beta*-adrenergic antagonism effects on the FPC during task-based fMRI.** (A) Functional connectivity and (B) global efficiency between ASD (dark grey) and control (light grey) groups across propranolol, nadolol and placebo are displayed. Error bars represent standard error and significant differences are indicated (*) as well if these differences were supported when only examining task blocks (**).



There was a significant drug by group interaction for functional connectivity, $F(2,44)=4.318$, $p=0.019$, and global efficiency, $F(2,44)=3.962$, $p=0.026$, which remained significant controlling for performance. Individuals with ASD exhibited a significant reduction in FC during propranolol ($M=0.36$, $SD=0.05$) compared to placebo ($M=0.42$, $SD=0.05$), $t(11)=2.490$, $p=0.030$, which exhibited a trend when only examining task blocks, $p=0.086$; whereas controls exhibited significantly higher FC during nadolol ($M=0.38$, $SD=0.05$) compared to placebo ($M=0.34$, $SD=0.03$), $t(11)=2.480$, $p=0.031$, which also exhibited a trend when only examining task blocks, $p=0.069$. Individuals with ASD also exhibited a significant reduction in *Eglobal* during propranolol ($M=0.27$, $SD=0.04$) compared to placebo ($M=0.31$, $SD=0.04$), $t(11)=2.406$, $p=0.035$; however propranolol effects on global efficiency in individuals with ASD were no longer significant when only examining task blocks. Controls exhibited significantly higher *Eglobal* during nadolol ($M=0.28$, $SD=0.03$) compared to placebo ($M=0.25$, $SD=0.02$), $t(11)=2.675$, $p=0.022$, which exhibited a trend when only examining task blocks, $p=0.067$, Figure 11. Propranolol significantly reduced functional connectivity in individuals with ASD whereas nadolol increased functional connectivity and global efficiency in controls. These effects were not related to performance or activation effects.

Examining responders and non-responders regardless of diagnostic group, there were no group by responder interactions for FC or *Eglobal*, $p>0.05$. Due to our *a priori* hypothesis of *beta*-adrenergic effects on control networks in individuals with ASD, we also assessed if the aforementioned drug-related changes in functional connectivity and global efficiency were different in individuals with ASD between responder groups. There were no baseline differences in FC or *Eglobal* between responder groups, $p>0.05$. There were no significant effects of drug in

the non-responder group, p>0.05; however, individuals with ASD who exhibited a performance benefit from propranolol displayed a significant reduction in FC during propranolol (M=0.34, SD=0.03) compared to placebo (M=0.42, SD=0.03), t(5)=4.129, p=0.009*, 95% CI [-0.14, -0.03] and a trend towards lower FC during propranolol compared to nadolol (0.42, SD=0.09), t(5)=2.246, p=0.075, 95% CI [-0.17, 0.01], but not propranolol compared to placebo, p>0.05. Individuals with ASD who exhibited a performance benefit from propranolol also exhibited lower *Eglobal* during propranolol (M=0.25, SD=0.02) compared to placebo (0.31, SD=0.02), t(5)=-4.566, p=0.006*, 95% CI [-0.10, -0.03] and a trend towards lower *Eglobal* during propranolol compared to nadolol (M=0.31, SD=0.07), t(5)=2.058, p=0.095, 95% CI [-0.13, 0.02], but not propranolol compared to placebo, p>0.05. The effects of propranolol compared to placebo remained significant for functional connectivity, p=0.05, and global efficiency, p=0.04, when only examining task blocks. The aforementioned effects of propranolol on functional connectivity and global efficiency in individuals with ASD were due to individuals who exhibited a performance benefit from propranolol on the semantic fluency task.

In the FPC, baseline functional connectivity across all timepoints was not associated with change in performance in response to propranolol, and there were no associations in the ASD or control groups, p>0.05. Individuals with ASD exhibited significantly higher functional connectivity and global efficiency in the FPC at baseline compared to controls and propranolol administration significantly reduced connectivity and global efficiency in individuals with ASD who exhibited a performance benefit from propranolol administration. Individuals with ASD who benefited from propranolol also exhibited a trend towards reduced functional connectivity and global efficiency compared to nadolol in; however these effects were related to activation

differences across conditions. Baseline functional connectivity of the FPC was not generally associated with behavioral benefit from propranolol.

**Dorsal Attention Network**

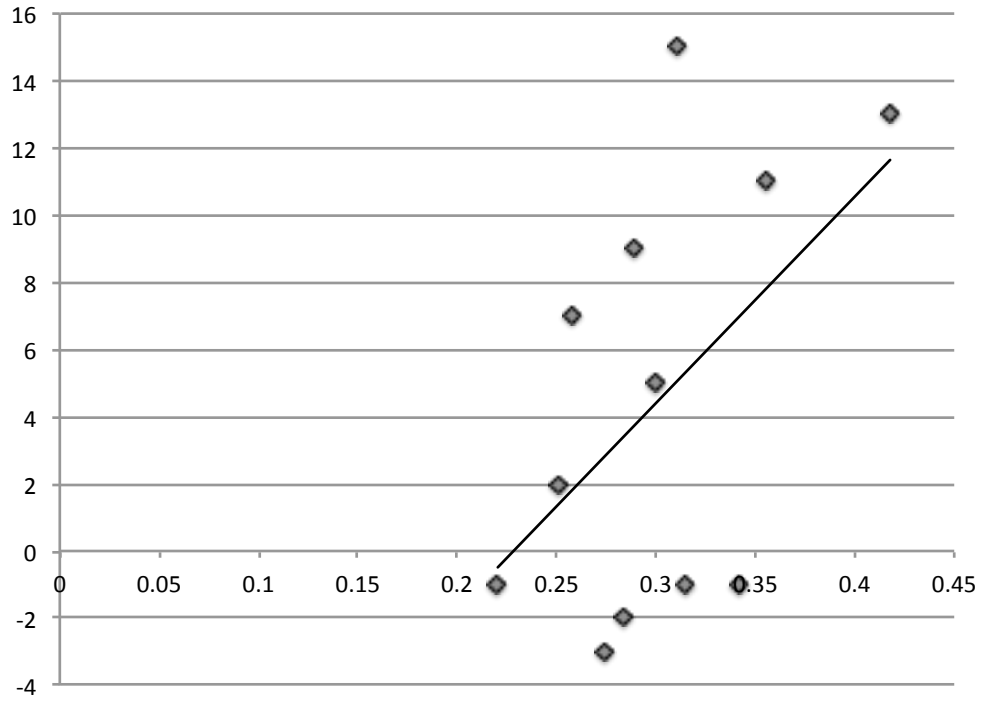
Characteristic path length exhibited a significant difference between ASD and control groups, F(1,22)=4.07, p=0.056, which was due to a significantly shorter path length *in the* ASD group (M=4.07, SD=0.54) at baseline, t(22)=2.79, p=0.011*, compared to the control group (M=4.84, SD=0.80); however *L* was no longer different between groups controlling for performance.. There was a trend towards an effect of drug for FC, F(2,44)=3.162, p=0.052, and a significant drug effect for *Eglobal*, F(2,44)=3.899, p=0.028, and *L*, F(2,44)=3.661, p=0.034; however these effects were also not significant when controlling for performance. Global processing differences between diagnostic groups at baseline and drug-related alterations in network coherence were related to performance effects on the DAN.

Examining responders and non-responders regardless of diagnostic group, there were no group by responder interactions for FC, global efficiency, or path length, p>0.05. Due to our *a priori* hypothesis of *beta*-adrenergic effects on attention networks in individuals with ASD, we also assessed if drug-related changes in FC, global efficiency, and path length were different in individuals with ASD between responder groups. There were no significant differences in FC, *Eglobal*, or *L* at baseline between responder groups, p>0.05, and no significant effects of drug in the non-responder group of individuals with ASD, p>0.05. Individuals with ASD who exhibited a performance benefit from propranolol displayed higher FC during nadolol (M=0.37, SD=0.07) compared to propranolol (M=0.32, SD=0.03), t(5)=3.050, p=0.028, 95% CI [-0.09, -0.008] and a trend towards higher *Eglobal* during nadolol (M=0.30, SD=0.05) compared to propranolol (M=0.27, SD=0.02), t(5)=2.483, p=0.056, 95% CI [-0.097, 0.022]; however there effects were no

longer significant when only examining task blocks, $p>0.05$. There were no differences between responder and nonresponder groups that are not accounted for by activation effects.

In the DAN, baseline functional connectivity exhibited a weak association with change in performance in response to propranolol, $r=0.209$, which was due to a weak association in the ASD group, $r=0.180$, but a moderate correlation in the control group, $r=0.508$, $p=0.092$, Figure 12. When examining only timepoints from stimulus blocks, this association was highly significant, $r=0.787$, $p=0.002$. Baseline FC of the DAN is associated with performance benefits in individuals unaffected by ASD such that individuals with higher baseline FC typically exhibit a larger performance benefit from propranolol.

**Figure 12 Baseline functional connectivity of the DAN in controls.** Baseline functional connectivity of the DAN is displayed for control individuals in relation to number of responses during propranolol administration compared to placebo.



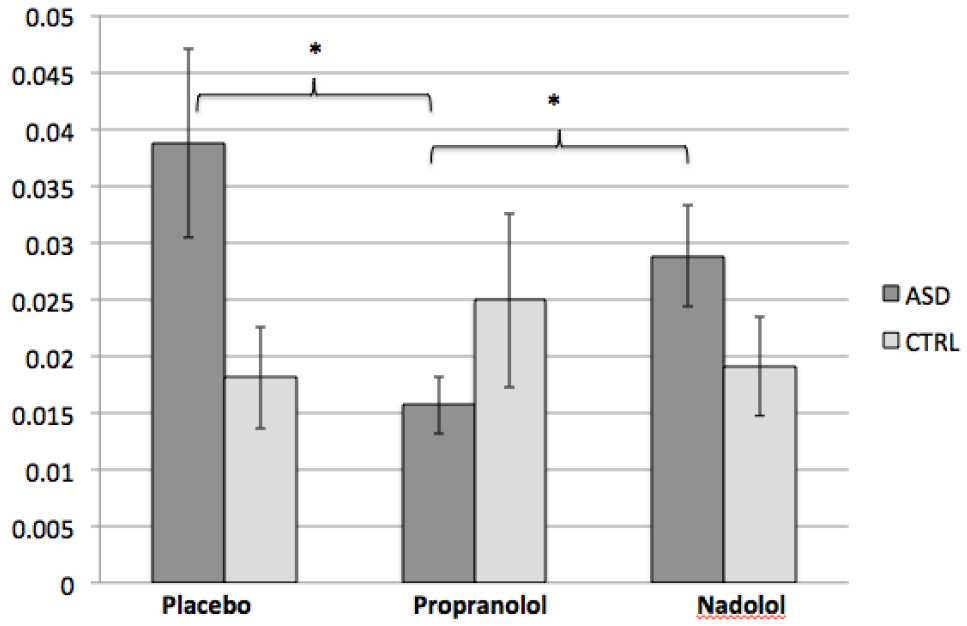
Individuals with ASD exhibited a significantly shorter path length in the DAN at baseline controlling for activation effects, suggesting potentially more global processing in the DAN; however these differences were related to performance. There were no significant effects of beta-adrenergic antagonism on network coherence in the DAN that was associated with performance or activation differences. Baseline FC in the DAN was not generally associated with behavioral benefits from propranolol in individuals with ASD but in controls higher baseline FC DAN was associated with a larger behavioral benefit from propranolol, potentially suggesting a benefit for individuals who required greater re-orientation to task at baseline.

**Default Mode Network**

There was a trend towards a drug by group interaction for clustering coefficient, $F(2,44)=2.690$, $p=0.079$, which also exhibited a trend when controlling for performance, $F(2,42)=3.094$, $p=0.056$. There was also a significant drug by group interaction for local efficiency, $F(2,44)=3.635$, $p=0.035$, which remained significant when controlling for task performance, $F(2,42)=3.397$, $p=0.043$. Diagnostic group by drug condition interactions on local processing in the DMN were not due to performance effects. The control group did not exhibit any significant drug-related changes in *C* or *Elocal*. *C* was significantly lower in the ASD group during propranolol (M=0.013, SD=0.008) compared to placebo (M=0.028, SD=0.019), $t(11)=2.726$, $p=0.020$, and there was a trend towards lower *C* during propranolol compared to nadolol (M=0.023, SD=0.012), $t(11)=1.942$, $p=0.078$. *Elocal* was also significantly lower in the ASD group during propranolol (M=0.016, SD=0.008) compared to placebo (M=0.039, SD=0.029), $t(11)=2.887$, $p=0.015$*, and significantly lower during propranolol compared to nadolol (M=0.029, SD=0.015), $t(11)=2.283$, $p=0.043$, Figure 13. *Beta*-adrenergic antagonism did not affect network coherence in the DMN when only focusing on task blocks, $p>0.05$; however

the DMN is downregulated during cognitive processing and therefore alterations in network coherence would primarily be expected during rest blocks interspersed between task. There were no significant associations between baseline FC in the DMN and performance response to propranolol.

**Figure 13 *Beta*-adrenergic antagonism effects on the DMN during task-based fMRI.** Local efficiency is displayed across the ASD (dark grey) and a control, CTRL, (light grey) groups when comparing propranolol, nadolol, and placebo. Error bars represent standard error and significant differences are indicated (*).




*Beta*-adrenergic antagonism selectively affected network coherence of the DMN in individuals with ASD, and these alterations remained significant after controlling for performance. Propranolol decreased local processing within the DMN during rest blocks.

Baseline FC of the DMN was not associated with behavioral benefits from propranolol on verbal fluency.

## Discussion

The purpose of this study was to assess the effects of *beta*-adrenergic antagonism on semantic fluency performance and intrinsic network coherence during cognitive processing and compare these effects between individuals with ASD and unaffected individuals. Semantic fluency was examined because language and communication abilities are affected in ASD and *beta*-adrenergic antagonism was previously shown to benefit semantic fluency performance.[185] Individuals with ASD produced significantly less semantically associated words during a fluency task across all conditions compared to unaffected individuals, including baseline. Following propranolol administration, a lipophilic *beta*-adrenergic antagonist, there was a main effect of drug such that participants produced significantly more semantically associated words compared to placebo. The number of words produced during propranolol administration was not significantly different than during nadolol, a hydrophilic *beta*-adrenergic antagonist, or between nadolol and placebo, suggesting the beneficial effects of *beta*-adrenergic agents on performance may be the result of combined CNS and PNS mechanisms. Previous research suggested an ASD specific effect of propranolol on semantic fluency compared to unaffected individuals[185]; however the testing environment may have contributed to the difference between these investigations. Semantic fluency assessment was conducted while the participant was in the MR scanner, which can be a somewhat stressful environment. Controls may have exhibited a performance benefit in the imaging environment due to a heightened stress reaction at baseline in the imaging environment but a reduced stress response following propranolol administration allowing better performance. A lower proportion of individuals with ASD exhibiting a

performance benefit in the imaging environment compared to a quiet testing room may have also been related to a heighten stress response causing greater competition for noradrenergic binding sites, which is especially relevant considering heightened stress reactivity in some individuals with ASD.[173,174] Overall, semantic processing is affected in ASD and *beta*-adrenergic can improve verbal fluency, at least in a subset of individuals, when centrally-acting *beta*-adrenergic agents are utilized. *Beta*-adrenergic antagonism was previously shown to alter network coherence in the brain[124] and we further illustrated that pharmacologically-mediated alterations in network coherence are associated with verbal processing improvements in individuals with ASD.

The default mode network (DMN) was previously examined during resting-state and we expanded on this investigation by assessing the DMN during cognitive processing. *Beta*-adrenergic antagonism altered resting state network coherence of the DMN across all participants regardless of diagnostic group; however during task-based fMRI of verbal processing there appeared to be an ASD specific effect of *beta*-adrenergic antagonism. Following propranolol administration, local processing was decreased in individuals with ASD compared to baseline whereas controls exhibited no significant alterations in network coherence. This ASD specific effect may be due inherent differences in DMN network coherence in ASD.[96,100,234,235] Previous reports have suggested both hypo-[100,235] and hyper- connectivity[235,249] between regions comprising the DMN. Although we did not find a baseline difference in the DMN, estimates of local processing in individuals with ASD following propranolol administration were more similar to controls at baseline. Reduced local processing is also especially relevant considering the DMN is typically down-regulated during cognitive processing tasks.

The frontoparietal control network (FPC) dynamically couples with the DMN during internally directed cognition and is important for allowing efficient network utilization[242]. Regions comprising the FPC are generally consistent with regions of the executive control and salience networks[250]; however these regions shift network affiliation depending on cognitive demand allowing the FPC to mediate interactions between large-scale brain networks. We hypothesized baseline hypoconnectivity in individuals with ASD due to previous reports of hypoconnectivty between the frontal and parietal lobes[251] as well as other regions comprising the FPC, such as the anterior insula[252]; however we found that individuals with ASD exhibited hyperconnectivity and greater global efficiency within the FPC compared to unaffected individuals. Examinations of the salience network, which include the anterior insula, in individuals with ASD have also reported hyperconnectivity compared to unaffected individuals and these patterns of connectivity discriminated individuals with ASD from controls.[249] The anterior insula serves as a hub mediating interactions between the FPC and other regions in the brain.[253] Hyperconnectivity of the anterior insula with other regions comprising the FPC at baseline may limit the ability of the insula to dynamically interact with other large-scale brain networks during different aspects of cognition processing. Propranolol significantly reduced connectivity and global processing in the FPC in individuals with ASD to levels comparable to unaffected individuals, which could increase the ability of regions within the FPC to couple with other networks and improve information processing. These pharmacologically-mediated effects were primarily due to individuals with ASD who exhibited a performance benefit from propranolol, even after accounting for performance and activation effects. Furthermore, individuals with ASD who did not behaviorally benefit from propranolol did not exhibit any significant alterations in network coherence following propranolol administration, further

supporting a link between altered network coherence and behavioral benefits in individuals with ASD. Hyperconnectivty of the salience network has been associated with reduced maturation of functional neuronal networks in individuals with ASD,[249] and *beta*-adrenergic antagonism of the CNS may mitigate some aspects of hyperconnectivity of the FPC in a subset of individuals with ASD. Reduced connectivity within the FPC may potentially allow better dynamic integration of other networks underlying cognitive processing, such as attention and language networks.

The dorsal attention network (DAN) is implicated across multiple cognitive domains and is associated with attention orienting during externally-directed cognition.[240,241] Primary language and associative processing regions are also preferentially activated during the processing of semantic associations between items, such as during verbal fluency tasks.[248] DAN and language regions typically exhibit disrupted connectivity in individuals with ASD, such as hypo-connectivity during attention orienting[243] and verbal fluency.[109] Global network integration of the DAN was significantly higher at baseline in the ASD group compared to unaffected individuals, but these differences in network coherence were related to performance effects across diagnostic groups. *Beta*-adrenergic modulation of network coherence in the DAN appeared to be primarily due to performance and activation differences between comparisons with no indications of significant *beta*-adrenergic effects on attention networks in individuals with ASD. Language regions were previously shown to exhibit significantly higher functional connectivity following *beta*-adrenergic antagonism in individuals with ASD during verbal processing. [124] Individuals with ASD in the current investigation exhibited significantly higher local efficiency in language regions at baseline, suggesting potentially greater reliance on local processing. Propranolol increased local efficiency and clustering relative to placebo in this investigation but these alterations appeared to be due to task performance effects on activation.

Previous analyses utilized larger ROIs localized to different regions associated with language processing and did not account for activation effects, which may explain these differences.

*Beta*-adrenergic antagonism did not significantly affect network coherence in the DAN or language networks; however the assessment of these networks may provide valuable information regarding prediction of treatment response. Baseline connectivity of the DAN in controls was associated with performance response to propranolol such that individuals exhibiting the highest connectivity at baseline in the DAN exhibited the largest performance benefit from propranolol. The DAN is primarily activated during attention re-orienting to external stimuli,[240,241] and this association suggests individuals typically requiring the most re-orienting during cognitive processing benefit the most from *beta*-adrenergic antagonism due to better attention to the task. Baseline connectivity of the associative language network was related to performance response in individuals with ASD such that individuals with the lowest baseline connectivity exhibited the largest behavioral benefit from propranolol. Although no group level differences were found in language regions during task performance, this association suggests that effects on language regions are involved with performance benefits of *beta*-adrenergic antagonism in individuals with ASD and those with the lowest network coherence of the language network may benefit most from this type of intervention.

*Beta*-adrenergic antagonism was previously shown to benefit cognitive processing, especially in verbal domains. We found that *beta*-adrenergic antagonism improved semantic associative processing in individuals with ASD and controls; however this was only the case when lipophilic agents were administered, suggesting these improvements were not due to peripheral hemodynamic mechanisms. The most robust effects of *beta*-adrenergic antagonism on network coherence in individuals with ASD were in the frontoparietal control network.

Individuals with ASD exhibited hyperconnectivity at baseline that was mitigated following propranolol administration. Individuals with ASD who exhibited altered network coherence in the FPC following *beta*-adrenergic antagonism also expressed verbal processing benefits. *Beta*-adrenergic modulation of network coherence in ASD has been posited to benefit associative processing by increasing access to lexical, semantic, and associative networks during a search of semantic associations.[199] The frontoparietal control network, including the anterior insula, acts a central hub allowing dynamic integration of large-scale neuronal networks and may therefore be ideally situated to augment a network search. Pharmacological modulation of network coherence allowing more efficient information processing may underlie the aforementioned propranolol-mediated benefits to cognitive processing and this is especially relevant regarding individuals with ASD due to the potential to alter inherent disturbances in network integration and optimize network coherence in these individuals.

In addition to the aforementioned effects of propranolol on network coherence in the central nervous system, propranolol also has anxiolytic effects and helps maintain homeostasis in the sympathetic nervous system. This is especially relevant for individuals with ASD because of prevalent comorbid diagnoses such as anxiety[151] and secondary manifestations such as heightened sympathetic nervous system arousal[170-172] and greater stress reactivity compared to unaffected individuals.[173,174] Propranolol may be able to benefit some core symptoms and secondary manifestations in individuals with ASD. Cumulatively, these findings support the potential efficacy of *beta*-adrenergic antagonists for some patients with ASD.

**LIMITATIONS**

Due to the pilot nature of our investigation, correction for multiple comparisons was minimally applied and the potential for increased Type I error should be considered regarding

interpretation of this work. Additionally, to more accurately assess the effects of propranolol on network coherence in ASD, a larger more representative sample including younger individuals with higher variability of disorder severity should be examined. Improved control matching including considerations for IQ subscales and additional cognitive domains would help account for additional variability between groups. Additional considerations should also be given to the potential stress-related effects of MR acquisition. Finally, due to the differences in time to peak effects between drugs, we were unable to fully blind all research staff. To alleviate as many confounds as possible, testing following drug administration was either conducted by a lab member blind to diagnostic group and drug condition or consisted of automated computer responses and questionnaires. Serial dose studies assessing the long-term effects of *beta*-adrenergic antagonism or double-blind placebo controlled studies with the additional administration of a placebo one hour after initial drug administration would address these concerns.

# CHAPTER 3:

# CEREBELLAR INFLUENCES ON NETWORK COHERENCE

## The Cerebellum and ASD

The cerebellum is an evolutionarily older part of the mammalian brain maturing before more frontal neocortical regions and is interconnected to the neocortex, exerting some regulatory control of cortical and subcortical neuronal circuits. Cerebellar circuits are important for providing regulatory feedback to other regions of the brain, and although traditionally thought to be exclusively involved with motor control, imaging and lesion studies have implicated higher-order cognitive domains as well. Patients with cerebellar lesions or atrophy exhibit deficits in attention,[126] executive function,[127,128,254] language,[127,128,255,256] working memory,[127] associative learning,[125,126,257] visuospatial processing,[127,128,130] and affective[127,128] and sensory processing.[125,127,128] Neuroimaging studies also report that the cerebellum is active during tasks within these processing domains,[131-133,142,258-272] supporting evidence from lesion studies that the cerebellum is involved in cognitive processing. Such deficits have been conceptualized into a cerebellar cognitive affective syndrome that is characterized by disturbances in executive function, impaired spatial cognition, personality change such as blunted affect, and linguistic difficulty.[127] Patients with cognitive affect syndrome often present impairments in planning and abstract reasoning, increased distractibility and perseveration, decreased working memory and associative processing, and changes in personality. The cerebellum appears to have an important role in complex cognitive functions such as attention, language, working memory, and sensory integration, in addition to motor control.

The cerebellum is divided into two hemispheres separated by a midline vermis, and was originally discovered to have a ubiquitous role in motor control and coordination. Lesion studies

indicated a potential zonal organization of the cerebellum such that medial zones, including the vermis, regulate tone, posture and locomotion whereas more lateral zones, such as the posterior hemispheres, regulate coordination of skilled movements.[273] With the advent of neuroimaging studies of the cerebellum, somatotopic organization has been localized to specific neuronal clusters with certain body representations found throughout the anterior and posterior hemispheres as well as the vermis.[274] The extensive role of the cerebellum in motor control and coordination, especially due to prominent ataxic outcomes following lesions, originally obscured the identification of cerebellar involvement in cognition. Considering the aforementioned involvement of the cerebellum in cognitive processing, additional domains have been attributed to subfields within the cerebellum. The anterior cerebellar lobes are primarily involved with conventional aspects of cerebellar function such as sensorimotor processing.[275-277] In addition to the motor and coordination aspects of the cerebellum, the posterior cerebellar lobes are also involved with cognitive processing,[126,127,277] and the vermis is involved with affective processing.[127,277-279] The cerebellum has a prominent role in motor control and coordination with somatotopic organization of cerebellar subfields contributing to different regions of the body and different aspects of tone, posture, and coordination of movement. More recently, topographical organization of the cerebellum has been found to exert modulatory effects on cognitive processing domains in addition to motor control.

Cerebellar neurons receive excitatory afferent inputs from the contralateral inferior olive, projecting to Purkinje cell dendrites, and from the pontine, tegmentum, medullar oblongata, and reticular formation, projecting to granule cell dendrites. Primary efferent cerebellar output is from Purkinje cells projections to the deep cerebellar nuclei, which in turn send contralateral transthalamaic projections to the neocortex, including the primary and pre-motor cortices,

prefrontal cortex, and medial temporal and parietal lobes.[280] Perturbations of specific cerebellar subfields are associated with distinct cognitive and behavioral profiles. In addition to affects on motor control,[273] disturbances in modulatory output from the posterior cerebellar hemispheres affects prefrontal/posterior parietal circuits and generally causes cognitive impairments, and disturbances in modulatory output from the vermis affects limbic circuits and generally causes affective dysfunction.[277] The universal cerebellar transform hypothesis conceptualizes these findings into a framework by which cerebellar modulation serves as an oscillation dampener to maintain a homeostatic baseline in neuronal processing. Alterations of these modulatory effects in domain specific subfields lead to dysmetria, which can result in what has been referred to as dysmetria of thought when the posterior cerebellar hemispheres or vermis is affected.[281] Overall, the cerebellum has been implicated in modulation of higher-order cognitive and behavioral domains in addition to motor control with specific topographical organization of modulatory effects on neocortical circuits.

Cerebellar modulation of cortical and subcortical circuits is of particular interest regarding the study of ASD because one of the most consistent postmortem findings associated with ASD are decreased Purkinje cells in the cerebellar hemispheres.[46-49,282] Some studies also report a bimodal distribution of hypo- or hyper- plasia in the cerebellar vermis,[283,284] whereas other studies found no difference compared to controls.[46-49] Nevertheless, cerebellar perturbations may underlie some aspects of ASD symptomatology, such as repetitive behaviors, stereotypy, and social-communication impairments, due to the aforementioned modulatory effects of these circuits on cortical and subcortical regions associated with cognitive processing domains associated with these symptoms. Only a single study has investigated the influence of functional connectivity between the cerebellum and cognitive networks in individuals with

ASD.[285] Individuals with ASD exhibited significant cerebrocerebellar hypo-connectivity with supramodal networks and increased cross network connectivity between the cerebellum and neocortex, suggesting altered segregation of cerebrocerebellar circuits in ASD. Further support for cerebellar involvement can be seen from studies examining patient groups at high risk for ASD. Approximately 30 percent of patients with fragile X syndrome (FXS) exhibit ASD-related behaviors and meet diagnostic criteria,[286] and FXS patients with ASD express vermal abnormalities whereas FXS patients without ASD do not.[284] As many as 36% of patients with Joubert syndrome, characterized by the absence or underdevelopment of the vemis, also exhibit ASD-related behaviors and meet diagnostic criteria.[287] In tuberous sclerosis (TS), a rare syndromic disorder characterized by hamartomas in the brain and other organs, approximately 40% of patients are diagnosed with ASD,[288] and TS patients with cerebellar lesions have more severe ASD symptoms compared to those without lesions.[289] ASD patients may also have an increased genetic susceptibility to cerebellar malformations. For example, ASD implicated genes such as EN2,[290] GABRB3,[291] and MET[292] are all involved with cerebellar development. Loss of EN2 in mice causes cerebellar malformations accompanied by deficits in motor and social behaviors.[293] Loss of GABRB3 in mice causes cerebellar vermal hypoplasia and deficits in social and exploratory behaviors,[294] and loss of MET in zebrafish causes cerebellar hypoplasia and reductions in granule cell numbers.[295] The cerebellum may be critically involved with the presentation of the ASD phenotype.

Decreased Purkinje cell output from the cerebellum in individuals with ASD[46-49,282] suggests that altered neuronal network dynamics in ASD may be due to an altered balance of excitation to inhibition disrupting network coherence.[144,145] Purkinje cells are a class of inhibitory neurons that provide the primary cerebellar output to the neocortex via connections

through the deep cerebellar nuclei. Decreased inhibitory output from Purkinje cells could therefore perturb the balance of excitation to inhibition in neocortical networks during cognitive processing. Glutamate and γ-aminobutyric acid (GABA) define the excitatory to inhibitory (E/I) balance within and between neuronal networks, which may affect coordinated functional utilization of distinct neuronal clusters important for carrying out different aspects of information processing. Determining the relationship between E/I balance and functional integrity of cerebrocerebellar connections will help elucidate the potential role of altered modulatory effects of the cerebellum in ASD. Glutamate is the primary excitatory neurotransmitter,[296] typically resulting in depolarization of the cell, and γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the mature nervous system,[297] typically resulting in hyperpolarization. GABAergic interneurons provide functional architecture to neuronal circuits via feedback and feedforward inhibitory control of neuronal glutamatergic excitability. Neuronal binding of glutamate and GABA shapes the spatiotemporal patterns of electrical signaling in the brain,[298] and the balance between neuronal excitability and inhibitory (E/I) control is crucial to neuronal circuit patterning. The balance between glutamate and GABA signaling shaping the balance between excitation and inhibition in the brain is important for neurodevelopment, cognitive processing, and shaping functional connectivity patterns within neuronal networks.

$GABA_A$ and $GABA_B$ receptor densities are reduced in ASD in the frontal, limbic and cerebellar cortices[299,300] and glutamatergic receptor density may also be affected in ASD.[301] Glutamic acid decarboxylases (GAD 65 and 67), enzymes that convert glutamate to GABA,[302-304] and reelin, a protein expressed in glutamatergic/GABAergic neurons,[305,306] are also reduced in individuals with ASD. These findings implicate the regulation of glutamatergic/GABAergic signaling and the affect on E/I balance as a potential mechanism within ASD. Investigations of

mouse models of ASD have reported that altered E/I in the cerebellum as well as in the prefrontal cortex affects cellular processing and causes impairments in social interactions and communication; whereas intervention reversing these alterations in E/I ameliorates behavioral impairment.[307,308] Post mortem studies have implicated altered E/I balance in individuals with ASD and mouse models link these disturbances to behavioral outcomes associated with the ASD phenotype. Defining the contributions of E/I effects in ASD patients with behavioral outcomes will help elucidate if alterations in E/I balance are common across patients with ASD.

Magnetic resonance spectroscopy (MRS) provides a means of quantifying certain molecules of interest utilizing non-invasive magnetic resonance imaging techniques. [309] $^{1}$H-MRS, based on the magnetic properties of hydrogen ions in tissue, quantifies metabolites including GABA, glutamine/glutamate, and N-acetylaspartate,[310] a marker of neuronal density/activity. Studies utilizing MRS to measure metabolite levels in ASD have revealed some discordant findings. Some report decreased NAA[311-321] whereas others report increased NAA[322-324] still others find no difference[323,325-328]. Aoki et al (2012)[329] conducted a meta-analysis of MRS studies focusing on ASD and found that NAA levels in the frontal, parietal, and temporal lobes were significantly reduced in children with ASD, but not adults, suggesting a developmental trajectory of altered neuronal density in individuals with ASD. Consistent patterns of GABA and glutamate in ASD were not revealed with these meta-analytic techniques due to the heterogeneity across studies and current limitations regarding assessment of these molecules in the brain. Increased glutamate levels have been reported in the amygdala-hippocampus[330] and anterior cingulate cortex[331] of individuals with ASD but decreased levels have also been found in the frontal lobes,[332] anterior cingulate cortex,[333] basal ganglia,[334] and cerebellum.[311] These findings suggest that the relationship between glutamate and ASD is more complex than just increased

transmission, which was originally theorized in earlier studies. Reduced GABA levels have been reported in the frontal lobes of ASD patients utilizing standard MRS sequences[332]; however measurement of GABA levels *in vivo* has proven difficult due to the spectral overlap of GABA resonances (1.9ppm and 3.0ppm) with other metabolites, effectively masking the signal. Spectral editing procedures, such as MEGA-PRESS,[335] allow for GABA signals to be separated from other metabolites and studies utilizing J-coupled editing procedures have corroborated reduced GABA levels in the frontal lobes in ASD[326] and additionally suggested reductions in the superior temporal[336,337] and pre-central[337] gyri. Thus, altered neuronal density, regionally specific increases/decreases in glutamatergic signaling and reductions in GABAergic signaling are implicated in ASD; however the cerebellum has not been as extensively evaluated as the cerebrum in individuals with ASD. To our knowledge, only two studies have evaluated the cerebellum utilizing MRS techniques, which reported reduced NAA and glutamate+glutamine levels in individuals with ASD compared to unaffected individuals.[311,320] These findings suggest reduced neuronal density and potentially altered E/I, but GABA levels would need to be quantified. Overall, MRS techniques have generally corroborated post mortem and animal model studies suggesting that E/I balance in the brain and neuronal density in the cerebellum is associated with the ASD phenotype; however the relationship between GABA and glutamate levels within the cerebellum has not yet been investigated.

Altered E/I balance in the brain in individuals with ASD is a prominent theory regarding outcomes of perturbations in circuit structure and network dynamics in the brain, especially regarding cerebellar pathology. Cerebellar abnormalities in ASD may perturb modulatory control inputs to neocortical circuits, such as efferent projections to the frontal, temporal, and parietal cortices and affect functional cerebrocerebellar connectivity. Functional connectivity between

cerebellar and neocortical circuits appears to be altered in ASD with general patterns of hyper-connectivity with motor regions but hypo-connectivity with supramodal regions,[285] which may affect cognitive processing; however, cognitive measures were limited to symptom severity ratings in the only study that has assessed cerebrocerebellar connectivity in ASD. Determining the relationship between cerebrocerebellar connectivity and E/I balance and their effects on cognitive and behavioral outcomes in individuals with ASD will help elucidate the role of neuropathological alterations in cerebellum on symptoms outcomes. Compared to the cerebrum, the cerebellum has not been as extensively researched in ASD even though it is consistently implicated in the disorder. Further research to understand cerebrocerebellar connectivity in individuals with ASD and how the balance between excitation and inhibition in distinct neuronal clusters affects these alterations and symptom outcomes is warranted.

Aim 1: Utilize resting-state fMRI to assess functional connectivity between the cerebellum and neocortex in individuals with and without ASD. This investigation will allow us to determine whether relationships exist between cerebrocerebellar connectivity and symptom outcomes in ASD.

Aim 2: Utilize magnetic resonance spectroscopy techniques to assess metabolites of glutamate and GABA in the cerebellum and determine whether differences are associated with cerebrocerebellar connectivity. This investigation will allow us to examine how network coherence alterations in ASD are associated with alterations in E/I balance.

## RESTING STATE fMRI & MR SPECTROSCOPY

One of the most consistent postmortem findings associated with ASD are decreased Purkinje cells in the cerebellar hemispheres.[46-49,282] Some studies also report a bimodal distribution of hypo- or hyper- plasia in the cerebellar vermis[283,284] and a potential reduction in the number of Purkinje cells;[50] however vermal abnormalities are not as consistently reported as cerebellar hemisphere neuropathology.[338] Although cerebellar perturbations are implicated in ASD, the majority of imaging studies in ASD assessing functional network coherence do not account for the potential influence of cerebellar modulation on neocortical networks. A task-based functional connectivity study reported that cerebrocerebellar networks exhibit significant hypo-connectivity during finger tapping[339] in individuals with ASD; however, the influence of cerebellar connections on cognitive neocortical circuits have only recently been investigated. To date only a single study has specifically investigated the influence of cerebellar connectivity on cognitive networks in individuals with ASD.[285] This study reported that individuals with ASD exhibited significant cerebrocerebellar hypo-connectivity with supramodal networks and increased cross network connectivity between the cerebellum and neocortex, suggesting altered segregation of cerebrocerebellar circuits in ASD. Cerebrocerebellar connectivity appears to be affected in ASD, with potentially diminished cerebellar modulation of non-motor systems. Further research into the mechanisms underlying perturbed cerebrocerebellar circuits in ASD is warranted.

Altered neuronal network dynamics associated with ASD may be due to an altered balance of excitation to inhibition disrupting network coherence.[144,145] Glutamate and γ-aminobutyric acid (GABA) define the excitatory to inhibitory (E/I) balance within and between neuronal networks, which may affect cerebellar modulation of distinct neuronal clusters

important for carrying out different aspects of information processing. GABA and glutamate receptor densities are reduced in the cerebellar cortices of individuals with ASD,[300,301] as well as enzymes that convert glutamate to GABA[302-304] and proteins expressed in glutamatergic/GABAergic neurons.[305,306] These findings implicate the regulation of glutamatergic/GABAergic signaling and E/I balance as a potential mechanism underlying altered cerebrocerebellar connectivity in individuals with ASD; however to date, no investigations have examined the influence of E/I on cerebrocerebellar connectivity in individuals with ASD. Determining the relationship between functional integrity of cerebrocerebellar connections and E/I balance in individuals with ASD will help elucidate the role of altered modulatory effects of the cerebellum on network coherence in ASD and provide critical insight into specific mechanisms that may underlie these perturbations.

The purpose of this study was to examine functional cerebrocerebellar connectivity in individuals with ASD and assess whether alterations in network coherence are related to E/I balance within the cerebellum. ASD and matched controls were administered measures of symptom severity and social and language competence. Following behavioral testing, resting-state fMRI and magnetic resonance spectroscopy data were acquired. Glutamate, GABA, and N-acetylaspartate[310], a marker of neuronal density/activity, were assessed in the right postereolateral cerebellar hemisphere, the cerebellar vermis, and the left dorsolateral prefrontal cortex, due to contralateral projections between the cerebellar hemispheres and neocortex and greater activation of the right cerebellum and left prefrontal cortex during language and social processing.[277,340] The right cerebellar hemisphere junction of crus I and crus II was chosen because this area is active during the processing of language and other higher order cognitive domains[262] and is functional connected with the left dorsolateral prefrontal cortex.[341] Due to the

potential vermal hyper- or hypo- plasia in individuals with ASD, we also examined the anterior lobe of the vermis.[283,284] Cerebrocerebellar connectivity and metabolite levels were quantified and associations between these measures with cognitive and behavioral outcomes were assessed across diagnostic groups.

We hypothesized that individuals with ASD would exhibit hypo- cerebrocerebellar connectivity between the right cerebellar hemisphere and left dorsolateral prefrontal cortex compared to unaffected controls, as previously reported,[285] and that cerebrocerebellar connection strength would be related to social and language competency. We also hypothesized that GABA concentrations in the cerebellum, especially in the right cerebellar hemisphere, of individuals with ASD would be reduced compared to typically developing controls with concurrent alterations in the balance between excitatory/inhibitory neurotransmitter metabolites. We propose that individuals with the lowest cerebrocerebellar connectivity, especially in individuals with ASD, will exhibit the largest reductions in GABA and alterations in E/I.

## Methods

### PARTICIPANTS

Fifteen individuals with ASD, confirmed from clinical report and the Autism Diagnostic Interview-Revised (ADI-R)[209] or Autism Diagnostic Observation Schedule (ADOS),[342] aged between 15 and 35 years were recruited from the University of Missouri Thompson Center for Autism and Neurodevelopmental Disorders. Fifteen gender, age, FSIQ and handedness matched controls without any previous major medical or psychiatric diagnoses were recruited from the surrounding community. IQ was estimated with the Wechsler Abbreviated Scale of Intelligence [210] and demographic information, including ethnicity, years of education, and socio-economic

status, were collected with questionnaires. All participants were consented in accordance with the University of Missouri Health Sciences Institutional Review Board.

**ENROLLMENT CRITERIA**

Following initial participant screening, all subjects were interviewed by a physician to ensure enrollment criteria. Inclusion criteria included: 1) an ASD diagnosis confirmed from clinical report and the ADI or ADOS and no previous major medical or psychiatric diagnoses, and 2) between 15 and 35 years old. Exclusion criteria included: 1) schizophrenia, 2) major depression, 3) bipolar disorder, 4) non-ASD related learning disability, 5) previous major head trauma, or 6) pregnancy. Participant medications were also screened. Control participants were not enrolled if currently or regularly taking any psychoactive medications. ASD participants were not enrolled if currently or regularly taking any medications directly affecting the GABAergic or glutamatergic systems.

**BEHAVIORAL ASSESSMENT**

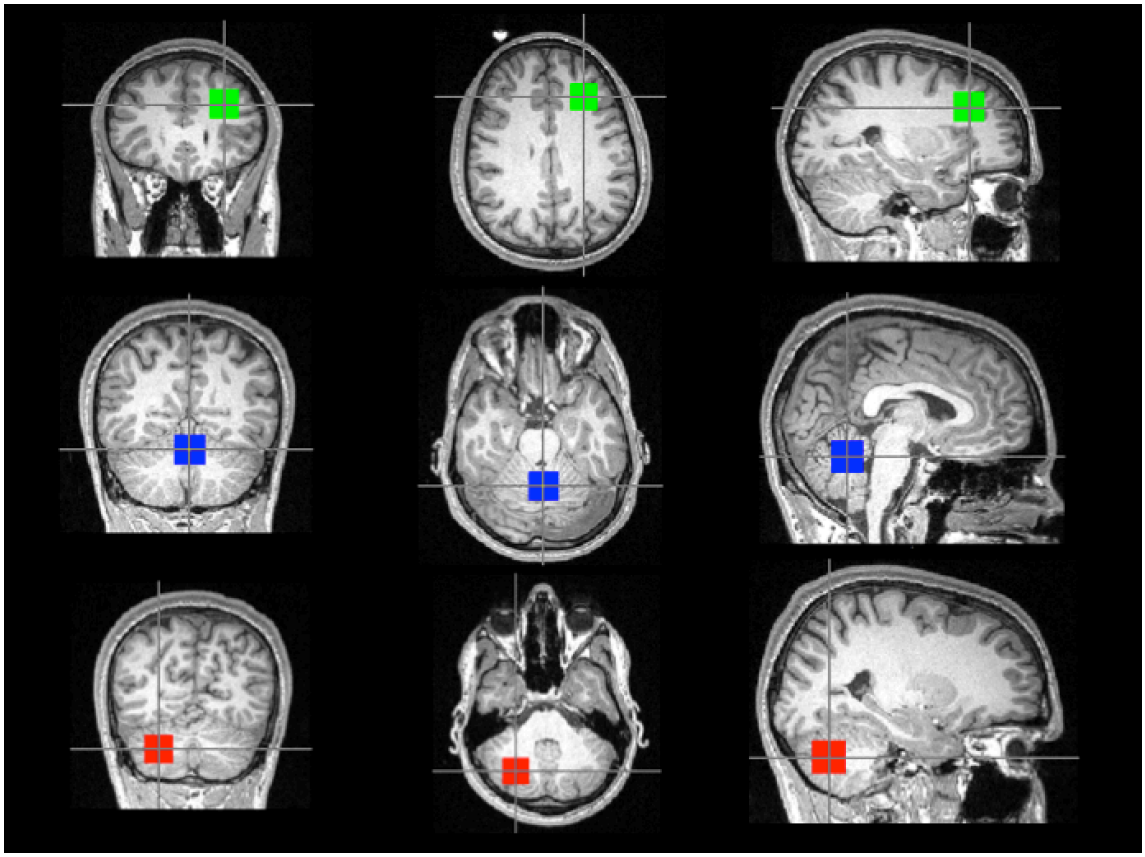
Questionnaires were directly administered or mailed to each participant's parent or caregiver to quantitatively assess ASD-related symptom presentation. The Social Responsiveness Scale (SRS) is a standardized form for assessing symptom severity over a 6 months period across communication skills, social functioning, and stereotyped behaviors and restricted interests.[343] The Aberrant Behavior Checklist (ABC) is a standardized form for assessing maladaptive behaviors in clinical populations.[344] Additional behavioral testing was conducted with each participant prior to MR scanning to assess current social and language competency in the experimental setting. Social competency was assessed with the General Social Outcomes Measures (GSOM),[345] which is a progress-monitoring tool developed to assess social skills in individuals with a PDD and provides measures of additional social domains not assessed with the

SRS. Language competency was assessed with the Test of Language Competence (TLC),[346] which is an assessment tool developed to measure higher-level language function for communication and that has previously been shown to distinguish meta-linguistic abilities in individuals with ASD.[347] Two independent raters that were blind to diagnostic group scored each measure.

**MRI ACQUISITION**

Magnetic resonance imaging was carried out at the Brain Imaging Center of the University of Missouri Department of Psychological Sciences utilizing a MAGNETOM Trio A Tim System package (Siemens, Malvern, PA). Structural T1-weighted images were acquired for anatomical localization (TR=1920 ms, TE=2.9 ms, Flip Angle=9 degrees, 176 sagittal slices at 1 $mm^3$) and functional T2*-weighted images were acquired for BOLD activation (TR=2200 ms, TE=30 ms, Flip Angle=90 degrees, 35 ACPC-aligned slices at 4 $mm^3$) during 5 minutes of passive rest in which the participant viewed a blank screen with a cross-hair fixation point. Single-voxel (20 $mm^3$) point-resolved spectroscopy spin-echo[348] sequences were used to detect metabolites of interest (TR = 2000 ms, TE = 80 ms, flip angle = 90°, 128 averages, weak water suppression at bandwidth = 50 Hz, delta frequency = -2.3 ppm, bandwidth = 1200 Hz) and were repeated without water suppression to allow absolute quantification of metabolites. [349] Single-voxel (20$mm^3$) MEGA-PRESS (TR = 2000 ms, TE= 68 ms, flip angle = 90°, water saturation bandwidth = 35 Hz, delta frequency = -1.7 ppm, bandwidth = 2000 Hz) sequences were used to detect GABA and were repeated without water suppression. Voxels were independently localized in each participant in the right postereolateral cerebellar hemisphere junction of crus I and crus II, the anterior lobe of the cerebellar vermis, and the left dorsolateral prefrontal cortex, with reference to the standardized MNI atlas,[220,221] Figure 14.

**Figure 14 Representative MRS voxel locations.** Voxel locations are displayed from a representative participant for the left dorsolateral prefrontal cortex [23], the anterior lobe of the vermis (dark blue), and the right postereolateral cerebellum hemisphere (red).



**MRI PREPROCESSING**

Preprocessing of fMRI data consisted of slice timing correction, rigid body realignment, intensity normalization, brain extraction and registration to the structural T1-weighted image with the FMRIB Software Library (FSL).[213,214] To account for spurious fluctuations in the BOLD signal, [215] translation and rotation parameters (x, y, z, pitch, roll, and yaw) from realignment were combined with average BOLD signals from the ventricles and white matter and their temporal derivatives and regressed out of the timeseries data with the REST toolkit.[216]. Global

signal regression was not included within these preprocessing procedures due to the small number of regions of interest being investigated and potential to create anticorrelations between cerebrocerebellar circuits. Although global signal regression removes noise associated with whole brain signal,[350] spurious anticorrelations may also be introduced,[351] which may confound the interpretation of cerebrocerebellar network coherence. Temporal band-pass filtering ($0.01 < f < 0.08$ Hz) was applied with the REST toolkit to reduce the effects of low-frequency drift and high-frequency noise and focus on intrinsic signal fluctuations.[217] fMRI data were then motion corrected as motion can substantially influence functional connectivity analyses. [352,353] BOLD acquisitions were scrubbed for excess motion and signal intensity using in-house MATLAB programs (The Mathworks, Inc., Natick, MA). Any acquisitions that exceeded 2 standard deviations from the within-subject within-run mean for any translation, rotation, or intensity parameter or exceeded motion of more than 2 mm in any direction were removed. Midpoint voxel coordinates from MRS acquisition in the right postereolateral cerebellum (R Cere Hemi), vermis, and left dorsolateral prefrontal cortex (L DLPFC) were used to construct 20 $mm^3$ ROIs. ROIs used for MRS and functional connectivity analyses were completely overlapping to allow an assessment of the correspondence between metabolite levels and functional connectivity between regions. To account for covariance and allow assessment of unique functional connectivity, partial correlation matrices containing all possible ROI pairs were generated for each participant. Fischer's r-to-z transformations were then applied to standardize the data.

Metabolite levels were quantified with LCModel.[354] Metabolite concentrations for glutamate[32], γ-aminobutyric acid (GABA), N-acetylaspartate (NAA), and creatine plus phosphocreatine (Cr+PCr) were computed within each individual. Concentrations are presented in institutional units, which provide a comparison between diagnostic groups and approximates

millimoles (mM) per liter but may vary by an unknown percentage from absolute mM. The balance between excitation and inhibition (E/I) was also computed by generating the ratio of glutamate to GABA within each ROI; however these metabolites were assessed across different acquisition protocols and thus only provided a relative comparison between groups, not an absolute ratio of mM concentrations between metabolites. E/I comparisons were log transformed to account for skewed distributions based on ratio comparisons; however the reported group averages and standard deviations reflect untransformed ratios. The FSL toolbox FAST was also used to segment anatomical brain tissue into gray matter, white matter, and cerebrospinal fluid (CSF) within the regions of interest, because differing gray/white matter proportions can affect spectra concentration.[355] Tissue composition was accounted for in all analyses involving metabolite concentrations.

**STATISTICAL ANALYSES**

One ASD participant was unable to complete the study due to an adverse reaction to the imaging environment and this subject's matched control was subsequently removed. Two ASD participants' IQ estimates were more than 2 standard deviations below the average and these subjects were not matched to typically developing controls. Statistical analyses were conducted on 14 individuals with ASD and 12 controls and 12 individuals with ASD and 12 matched controls to compare matching and sample size effects. Analyses consisted of an analysis of variance analysis of variance, ANOVA, approach with an additional $\chi^2$ (chi-squared) for categorical variables.

Analyses included a set of one-way ANOVAs between groups to assess 1) continuous demographic variables (age, IQ, and years of education) and a $\chi^2$ (chi-squared) for categorical demographic variables (gender, ethnicity, handedness, and family income), 2) measures of

social-communication symptom severity (Total SRS and subscales including social awareness, social cognition, social communication, and social motivation) and restricted interests/repetitive behaviors (SRS subscale autistic mannerisms and Total ABC including subscales irritability, lethargy, stereotypy, hyperactivity and inappropriate speech), 3) measures of social competence (Total GSOM and subscales including conversational reciprocity, facial expressions, social problem solving and emotional perspective taking) and language competence (Total TLC and subscales including ambiguous sentences, listening comprehension, oral expression, and figurative language), 4) functional connectivity estimates between ROI pairs (R Cere Hemi, vermis, and L DLPFC), and 5) a set of three ANCOVAs (one per MRS ROI) controlling for proportion of grey matter within each region of interest.

Signal to noise ratio of PRESS and MEGA PRESS sequences were used to determine the validity of metabolite concentrations from each participant. Functional connectivity estimates from resting-state were also regressed across log transformed E/I ratios, controlling for tissue composition and signal to noise, to determine if any significant relationships exist between excitatory/inhibitory balance and cerebrocerebellar connectivity. The associations between symptom severity and behavioral outcomes with cerebrocerebellar connectivity were also examined to determine if cerebellar modulatory influences are associated with cognitive and behavioral outcomes in individuals with ASD.

Analyses were carried out with IBM SPSS Statistics Software.[230] Due to the small sample size and pilot nature of this investigation, correction for multiple comparisons was only completed across regions of interest, which may increase Type I error but will allow hypothesis generation for future investigations. Correction for multiple comparisons was completed by

controlling for the false discovery rate (FDR).[231] Significance following FDR correction is indicated (*).

**Table 3 Demographic and diagnostic information undergoing magnetic resonance spectroscopy.** Data represents average scores +/- standard deviation or number of subjects within each categorical group for matched subjects. Categorical groups include males and females (M/F), white, black, Hispanic and other (W/B/H/O), right and left (R/L), and most frequently reported income bracket (mode).

| | **ASD** | **CTRL** | **Statistics** | **p** |
|---|---|---|---|---|
| ***Demographics*** | (n=12) | (n=12) | | |
| Age (years) | 22.17 +/- 4.59 | 23.18 +/- 3.04 | F(1,22)=0.40 | 0.536 |
| Gender (M/F) | 10/2 | 10/2 | $\chi^{2223}$=0.00 | 1.000 |
| Race (W/B/H/O) | 10/0/0/2 | 12 | $\chi^2$(2)=2.18 | 0.336 |
| Handedness (R/L) | 11/1 | 11/1 | $\chi^{2223}$=0.00 | 1.000 |
| Education (years) | 14.83 +/- 4.39 | 15.92 +/- 1.62 | F(1,22)=0.64 | 0.431 |
| Family Income (mode) | Don't know | $100,000/more | $\chi^2$(7)=12.12 | 0.097 |
| ***Intelligence Quotients*** | | | | |
| VIQ | 100.00 +/- 23.11 | 112.42 +/- 5.66 | F(1,22)=3.27 | 0.084 |
| PIQ | 105.75 +/- 21.14 | 106.50 +/- 11.67 | F(1,22)=0.01 | 0.915 |
| FSIQ | 103.33 +/- 19.08 | 110.67 +/- 8.21 | F(1,22)=1.49 | 0.235 |
| ***Diagnostics (cutoff)*** | | | | |
| ADI Social [223] | 20.00 +/- 5.10 | - | | |
| ADI Communication (8) | 15.00 +/- 4.77 | - | | |
| ADI Repetitive (3) | 7.66 +/- 2.18 | - | | |
| ADI Abnormality [223] | 3.89 +/- 0.93 | - | | |

# Results

## PARTICIPANTS

Control participants provided an adequate comparison group without significant contributions from confounding demographic influences. Comparing only matched participants, there were no significant group differences in age, verbal IQ (VIQ), performance IQ (PIQ), full scale IQ (FSIQ), or years of education, $p>0.05$, nor gender, ethnicity, handedness or family income, $p>0.05$, Table 3. VIQ approached significance, $p=0.084$, which was primarily due to matching participants based on FSIQ. Comparing all participants, FSIQ exhibited a trend towards a difference between groups, $F(1,24)=3.244$, $p=0.084$, with higher FSIQ in the control group, ($M=110.67$, $SD=8.22$) compared to individuals with ASD ($M=98.64$, $SD=21.78$) and verbal IQ was significantly higher, $F(1,24)=5.258$, $p=0.031$, in the control group ($M=112.42$, $SD=5.66$) compared to individuals with ASD. ($M=96.21$, $SD=23.84$) Considering that language abilities in individuals with ASD are often affected, moderate differences in verbal IQ would not be unexpected.

## BEHAVIORAL ASSESSMENT

Behavioral assessment of individuals with ASD indicated the anticipated pattern of cognitive and behavioral symptoms regarding the typical presentation of the ASD phenotype. Individuals with ASD displayed significantly diminished social cognition and more prevalent presentations of aberrant behaviors compared to unaffected individuals. Social and language competency were also affected such that individuals with ASD displayed less naturalistic conversational skills and impaired listening comprehension. Consistent with the heterogeneity of symptom presentation across the autism spectrum, individuals with ASD displayed greater variability of skills in social and language domains compared to controls.

**Social Responsiveness Scale**

Individuals with ASD exhibited more ASD-related symptoms compared to controls, with all SRS subscales in the mild to moderate range of severity. Average SRS scores from controls were within the normal range for individuals in the general population unaffected by ASD. Individuals with ASD exhibited higher SRS scores compared to control participants in the total SRS ($M=72.54$, $SD=14.25$; $M=46.33$, $SD=14.4$), $F(1,23)=20.872$, $p<0.001$, and social awareness ($M=62.46$, $SD=14.63$; $M=46.58$, $14.29$), $F(1,23)=7.516$, $p=0.012$, social cognition ($M=70.23$, $SD=3.07$; $M=46.33$, $SD=10.22$), $F(1,23)=25.621$, $p<0.001$, social communication ($M=69.07$, $SD=12.62$; $M=46.92$, $M=15.17$), $F(1,23)=15.867$, $p=0.001$, social motivation ($M=70.00$, $SD=15.13$; $M=46.42$, $SD=9.93$), $F(1,23)=20.825$, $p<0.001$, and autistic mannerisms ($M=74.92$, $SD=16.65$, $M=48.00$, $SD=16.58$), $F(1,23)=16.380$, $p=0.001$, subscales, which remained significant when only comparing matched participants, Figure 15. One ASD participant's caregiver elected not to complete the SRS.

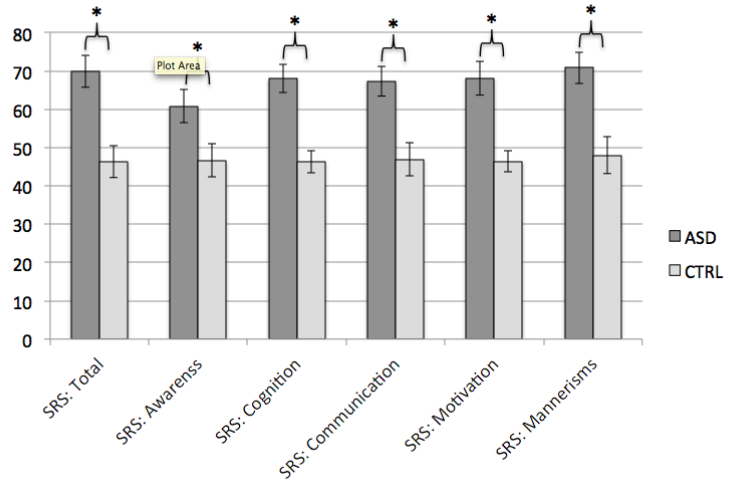
**Aberrant Behavior Checklist**

Individuals with ASD presented more aberrant behaviors compared to controls with more behaviors suggestive of lethargy, stereotypy, and inappropriate speech, which would be expected for individuals with a neurodevelopmental disorder. Controls displayed generally low occurrences of aberrant behaviors indicating minimal presentation of clinically relevant symptoms. Individuals with ASD exhibited higher ABC scores compared to control participants in the total ABC ($M=22.36$, $SD=14.80$; $M=4.83$, $7.83$), $F(1,24)=13.518$, $p=0.001$, and lethargy ($M=10.86$, $SD=7.38$; $M=0.67$, $SD=1.78$), $F(1,24)=21.692$, $p<0.001$, stereotypy ($M=2.86$, $SD=2.74$; $M=0.25$, $SD=0.87$), $F(1,24)=9.948$, $p=0.004$, and inappropriate speech ($M=2.21$, $SD=2.11$; $M=0.17$, $SD=0.039$). $F(1,24)=10.832$, $p=0.003$, subscales, which remained significant

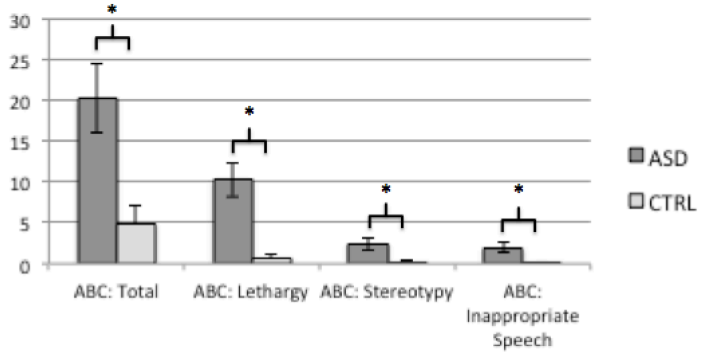
when only examining matched participants, Figure 15. There were no differences in irritability or hyperactivity, $p>0.05$, and all individuals generally presented few behavioral symptoms within these domains across diagnostic groups.

**Figure 15 Measures of symptom severity.** The (A) Social Responsiveness Scale and (B) Aberrant Behavior Checklist (ABC) and subscales of interest between ASD (dark grey) and control, CTRL, (light grey) groups are displayed. Error bars represent standard error and significant differences are indicated (*).

A




B



**General Social Outcomes Measure**

Individuals with ASD also expressed less general social competency compared to unaffected individuals, especially in a conversational setting. Individuals with ASD exhibited significantly lower GSOM scores compared to control participants for the total GSOM (M=70.79, SD=9.92; M=80.42, SD=4.91), $F(1,24)=9.321$, $p=0.005$, and conversational reciprocity subscale (M=22.35, SD=3.80; M=26.75, SD=2.18), $F(1,24)=12.497$, $p=0.002$, which remained significant when only comparing matched participants, Figure 16. There were no differences in interpretation of facial expressions, social problem solving, or emotional perspective taking, $p>0.05$, between individuals with ASD and controls. The GSOM was primarily designed for use with younger individuals, 10-15 years of age, and has not been systematically evaluated in older populations. Age-group effects should be considered with the interpretation of these comparisons.

**Test of Language Competence**

Individuals with ASD exhibited lower general language competency compared to unaffected individuals, especially in listening comprehension. Individuals with ASD exhibited significantly lower TLC scores compared to controls for the total TLC (M=143.36, SD=35.35; M=172.50, SD=11.50), $F(1,24)=7.439$, $p=0.012$, and ambiguous sentences (M=28.83, SD=9.21; M=33.67, SD=5.00), $F(1,24)=4.468$, $p=0.045$, listening comprehension (M=27.67, SD=6.61; M=33.08, SD=3.45), $F(1,24)=6.538$, $p=0.017$, oral expression (M=63.25, SD=18.15; M=74.00, SD=3.8), $F(1,24)=4.329$, $p=0.048$, and figurative language (M=26.17, SD=11.28; M=31.75, SD=5.66), $F(1,24)=3.267$, $p=0.083$, subscales. Comparing only matched participants, total TLC and listening comprehension remained significantly lower in individuals with ASD compared to controls and oral expression exhibited a trend, $p=0.057$, Figure 16; whereas ambiguous sentences and figurative language interpretation were no longer different between groups, $p>0.05$. Due to

the advanced age of participants within this sample compared to available standardized scores of TLC, raw scores were utilized in these analyses. These effects were also examined with standardized data based on the nearest age group and were consistent across analyses.

**Figure 16 Measures of social and language competency.** (A) General social outcomes measure (GSOM) and (B) the Test of Learning Competence (TLC) total scores and subscales of interest are displayed between ASD (dark grey) and control, CTRL, (light grey) groups. Error bars represent standard error and significant differences are indicated (*).

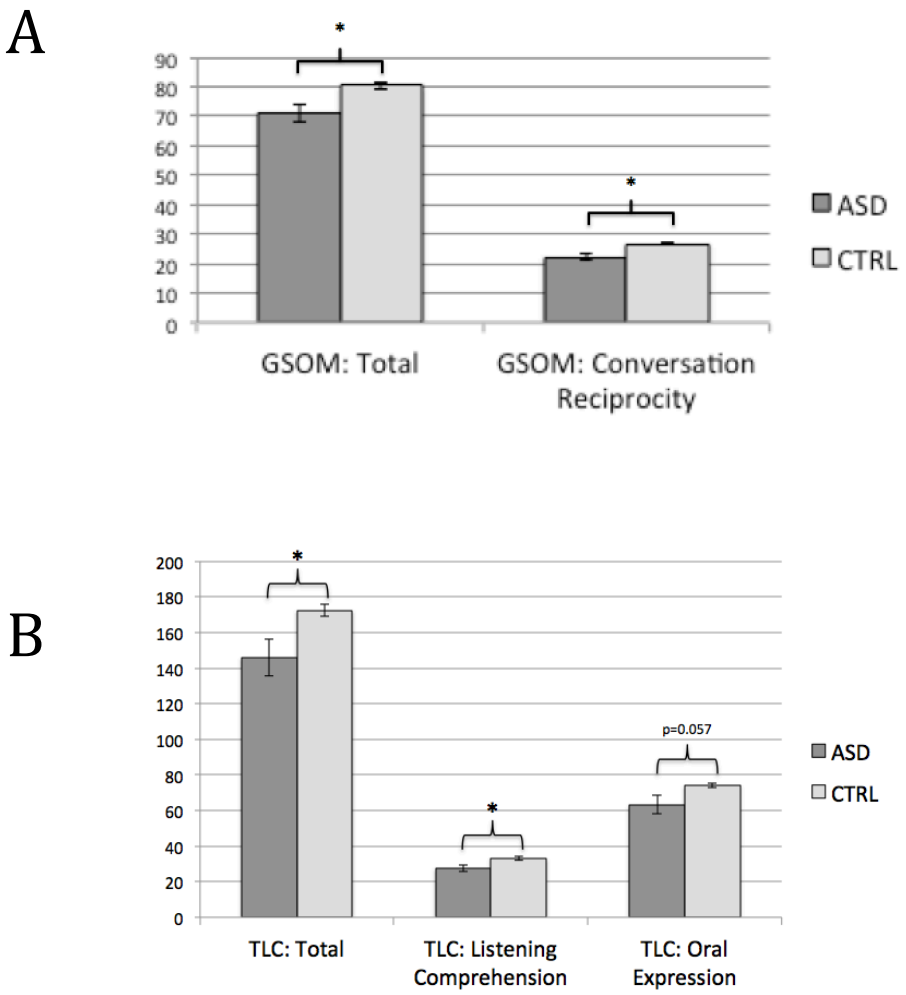
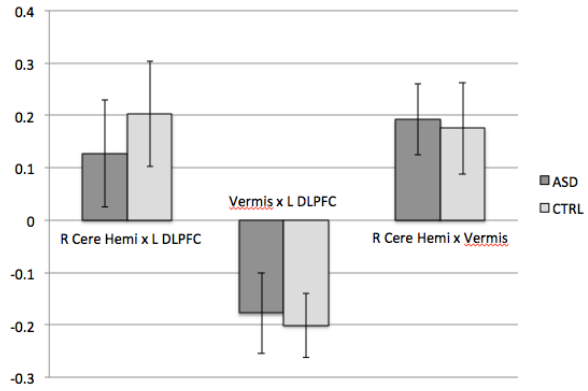
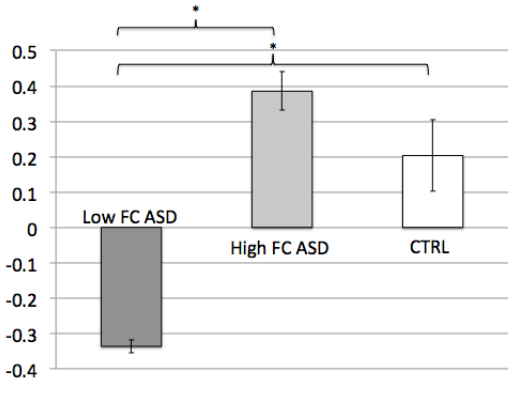


**Figure 17 Cerebro-cerebellar functional connectivity.** (A) Functional connectivity between the right postereolateral cerebellar hemisphere (R Cere Hemi), left dorsolateral prefrontal cortex (L DLPFC), and vermis are displayed across the ASD (dark grey) and control, CTRL, (light grey) groups. (B) Functional connectivity between the right postereolateral cerebellar hemisphere and left dorsolateral prefrontal cortex is displayed for the low FC and high FC ASD groups compared to controls (C) as well as individual participant  Error bars represent standard error and significant differences are indicated (*).

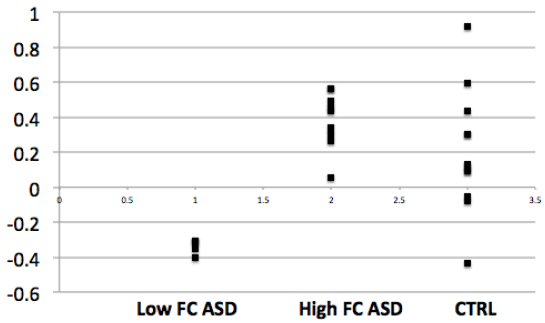
A




B




C



## FUNCTIONAL CONNECTIVITY

There were no significant differences in cerebrocerebellar functional connectivity between individuals with ASD and controls when comparing diagnostic groups, $p>0.05$, which remained non-significant when examining only matched participants, Figure 17. There was a single outlier identified in the control group when examining connectivity between the right cerebellar hemisphere and vermis, which exhibited an FC estimate greater than 3 SD from the within group mean. Analyses were examined excluding this outlier and group level connectivity estimates remained non-significant between groups, $p>0.05$. FC between the R Cere Hemi was significantly associated with listening comprehension. Across all participants regardless of diagnostic group, listening comprehension was significantly associated with cerebrocerebellar connectivity, $r=0.436$, $p=0.026$; however this association appeared to be driven by individuals with ASD, $r=0.588$, $p=0.027$, Figure 18, as controls showed no association, $r=0.086$, $p=0.790$. Individuals with ASD exhibited significantly reduced performance on the listening comprehension task compared to unaffected individuals and this performance effect was related to functional connectivity between the cerebellar hemisphere and prefrontal cortex. Symptom severity and social competency were not generally associated with cerebrocerebellar connectivity.

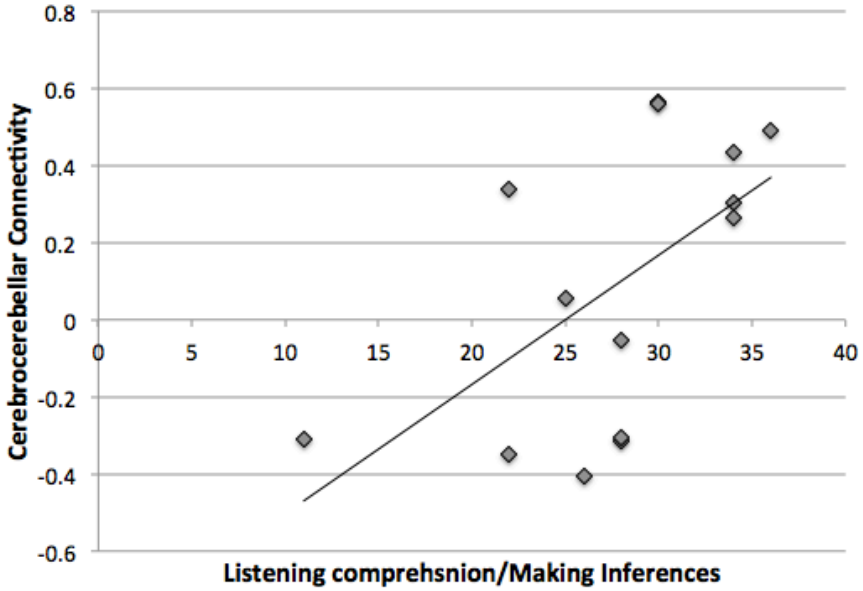
Although there were no group level differences in cerebrocerebellar FC between individuals with ASD and controls, there was a statistically significant subset of individuals with ASD, as described below, whom exhibited significantly lower, anticorrelated, cerebrocerebellar connectivity, Figure 17. Functional connectivity estimates generally displayed a normal distribution across individuals; however connectivity between the R Cere Hemi and L DLPFC exhibited a bimodal distribution in individuals with ASD. Potential bimodality of this distribution was assessed with the Dip test, which quantitatively assesses a unimodal distribution

for significant flat steps in the distribution function indicating violations of unimodality.[356] The ASD and control group distributions were examined and the Dip test indicated a significant unimodal distribution in controls, D=0.076, p=0.84, but a significant non-unimodal distribution in individuals with ASD, D=0.148, p=0.004. Bimodal normal distributions were fitted in R[357] to identify a relevant breakpoint, which was indicated between -0.307 and 0.057. This breakpoint segregated 5 individuals with ASD into the low FC group and 9 individuals into the high FC group. Groups were separated and compared to controls with a one-way ANOVA. There was a significant difference in cerebrocerebellar connectivity across groups, $F(2,25)=11.93$, $p<0.001$, due to lower cerebrocerebellar connectivity in the low FC ASD group (M=-0.34, SD=0.04) compared to the high FC ASD group (M=0.37, SD=0.16), $t(12)=9.368$, $p<0.001$*, and compared to the control group (M=0.22, SD=0.35), $t(15)=3.410$, $p=0.004$*. There were no differences in cerebrocerebellar FC between the high FC ASD and control groups, $p>0.05$, suggesting a subset of individuals with ASD that exhibit significant anticorrelated cerebrocerebellar connectivity.

The low FC ASD group and high FC ASD groups were then compared across diagnostic and behavioral measures utilizing one-way ANOVAs and a chi-squared to determine if differences in demographics or symptom severity segregates with anticorrelated cerebrocerebellar connectivity. There were no differences in age, IQ, or years of education nor gender, ethnicity, handedness, or family income between, $p>0.05$. Symptom severity, as assessed with the SRS and ABC, did not significantly differ between low FC ASD and high FC ASD groups, $p>0.05$. There were also no differences in social competency, as assessed with the GSOM, and generally no differences in language competency, as assessed with the TLC; however listening comprehension was significantly lower in the low FC ASD group (M=23.00,

SD=7.14) compared to the high FC ASD group (M=30.33, SD=4.69), F(1,13)=5.459, p=0.038, which is especially interesting considering the role of the R Cere Hemi in language processing.

**Figure 18 Cerebrocerebellar connectivity and listening comprehension.** Functional connectivity between the left dorsolateral prefrontal cortex and right postereolateral cerebellum hemisphere is associated with scores on the listening comprehension subscale of the test of language competence in individuals with ASD.




**METABOLITE LEVELS**

Comparing individuals with ASD and controls, there were generally no differences in metabolite levels. One ASD participant was removed from analysis of L DLPFC metabolite levels due to an insufficient signal to noise ratio. L DLPFC metabolite levels and E/I did not significantly differ between ASD and control participants, $F(14,9)=0.613$, $p>0.05$. One ASD participant and two control participants were removed from analysis of vermis metabolite levels due to an insufficient signal to noise ratio. Vermis metabolite levels and E/I did not significantly differ between ASD and control participants, $F(14,7)=0.872$, $p>0.05$. Two ASD participants were removed from analysis of R Cere Hemi metabolite levels due to an insufficient signal to

noise ratio. R Cere Hemi metabolite levels and E/I did not significantly differ between ASD and control participants, $F(13,9)=1.153$, $p>0.05$. Metabolite levels are displayed in Figure 19 and E/I ratios are displayed in Figure 20.

**Figure 19 Metabolite concentrations.** Metabolite concentrations for glutamate [32], GABA, n-acetyl aspartate (NAA), and creatine+phosphocreatine (Cr+PCr) are displayed for the (A) left dorsolateral prefrontal cortex (DLPFC), (B) right postereolateral cerebellum hemisphere (R Cere Hemi), and (C) vermis separately for the ASD (dark grey) and control, CTRL, (light grey) groups. Error bars represent standard error and significant differences are indicated (*).

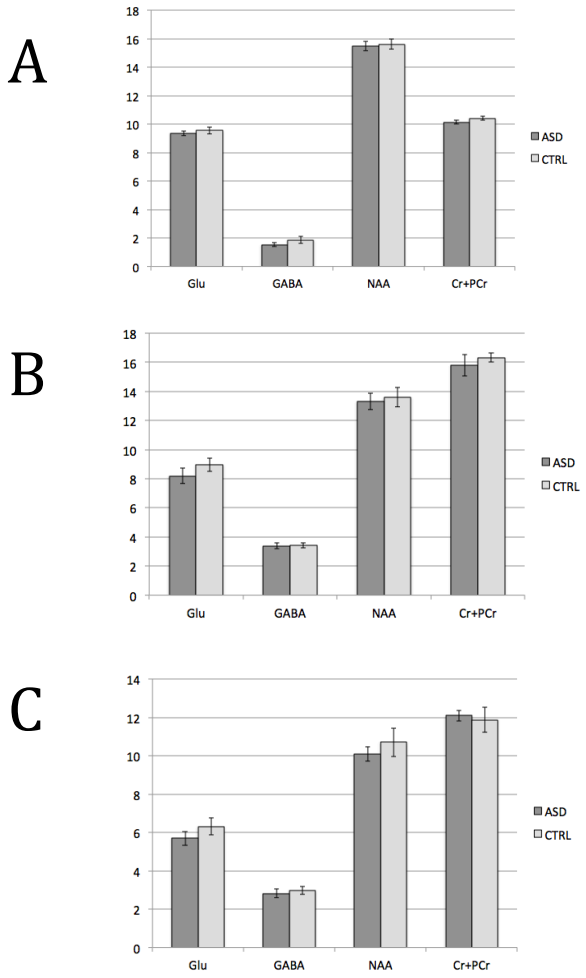
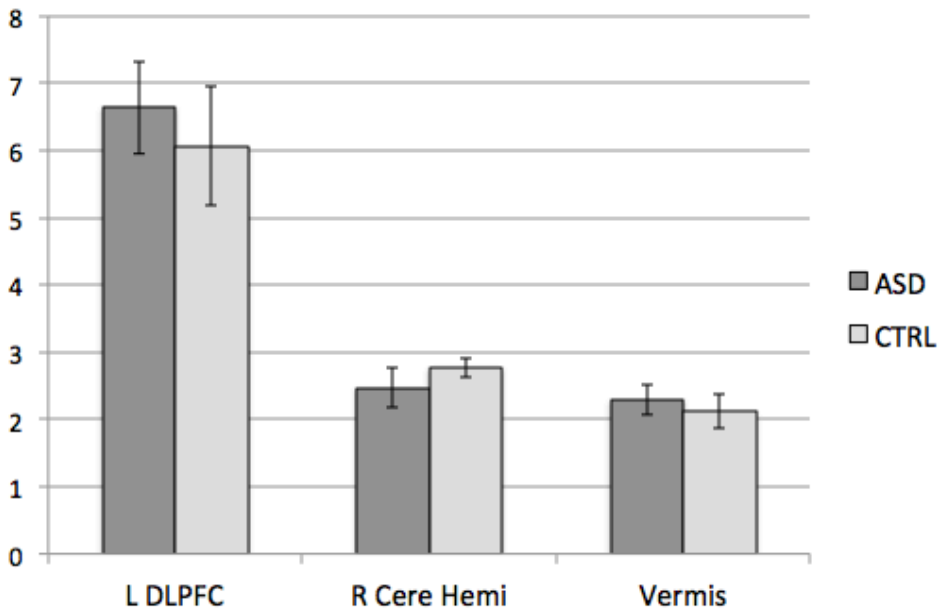


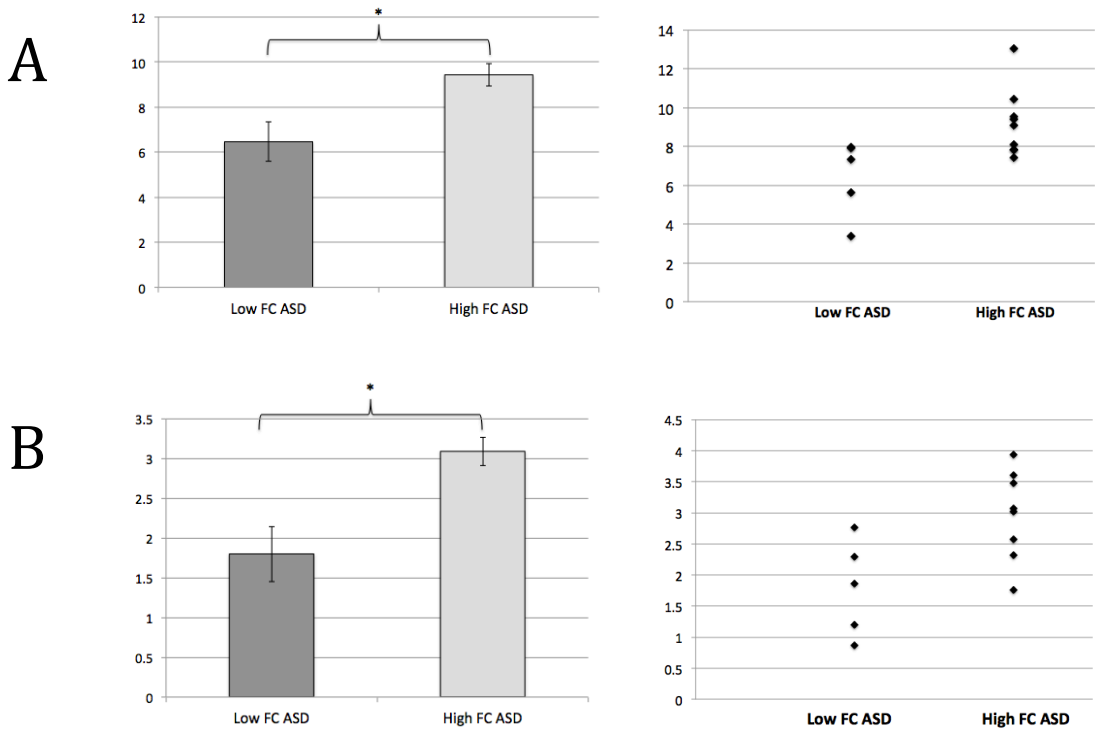
**Figure 20 Excitatory to inhibitory ratios.** Excitatory to inhibitory, glutamate/GABA, ratios are displayed for left the dorsolateral prefrontal cortex (DLPFC), right postereolateral cerebellum hemisphere (R Cere Hemi), and vermis separately for the ASD (dark grey) and control, CTRL, (light grey) groups. Error bars represent standard error and significant differences are indicated (*).




Individuals with ASD that expressed significantly lower, anticorrelated, FC also displayed significantly lower glutamate levels, with concurrent reductions in E/I balance, compared to higher FC individuals with ASD. Due to the bimodal distribution of cerebro-cerebellar functional connectivity in individuals with ASD, metabolite levels of the L DLPFC and R Cere Hemi were compared between low and high FC ASD groups to determine if metabolite levels segregate with FC differences in individuals with ASD. L DLPFC concentrations of GABA, Glu, NAA, and Cr+PCr did not significantly differ across low and high FC ASD participants, $F(5,6)=0.633$, $p>0.05$; however glutamate levels were significantly lower

in the low FC ASD group (M=6.46, SD=1.94) compared to the high FC ASD group (M=9.43, SD=1.71) in the R Cere Hemi, F(1,12)=5.180, p=0.049, and E/I ratios were also significantly lower in the low FC ASD group (M=1.80, SD=0.35) compared to the high FC ASD group (M=3.09, SD=0.18), F(1,12)=7.091, p=0.026, Figure 21. Altered glutamate levels in the R Cere Hemi in these individuals suggest potentially less cerebellar modulatory influence on prefrontal circuits during cognitive processing.

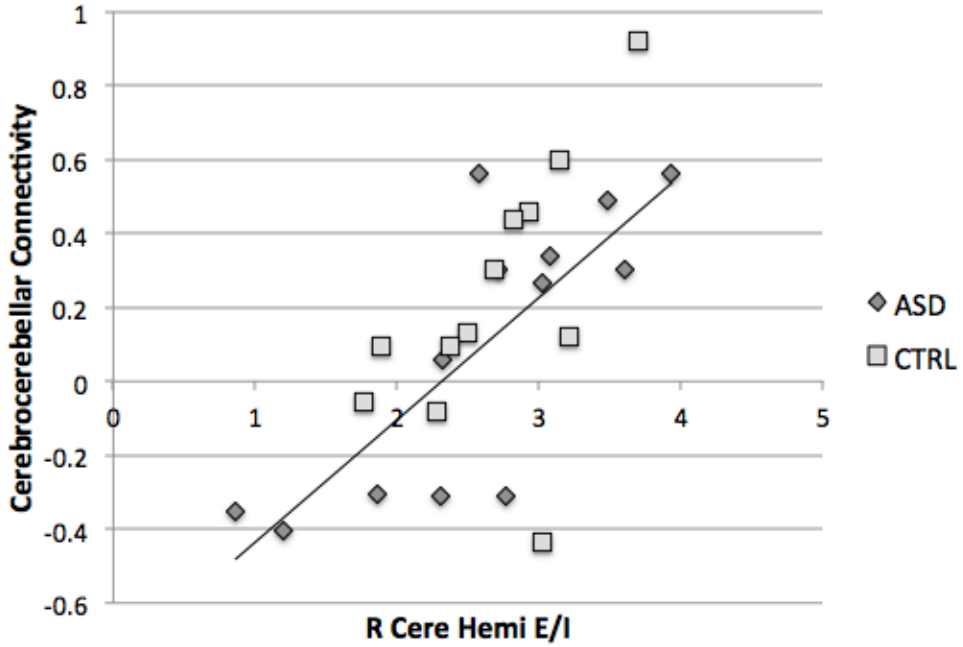
**Figure 21 Glutamate and E/I in ASD.** (A) Metabolite concentrations of glutamate and (B) excitatory/inhibitory (E/I) balance of glutamate/GABA in the right postereolateral cerebellum hemisphere are displayed for individuals with ASD expressing low (dark grey) and high (light grey) cerebro-cerebellar functional connectivity. Individual participant data is also displayed. Error bars represent standard error and significant differences are indicated (*).



**Metabolite Levels and Functional Connectivity**

Across all individuals regardless of diagnostic group, cerebrocerebellar connectivity between the R Cere Hemi and L DLPFC was significantly associated with E/I in right postereolateral cerebellar hemisphere, r=0.603, p=0.003. Individuals with ASD, r=0.677, p=0.031, and controls, r=0.606, p=0.084, contributed to these effects, Figure 22. However, cerebrocerebellar connectivity between the R Cere Hemi and L DLPFC was not significantly associated with E/I of the L DLPFC, r=0.082, p=0.711, with non-significant effects in both the ASD and control groups, p>0.05.

**Figure 22 Cerebrocerebellar connectivity and E/I in the cerebellum.** Functional connectivity between the right postereolateral cerebellum hemisphere (R Cere Hemi) and left dorsolateral prefrontal cortex is associated with the balance between glutamate and GABA (E/I) in the R Cere Hemi in both individuals with ASD (dark grey triangle) and controls, CTRL, (light grey squares).



## Discussion

The purpose of this study was to examine functional cerebrocerebellar connectivity in individuals with ASD and assess if alterations in network coherence were related to the balance between excitation and inhibition in the cerebellum. A subset of individuals with ASD exhibited anticorrelated cerebrocerebellar connectivity between the right postereolateral cerebellar hemisphere and left dorsolateral prefrontal cortex, implicating reduced modulatory influences of the cerebellum on neural circuit dynamics. Cerebellar modulation is theorized to serves as an oscillation dampener to maintain a homeostatic baseline in neuronal processing such that disturbances in cerebellum modulation can have deleterious on cognitive processing and behavior.[281] Individuals with ASD that exhibited anticorrelated cerebrocerebellar connectivity also displayed diminished listening comprehension skills and deficits in the ability to make inferences from verbal information, which is especially relevant considering the role of the posterior cerebellum hemispheres in language processing. Altered cerebrocerebellar connectivity was also associated with a reduced balance between excitation and inhibition in the cerebellum due to reduced concentrations of glutamate, indicating a potential mechanism underlying downregulation of cerebrocerebellar connectivity in these individuals. Neuropathological alterations in the cerebellum have been noted for decades in individuals with ASD[46-49,282] but have only recently been incorporated into neuroimaging studies of functional connectivity in ASD.[285] The cerebellum is implicated in an array of higher-order cognitive domains,[277] such as language processing, and neuropathological perturbations of the cerebellum may be associated with the presentation of symptoms related to the ASD phenotype. Although our pilot study only implicates a subset of individuals with ASD, this is the first investigation to suggest a relationship between potential neurochemical alterations in the cerebellum with effects on

cerebrocerebellar connectivity and behavioral outcomes in individuals with ASD, which warrants further investigation.

The cerebellum exhibits altered patterns of functional activation individuals with ASD depending on task demands. The cerebellum appears to be hyper-active during motor processing but hypo-active during attention orientating in individuals with ASD,[358] suggesting potential differing functional outcomes of the previously reported cerebellar abnormalities depending on neural system targets and processing demands. To date, only a single study has specifically investigated functional network coherence between cerebellar subfields and neocortical networks in individuals with ASD.[285] These researchers discovered that individuals with ASD exhibited global cerebrocerebellar hyper-connectivity driven by hyper-connectivity to sensorimotor networks but hypo-connectivity to supramodal networks, including the prefrontal cortex. The functional relationships between the cerebellum and neocortical circuits involved with cognitive processing appear to be affected in ASD and these alterations warrant further investigation. We found that a subset of individuals with ASD exhibited anticorrelated functional connectivity between the right cerebellar hemisphere and left dorsolateral prefrontal cortex, which may have contributed to the previous report of hypo- cerebrocerebellar connectivity of supramodal networks.[285] The prefrontal cortex has been theorized to maintain patterns of activity in the neocortex by guiding activity patterns in other brain structures to support optimized cognitive processing based on task demands.[359] Feedforward control from the cerebellum modulates neuronal circuit dynamics in the neocortex,[360] and disturbances in modulatory output from the posterior cerebellar hemispheres affects prefrontal circuits and generally results in cognitive impairments.[277] Altered cerebellar modulation may therefore perturb neocortical circuit dynamics and lead to some aspects of the ASD phenotype. The right posterolateral hemispheres

of the cerebellum typically exhibit functional connections with the dorsolateral prefrontal cortex, a region within the default mode network (DMN), during passive rest and internal mentation.[361] The DMN is typically upregulated during passive rest but downregulated during over cognitive processing.[98,99] We found a similar profile of cerebrocerebellar connectivity between these regions in controls and most individuals with ASD in the current sample, but an additional subset of individuals with ASD with a markedly different pattern of cerebrocerebellar connectivity. Heterogeneity across the autism spectrum suggests etiological subgroups most likely exist and our findings support a subgroup of individuals with ASD in which some aspects of the ASD phenotype are associated with altered functional integration between cerebellar and cerebral circuits. Additionally within the previous report of hypo-connectivity in the cerebellum during attention orienting, there appeared to be a subset of individuals with ASD that exhibited deactivation within the cerebellum, suggesting potential downregulation of the cerebellum in these individuals and supporting our finding of an anticorrelation between cerebrocerebellar circuits.

Individuals with ASD exhibiting anticorrelated cerebrocerebellar connectivity displayed concurrent deficits in language comprehension and inference abilities. High functioning verbal individuals with ASD have relatively intact basic language skills but diminished interpretive linguistic abilities.[347] We found that these interpretive linguistic abilities were decreased in individuals with ASD exhibiting perturbed cerebrocerebellar connectivity. The role of the cerebellum in these abilities is further supported by reports that the cerebellum is activated during language processing,[131,132,262,263] and patients with cerebellar lesions exhibit language deficits.[127,128,255,256] Additionally, patients with cerebellar lesions also exhibit deficits in the same domains and with the same assessment measure as utilized in this investigation.[362] Thus,

weakened functional coherence between cerebellar and prefrontal regions may affect network coherence during cognitive processing of verbally-mediated demands and alter the capacity to infer meaning. This is especially relevant regarding individuals with ASD because language comprehension and inference abilities are theorized to mediate the capacity to take into account the mental state of other individuals.[338] Mental state inference deficits in individuals with ASD have been conceptualized into a prominent theory of mind hypothesis suggesting individuals with ASD are impaired in their ability to impute the beliefs of others,[363] which can have profound effects on social communication.

Cerebrocerebellar connectivity appears to be associated with language processing[131] in individuals with ASD; however the mechanisms underlying these connectivity differences remain in question. Glutamate and GABA define the excitatory to inhibitory (E/I) balance within and between neuronal networks, and neuronal network dynamics in ASD may be affected by altered E/I balance disrupting network coherence.[144,145] Glutamate and GABA levels in the postereolateral cerebellum and dorsolateral prefrontal cortex were assessed with MRS to determine whether alterations in E/I within these regions are associated with altered connectivity. Individuals with ASD that displayed anticorrelated cerebrocerebellar connectivity also exhibited reduced glutamate concentrations in the postereolateral cerebellum with a concurrent reduction in E/I balance but no associated neurochemical alterations in the prefrontal cortex. Increased glutamatergic signaling has been previously suggested to underlie some aspects of the ASD phenotype[145,330,364,365]; however reduced glutamatergic signaling capabilities are also supported by previous MRS reports of reduced glutamate and glutamine in the cerebellum of individuals with ASD[311] and neuropathological alterations including reduced glutamate receptor densities[300,301] and lower levels of proteins expressed in glutamatergic neurons.[305,306]

Glutamatergic neurons in the cerebellar hemispheres include granule cells and unipolar brush cells as well as afferent mossy and climbing fiber projections. Reductions in glutamatergic signaling capabilities in the cerebellar hemispheres may affect the ability of these interneurons and projections to provide excitatory input to Purkinje cells and thus affect afferent projections to the deep cerebellar nuclei and resulting contralateral projections to the neocortex. For example, Lurcher mutant mice exhibit postnatal degeneration of cerebellar Purkinje and granule cells[366] and display some behaviors associated with the ASD phenotype such as repetitive behaviors and hyperactivity.[367] The degeneration of these cerebellar neurons causes a significant reduction in glutamate release of efferent cerebellar projections that ultimately modulate PFC activity.[368] Thus, altered signaling capabilities within the cerebellar hemispheres implicate a mechanism by which functional cerebrocerebellar connectivity may be affected in these individuals with ASD.

Altered glutamate levels in the cerebellum are associated with cerebrocerebellar connectivity and some aspects of the ASD phenotype. This is the first investigation to provide a link between neurochemical alterations in the cerebellum, functional cerebrocerebellar connectivity and behavioral outcomes in individuals with ASD. These findings suggest a potential neural systems outcome of the previously reported neuropathological alterations in the cerebellum of individuals with ASD and that neuroimaging investigations of ASD should also examine the influence of cerebellar modulatory effects on neocortical networks. Furthermore, the identification of a subset of individuals with ASD exhibiting altered glutamate levels suggests that the failure of clinical trials of glutamatergic agents in individuals with ASD may be due to the heterogeneity of these perturbations within the ASD population. Research into stratification markers to identify these individuals, such as standardized MRS assessment, is warranted.

**LIMITATIONS**

We hypothesized a reduction in GABA in the cerebellum due to previous reports of consistent reductions in Purkinje cells in the cerebellum of individuals with ASD.[46-49,282] The spectral overlap of GABA resonances with other metabolites and relatively low signal-to- noise ratio in our MRS assessments may have limited our ability to accurately assess GABA concentrations within this participant sample. Future investigations should address this issue by increasing the number of averages during acquisition when examining cerebellar regions. Additionally, the main implications from these analyses were within a subset of individuals with ASD, suggesting a heterogeneous subgroup compared to the overall population of individuals with ASD. A larger more representative sample including younger individuals with higher variability of disorder severity should be examined to further elucidate the consistency of these effects within the ASD population. Finally due to the pilot nature of our investigation, correction for multiple comparisons was minimally applied and the potential for increased Type I error should be considered regarding interpretation of this work.

# CHAPTER 4:

# IMPLICATIONS

Autism spectrum disorder is a behaviorally defined neurodevelopmental disorder that affects approximately 1 in every 68 children in the United States.[13] Individuals with ASD exhibit deficits in social communication and the presentation of stereotyped interests and repetitive behaviors. ASD is extremely heterogeneous with differences in symptom severity and effects on quality of life within the patient population. Genetic susceptibility appears to play a major role in the development of ASD-related symptoms and the majority of these genetic contributions involve genes associated with neuronal development and signaling mechanisms.[25] ASD is associated with microstructural abnormalities in neuronal circuit structure,[51] and these altered circuits in the brain may affect functional utilization of neuronal clusters during cognitive processing causing cognitive and behavioral outcomes associated with ASD. Structural and functional connectivity within networks in the brain of individuals with ASD exhibit hyper-connectivity between adjacent neuronal clusters and hypo-connectivity between more remote neuronal clusters,[94] as well as general patterns of reduced network integration and less network segregation during neuronal network development[121] These findings are suggestive of underlying disturbances in neuronal structure and function in individuals with ASD, but the identification of ASD-specific neuropathology or biomarkers has proven difficult and is only beginning to be elucidated, which makes mechanistically-directed intervention extremely difficult.

The only currently FDA approved pharmacological interventions for ASD are antipsychotics, risperidone and aripiprazole; however, antipsychotics do not treat the core symptoms of the disorder and can have moderate to severe side effects. Pharmacological agents affecting distinct mechanistic pathways have been investigated in the treatment of ASD but there

are currently no pharmacological interventions for the core symptoms of the disorder. There are some mechanisms of action that exhibit promise in initial trials in individuals with ASD and warrant further investigation. *Beta*-adrenergic antagonism can benefit individuals with ASD by reducing aggressiveness and providing some improvements in speech, language, associative processing, and working memory abilities. [239] Large-scale clinical trials have not yet been conducted and will be necessary to determine the clinical efficacy of this type of intervention across the ASD population; however *beta*-adrenergic antagonism is especially relevant for the treatment of ASD because it may be able to affect network coherence in the brain, which is one of the most prominent neural correlates identified in the disorder.

Previous reports have shown that propranolol administration, a lipophilic *beta*-adrenergic antagonist with effects on noradrenergic receptors in the brain, is associated with altered functional connectivity within language networks during verbal processing.[124] Our first experiment expanded on this work by examining the effects of propranolol on functional connectivity and network coherence during passive rest to explore the effects of propranolol administration in both individuals with ASD and unaffected controls. We found that the effect of propranolol on network coherence is more complex than just increased functional connectivity and that *beta*-adrenergic antagonism may be able to up- or down- regulate specific subnetworks in the brain. We focused on the default mode network (DMN) because this is the primary network active during passive rest and internal mentation[98] and has been previously shown to exhibit altered network coherence in individuals with ASD.[96] During internal mentation, propranolol exhibited general effects across individuals with ASD and controls. Propranolol decreased functional connectivity and global processing in the DMN in the dorsal medial prefrontal cortex (dMPFC) subnetwork and increased functional connectivity and global

processing within the medial temporal lobe (MTL) subnetwork of the DMN. Reduced network coherence in the dMPFC could potentially support cognitive benefits because the DMN dissociates from task-related networks during task performance, which may allow greater network integration and better segregation between networks during cognitive processing. Increased network access to MTL regions, such as the hippocampus, may also support cognitive benefits due to the role of the hippocampus across different cognitive processing domain, such as memory and affective processing. Although we did not find any baseline differences in network coherence of the DMN between individuals with ASD and unaffected individuals, these findings support the potential of *beta*-adrenergic antagonism to alter inherent disturbances in network coherence in individuals with ASD during cognitive processing, which may be relevant for treating some of the core symptoms of the disorder. Additionally, the different effects across subnetworks of the DMN suggests that *beta*-adrenergic antagonism may be able to alter both hyper- and hypo- connectivity states in individuals with ASD, which could improve integration within and segregation between networks. Altered network coherence may be related to the aforementioned behavioral benefits of *beta*-adrenergic antagonism; however the effects of on propranolol performance have not yet been examined during a cognitive processing task.

*Beta*-adrenergic antagonism during cognitive processing was examined in experiment 2 to assess the effects of propranolol on network coherence and determine whether alterations in network coherence are related to performance benefits. Previous research reported altered functional connectivity in individuals with ASD during the processing of verbal information but did not include a measure of performance.[124] Additionally, this investigation only examined regions associated with the processing of verbal information. Networks in the brain must work in concert to carry out different aspects of information processing. Language regions are directly

involved with processing verbal information; however attention and control networks are also important for orienting to the external stimulus and allowing efficient shifting between networks during different aspects of cognitive processing.[247] Internal mentation is also important for allowing reflection during periods of rest between overt cognitive demands. Therefore in experiment 2, we examined the effects of *beta*-adrenergic antagonism on multiple networks involved with different aspects of cognitive processing to identify whether propranolol selectively influences language networks or has general modulatory effects that are applicable to multiple domains associated with cognition.

Following propranolol administration, participants completed a semantic fluency task. Individuals with ASD have previously been shown to exhibit a behavioral benefit from propranolol during semantic fluency and this task involves cognitive processing across multiple domains including language and associative processing.[185] We found that *beta*-adrenergic antagonism improved semantic associative processing in a subset of individuals with ASD and controls. Functional connectivity and network coherence was also affected individuals with ASD who exhibited a performance benefit. The most robust effects on network coherence in individuals with ASD were in the frontoparietal control (FPC) network. The FPC mediates the shifting of access to different networks involved with cognitive processing, especially between the DMN and attention networks.[247] At baseline, individuals with ASD exhibited hyperconnectivity in the FPC compared to controls. Following propranolol administration, this hyperconnectivity was reduced in the individuals with ASD who performed better on the semantic fluency task following *beta*-adrenergic antagonism. Therefore, we found a baseline difference in network coherence in individuals with ASD that was mitigated with propranolol,

which improved a cognitive processing domain that is associated with the core symptoms of the disorder.

*Beta*-adrenergic modulation of network coherence in ASD has been posited to benefit associative processing by increasing access to lexical, semantic, and associative networks during a search of semantic associations.[199] Our findings support this theory by showing that a network involved with shifting network access during cognitive processing is affected by *beta*-adrenergic antagonism and that altered network coherence is associated with improved performance. The beneficial performance effects of altered network coherence in the FPC may be specifically involved with the role of the anterior insula within this network. The anterior insula acts a central hub allowing dynamic integration of large-scale neuronal networks and is ideally situated to augment a network search.[253] Altered integration and segregation of functional connectivity of the insula with other regions of the brain has been theorized to be centrally involved with multiple domains affected in ASD.[249,252] In addition to beta-adrenergic effects on the FPC, network coherence of the DMN was also affected in individuals with ASD during cognitive processing. The DMN is typically downregulated during cognitive processing due to the FPC shifting of network access away from the DMN. [98,247] Individuals with ASD exhibited increased local processing in the DMN during periods of internal mentation, which were interspersed between verbal processing demands. Propranolol reduced this reliance on local processing, which may have supported improved performance by allowing more efficient shifting from the DMN to networks involved with more overt cognitive processing demands. Overall, individuals with ASD exhibit inherent disturbances in functional network utilization in the brain and *beta*-adrenergic antagonism may be able to mitigate some of these effects and allow greater access to domain-relevant networks during cognitive processing, at least in a subset of individuals.

Propranolol is a generic *beta*-adrenergic antagonist that is generally well tolerated and has less severe side effects than any of the currently approved pharmacological interventions for ASD. In addition to the aforementioned effects of propranolol on network coherence in the central nervous system, propranolol also has anxiolytic effects and helps maintain homeostasis in the sympathetic nervous system. This is especially relevant for individuals with ASD because of common comorbid diagnoses such as anxiety and secondary manifestations such as heightened sympathetic nervous system arousal and greater stress reactivity compared to unaffected individuals. We examined *beta*-adrenergic effects on anxiety within our ASD and control samples; however we did not find any group or drug-related differences. The absence of drug-related changes in anxiety measures may be due to insufficient self-report sensitivity within the time domain of assessment, and difficulty individuals with ASD exhibit with emotional introspection.[233] Therefore, propranolol may also be able to benefit secondary manifestations in individuals with ASD. Cumulatively, these findings support the potential utility of trials of *beta*-adrenergic antagonists for some patients with ASD.

Our findings also support the potential clinical utility of neuroimaging assessment of functional connectivity in individuals with ASD. Unfortunately, current functional imaging techniques are not reliable as diagnostic markers for ASD due to the high cost, differences in acquisition across sites, methodological differences in analysis, and lack of consistent diagnostic targets. However, these techniques may be applicable as patient stratification and treatment markers. Clinical trials of pharmacological agents in individuals with ASD have typically failed to pass Phase II trials due to inconsistent results within the population. These failures are most likely related to the heterogeneity of individuals across the autism spectrum. Functional neuroimaging may provide a means of identifying individuals with ASD expressing

abnormal function within specific networks that may specifically benefit from certain types of treatment. For example, we found that individuals with ASD expressed baseline differences in network coherence of the FPC but only a subset of these individuals exhibited altered network coherence in response to drug. A single dose administration followed by fMRI and functional connectivity analysis may aid in identification of subjects most likely to benefit from propranolol and allow stratification of individuals who could then be enrolled in a double-blinded controlled trial of the long terms effects of this type of intervention. Additionally, functional neuroimaging to identify subgroups exhibiting alterations in network coherence in response to drug may help with stratification research such that more clinically feasible markers could potentially be identified following identification. Functional neuroimaging may also be used to track treatment-related changes in the brain and allow researchers to identify target networks exhibiting the most robust alterations in response to pharmacological intervention. Functional neuroimaging acquisition and analysis techniques would need to be more standardized but may allow patient stratification and better progress monitoring in individuals with ASD.

The first two experiments outlined in this manuscript assessed disturbances in functional utilization of cerebral circuits in individuals with ASD and a pharmacological intervention that can augment these disturbances. There are other relevant considerations regarding network coherence and functional utilization of neuronal clusters in the brain in individuals with ASD. Abnormalities in cerebellar circuits in individuals with ASD are one of the most consistently reported neuropathological perturbations.[46-50] Cerebellar circuits are important for providing regulatory feedback to other regions of the brain, and although traditionally thought to be exclusively involved with motor control, imaging and lesion studies have implicated higher-

order cognitive domains as well.[125-128,130,254-257] The cerebellum is involved with complex cognitive processing such as attention, language, working memory, and sensory integration, in addition to motor control. The cerebellum interacts with neuronal circuits in the cerebrum through contralateral transthalamaic projections, including the primary and pre-motor cortices, prefrontal cortex, and medial temporal and parietal lobes.[280] Additionally, perturbations of specific cerebellar subfields are associated with distinct cognitive and behavioral outcomes, which suggest topographical organization of cerebellar circuit output to cortical and subcortical neuronal clusters. The universal cerebellar transform hypothesis conceptualizes these findings into a framework by which cerebellar modulation serves as an oscillation dampener to maintain a homeostatic baseline in neuronal processing.[281] Due to the prevalent associations of cerebellar disturbances in individuals with ASD and role of the cerebellum in modulating baseline coherence in neuronal processing, we also examined functional connectivity between the cerebrum and cerebellum in individuals with ASD to determine whether cognitive and behavioral outcomes are associated with cerebrocerebellar network coherence. To date, only a single investigation has examined cerebrocerebellar connectivity in individuals with ASD.[285] Hyperconnectivity was reported between cerebrocerebellar motor regions but hypoconnectivity between cerebrocerebellar supramodal regions, including areas involved with cognitive processing such as the prefrontal cortex and superior parietal lobes,[285] Functional connectivity between the cerebellum and cerebrum seems to be affected in individuals with ASD.

In experiment 3, cerebrocerebellar connectivity was assessed between the postereolateral cerebellar hemisphere, the region of the cerebellum most implicated in cognitive processing, and the dorsolateral prefrontal cortex, a region in the cerebrum with transthalamic connections from the cerebellum that is involved with an array cognitive processing domains such as executive

control, working memory, and cognitive flexibility.[361] We found that a subset of individuals with ASD exhibited anticorrelated cerebrocerebellar connectivity between the right postereolateral cerebellar hemisphere and left dorsolateral prefrontal cortex; whereas unaffected controls and the remaining individuals with ASD displayed positive connectivity between these regions, with similar connectivity strength to previous reports.[361] The large amount of heterogeneity across the autism spectrum suggests that etiological subgroups most likely exist and our findings indicate that at least a subgroup of individuals with ASD exhibit altered cerebrocerebellar connectivity. These findings also support a potential functional network outcome from the aforementioned neuropathological alterations in neuronal circuit structure in the cerebellum of individuals with ASD. However, this experiment does not provide a direct link between underlying mechanisms in the cerebellum and effects on cerebrocerbellar connectivity. Therefore, in experiment 4 we examined specific mechanisms that may be affected in the cerebellum, such as levels of glutamate and GABA.

Glutamate is the primary excitatory neurotransmitter and γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the mature nervous system. Neuronal binding of glutamate and GABA shapes the spatiotemporal patterns of electrical signaling in the brain[298], and the balance between neuronal excitability and inhibitory (E/I) control is crucial to neuronal circuit patterning. E/I balance in the brain is important for neurodevelopment, cognitive processing, and shaping functional connectivity patterns within neuronal networks. E/I may be affected in ASD and underlie some of the aforementioned disturbances in network coherence.[145] For example, Purkinje cells are GABAergic and the primary output from the cerebellar hemispheres. Purkinje cells are consistently reduced in the cerebellum of individuals with ASD.[46-50] GABAergic and glutamatergic receptor densities are also reduced in cerebellum of

individuals with ASD,[299,300] as well as enzymes that convert glutamate to GABA[302-304] and proteins expressed in glutamatergic/GABAergic neurons.[305,306] Overall, converging evidence suggests that altered neuronal network dynamics in ASD may be due to an altered balance of excitation to inhibition disrupting network coherence.[144,145] This is especially relevant regarding cerebrocerebellar connectivity due to the multiple GABAergic and glutamatergic pathways that are affected in the cerebellum of individuals with ASD. Examining the relationship between glutamate and GABA, E/I balance, and functional integrity of cerebrocerebellar connections will help elucidate the potential role of altered network coherence of the cerebellum in ASD and provide an assessment of potential mechanisms underlying the previously reported disturbances in cerebrocerebellar connectivity.

In experiment 4, GABA and glutamate concentrations were assessed between the postereolateral cerebellar hemisphere and the dorsolateral prefrontal cortex. We found that individuals with ASD who exhibited altered cerebrocerebellar connectivity also displayed reduced glutamate concentrations and E/I balance in the cerebellum relative to individuals with ASD who did not exhibit altered cerebrocerebellar connectivity, implicating a neurochemical mechanism by which cerebrocerebellar connectivity may be affected. We anticipated a reduction in GABA in the cerebellum due to previous reports of Purkinje cells loss[47-50,280]; [46-49,282] however the spectral overlap of GABA resonances with other metabolites and relatively low signal-to-noise ratio in our MRS assessments may have limited our ability to accurately assess GABA concentrations within this participant sample. Nevertheless, this is the first investigation to provide a link between neurochemical alterations in the cerebellum and functional cerebrocerebellar connectivity in individuals with ASD.

ASD is a behaviorally defined disorder and therefore the impact of these alterations on symptom presentation is paramount to the salience of these findings. In experiment 3 and 4, we also assessed symptom severity and cognitive and behavioral outcomes in individuals with ASD. Our patient population was primarily higher functioning individuals with ASD and all participants within this study were verbal. Language and communication in individuals with ASD is often affected; however high functioning verbal individuals with ASD have relatively intact basic language skills. Therefore we examined higher-level meta-linguistic abilities because these domains are affected in high functioning individuals with ASD[347] as well as individuals with cerebellar lesions,[362] suggesting a potential cognitive domain that is affected in individuals with ASD exhibiting altered cerebrocerebellar connectivity. We found that individuals with ASD who exhibited altered cerebrocerebellar connectivity and lower levels of glutamate in the cerebellum also expressed reduced language comprehension, which was suggestive of reduced inference abilities. This is especially relevant in individuals with ASD, in which a core feature is social and communication impairments, because inference abilities are thought to mediate the capacity to infer the mental state of other individuals[338] Mental state inference impairment have been conceptualized into a prominent theory of mind hypothesis suggesting that core deficits in ASD are due to the inability to impute the beliefs of others.[363] Therefore, our findings support a link between reduced potential for excitation within the cerebellum, perturbed cerebrocerebellar connectivity between the cerebellar hemispheres and prefrontal cortex, and cognitive outcomes in individuals with ASD that may be associated with core deficits of the disorder.

The identification of a subgroup of individuals with ASD who exhibit specific alterations in glutamate is also potentially beneficial to clinical trials in individuals with ASD. As previously mentioned, large scale clinical trials have largely failed due to heterogeneity across

the autism spectrum. Glutamatergic agents that have been investigated in individuals with ASD such as memantine, mostly exert antagonist effects.[160] The primary mechanism of action of these drugs is to reduce glutamatergic binding in the CNS. We identified a subset of individuals with ASD exhibiting reduced glutamate levels that would most likely not benefit from agents further reducing the effects of glutamate. Standardized MRS assessment of metabolites such as glutamate and NAA has relatively robust signal-to-noise, which could benefit clinicians and researchers by identifying individuals who would be most likely benefit from this type of intervention. MRS protocols for the assessment of GABA do not currently exhibit enough external validity to be utilized in clinical populations; however additional research into methods for optimizing these techniques is warranted.

Our main goal with this investigation was to examine functional connectivity and network coherence in individuals with ASD. We identified cerebrocerebellar circuits that exhibit perturbed network coherence in some individuals with ASD and found evidence suggesting a connection between altered cerebrocerebellar connectivity and underlying neurochemical mechanisms. We also identified alterations in network coherence that are associated with behavioral improvements and provided evidence for a potential pharmacological intervention for ASD that can influence inherent disturbances in functional connectivity. Additionally, we provide some support for the clinical assessment of network coherence and neurochemical profiles in individuals with ASD that may provide relevant information to aid in stratification of individuals with ASD for clinical trials. These findings support the utility of the assessment of network coherence in the brain of individuals with ASD to provide clinically relevant information.

# Bibliography


1. AmericanPsychiatricAssociation. *Diagnostic and Statistical Manual of Mental Disorders: DSM-V.* Arlington, VA2013.
2. E. B. Die Prognose der Dementia praecox (Schizophreniegruppe). *Allgemeine Zeitschrift fur Psychiatrie und psycholische Medizin.* 1908;65:436-464.
3. Kanner L. Autistic disturbances of affective contact. *Nervous child.* 1943;2(3):217-250.
4. Asperger H. Die Autistischen Psychopathen im Kindesalter. *Archiv fur Psychiatrie und Nervenkrankheiten.* 1944;117(1):76-136.
5. Rimland B. *Infantile Autism: The Syndrome and Its Implications for a Neural Theory of Behavior.* Englewood Cliffs, NJ: Prentice-Hall; 1964.
6. Lotter V. Epidemiology of autistic conditions in young children. *Social Psychiatry.* 1966;1(3):124-135.
7. AmericanPsychiatricAssociation. *Diagnostic and statistical manual of mental disorders (3rd ed.).* Washington, D.C.: Author; 1980.
8. AmericanPsychiatricAssociation. *Diagnostic and statistical manual of mental disorders (3rd ed. revised).* Washington, D.C.: Author; 1987.
9. AmericanPsychiatricAssociation. *Diagnostic and statistical manual of mental disorders (4th ed.).* Washington, D.C.: Author; 1994.
10. Wing L. Asperger's syndrome: a clinical account. *Psychological Medicine.* 1981;11:115-129.
11. Frith U. *Autism and Asperger syndrome.* Cambridge University Press; 1991.
12. Investigators AaDDMNSYP. Prevalence of Autism Spectrum Disorders --- Autism and Developmental Disabilities Monitoring Network, Six Sites, United States, 2000. *MMWR CDC Surveillance Summaries.* 2007;56(2201):1-11.
13. Baio J. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *Morb Mortal Wkly Rep.* 2014;63:1-21.
14. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of Autism in a US Metropolitan Area. *The Journal of the American Medical Association.* 2003;289(1):7.
15. Leyfer OT, Folstein SE, Bacalman S, et al. Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. *J Autism Dev Disord.* 2006;36:849-861.
16. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry.* 2008;47(8):921-929.
17. Bailey A, Le Couteur A, Gottesman I, al. e. Autism as a Strongly Genetic Disorder: Evidence from a Britist Twin Study. *Psychol Med.* 1995;25:63-77.
18. Folstein SE, Rutter M. Infantile Autism: A Genetic Study of 21 Twin Pairs. *Journal of Child Psychology and Psychiatry.* 2006;18(4):297-321.

19. Steffenburg S, Gillberg C, Kellgren L, et al. A Twin Study of Autism in Denmark, Finland, Iceland, Norway and Sweden. *Journal of Child Psychology and Psychiatry.* 2006;30(3):405-416.
20. Hallmayer J, Cleveland S, Torres A, et al. GEnetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry.* 2011;68(11):1095-1102.
21. Bailey A, Le Couteur A, Gottesman I, et al. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychological Medicine.* 1995;25(01):63-77.
22. Losh M, Sullivan PF, Trembath D, Piven J. Current Developments in the Genetics of Autism: From Phenome to Genome. *The Jounral of Neuropathological and Experimental Neurology.* 2008;67(9):829-837.
23. Szatmari P, Paterson AD, Zwaigenbaum L, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nature genetics.* Mar 2007;39(3):319-328.
24. Sullivan PF, Daly MJ, O'Donovan M. Genetic architectures of psychiatric disorders: the emerging picture and its implications. *Nature reviews. Genetics.* Aug 2012;13(8):537-551.
25. Rubenstein JL. Three hypotheses for developmental defects that may underlie some forms of autism spectrum disorder. *Current opinion in neurology.* Apr 2010;23(2):118-123.
26. Kelleher RJ, Geigenmuller U, Hovhannisyan H, et al. High-Throughput Sequencing of mGluR Signaling Pathway Genes Reveals Enrichment of Rare Variants in Autsim. *PLoS One.* 2012;7(4):e35003.
27. Smalley SL. Autism and Tuberous Sclerosis. *Journal of Autism and Developmental Disorders.* 1998;28(5):407-414.
28. Ascano M, Jr., Mukherjee N, Bandaru P, et al. FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature.* Dec 20 2012;492(7429):382-386.
29. Gillberg C, Wahlstrom J, Hagberg B. A "New" Chromosome Marker Common to the Rett Syndrome and Infantile Autism? The Frequency of Fragile Sites at X P22 in 81 Children with Infantile Autism, Childhood Psychosis and the Rett Syndrome. *Brain Dev.* 1985;7:365-367.
30. Jamain S, Quach H, Betancur C, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nature genetics.* May 2003;34(1):27-29.
31. Feng J, Schroer R, Yan J, et al. High frequency of neurexin 1beta signal peptide structural variants in patients with autism. *Neuroscience letters.* Nov 27 2006;409(1):10-13.
32. Singh SK, Eroglu C. Neuroligins provide molecular links between syndromic and nonsyndromic autism. *Science signaling.* 2013;6(283):re4.
33. Cook Jr. EH, Courchesne R, Lord C, et al. Evidence of linkage between the serotonin transporter and autistic disorder. *Molecular Psychiatry.* 1997;2:247-250.
34. Kinast K, Peeters D, Kolk SM, Schubert D, Homberg JR. Genetic and pharmacological manipulations of the serotonergic system in early life: neurodevelopmental underpinnings of autism-related behavior. *Frontiers in cellular neuroscience.* 2013;7:72.

35. Ji L, Chauhan A, Brown WT, Chauhan V. Increased activities of Na+/K+-ATPase and Ca2+/Mg2+-ATPase in the frontal cortex and cerebellum of autistic individuals. *Life sciences.* Dec 16 2009;85(23-26):788-793.
36. Steiner CE, Acosta AX, Guerrerio MM, Marques-de-Faria AP. Genotype and natural history in unrelated individual with phenylketonuria and autistic behavior. *Arq. Neuro-Psiquiatr.* 2007;65(2A).
37. Baieli S, Pavone L, Meli C, Fiumara A, Coleman M. Autism and Phenylketonuria. *Journal of Autism and Developmental Disorders.* 2003;33(2):201-204.
38. Amir RB, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature genetics.* 1999;23:185-188.
39. Samaco RC, McGraw CM, Ward CS, Sun Y, Neul JL, Zoghbi HY. Female Mecp2(+/-) mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre-clinical studies. *Human molecular genetics.* Jan 1 2013;22(1):96-109.
40. Alvarez Retuerto AI, Cantor RM, Gleeson JG, et al. Association of common variants in the Joubert syndrome gene (AHI1) with autism. *Human molecular genetics.* Dec 15 2008;17(24):3887-3896.
41. Tuz K, Hsiao YC, Juarez O, et al. The Joubert syndrome-associated missense mutation (V443D) in the Abelson-helper integration site 1 (AHI1) protein alters its localization and protein-protein interactions. *The Journal of biological chemistry.* May 10 2013;288(19):13676-13694.
42. Mandy WP, Skuse DH. Research Review: What is the association between the social‐communication element of autism and repetitive interests, behaviours and activities? *Journal of Child Psychology and Psychiatry.* 2008;49(8):795-808.
43. Ronald A, HappÉ F, Bolton P, et al. Genetic Heterogeneity Between the Three Components of the Autism Spectrum: A Twin Study. *Journal of the American Academy of Child & Adolescent Psychiatry.* 6// 2006;45(6):691-699.
44. Ronald A, HappÉ F, Price TS, Baron-Cohen S, Plomin R. Phenotypic and Genetic Overlap Between Autistic Traits at the Extremes of the General Population. *Journal of the American Academy of Child & Adolescent Psychiatry.* 10// 2006;45(10):1206-1214.
45. Ronald A, Happé F, Plomin R. The genetic relationship between individual differences in social and nonsocial behaviours characteristic of autism. *Developmental Science.* 2005;8(5):444-458.
46. Bailey A, Luthert P, Dean A, et al. A clinicopathological study of autism. *Brain.* 1998;121:889-905.
47. Bauman ML, Kemper TL. Histoanatomic observations of the brain in early infantile autism. *Neurology.* 1985;35:866-874.
48. Arin DM, Bauman ML, Kemper TL. The distribution of Purkinje cell loss in the cerebellum in autism. *Neurology.* 1991;41(Suppl):307.
49. Whitney ER, Kemper TL, Bauman ML, Rosene DL, Blatt GJ. Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: a stereological experiment using calbindin-D28k. *Cerebellum.* 2008;7(3):406-416.

50. Ritvo ER, Freeman BJ, Scheibel AB, et al. Lower Purkinje Cell Counts in the Cerebella of Four Autitic Subjects: Initial Findings of the UCLA-NSAC Autopsy Research Report. *American Journal of Psychiatry.* 1986;143:862-866.
51. Cassanova MF, Buxhoeveden DP, Switala AE, Roy E. Minicolumnar pathology in autism. *Neurology.* 2002;58:428-432.
52. Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N. Effects of age on brian volume and head circumference in autism. *Neurology.* 2002;59(2):175-183.
53. Courchesne E, Karns CM, Davis HR, et al. Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology.* 2001;57(2):245-254.
54. Hardan AY, Minshew NJ, Mallikarjuhn M, Keshavan MS. Brain volume in autism. *Journal of Child Neurology.* 2001;16(6):421-424.
55. Piven J, Arndt S, Bailey J, Havercamp S, Andreasen NC, Palmer P. An MRI study of brain size in autism. *American Journal of Psychiatry.* 1995;152(8):1145-1149.
56. Sparks BF, Friedman SD, Shaw DW, et al. Brain structural abnormalities in young children with autism spectrum disorder. *Neurology.* 2002;59(2):184-192.
57. Abell F, Krams M, Ashburner J, et al. The neuroanatomy of autism: A voxel-based whole brain analysis of structural scans. *NeuroReport.* 1999;10(8):1647-1651.
58. Carper RA, Moses P, Tigue ZD, Courchesne E. Cerebral lobes in autism: Early hyperplasia and abnormal age effects. *NeuroImage.* 2002;16(4):1038-1051.
59. Piven J, Arndt S, Bailey J, Andreasen N. Regional Brain Enlargement in Autism: A Magnetic Resonance Imaging Study. *Journal of the American Academy of Child & Adolescent Psychiatry.* 4// 1996;35(4):530-536.
60. Piven J, Saliba K, Bailey J, Arndt S. An MRI study of autism: The cerebellum revisited. *Neurology.* 1997;49(2):546-551.
61. Brambilla P, Hardan A, di Nemi SU, Perez J, Soares JC, Barale F. Brain anatomy and development in autism: review of structural MRI studies. *Brain Research Bulletin.* 10/15/ 2003;61(6):557-569.
62. Aylward EH, Minshew NJ, Goldstein G, et al. MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology.* 1999;53(9):2145-2150.
63. Saitoh O, Karns CM, Courchesne E. Development of the hippocampal formation from 2 to 42 years: MRI evidence of smaller area dentata in autism. *Brain.* 2001;124(7):1317-1324.
64. Howard MA, Cowell PE, Boucher J, et al. Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. *NeuroReport.* 2000;11(13):2931-2935.
65. Brodmann K. Vergleichende Lokalisationslehre der Groshirnrinde. *Leipzig: Barth.* 1909.
66. Le Bihan D, Mangin J-F, Poupon C, et al. Diffusion tensor imaging: Concepts and applications. *Journal of Magnetic Resonance Imaging.* 2001;13(4):534-546.
67. Shukla DK, Keehn B, Lincoln AJ, Müller R-A. White Matter Compromise of Callosal and Subcortical Fiber Tracts in Children With Autism Spectrum

Disorder: A Diffusion Tensor Imaging Study. *Journal of the American Academy of Child & Adolescent Psychiatry.* 12// 2010;49(12):1269-1278.e1262.
68. Bloemen OJN, Deeley Q, Sundram F, et al. White matter integrity in Asperger syndrome: a preliminary diffusion tensor magnetic resonance imaging study in adults. *Autism Research.* 2010;3(5):203-213.
69. Jou RJ, Mateljevic N, Kaiser MD, Sugrue DR, Volkmar FR, Pelphrey KA. Structural neural phenotype of autism: preliminary evidence from a diffusion tensor imaging study using tract-based spatial statistics. *American Journal of Neuroradiology.* 2011;32(9):1607-1613.
70. Groen WB, Buitelaar JK, van der Gaag RJ, Zwiers MP. Pervasive microstructural abnormalities in autism: a DTI study. *Journal of Psychiatry and Neuroscience.* 2011;36(1):32-40.
71. Ben Bashat D, Kronfeld-Duenias V, Zachor DA, et al. Accelerated maturation of white matter in young children with autism: A high b value DWI study. *NeuroImage.* 8/1/ 2007;37(1):40-47.
72. Weinstein M, Ben-Sira L, Levy Y, et al. Abnormal white matter integrity in young children with autism. *Human Brain Mapping.* 2010;32(4):534-543.
73. Travers BG, Adluru N, Ennis C, et al. Diffusion Tensor Imaging in Autism Spectrum Disorder: A Review. *Autism Research.* 2012;5(5):289-313.
74. Barnea-Goraly N, Kwon H, Menon V, Eliez S, Lotspeich L, Reiss AL. White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biological Psychiatry.* 2/1/ 2004;55(3):323-326.
75. Alexander AL, Lee JE, Lazar M, et al. Diffusion tensor imaging of the corpus callosum in Autism. *NeuroImage.* 1/1/ 2007;34(1):61-73.
76. Brito AR, Vasconcelos MM, Domingues RC, et al. Diffusion tensor imaging findings in school-aged autistic children. *Journal of Neuroimaging.* 2009;19(4):337-343.
77. Shukla DK, Keehn B, Muller A. Tract-specific analyses of diffusion tensor imaging show widespread white matter compromise in autism spectrum disorder. *Journal of Child Psychology and Psychiatry.* 2010;52(3):286-295.
78. Jeong JW, Kumar AK, Sundaram SK, Chugani HT, Chugani DC. Sharp curvature of frontal lobe white matter pathways in children with autism spectrum disorders: tract-based morphometry analysis. *American Journal of Neuroradiology.* 2011;32(9):1600-1606.
79. Noriuchi M, Kikuchi Y, Yoshiura T, et al. Altered white matter fractional anisotropy and social impairment in children with autism spectrum disorder. *Brain research.* 2010;1362:141-149.
80. Barnea-Goraly N, Lotspeich LJ, Reiss AL. Similar white matter aberrations in children with autism and their unaffected siblings: A diffusion tensor imaging study using tract-based spatial statistics. *Archives of General Psychiatry.* 2010;67(10):1052-1060.
81. Poustka L, Jennen-Steinmetz C, Henze R, Vomstein K, Haffner J, Sieltjes B. Fronto-temporal disconnectivity and symptom severity in children with autism spectrum disorder. *World Journal of Biological Psychiatry.* 2012;13(4):269-280.

82. Lee JE, Chung MK, Lazar M, et al. A study of diffusion tensor imaging by tissue-specific, smoothing-compensated voxel-based analysis. *NeuroImage.* 2/1/ 2009;44(3):870-883.
83. Honey CJ, Sporns O, Cammoun L, et al. Predicting human resting-state functional connectivity from structural connectivity. *Proceedings of the National Academy of Sciences.* 2009;106(6):2035-2040.
84. Chklovskii DB, Schikorski T, Stevens CF. Wiring optimization in cortical circuits. *Neuron.* 2002;34:341-347.
85. Kaiser M, HIlgetag CC. Nonoptimal component placement, but short processing paths, due to long-distance projections in neural systems. *PLoS Computational Biology.* 2006;2(7):e95.
86. Logothetis NK, Wandell BA. Interpreting the BOLD signal. *Annu Rev Physiol.* 2004;66:735-769.
87. Friston KJ. Functional and effective connectivity in neuroimaging: A synthesis. *Human brain mapping.* 1994;2:56-78.
88. Fair DA, Cohen AL, Power JD, et al. Functional brain networks develop from a "local to distributed" organization. *PLoS Computational Biology.* 2009;5(5):e1000381.
89. Courchesne E, Pierce K. Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. *Current opinion in neurobiology.* Apr 2005;15(2):225-230.
90. Herbert MR, Ziegler DA, Makris N, et al. Localization of white matter volume increase in autism and developmental language disorder. *Annals of neurology.* Apr 2004;55(4):530-540.
91. Kana RK, Keller TA, Minshew NJ, Just MA. Inhibitory control in high-functioning autism: decreased activation and underconnectivity in inhibition networks. *Biological psychiatry.* Aug 1 2007;62(3):198-206.
92. Anderson JS, Druzgal TJ, Froehlich A, et al. Decreased Interhemispheric Functional Connectivity in Autism. *Cerebral Cortex.* October 12, 2010 2010.
93. Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and Anatomical Cortical Underconnectivity in Autism: Evidence from an fMRI Study of an Executive Function Task and Corpus Callosum Morphometry. *Cerebral Cortex.* April 1, 2007 2007;17(4):951-961.
94. Wass S. Distortions and disconnections: disrupted brain connectivity in autism. *Brain and Cognition.* 2011;75(1):18-28.
95. Just MA, Cherkassky VL, Keller TA, Minshew NJ. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain.* 2004;127(8):1811-1821.
96. Cherkassky VL, Kana RK, Keller TA, Just MA. Functional connectivity in a baseline resting-state network in autism. *Neuroreport.* 2006;17(16):1687-1690.
97. Uddin LQ, Supekar K, Menon V. Reconceptualizing functional brain connectivity in autism from a developmental perspective. *Frontiers in Human Neuroscience.* 2013-August-7 2013;7.
98. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *PNAS.* 2000;98(2):676-682.

99. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: Anatomy, function, and relevance to disease. *Ann. N.Y. Acad. Sci.* 2008;1124:1-38.
100. Assaf M, Jagannathan K, Calhoun VD, et al. Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *Neuroimage.* 2010;53:247-256.
101. Kennedy DP, Courchesne E. Functional abnormalities of the default network during self-and other-reflection in autism. *Social Cognitive and Affective Neuroscience.* 2008;3(2):177-190.
102. Monk CS, Peltier SJ, Wiggins JL, et al. Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *NeuroImage.* 8/15/ 2009;47(2):764-772.
103. von dem Hagen EAH, Stoyanova RS, Baron-Cohen S, Calder AJ. Reduced functional connectivity within and between 'social' resting state networks in autism spectrum conditions. *SCAN.* 2013;8:694-701.
104. Weng S-J, Wiggins JL, Peltier SJ, et al. Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. *Brain research.* 2/8/ 2010;1313(0):202-214.
105. Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V. Default Mode Network in Childhood Autism: Posteromedial Cortex Heterogeneity and Relationship with Social Deficits. *Biological Psychiatry.* 8/1/ 2013;74(3):212-219.
106. Washington SD, Gordon EM, Brar J, et al. Dysmaturation of the default mode network in autism. *Human Brain Mapping.* 2014;35(4):1284-1296.
107. Just MA, Cherkassky VL, Keller TA, Minshew N. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain.* 2004;127:1811-1821.
108. Solomon M, Ozonoff SJ, Ursu S, et al. The neural substrates of cognitive control deficits in autism spectrum disorders. *Neuropsychologia.* 10// 2009;47(12):2515-2526.
109. Jones TB, Bandettini PA, Kenworthy L, et al. Sources of group differences in functional connectivity: An investigation applied to autism spectrum disorder. *NeuroImage.* 1/1/ 2010;49(1):401-414.
110. Koshino H, Carpenter PA, Minshew NJ, Cherkassky VL, Keller TA, Just MA. Functional connectivity in an fMRI working memory task in high-functioning autism. *NeuroImage.* 2/1/ 2005;24(3):810-821.
111. Koshino H, Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. fMRI Investigation of Working Memory for Faces in Autism: Visual Coding and Underconnectivity with Frontal Areas. *Cerebral Cortex.* 2008;18(2):289-300.
112. Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. *Brain.* 2006;129(9):2484-2493.
113. Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. Atypical frontal-posterior synchronization of Theory of Mind regions in autism during mental state attribution. *Social Neuroscience.* 2009/04/01 2009;4(2):135-152.
114. Villalobos ME, Mizuno A, Dahl BC, Kemmotsu N, Müller R-A. Reduced functional connectivity between V1 and inferior frontal cortex associated with visuomotor performance in autism. *NeuroImage.* 4/15/ 2005;25(3):916-925.

115. Turner KC, Frost L, Linsenbardt D, McIlroy JR, Muller A. Atypically diffuse functional connectivity between caudate nuclei and cerebral cortex in autism. *Behavioral and Brain Functions.* 2006;2(34).
116. Mostofsky SH, Powell SK, Simmonds DJ, Goldberg MC, Caffo B, Pekar JJ. Decreased connectivity and cerebellar activity in autism during motor task performance. *Brain.* Sep 2009;132(Pt 9):2413-2425.
117. Shih P, Shen M, Öttl B, Keehn B, Gaffrey MS, Müller R-A. Atypical network connectivity for imitation in autism spectrum disorder. *Neuropsychologia.* 8// 2010;48(10):2931-2939.
118. Noonan SK, Haist F, Müller R-A. Aberrant functional connectivity in autism: Evidence from low-frequency BOLD signal fluctuations. *Brain research.* 3/25/ 2009;1262(0):48-63.
119. Welchew DE, Ashwin C, Berkouk K, et al. Functional disconnectivity of the medial temporal lobe in Asperger's syndrome. *Biological Psychiatry.* 5/1/ 2005;57(9):991-998.
120. Mizuno A, Villalobos ME, Davies MM, Dahl BC, Müller R-A. Partially enhanced thalamocortical functional connectivity in autism. *Brain research.* 8/9/ 2006;1104(1):160-174.
121. Wass S. Distortions and disconnections: disrupted brain connectivity in autism. *Brain and cognition.* Feb 2011;75(1):18-28.
122. Muller RA, Shih P, Keehn B, Deyoe JR, Leyden KM, Shukla DK. Underconnected, but How? A Survey of Functional Connectivity MRI Studies in Autism Spectrum Disorders. *Cerebral Cortex.* 2011;21:2233-2243.
123. Ratey JJ, Bemporad J, Sorgi P, et al. Brief report: Open trial effects of beta-blockers on speech and social behaviors in 8 autistic adults. *Journal of Autism and Developmental Disorders.* 1987;17(3):439-446.
124. Narayanan A, White C, Saklayen S, et al. Effect of Propranolol on Functional Connectivity in Autism Spectrum Disorder—A Pilot Study. *Brain Imaging and Behavior.* 2010/06/01 2010;4(2):189-197.
125. Bracke-Tolkmitt R, Linden A, Canavan GM, et al. The Cerebellum Contributes to Mental Skills. *Behavioral Neuroscience.* 1989;103(2):442-446.
126. Akshoomoff N, Courchesne E. A New Role for the Cerebellum in Cognitive Operations. *Behavioral Neuroscience.* 1992;106(5):731-738.
127. Schmahmann JD. The cerebellar cognitive affective syndrome. *Brain.* Sherman, J. C.;121:561-579.
128. Levisohn L, Cronin-Golomb A, Schmahmann JD. Neuropsychological consequences of cerebellar tumour resection in children. *Brain.* 2000;123(5):1041-1050.
129. Appollonio IM, Grafman J, Schwartz V, Massaquoi S, Hallet M. Memory in patient with cerebellar degeneration. *Neurology.* 1993;43(8):1536.
130. Wallesch C-W, Horn A. Long-term effects of cerebellar pathology on cognitive functions. *Brain and Cognition.* 9// 1990;14(1):19-25.
131. Petersen SE, Fiez JA. The Processing of Single Words Studied with Positron Emission Tomography. *Annual Review of Neuroscience.* 1993;16:509-530.
132. Frings M, Dimitrova A, Schorn CF, et al. Cerebellar involvement in verb generation: An fMRI study. *Neuroscience letters.* 11/27/ 2006;409(1):19-23.

133. Schlösser R, Hutchinson M, Joseffer S, et al. Functional magnetic resonance imaging of human brain activity in a verbal fluency task. *Journal of Neurology, Neurosurgery & Psychiatry.* 1998;64(4):492-498.
134. Schweizer TA, Alexander MP, Gillingham BAS, Cusimano M, Stuss DT. Lateralized cerebellar contributions to word generation: a phonemic and semantic fluency study. *Behavioural Neurology.* 2010;23(1-2):31-37.
135. Stoodley CJ, Valera EM, Schmahmann JD. An fMRI study of intra-individual functional topography in the human cerebellum. *Behavioral Neurology.* 2010;23(1-2):65-79.
136. Lee TMC, Liu H-L, Hung KN, et al. The cerebellum's involvement in the judgment of spatial orientation: A functional magnetic resonance imaging study. *Neuropsychologia.* // 2005;43(13):1870-1877.
137. Bonda E, Petrides M, Frey S, Evans A. Neural correlates of mental transformations of the body-in-space. *Proceedings of the National Academy of Sciences.* 1995;92(24):11180-11184.
138. Lee GP, Meador KJ, Loring DW, et al. Neural Substrates of Emotion as Revealed by Functional Magnetic Resonance Imaging. *Cognitive and Behavioral Neurology.* 2004;17(1):9-17.
139. George MS, Ketter TA, Gill DS, et al. Brain regions involved in recognizing facial emotion or identity: An oxygen-15 PET study. *The Journal of Neuropsychiatry and Clinical Neurosciences.* 1993;5(4):384-394.
140. Chen SHA, Desmond JE. Temporal dynamics of cerebro-cerebellar network recruitment during a cognitive task. *Neuropsychologia.* // 2005;43(9):1227-1237.
141. Hautzel H, Mottaghy FM, Specht K, Müller H-W, Krause BJ. Evidence of a modality-dependent role of the cerebellum in working memory? An fMRI study comparing verbal and abstract n-back tasks. *NeuroImage.* 10/1/ 2009;47(4):2073-2082.
142. Schall U, Johnston P, Lagopoulos J, et al. Functional brain maps of Tower of London performance: a positron emission tomography and functional magnetic resonance imaging study. *NeuroImage.* 10// 2003;20(2):1154-1161.
143. Lie C-H, Specht K, Marshall JC, Fink GR. Using fMRI to decompose the neural processes underlying the Wisconsin Card Sorting Test. *NeuroImage.* 4/15/ 2006;30(3):1038-1049.
144. Pizzarelli R, Cherubini E. Alterations of GABAergic signaling in autism spectrum disorders. *Neural plasticity.* 2011;2011:297153.
145. Rubenstein JLR, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes, Brain and Behavior.* 2003;2:255-267.
146. Myers SM, Johnson CP, Disabilities tCoCW. Management of Children With Autism Spectrum Disorders. *Pediatrics.* November 1, 2007 2007;120(5):1162-1182.
147. McDougle CJ, Holmes JP, Carlson DC, Pelton GH, Cohen DJ, Price LH. A double-blind, placebo-controlled study of risperidone in adults with autistic disorder and other pervasive developmental disorders. *Archives of General Psychiatry.* 1998;55(7):633-641.

148. Marcus RN, Owen R, Kamen L, et al. A Placebo-Controlled, Fixed-Dose Study of Aripiprazole in Children and Adolescents With Irritability Associated With Autistic Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry.* 11// 2009;48(11):1110-1119.
149. ÜÇOk ALP, Gaebel W. Side effects of atypical antipsychotics: a brief overview. *World Psychiatry.* 2008;7(1):58-62.
150. Ray WA, Meredith S, Thapa PB, Meador KG, Hall K, Murray KT. ANtipsychotics and the risk of sudden cardiac death. *Archives of General Psychiatry.* 2001;58(12):1161-1167.
151. White SW, Oswald D, Ollendick T, Scahill L. Anxiety in children and adolescents with autism spectrum disorders. *Clinical Psychology Review.* 2009;29(3):216-229.
152. Richdale AL. Sleep problems in autism: prevalence, cause, and intervention. *Developmental Medicine & Child Neurology.* 1999;41(01):60-66.
153. Wang L, Tancredi DJ, Thomas DW. The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. *Journal of Developmental and Behavioral Pediatrics.* 2011;32(5):351-360.
154. Moore ML, Eichner SF, Jones JR. Treating functional impairment of autism with selective serotonin-reuptake inhibitors. *Ann Pharmacother.* 2004;38(9):1515-1519.
155. Kolevzon A, Mathewson KA, Hollander E. Selective serotonin reuptake inhibitors in autism: a review of efficacy and tolerability. *J Clin Psychiatry.* 2006/03// 2006;67(3):407-414.
156. Hollander E, Phillips A, Chaplin W, et al. A Placebo Controlled Crossover Trial of Liquid Fluoxetine on Repetitive Behaviors in Childhood and Adolescent Autism. *Neuropsychopharmacology.* 12/15/online 2004;30(3):582-589.
157. McDougle CJ, Naylor ST, Cohen DJ, Volkmar FR, Heninger GR, Price LH. A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. *Archives of General Psychiatry.* 1996;53(11):1001-1008.
158. King BH, Hollander E, Sikich L, et al. Lack of efficacy of citalopram in children with autism spectrum disorders and high levels of repetitive behavior: Citalopram ineffective in children with autism. *Archives of General Psychiatry.* 2009;66(6):583-590.
159. Posey DJ, Erickson CA, McDougle CJ. Developing Drugs for Core Social and Communication Impairment in Autism. *Child and Adolescent Psychiatric Clinics of North America.* 10// 2008;17(4):787-801.
160. Rossignol DA, Frye RE. The Use of Medications Approved for Alzheimer's Disease in Autism Spectrum Disorder: A Systematic Review. *Frontiers in Pediatrics.* 08/22/2014;2:87.
161. Ghaleiha A, Asadabadi M, Mohammadi M-R, et al. Memantine as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. *International Journal of Neuropsychopharmacology.* 2013;16(4):783-789.
162. Belsito K, Law P, Kirk K, Landa R, Zimmerman A. Lamotrigine Therapy for Autistic Disorder: A Randomized, Double-Blind, Placebo-Controlled Trial. *Journal of Autism and Developmental Disorders.* 2001/04/01 2001;31(2):175-181.

163. Lemonnier E, Degrez C, Phelep M, et al. A randomised controlled trial of bumetanide in the treatment of autism in children. *Transl Psychiatry.* 11/27/online 2012;2:e197.
164. Delahunty C, Walton-Bowen K, Kuriyama N, et al. Randomized, Controlled, Phase 2 Trial of STX209 (Arbaclofen) for Social Function in ASD. Paper presented at: American Academy of Pediatrics; October 28, 2013, 2013; Orlando.
165. Heinrichs M, Domes G. Neuropeptides and social behaviour: effects of oxytocin and vasopressin in humans. In: Inga DN, Rainer L, eds. *Progress in Brain Research.* Vol Volume 170: Elsevier; 2008:337-350.
166. Hollander E, Bartz J, Chaplin W, et al. Oxytocin increases retention of social cognition in autism. *Biol Psychiatry.* 2007;61(4):498-503.
167. Anagnostou E, Soorya L, Chaplin W, et al. Intranasal oxytocin versus placebo in the treatment of adults with autism spectrum disorders: a randomized controlled trial. *Molecular Autism.* 2012;3(1):16.
168. Hollander E, Novotny S, Hanratty M, et al. Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. *Neuropsychopharmacology.* 2003;28:193-198.
169. Guastella AJ, Gray KM, Rinehart NJ, et al. The effects of a course of intranasal oxytocin on social behaviors in youth diagnosed with autism spectrum disorders: a randomized controlled trial. *Journal of Child Psychology and Psychiatry.* 2014;56(4):444-452.
170. Hirstein W, Iversen P, Ramachandran VS. Autonomic responses of autistic children to people and objects. *Proceedings of the Royal Society of London. Series B: Biological Sciences.* September 22, 2001 2001;268(1479):1883-1888.
171. Kushki A, Drumm E, Mobarak M, et al. Investigating the autonomic nervous system response to anxiety in children with autism spectrum disorders. *PLoS one.* 2013;8(4):59730.
172. Ming X, Julu P, Brimacombe M, Connor S, Daniels M. Reduced cardiac parasympathetic activity in children with autism. *Brain Dev.* 2005;27(7):509 - 516.
173. Corbett B, Schupp C, Simon D, Ryan N, Mendoza S. Elevated cortisol during play is associated with age and social engagement in children with autism. *Mol Autism.* 2010;1(1):13.
174. Corbett BA, Mendoza S, Abdullah M, Wegelin JA, Levine S. Cortisol circadian rhythms and response to stress in children with autism. *Psychoneuroendocrinology.* 2006;31(1):59-68.
175. Tordjman S, McBride PA, Hertzig ME, et al. Plasma β-Endorphin, Adrenocorticotropin Hormone, and Cortisol in Autism. *Journal of Child Psychology and Psychiatry.* 1997;38(6):705-715.
176. Lake C, Ziegler MG, Murphy DL. Increased norepinephrine levels and decreased dopamine-β-hydroxylase activity in primary autism. *Archives of General Psychiatry.* 1977;34(5):553-556.
177. Minderaa RB, Anderson GM, Volkmar FR, Akkerhuis GW, Cohen DJ. Noradrenergic and adrenergic functioning in autism. *Biological Psychiatry.* 1994;36(4):237-241.
178. Biederman J, Spencer T. Attention-deficit/hyperactivity disorder (adhd) as a noradrenergic disorder. *Biological Psychiatry.* 1999;46(9):1234-1242.

179. Bremenr JD, Krystal JH, Southwith SM, Charney DS. Noradrenergic mechanisms in stress and anxiety: II. clinical studies. *Synapse.* 1996;23:39-51.
180. Marco EJ, Hinkley LBN, HIll SS, Nagarajan SS. Sensory processing in autism: a review of neurophysiologic findings. *Neuropsychiatric Disorders and Pediatric Psychiatry.* 2011;69:48R-54R.
181. Jaselskis CA, Cook EHJ, Fletcher KE, Leventhal BL. Clonidine Treatment of Hyperactive and Impulsive Children with Autistic Disorder. *Journal of Clinical Psychopharmacology.* 1992;12(5).
182. Handen BL, Sahl R, Hardan AY. Guanfacine in Children with Autism and/or Intellectual Disabilities. *Journal of Developmental & Behavioral Pediatrics.* 2008;29(4).
183. Fankhauser MP, Karumanchi VC, German ML, Yates A, Karumanchi SD. A double-blind, placebo-controlled study of the efficacy of transdermal clonidine in autism. *Journal of Clinical Psychiatry.* 1992;53(3):77-82.
184. Beversdorf DQ, Carpenter AL, Miller RF, Cios JS, Hillier A. Effect of propranolol on verbal problem solving in autism spectrum disorder. *Neurocase.* 2008;14(4):378-383.
185. Beversdorf DQ, Saklayen S, Higgins KF, Bodner KE, Kanne SM, Christ SE. Effect of propranolol on word fluency in autism. *Cognitive and Behavioral Neurology.* 2011;24(1):11-17.
186. Bodner KE, Beversdorf DQ, Saklayen SS, Christ SE. Noradrenergic moderation of working memory impairments in adults with autism spectrum disorder. *Journal of the International Neuropsychological Society.* 2012;18(03):556-564.
187. Zamzow RM, Ferguson BJ, Hegarty II JP, et al. Effects of a beta-adrenergic antagonist on social and cognitive functioning in autism spectrum disorder. Paper presented at: International Meeting for Autism Research2014; Atlanta, GA.
188. Prichard BNC, Gillam PMS. Treatment of hypertension with propranolol. *The British Medical Journal.* 1969;1:7-16.
189. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews.* 2003;42:33-84.
190. Bouret S, Sara SJ. Network reset: a simplified overarching theory of locus coeruleus noradrenaline function. *Trends in Neuroscience.* 2005;28(11):574-582.
191. Jones BE. Activity, modulation and role of basal forebrain cholinergic neurons innvervating the cerebral cortex. . *Prog Brain Res.* 2004;145:157-169.
192. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Rev.* 2003;42(33-84).
193. Samuels ER, Szabadi E. Functional Neuroanatomy of the Noradrenergic Locus Coeruleus: Its Roles in the Regulation of Arousal and Autonomic Function Part I: Principles of Functional Organisation. *Current Neuropharmacology.* 12/11/2008;6(3):235-253.
194. Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual Review of Neuroscience.* 2005;28:403-450.

195. Valentino RJ, Van Bockstaele E. Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology.* 4/7/ 2008;583(2–3):194-203.
196. Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. *Nat Rev Neurosci.* 03//print 2009;10(3):211-223.
197. Beversdorf DQ, Hughes JD, Steinberg BA, Lewis LD, Heilman KM. Noradrenergic modulation of cognitive flexibility in problem solving. *NeuroReport.* 1999;10(13).
198. Hasselmo ME. *Noradrenergic Suppression of Synaptic Transmission May Influence Cortical Signal-to-Noise Ratio.* Vol 771997.
199. Beversdorf DQ, Hughes JD, Steinberg BA, Lewis LD, Heilman KM. Noradrenergic modulation of cognitive flexibility in problem solving. *NeuroReport.* 1999;10(13):2763-2767.
200. Alexander JK, Hillier A, Smith RM, Tivarus ME, Beversdorf DQ. Beta-adrenergic modulation of cognitive flexibility during stress. *Journal of Cognitive Neuroscience.* 2007/03/01 2007;19(3):468-478.
201. Campbell HL, Tivarus ME, Hillier A, Beversdorf DQ. Increased task difficulty results in greater impact of noradrenergic modulation of cognitive flexibility. *Pharmacol Biochem Be.* 2008;88(3):222-229.
202. Hermans EJ, van Marle HJF, Ossewaarde L, et al. Stress-Related Noradrenergic Activity Prompts Large-Scale Neural Network Reconfiguration. *Science.* 2011;334(6059):1151-1153.
203. Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga-Barke EJS. Default-mode brain dysfunction in mental disorders: A systematic review. *Neuroscience & Biobehavioral Reviews.* 2009;33:279-296.
204. Mitchell JP. Mentalizing and Marr: An information processing approach to the study of social cognition. *Brain Research.* 3/24/ 2006;1079(1):66-75.
205. Spreng RN, Mar RA, Kim ASN. The Common Neural Basis of Autobiographical Memory, Prospection, Navigation, Theory of Mind, and the Default Mode: A Quantitative Meta-analysis. *Journal of Cognitive Neuroscience.* 2009/03/01 2008;21(3):489-510.
206. Baron-Cohen S. Theory of mind and autism: a review. *International review of research in mental retardation.* 2001;23:169-184.
207. Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL. Functional-anatomic fractionation of the brain's default network. *Neuron.* 2010;65:550-562.
208. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci.* 03//print 2009;10(3):186-198.
209. Le Couteur A, Lord C, Rutter M. *The autism diagnostic interview-revised (ADI-R).* Los Angeles, CA: Western Psychological Services; 2003.
210. Wechsler D. *WASI manual.* San Antonio: Psychological Corporation; 1999.
211. Beversdorf DQ, White DM, Chever DC, Hughes JD, Bornstein RA. Central beta-adrenergic modulation of cognitive flexibility. *NeuroReport.* 2002;13(18):2505-2507.
212. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: Psychometric properties. *Journal of Consulting and Clinical Psychology.* 1988;56(6):893-897.

213. Jenkinson M, Beckman CF, Behrens TE, Woolrich MW, Smith SM. FSL. *Neuroimage.* 2012;62:782-790.
214. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage.* 2004;23(Supplement 1):S208-S219.
215. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature Reviews.* 2007;8:700-711.
216. Song X-W, Dong Z-Y, Long X-Y, et al. REST: A toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS one.* 2011;6(9):E25031.
217. Lowe MJ, Mock BJ, Sorenson JA. Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage.* 1998;7:119-132.
218. Van Dijk KRA, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage.* 2012;59(2):431-438.
219. Power JD, Barnes KA, Synder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivty MRI networks arise from subject motion. *Neuroimage.* 2012;59(3):2142-2154.
220. Mazziotta J, Toga A, Evans AC, et al. A probabilistic atlas and reference system for the human brain: International consortium for brain mapping (ICBM). *Phil. Trans. R. Soc. Lond.* 2001;356:1293-1322.
221. Talairach J, Tournoux P. *Co-planar stereotaxic atlas of the human brain.* New York: Thieme; 1988.
222. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage.* Jan 2002;15(1):273-289.
223. Investigators AaDDMNSYP. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years --Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States 2010. *MMWR CDC Surveillance Summaries.* 2014;63(SS02):1-21.
224. Xia M, Wang J, He Y. BrainNet Viewer: a network visualization tool for human brain connectomics. *PLos ONE.* 2013;8(7):e68910.
225. Van Dijk KRA, Hedden T, Venkataraman A, Evans KC, Lazar SW, Buckner RL. Intrinsic functional connectivity as a tool for human connectomics: Theory, properties, and optimization. *Journal of Neurophysiology.* 2010;103(1):297-321.
226. Weissenbacher A, Kasess C, Gerstl F, Lanzenberger R, Moser E, Windischberger C. Correlations and anticorrelations in resting-state functional connectivity MRI: A quantitative comparison of preprocessing strategies. *Neuroimage.* 2009;47:1408-1416.
227. Guye M, Bettus G, Bartolomei F, Cozzone PJ. Graph theoretical analysis of structural and functional connectivity MRI in normal and pathological brain networks. *Magn Reson Mater Phy.* 2010;23:409-421.
228. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. *Neuroimage.* Sep 2010;52(3):1059-1069.
229. Power Jonathan D, Cohen Alexander L, Nelson Steven M, et al. Functional Network Organization of the Human Brain. *Neuron.* 11/17/ 2011;72(4):665-678.

230. *IBM SPSS Statistics for Macintosh* [computer program]. Version 22. Armonk, NY: IBM Corp.; 2013.
231. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society.* 1995;57(1):289-300.
232. McLeod DR, Hoehn-Saric R, Stefan RL. Somatic symptoms of anxiety: Comparison of self-report and physiological measures. *Biological Psychiatry.* 3// 1986;21(3):301-310.
233. Hill E, Berthoz S, Frith U. Brief Report: Cognitive Processing of Own Emotions in Individuals with Autistic Spectrum Disorder and in Their Relatives. *Journal of Autism and Developmental Disorders.* 2004/04/01 2004;34(2):229-235.
234. Kennedy DP, Courchesne E. The intrinsic functinal organization of the brain is altered in autism. *Neuroimage.* 2008;39(4):1877-1885.
235. Monk CS, Peltier SJ, Wiggins JL, et al. Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *Neuroimage.* 2009;47(2):764-772.
236. Lind SE, Bowler DM. Episodic memory and episodic future thinking in adults with autism. *Journal of abnormal psychology.* 2010;119(4):896.
237. Graziano P, Derefinko K. Cardiac vagal control and children's adaptive functioning: a meta-analysis. *Biol Psychol.* 2013;94(1):22 - 37.
238. Rudie JD, Brown JA, Beck-Pancer D, et al. Altered functional and structural brain network organization in autism. *NeuroImage: Clinical.* // 2013;2(0):79-94.
239. Ratey J, Bemporad J, Sorgi P, et al. Brief report: Open trial effects of beta-blockers on speech and social behaviors in 8 autistic adults. *Journal of Autism and Developmental Disorders.* 1987/09/01 1987;17(3):439-446.
240. Corbetta M, Shulman GL. Control of goal-directed and stimulusd-driven attention in the brain. *Nature Reviews Neuroscience.* 2002;3(3):201-215.
241. Fox MD, Synder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(27):9673-9678.
242. Vincent JL, Kahn I, Syndeer AZ, Raichle ME, Buckner RL. Evidence for a Frontoparietal Control System Revealed by Intrinsic Functional Connectivity. *J Neurophysiol.* 2008;100:3328-3342.
243. Fitzgerald J, Johnson K, Kehoe E, et al. Disrupted functional connectivity in dorsal and ventral attention networks during orienting in autism spectrum disorders. *Autism Res.* 2015;8(2):136-152.
244. Kanna RK, Keller TA, Minshew NJ, Just MA. Inhibitory Control in High-Functioning Autism: Decreased Activation and Underconnectivity in Inhibtion Networks. *Biol Psychiatry.* 2007;62:198-206.
245. Solomon M, Ozonoff SJ, Ursu S, et al. The neural substrates of cognitive control deficits in autism spectrum disorders. *Neuropsychologia.* 2009;47:2515-2526.
246. Anderson JS, Lange N, Froehlich A, et al. Decreased Left Posterior Insular Activity during Auditory Language in Autism. *American Journal of Neuroradiology.* 2010;31(1):131-139.

247. Spreng RN, Sepulcre J, Turner GR, Stevens WD, Schacter DL. Intrinsic architecture underlying the relations among the default, dorsal attention, and frontoparietal control networks of the human brain. *J Cogn Neurosci.* 2013;25(1).
248. Wagner S, Sebastian A, Lieb K, Tuscher O, Tadic A. A coordinate-based ALE functional MRI meta-analysis of brain activation during verbal fluency tasks in healthy control subjects. *BMC Neuroscience.* 2014;15(19).
249. Uddin LQ, Supekar K, Lynch CJ, et al. SAlience network–based classification and prediction of symptom severity in children with autism. *JAMA Psychiatry.* 2013;70(8):869-879.
250. Seeley WW, Menon V, Schatzberg AF, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. *The Journal of Neuroscience.* 2007;27(9):2349-2356.
251. Just MA, Keller TA, Malave VL, Kana RK, Varma S. Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. *Neuroscience and biobehavioral reviews.* Apr 2012;36(4):1292-1313.
252. Uddin LQ, Menon V. The anterior insula in autism: under-connected and under-examined. *Neurosci Biobehav Rev.* 2009;33(8):1198-1203.
253. Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of the insula. *Brain Structure and Function.* 2010;214(5-6):655-667.
254. Grafman J, Litvan I, Massaquoi S, Stewart M, Sirigu A, Hallet M. Cognitive planning deficit in patients with cerebellar atrophy. *Neurology.* 1992;42(8):1493.
255. Silveri Mc, Leggio MG, Molinari M. The cerebellum contributes to linguistic production: a case of agrammatic speech following a right cerebellar lesion. *Neurology.* 1994;44(11):2001-2005.
256. Kingma A, Mooij JJA, Metzemaekers JDM, Leeuw JA. Transient mutism and speech disorders after posterior fossa surgery in children with brain tumours. *Acta Neurochirurgica.* 1994;131(1-2):74-79.
257. Drepper J, Timmann D, Kolb FP, Diener H-C. Non-motor associateve learning in patients with isolated degenerative cerebellar disease. *Brain.* 1999;122(1):87-97.
258. Le TH, Pardo JV, Hu X. 4T fMRI study of nonspatial shifting of selective attention: cerebellar and parietal contributions. *Journal of Neurophysiology.* 1998;79(3):1535-1548.
259. Allen G, Buxton RB, Wong EC, Courchesne E. Attentional activation of the cerebellum independent of motor involvement. *Science.* 1997;1997(275):5308.
260. Rao SM, Bobholz JA, Hammeke TA, et al. Functional MRI evidence for subcortical participation in conceptual reasoning skills. *NeuroReport.* 1997;8(8).
261. Liddle PF, Kiehl KA, Smith AM. Event-related fMRI study of response inhibition. *Human Brain Mapping.* 2001;12(2):100-109.
262. Stoodley CJ, Valera EM, Schmahmann JD. Functional topography of the cerebellum for motor and cognitive tasks: an fMRI study. *Neuroimage.* 2012;59(2):1560-1570.
263. Raichle ME, Fiez JA, Videen T, et al. Practice-related changes in human functional anatomy during nonmotor learning. *Cereb Cortex.* 1994;4:8-26.
264. Desmond JE, Gabrieli JDE, Wagner AD, Ginier BL, Glover GH. Lobular patterns of cerebellar activation in verbal working-memory and finger-tapping tasks as

revealed by functional MRI. *The Journal of Neuroscience.* 1997;17(24):9675-9685.
265. LaBar KS, Gitelman DR, Parrish TB, Mesulam MM. Neuroanatomic Overlap of Working Memory and Spatial Attention Networks: A Functional MRI Comparison within Subjects. *NeuroImage.* 12// 1999;10(6):695-704.
266. Gurd JM, Amunts K, Weiss PH, et al. Posterior cortex is implicated in continuous switching between verbal fluency tasks: an fMRI study with clinical implications. *Brain.* 2002;125:1024-1038.
267. Ryding E, Decety J, Sjoholm H, Stenberg G, Ingvar DH. Motor imagery activates the cerebellum regionally. A SPECT rCBF study with 99mTc-HMPAO. *Cognitive Brain Research.* 1993;1(2):94-99.
268. Imaizumi S, Mori K, Kiritani S, et al. Vocal identification of speaker and emotion activates differerent brain regions. *NeuroReport.* 1997;8(12).
269. Habel U, Klein M, Kellermann T, Shah NJ, Schneider F. Same or different? Neural correlates of happy and sad mood in healthy males. *NeuroImage.* 5/15/ 2005;26(1):206-214.
270. Gündel H, O'Connor M-F, Littrell L, Fort C, Lane RD. Functional Neuroanatomy of Grief: An fMRI Study. *American Journal of Psychiatry.* 2003/11/01 2003;160(11):1946-1953.
271. Gao J-H, Parsons L, Bower J, Xiong J, Li J, Fox P. Cerebellum implicated in sensory acquistion and discrimination rather than motor control. *Science.* 1996;272:545-547.
272. Hadjikhani N, Roland PE. Cross-modal transfer of information between the tactile and the visual representations in the human brain: a positron emission tomographic study. *The Journal of Neuroscience.* 1998;18(3):1072-1084.
273. Chambers WW, Sprague JM. Functional localization in the cerebellum: Somatotopic organization in cortex and nuclei. *A.M.A. Archives of Neurology & Psychiatry.* 1955;74(6):653-680.
274. Manni E, Petrosini L. A century of cerebellar somatotopy: a debated representation. *Nat Rev Neurosci.* 03//print 2004;5(3):241-249.
275. Nitschke MF, Kleinschmidt A, Wessel K, Frahm J. Somatotopic motor representation in the human anterior cerebellum: a high-resolution functional MRI study. *Brain.* 1996;119:1023-1029.
276. Kelly RM, Strick PL. Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *The Journal of Neuroscience.* 2003;23(23):8432-8444.
277. Stoodley CJ, Schmahmann JD. Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex.* 7// 2010;46(7):831-844.
278. Tavano A, Grasso R, Gagliaradi C, et al. Disorders of cognitive and affective development in cerebellar malformations. *Brain.* 2007;130:2646-2660.
279. Takagi M, Zee DS, Tamargo R. Effects of lesions of the oculomotor vermis on eye movements in primate: saccades. *Journal of Neurophysiology.* 1998;80(4):1911-1931.
280. D'Angelo E, Casali S. Seeking a unified framework for cerebellar function and dysfunction: from circuit operations to cognition. *Frontiers in neural circuits.* 2012;6:116.

281. Schmahmann JD. Disorders of the Cerebellum: Ataxia, Dysmetria of Thought, and the Cerebellar Cognitive Affective Syndrome. *The Journal of Neuropsychiatry and Clinical Neurosciences.* 2004/08/01 2004;16(3):367-378.
282. Wood JJ, Gadow KD. Exploring the Nature and Function of Anxiety in Youth with Autism Spectrum Disorders. *Clinical Psychology: Science and Practice.* 2010;17(4):281-292.
283. Courchesne E, Saitoh O, Yeung-Courchesne R, et al. Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: identification of hypoplastic and hyperplastic subgroups with MR imaging. *Am J Roentgenol.* 1994;162(1):123-130.
284. Kaufmann WE, Cooper KL, Mostofsky SH, et al. Specificity of Cerebellar Vermian Abnormalities in Autism: A Quantitative Magnetic Resonance Imaging Study. *J Child Neurol.* 2003;18(7):463-470.
285. Khan AJ, Nair A, Keown CL, Datko MC, Lincoln AJ, Muller RA. Cerebro-cerebellar resting state functional connectivity in children and adolscents with autism spectrum disorder. *Biol Psychiatry.* 2015.
286. Hagerman RJ, Jackson III AW, Levitas A, Rimland B, Braden M. An analysis of autism in fifty males with fragile X syndrome. *Am J Med Genet.* 1986;23(1-2):359-374.
287. Ozonoff S, Williams BJ, Gale S, Miller JN. Autism and Autistic Behavior in Joubert Syndrome. *J Child Neurol.* 1999;14(10):636-641.
288. Hunt A, Shepherd C. A prevalence of study of autism in tuberous sclerosis. *J Autism Dev Disord.* 1993;23(2):323-339.
289. Weber AM, Egelhoff JC, McKellop JM, Franz DN. Autism and the Cerebellum: Evidence from Tuberous Sclerosis. *Journal of Autism and Developmental Disorders.* 2000;30(6):511-517.
290. Benayed R, Gharani N, Rossman I, et al. Support for the Homeobox Transcription Factor Gene ENGRAILED 2 as an Autism Spectrum Disorder Susceptibility Locus. *The American Journal of Human Genetics.* 11// 2005;77(5):851-868.
291. Buxbaum JD, Silverman JM, Smith CJ, et al. Association between a GABRB3 polymorphism and autism. *Molecular Psychiatry.* 2002;7(3):311.
292. Campbell DB, Sutcliffe JS, Ebert PJ, et al. A genetic variant that disrupts MET transcription is associated with autism. *Proceedings of the National Academy of Sciences.* 2006;103(45):16834-16839.
293. Cheh MA, Millonig JH, Roselli LM, et al. En2 knockout mice display neurobehavioral and neurochemical alterations relevant to autism spectrum disorder. *Brain research.* Oct 20 2006;1116(1):166-176.
294. DeLorey TM, Sahbaie P, Hashemi E, Homanics GE, Clark JD. Gabrb3 gene deficient mice exhibit impaired social and exploratory behaviors, deficits in non-selective attention and hypoplasia of cerebellar vermal lobules: a potential model of autism spectrum disorder. *Behavioural brain research.* Mar 5 2008;187(2):207-220.
295. Elsen GE, Choi LY, Prince VE, Ho RK. The autism susceptibility gene met regulates zebrafish cerebellar development and facial motor neuron migration. *Developmental biology.* Nov 1 2009;335(1):78-92.

296. Fonnum F. Glutamate: A Neurotransmitter in Mammlian Brain. *Journal of Neurochemistry.* 1984;42(1):1-11.
297. Luhmann HJ, Prince DA. Postnatal maturation of the GABAergic system in rat neocortex. *J Neurophysiol.* 1991;65:247-263.
298. Huang ZJ, Di Cristo G, Ango F. Development of GABA innervation in the cerebral and cerebellar cortices. *Nat Rev Neurosci.* Sep 2007;8(9):673-686.
299. Blatt GJ, Fitzgerald CM, Guptill JT, Booker AB, Kemper TL, Bauman ML. Density and Distribution of Hippocampal Neurotransmitter Receptors in Autism: An Autoradiographic Study. *Journal of Autism and Developmental Disorders.* 2001;31(6):537-543.
300. Fatemi SH, Reutiman TJ, Folsom TD, Rooney RJ, Patel DH, Thuras PD. mRNA and protein levels for GABAAalpha4, alpha5, beta1 and GABABR1 receptors are altered in brains from subjects with autism. *J Autism Dev Disord.* Jun 2010;40(6):743-750.
301. Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. *Neurology.* 2001;57:1618-1628.
302. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autisc parietal and cerebellar cortices. *Biol Psychiatry.* 2002;52(8):805-810.
303. Yip J, Soghomonian JJ, Blatt GJ. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. *Acta neuropathologica.* May 2007;113(5):559-568.
304. Yip J, Soghomonian JJ, Blatt GJ. Decreased GAD65 mRNA levels in select subpopulations of neurons in the cerebellar dentate nuclei in autism: an in situ hybridization study. *Autism Res.* Feb 2009;2(1):50-59.
305. Fatemi SH, Stary JM, Halt AR, Realmuto GR. Dysregulation of Reelin and Bcl-2 Proteins in Autistic Cerebellum. *Journal of Autism and Developmental Disorders.* 2001;31(6):529-535.
306. Persico AM, D'Agruma L, Maiorano N, et al. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Molecular Psychiatry.* 2001;6:150-159.
307. Yizhar O, Fenno LE, Prigge M, et al. Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature.* 09/08/print 2011;477(7363):171-178.
308. Tsai PT, Hull C, Chu Y, et al. Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature.* 08/30/print 2012;488(7413):647-651.
309. Kato T, Inubushi T, Kato N. Magnetic Resonance Spectroscopy in Affective Disorders. *Journal of Neuropsychiatry.* 1998;10:133-147.
310. Kampman KM, Volpicelli JR, Mulvaney F, et al. Effectiveness of propranolol for cocaine dependence treatment may depend on cocaine withdrawal symptom severity. *Drug and alcohol dependence.* 2001;63(1):69-78.
311. DeVito TJ, Drost DJ, Neufeld RW, et al. Evidence for cortical dysfunction in autism: a proton magnetic resonance spectroscopic imaging study. *Biol Psychiatry.* Feb 15 2007;61(4):465-473.

312. Endo T, Shioiri T, Kitamura H, et al. Altered chemical metabolites in the amygdala-hippocampus region contribute to autistic symptoms of autism spectrum disorders. *Biol Psychiatry.* Nov 1 2007;62(9):1030-1037.
313. Friedman SD, Shaw DW, Artru AA, et al. Regional brain chemical alterations in young children with autism spectrum disorder. *Neurology.* 2003;60:100-107.
314. Fuji E, Mori K, Miyazaki M, Hashimoto T, Harada M, Kagami S. Function of the frontal lobe in autistic individuals: a proton magnetic resonance spectroscopic study. *J Med Invest.* 2010;57:35-44.
315. Gabis L, Wei H, Azizian A, et al. 1H-magnetic resonance spectroscopy markers of cognitive and language ability in clinical subtypes of autism spectrum disorders. *J Child Neurol.* Jul 2008;23(7):766-774.
316. Hardan AY, Minshew NJ, Melhem NM, et al. An MRI and proton spectroscopy study of the thalamus in children with autism. *Psychiatry research.* Jul 15 2008;163(2):97-105.
317. Hisaoka S, Harada M, Nishitani H, Mori K. Regional magnetic resonance spectroscopy of the brain in autistic individuals. *Neuroradiology.* 2001;43(6):496-498.
318. Kleinhans NM, Schweinsburg BC, Cohen DN, Muller RA, Courchesne E. N-acetyl aspartate in autism spectrum disorders: regional effects and relationship to fMRI activation. *Brain research.* Aug 8 2007;1162:85-97.
319. Levitt JG, O'Neill J, Blanton RE, et al. Proton magnetic resonance spectroscopic imaging of the brain in childhood autism. *Biol Psychiatry.* 2003;54(12):1355-1366.
320. Otsuka H, Harada M, Mori K, Hisaoka S, Nishitani H. Brain metabolites in the hippocampus-amygdala region and cerebellum in autism: an 1 H-MR spectroscopy study. *Neuroradiology.* 1999;41:496-498.
321. Suzuki K, Nishimura K, Sugihara G, et al. Metabolite alterations in the hippocampus of high-functioning adult subjects with autism. *Int J Neuropsychopharmacol.* May 2010;13(4):529-534.
322. Murphy DGM, Critchley HD, Schmitz N, et al. Asperger Syndrome: A Proton Magnetic Resonance Spectroscopy Study of Brain. *Archiave of General Psychiatry.* 2002;59:885-891.
323. O'Brien FM, Page L, O'Gorman RL, et al. Maturation of limbic regions in Asperger syndrome: a preliminary study using proton magnetic resonance spectroscopy and structural magnetic resonance imaging. *Psychiatry research.* Nov 30 2010;184(2):77-85.
324. Oner O, Devrimci-Ozguven H, Oktem F, Yagmurlu B, Baskak B, Munir KM. Proton MR spectroscopy: higher right anterior cingulate N-acetylaspartate/choline ratio in Asperger syndrome compared with healthy controls. *AJNR. American journal of neuroradiology.* Sep 2007;28(8):1494-1498.
325. Friedman SD, Shaw DWW, Artru AA, Dawson G, Petropoulos H, Dager SR. Gray and White Matter Brain Chemistry in Young Children With Autism. *Archiave of General Psychiatry.* 2006;63(7):786-794.
326. Harada M, Taki MM, Nose A, et al. Non-invasive evaluation of the GABAergic/glutamatergic system in autistic patients observed by MEGA-editing

proton MR spectroscopy using a clinical 3 tesla instrument. *J Autism Dev Disord.* Apr 2011;41(4):447-454.
327. Kleinhans NM, Richards T, Weaver KE, Liang O, Dawson G, Aylward E. Brief report: biochemical correlates of clinical impairment in high functioning autism and Asperger's disorder. *J Autism Dev Disord.* Jul 2009;39(7):1079-1086.
328. Vasconcelos MM, Brito AR, Domingues RC, et al. Proton magnetic resonance spectroscopy in school-aged autistic children. *Journal of neuroimaging : official journal of the American Society of Neuroimaging.* Jul 2008;18(3):288-295.
329. Aoki Y, Kasai K, Yamasue H. Age-related change in brain metabolite abnormalities in autism: a meta-analysis of proton magnetic resonance spectroscopy studies. *Transl Psychiatry.* 2012;2:e69.
330. Page LA, Daly E, Schmitz N, et al. In Vivo 1H-Magnetic Resonance Spectroscopy Study of Amygdala-Hippocampal and Parietal Regions in ASD. *Am J Psychiatry.* 2006;163(12):2189-2192.
331. Bejjani A, O'Neill J, Kim JA, et al. Elevated Glutamatergic Compounds in Pregenual Anterior Cingulate in Pediatric Autism Spectrum Disorder Demonstrated by 1H MRS and 1H MRSI. *PLoS One.* 2012;7(7):e38786.
332. Kubas B, Kulak W, Sobaniec W, Tarasow E, Lebkowska U, Walecki J. Metabolite alterations in autistic children: a 1H MR spectroscopy study. *Advances in Medical Sciences.* 2012;57(1):152-156.
333. Bernardi S, Anagnostou E, Shen J, et al. In vivo 1H-magnetic resonance spectroscopy study of the attentional networks in autism. *Brain research.* Mar 22 2011;1380:198-205.
334. Horder J, Lavender T, Mendez MA, et al. Reduced subcortical glutamate/glutamine in adults with autism spectrum disorders: a [(1)H]MRS study. *Transl Psychiatry.* 2013;3:e279.
335. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR in Biomedicine.* 1998;11:266-272.
336. Rojas DC, Singel D, Steinmetz S, Hepburn S, Brown MS. Decreased left perisylvian GABA concentration in children with autism and unaffected siblings. *Neuroimage.* Jan 28 2013.
337. Gaetz W, Bloy L, Wang DJ, et al. GABA estimation in the brains of children on the autism spectrum: Measurement precision and regional cortical variation. *Neuroimage.* May 24 2013.
338. Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience.* Apr-May 2005;23(2-3):183-187.
339. Mostofsky SH, Powell SK, Simmonds DJ, Goldberg MC, Caffo B, Pekar JJ. Decreased connectivity and cerebellar activity in autism during motor task performance. *Brain : a journal of neurology.* 2009;132:2413-2425.
340. Baron-Cohen S, Ring H, Wheelwright S, et al. Social intelligence in the normal and autistic brain: an fMRI study. *Eur J Neurosci.* 1999;11(6):1891 - 1898.
341. O'Reilly JX, Beckmann CF, Tomassini V, Ramnani N, Johansen-Berg H. Distinct and overlapping functional zones in the cerebellum defined by resting state functional connectivity. *Cereb Cortex.* 2010;20(4):953-965.

342. Lord C, Risi S, Lambrecht L, et al. The Autism Diagnostic Observation Schedule-Generic: A Standard Measure of Social and Communication Deficits Associated with the Spectrum of Autism. *Journal of Autism and Developmental Disorders.* 2000;30(3):205-223.
343. Constantino JN, Gruber CP. The social responsiveness scale. *Los Angeles: Western Psychological Services.* 2002.
344. Aman MG, Singh NN, Stewart AW, Field CJ. The aberrant behavior checklist: A behavior rating scale for the assessment of treatment effects. *American Journal of Mental Deficiency.* 1985;89(5):485-491.
345. Stichter JP, Herzog MJ, O'Connor KV, Schmidt C. A Preliminary Examination of a General Social Outcome Measure. *Assessment for Effective Intervention.* 2012;38(1):40-52.
346. Wiig EH, Secord W. *Test of language competence (TLC): Expanded edition.* Psychological Corporation; 1989.
347. Minshew N, Goldstein G, Siegel DJ. Speech and Language in High-Functioning Autistic Individuals. *Neuropsychology.* 1995;9(2):255-261.
348. Courchesne E, Saitoh O, Townsend JP, et al. Cerebellar hypoplasia and hyerplasia in infantile autism. *Lancet.* 1994;343:63-64.
349. Roth K, Hubesch B, Meyerhoff DJ, et al. Noninvasive quantification of phosphorus metabolites in human tissue by NMR spectroscopy. *Journal of Magnetic Resonance.* 1969;81(2):299-311.
350. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. *NeuroImage.* 1/1/ 2014;84:320-341.
351. Murphy K, Birn RM, Handwerker DA, Jones TB, Bandettini PA. The impact of global signal regression on resting state correlations: Are anti-correlated networks introduced? *NeuroImage.* 2/1/ 2009;44(3):893-905.
352. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage.* Feb 1 2012;59(3):2142-2154.
353. Tyszka JM, Kennedy DP, Paul LK, Adolphs R. Largely typical patterns of resting-state functional connectivity in high-functioning adults with autism. *Cerebral Cortex.* 2014;4(24):1894-1905.
354. Provencher SW. Automatic quantitation of localized in vivo 1H spectra with LCModel. *NMR Biomed.* Jun 2001;14(4):260-264.
355. Gussew A, Erdtel M, Hiepe P, Rzanny R, Reichenbach JR. Absolute quantitation of brain metabolites with respect to heterogeneous tissue compositions in (1)H-MR spectroscopic volumes. *MAGMA.* Oct 2012;25(5):321-333.
356. Hartigan JA, Hartigan PM. The dip test of unimodality. *The Annals of Statistics.* 1985:70-84.
357. *R: A language and environment for statistical computing* [computer program]. Vienna, Austria: R Foundation for Statistical Computing; 2012.
358. Allen G, Courchesne E. Differential Effects of Developmental Cerebellar Abnormality on Cognitive and Motor Functions in the Cerebellum: An fMRI Study of Autism. *American Journal of Psychiatry.* 2003/02/01 2003;160(2):262-273.

359. Miller EK, Cohen JD. AN INTEGRATIVE THEORY OF PREFRONTAL CORTEX FUNCTION. *Annual Review of Neuroscience.* 2001/03/01 2001;24(1):167-202.
360. Ohyama T, Nores WL, Murphy M, Mauk MD. What the cerebellum computes. *Trends in neurosciences.* 4// 2003;26(4):222-227.
361. Krienen FM, Buckner RL. Segregated Fronto-Cerebellar Circuits Revealed by Intrinsic Functional Connectivity. *Cerebral Cortex.* 2009;19(10):2485-2497.
362. Guell X, Hoche F, Schmahmann JD. Metalinguistic Deficits in Patients with Cerebellar Dysfunction: Empirical Support for the Dysmetria of Though Theory. *Cerebellum.* 2015;14:50-58.
363. Baron-Cohen S, Leslie AM, Frith U. Does the autistic child have a "theory of mind" ? *Cognition.* 10// 1985;21(1):37-46.
364. Aldred S, Moore K, Fitzgerald M, Waring R. Plasma Amino Acid Levels in Children with Autism and Their Families. *Journal of Autism and Developmental Disorders.* 2003/02/01 2003;33(1):93-97.
365. Shinohe A, Hashimoto K, Nakamura K, et al. Increased serum levels of glutamate in adult patients with autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.* 12/30/ 2006;30(8):1472-1477.
366. Vogel MW, Caston J, Yuzaki M, Mariani J. The Lurcher mouse: Fresh insights from an old mutant. *Brain Research.* 4/6/ 2007;1140:4-18.
367. Martin LA, Goldowitz D, Mittleman G. Repetitive behavior and increased activity in mice with Purkinje cell loss: a model for understanding the role of cerebellar pathology in autism. *The European journal of neuroscience.* 01/25 2010;31(3):544-555.
368. McKimm E, Corkill B, Goldowitz D, et al. Glutamate Dysfunction Associated with Developmental Cerebellar Damage: Relevance to Autism Spectrum Disorders. *The Cerebellum.* 2014/06/01 2014;13(3):346-353.

# VITA

John Hegarty was born in Winston-Salem, North Carolina, and grew up in Greenville, South Carolina, where he attended Riverside Highschool. He completed a Bachelor of Science in psychology, with a minor in biology, at the Calhoun Honors College of Clemson University in 2006. He obtained a Master of Arts in psychology, with an emphasis in behavioral and cognitive neuroscience, at San Diego State University in 2010. He then enrolled at the University of Missouri where he earned a Doctor of Philosophy in neuroscience in 2015. In the fall of 2015, John will begin a postdoctoral position with Dr. Antonio Hardan in the Autism and Developmental Disorders Research Program at Stanford University.