UC San Diego

UC San Diego Electronic Theses and Dissertations

Title

Functional abnormalities of the default network in autism

Permalink

https://escholarship.org/uc/item/4gv0v4sg

Author

Kennedy, Daniel P.

Publication Date

2007

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA, SAN DIEGO

Functional Abnormalities of the Default Network in Autism

A Dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Neurosciences

by

Daniel P. Kennedy

Committee in charge:

Professor Eric Courchesne, Chair
Professor Patricia S. Churchland
Professor Martin P. Paulus
Professor Katerina Semendeferi
Professor Larry R. Squire

2007

Copyright

Daniel P. Kennedy, 2007

All rights reserved.

The Dissertation of Daniel P. Kennedy is approved, and it is acceptable in quality and form for publication on microfilm:

_____

_____

_____

_____

_____

Chair

University of California, San Diego

2007

DEDICATION

To my parents, for always encouraging me to pursue my interests; and to Tanya, for her love, support, and for always being there.

TABLE OF CONTENTS

Signature Page iii

Dedication iv

Table of Contents v

List of Abbreviations vii

List of Figures viii

List of Tables ix

Acknowledgements x

Vita, Publications xii

Abstract xiv

Chapter I Introduction 1
- A cognitive neuroscience approach to autism 2
- Metabolic and functional properties of the default network 5
- The default network and autism 8
- Aims 9
- References 11

Chapter II Failing to deactivate: Resting functional abnormalities in autism 16
- Abstract 17
- Introduction 18
- Methods 21
  - Participants 21
  - Stimuli 23
  - Behavioral data acquisition and analysis 23
  - Functional imaging data acquisition and analysis 24
- Results 28
  - Behavior 28
  - Functional Imaging 30
- Discussion 39
- Acknowledgements 46
- References 47

Chapter III The intrinsic functional organization of the brain is altered in autism 54
- Abstract 55

Introduction 56
Methods 58
Results 64
Discussion 71
Acknowledgements 77
References 78

Chapter IV Default network activity during self- and other-reflection in autism ... 84
Abstract 85
Introduction 86
Methods 88
Participants 88
Stimuli 89
Behavioral Data Acquisition and Analysis 91
Functional Imaging Data Acquisition and Analysis 92
Results 94
Behavioral Results 94
Functional Imaging Results 95
Discussion 102
Acknowledgements 108
References 109

Chapter V Discussion 113
Summary 114
Conclusions 114
References 118

LIST OF ABBREVIATIONS

ASD, Autism Spectrum Disorder

fMRI, functional magnetic resonance imaging

fcMRI, functional connectivity magnetic resonance imaging

MPFC, medial prefrontal cortex

rACC, rostral anterior cingulate cortex

PCC, posterior cingulate cortex

PrC, precuneus

RSC, retrosplenial cortex

ANG, angular gyrus

TNN, task-negative network

TPN, task-positive network

BOLD, blood oxygenation level-dependent

ADOS, Autism Diagnostic Observation Schedule

ADI-R, Autism Diagnostic Interview – Revised

LIST OF FIGURES

Figure 2.1: Behavioral data for control and autism groups 29

Figure 2.2: Significant functional activity derived from group whole-brain analyses 31

Figure 2.3: A between group comparison for the NUMBER vs. NEUTRAL contrast 35

Figure 2.4: Correlation between functional activity in a ventral MPFC cluster and score on the social subscale of the ADI-R in the ASD group 36

Figure 2.5: Individual subject analyses 38

Figure 3.1: Intrinsically-defined functional connectivity maps of the TNN 65

Figure 3.2: Intrinsically-defined functional connectivity maps of the TPN 66

Figure 3.3: Localized abnormalities of the TNN in autism 69

Figure 4.1: Areas of functional overlap between control and autism groups in the MENTAL (all 4 conditions) vs. MATH contrast 96

Figure 4.2: Functional activity for control and autism groups across conditions and regions 99

Figure 4.3: Bar graphs depicting percent signal change by region in control and autism groups during PSYC and PHYS judgments, and SELF and OTHER judgments 100

LIST OF TABLES

Table 2.1:	Clinical information for autism ASD subjects.....	22
Table 2.2:	Regions of activation and deactivation in control and ASD groups	32
Table 2.3:	Regions of significant difference between ASD and control groups	33
Table 3.1:	Clinical information for autism and control participants	60
Table 3.2:	Talairach locations of peak significance of the intrinsically-defined TNN and TPN functional connectivity maps for control and autism groups.....	67
Table 4.1:	Clinical information for autism and control participants	90
Table 4.2:	Talairach locations of peak significance for the MENTAL (all 4 conditions) vs. MATH contrast in control and autism groups.....	97

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Eric Courchesne. His dedication to autism research and his passion for science and discovery are truly inspiring. He allowed me the freedom to make my own mistakes, he encouraged me to pursue even my craziest ideas, and he pushed me to continually expand my knowledge and thinking. I gained an immeasurable amount from my time in his lab, and thoroughly enjoyed my graduate experience.

I am also deeply indebted to the members of my dissertation committee – Pat Churchland, Martin Paulus, Katerina Semendeferi, and Larry Squire. I have learned so much from all of them, and our conversations have largely influenced this work and, more generally, my approach to studying the brain.

Finally, I consider myself fortunate to have had the opportunity to interact with all the current and previous members of the Courchesne Lab. They all have made my graduate experience truly special. However, three people deserve individual mention.

Nearly every aspect in this dissertation, from study design to the colors and layout of figures, has benefited from my discussions with Elizabeth Redcay, a fellow graduate student and co-first author on Chapter II. She is always willing to devote her complete attention to help me troubleshoot any problem, to help me further develop ideas, or to give me feedback on my writing or presentations. This dissertation is as much a product of her efforts as it is of mine, and I will be forever grateful for her contributions and, more importantly, her friendship.

Dr. Cindy Carter acquired nearly all the clinical measures reported in these studies – and always with enthusiasm and a smile. Often, she went above and beyond to

help, whether that meant staying at work late to test a difficult-to-schedule subject or to dig up a reference that related to one of our discussions. This dissertation would not have been completed without her help.

Graham Wideman has always been willing to help with any aspect of my research, including stimulus programming, data analysis, and discussion of findings. I viewed each conversation with him as an opportunity to learn something entirely new, whether it was about principles of data management, repairing broken radiators, or creating and compressing digital images. I thoroughly enjoyed and benefited from our interactions.

Chapter 2, in full, is a reprint of the material as it appears in *PNAS* 2006; Kennedy, DP, Redcay, E, and Courchesne, E; 103(21); 8275-8280. The dissertation author was co-primary author on this paper. The secondary author was the thesis advisor.

Chapter 3, in full, has been submitted for publication; Kennedy, DP, and Courchesne, E. The dissertation author was primary author on this paper. The secondary author was the thesis advisor.

Chapter 4, in full, has been submitted for publication; Kennedy, DP, and Courchesne, E. The dissertation author was primary author on this paper. The secondary author was the thesis advisor.

VITA

1998-2002	Binghamton University B.S. w/Honors in Psychobiology
2002-2007	University of California, San Diego Ph.D. in Neurosciences

PUBLICATIONS

Di Lorenzo, P.M., Hallock, R.M., & Kennedy, D.P. (2003). Temporal coding of sensation: mimicking taste with electrical stimulation of the brain. *Behavioral Neuroscience*, 117(6): 1423-1433.

Courchesne, E., Redcay, E., & Kennedy, D.P. (2004). The autistic brain: birth through adulthood. *Current Opinion in Neurology*, 17(4): 489-496.

Courchesne, E., Redcay, E., Morgan, J.T., & Kennedy, D.P. (2005). Autism at the beginning: Microstructural and growth abnormalities underlying the cognitive and behavioral phenotype of autism. *Development and Psychopathology*, 17(3): 577-97.

Kennedy, D.P., Redcay, E., & Courchesne, E. (2006). Failing to deactivate: Resting functional abnormalities in autism. *PNAS*, 103: 8275-80.

Kennedy, D.P., Semendeferi, K., & Courchesne, E. (2007). No reduction of spindle neuron number in frontoinsular cortex in autism. *Brain & Cognition*, 64: 124-129.

Kennedy, D.P. & Squire L.R. (in press). An analysis of calendar performance in two autistic calendar savants. *Learning & Memory*.

Redcay, E., Kennedy, D.P., & Courchesne, E. (under review). Functional MRI during natural sleep as a method to study early functional brain development.

Kennedy, D.P. & Courchesne, E. (under review). The intrinsic functional organization of the brain is altered in autism.

Kennedy, D.P. & Courchesne, E. (submitted). Default network activity during self- and other-reflection in autism.

CONFERENCE ABSTRACTS

Hallock, R.M., Kennedy, D.P., & Di Lorenzo, P.M. Temporal pattern of lick-contingent electrical stimulation of the nucleus of the solitary tract predicts behavioral rejection. *Association for Chemoreception Sciences*, Sarasota, Florida. April 2001.

Kennedy, D.P. & Parvizi, J. Efferent projections from the temporal lobe to the prefrontal cortex: evolution and the functional anatomy of language. *Society for Neuroscience*, November 2002.

Redcay, E., Kennedy, D.P., & Courchesne, E. Differential effects of cognitive and emotional processing on resting network activity. *Cognitive Neuroscience Society*, San Francisco, April 2004.

Kennedy, D.P., Redcay, E., & Courchesne, E. Cognition, emotion, and the resting state: an fMRI study of neurofunctional abnormalities in autism. *IMFAR*, Sacramento, May 2004.

Kennedy, D.P., Semendeferi, K., & Courchesne, E. Are there abnormal numbers of spindle neurons in autism? A quantitative stereological investigation. *Society for Neuroscience*, San Diego, November 2005.

Kennedy, D.P., & Courchesne, E. Disrupted and intact functional organization of large-scale brain networks in autism. *IMFAR*, Seattle, May 2007.

Redcay, E., Kennedy, D.P., & Courchesne, E. Differential response to auditory and visual stimuli during sleep fMRI in 3-4 year old children. *Cognitive Neuroscience Society*, New York City, May 2007.

Kennedy, D.P., & Courchesne, E. The intrinsic functional organization of the human brain is altered in autism. *Cognitive Neuroscience Society*, New York City, May 2007.

ABSTRACT OF THE DISSERTATION

Functional Abnormalities of the Default Network in Autism

by

Daniel P. Kennedy

Doctor of Philosophy in Neurosciences

University of California, San Diego, 2007

Professor Eric Courchesne, Chair

One of the most striking and debilitating features of autism is the profound impairment in social and emotional functioning. In recent years, the emergence of modern cognitive neuroscience techniques has led to a greater understanding of the neural bases of such abilities in healthy control subjects. However, very little is known regarding the neural bases of the impaired social and emotional functioning in individuals with autism.

In the present series of studies, the functioning of the default network was examined across various task and non-task conditions. This network is comprised of the medial prefrontal cortex (MPFC), retrosplenial cortex/posterior cingulate cortex (RSC/PCC), precuneus (PrC), and angular gyrus (ANG), among other regions, and typically activates during performance of tasks of a social, emotional, or self-referential introspective nature (e.g., social perception, emotion processing, mentalizing tasks, autobiographical recall, etc.). Furthermore, the default network maintains high levels of metabolic activity at rest, in the absence of any task demands, suggesting that ongoing unconstrained thoughts of a social, emotional, and introspective nature might be the natural, default state of the mind.

In Chapter II, the default network activity in autism was examined during a resting condition and an implicit emotion processing condition. In both of these conditions, reduced levels of activity were found in regions of the default network. Furthermore, the degree of resting functional abnormality in the MPFC correlated to a clinical measure of social impairment. In Chapter III, these findings were extended by demonstrating reduced levels of functional connectivity within the default network at rest, with the reduction being particularly pronounced in the MPFC and left ANG. These reductions were not a pervasive feature of the resting brain, as no abnormality was found in a different network (i.e., the dorsal attention network). In Chapter IV, in order to determine whether default network regions are capable of being engaged under particular experimental circumstances, activity was measured during an explicitly-defined and experimentally-constrained self- and other-reflection task. Although subtle task-specific

abnormalities in autism were found, the overall level of functional activity across all conditions was remarkably similar between groups.

All together, these findings suggest that while the default network often does not engage under typical circumstances in individuals with autism, it is in fact capable of responding, given particular highly-specified experimental constraints. Further investigation of the nature of this engagement deficit might prove instrumental in elucidating the causes of autism, and may help to refine behavioral treatments.

CHAPTER I

Introduction

A Cognitive Neuroscience Approach to Autism

Autism was first brought to clinical awareness by Leo Kanner in 1943, a child psychiatrist at Johns Hopkins University (Kanner, 1943). He recognized that several children in his clinic presented with a common collection of symptoms that had not been previously described together, and thus were different from any other known disorder at the time. Among the many peculiarities displayed by these children, he identified three primary domains of abnormality shared by all of them – 1) social/emotional impairments, 2) language abnormalities (usually early language delay, but also abnormal features of language), and 3) the presence of restricted and repetitive interests and behaviors. These three domains of abnormality still serve as the defining features of autism today (Lord et al., 1994; Lord et al., 2000). Around the same time, Hans Asperger independently recognized a similar collection of abnormalities in a group of children with higher cognitive abilities than those described by Kanner, and without early language delay (reviewed in Frith, 2004). These children eventually received the distinct label of Asperger's Syndrome. Finally, a third related disorder has now been recognized, termed Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS). This disorder includes all the same clinical features of autism, but is considered a less severe form. Today, the term autistic disorders or Autism Spectrum Disorders (ASD) is often used as an umbrella term which encompasses these three disorders (i.e., autism, Asperger's Syndrome, and PDD-NOS, among several additional disorders), and reflects the prevailing idea that these disorders are somehow related and may differ mainly in severity, not etiology (Frith, 2004). For the remainder of this thesis, unless referring to specific participants in Chapters 2-4, the term autism is used interchangeably with ASD

to refer to the collection of disorders (i.e., autism, Asperger's Sydrome, and PDD-NOS) that exist on the autism spectrum.

Although autism is defined solely by behavior, we know that it's causes are biological, and likely varied. For instance, it is well-established that autistic-like behaviors can result from a number of different biological factors with different biological mechanisms of action, including specific environmental toxins (e.g., prenatal exposure to thalidomide, mercury poisoning), infectious diseases (e.g., prenatal rubella infection), and specific genetic mutations (e.g., FMR1 gene mutation, MECP2 mutation). Unfortunately, the biological bases for the majority of cases of autism remain elusive, although there are some clues. Among the cases whose causes are unknown, there is an extremely strong genetic component. In fact, autism is one of the most heritable neuropsychiatric disorders of unknown cause, with a 65-90% concordance rate in identical twins. (Concordance is only 5-15% for fraternal twins (Bailey et al., 1995)). However, the search for the genetic and biological bases of autism is made more difficult by the fact that there are presumably multiple genetic and biological factors that interact to produce autism in a single person, complicated by complex biology-environment interactions (i.e., there is not 100% penetrance, either because of a trigger or protective factor in the environment), and by the putative existence of several currently-indistinguishable subtypes of autism. Researchers are left with a rather daunting challenge of trying to associate a collection of behaviors with often far-removed and multiple biological factors (e.g., genes, environmental toxins, microscopic neuroanatomical features, etc.).

The relatively new field of cognitive neuroscience – created from the merging of psychology and the neurosciences – is well-positioned to make significant advances toward our understanding of the biological bases of autism. With the goal of revealing the neural underpinnings of behavior and cognition, cognitive neuroscience is also rather uniquely poised to reveal neural underpinnings of abnormal behavior and cognition. This is particularly important for disorders like autism, which are still only defined by their shared behavioral and cognitive abnormalities. Regardless of whether these abnormalities are caused by any number of genetic alterations, environmental agents, or cellular abnormalities, the common behavioral and cognitive abnormalities that characterize the disorder will undoubtedly be manifested in abnormal brain functioning. Thus, examination of brain functioning provides one of the clearest and most direct links to the defining behavioral and cognitive features of autism. It is expected that findings from the cognitive neuroscience approach to autism will be important for determining which, where, or how particular brain networks are disrupted – information likely to be useful in guiding subsequent investigations of the genetic, molecular, and cellular bases of the disorder. This view, that cognitive neuroscience has much to inform about the biological bases of autism, is what motivated and guided the following series of studies (described in Chapters II-IV).

Unfortunately, research on autism (and the cognitive neuroscience of autism) is still in it's infancy. In fact, even the brain regions and networks that are predominantly affected, and the neural mechanisms underlying their dysfunction, are largely unknown.[1]

[1] This was particularly evident in when this research began in 2003, as there were only 13 published fMRI studies on autism.

The following series of functional magnetic resonance imaging (fMRI) studies examined the functioning of one particular brain network in autism – the default network (also termed the resting network, or task-negative network). Below, I provide background information describing the typical functioning and unique metabolic characteristics of this network, and provide the rationale for studying the functionality of the default network in autism.

Metabolic and functional properties of the default network

Recently, researchers have described a rather unique network of brain regions, which includes the medial prefrontal cortex (MPFC), the retrosplenial cortex/posterior cingulate cortex (RSC/PCC), and precuneus (PrC), among other regions. Unlike any other brain network, this network maintains high metabolic activity during a resting or no-task condition – for instance, passive fixation, resting with eyes open, resting with eyes closed, etc. Because of it's resting metabolic properties, Raichle and colleagues termed this network of brain regions the *default network* (Raichle et al., 2001), but it has since been referred to as the *resting network* or the *task-negative network* (or TNN).[2] The high level of resting activity of the default network is often attenuated during the performance of goal-directed, attention-demanding cognitive tasks, resulting in the typically under-reported and sometimes entirely ignored deactivations in functional imaging studies. This pattern of functional activity is unlike the more commonly reported pattern of brain activity in which increasing task demands results in increased

[2] All three of these names will used at various points in this text, as each name refers to and emphasizes a particular aspect of the network.

(rather than decreased) metabolic demands. In fact, for the default network, the exact opposite pattern holds, in that the more cognitively-demanding the task, the greater the level of deactivation (McKiernan et al., 2003). Importantly, these deactivations provide a way to measure the functioning of the default network at rest using fMRI (as will be discussed and illustrated in Chapter II).

Although performance of many types of cognitively-demanding tasks reduces activity of the default network, there are specific cognitive and behavioral tasks that actually engage the default network – namely, social, emotion, and self-reflective (i.e., introspective) tasks. Performance of tasks of this nature often results in default network activity at levels similar to or even greater than that seen during rest, and much greater than the levels seen during a cognitively comparable task not of a social, emotional, or introspective nature. Activation of the default network has repeatedly been observed across a wide variety of such tasks, including self- and other-person judgments (Fletcher et al., 1995; Gusnard et al., 2001; Vogeley et al., 2001; Gallagher et al., 2002; Johnson et al., 2002; Kelley et al., 2002; Kjaer et al., 2002; Fossati et al., 2003; Gallagher and Frith, 2003; Lou et al., 2004; Macrae et al., 2004; D'Argembeau et al., 2005; Mitchell et al., 2005a; Mitchell et al., 2005b; Ochsner et al., 2005), person familiarity judgments (Maddock et al., 2001; Pierce et al., 2004), emotion processing (Whalen et al., 1998; Maddock et al., 2003; Cato et al., 2004), perspective-taking (Vogeley et al., 2001; Vogeley et al., 2004), passive observation of social interactions vs non-social interactions (Iacoboni et al., 2004), relaxation based on interoceptive biofeedback (Critchley et al., 2001; Nagai et al., 2004), conceptual judgments (based on internal knowledge stores) vs perceptual judgments (Binder et al., 1999), episodic memory tasks (Andreasen et al.,

1995), moral decision making (Greene et al., 2001), joint attention experience (Williams et al., 2005), and pleasantness judgments (Paulus and Frank, 2003), among others.

It has been hypothesized that these two seemingly unrelated features of the default network – i.e., the high resting activity and the task-evoked activation during social, emotional, and introspective processes - are actually functionally related. It is currently thought that the high default network activity reflects the natural propensity of individuals to mind-wander, often toward social, emotional, and introspective thoughts regarding the past, present and future (Buckner and Carroll, 2007). Furthermore, the deactivation of the default network during goal-directed attention-demanding cognitive tasks is thought to index the interruption of these types of unconstrained resting thoughts (Shulman et al., 1997; Gusnard et al., 2001; Raichle et al., 2001; McKiernan et al., 2003; Cavanna and Trimble, 2006). This may explain why the amount of deactivation is attenuated when the task is of a social, emotional, or introspective nature (Fox et al., 2005).

Self-reports obtained from subjects, which is the primary methodology for examining the contents of the mind, support this hypothesis. For instance, one study reports that a majority of subjects at rest describe mental images and inner speech, most often pertaining to "autobiographical reminiscences" (Mazoyer et al., 2001). Similarly, D'Argembeau (2005) examined self-reports of subjects following 2 minute resting periods, and found that subjects often reported frequent thoughts regarding themselves and others. Furthermore, using the ability to reflect on the content of our own minds, most of us agree that social, emotional, and self-oriented thoughts regarding the past,

present, and future comprise a large portion of our resting thoughts (e.g., when waiting at a traffic light; when trying to fall asleep, etc.).

However, experimental difficulty arises in trying to directly examine how the contents of ones' mind relates to default network brain activity, as the very act of self-reporting (or knowing one might have to self-report) alters the content of the mind. This makes studying the moment to moment relationships between mental activity and brain activity impossible. Nonetheless, two creative studies have attempted to address the relationship between default network activity and unconstrained mental activity. First, D'Argembeau et al. (2005) identified a correlation between the frequency of self-oriented thoughts across of number of rest and non-resting tasks (assessed after scanning) and activity of the MPFC, one region of the default network. More recently, Mason and colleagues (2007) found that the level of activity of the default network correlated to subjects' self-reported propensity to mind-wander – specifically, subjects with a higher frequency of mind-wandering had a higher level of default network activity. Thus, it seems reasonable to assume that high default network activity at rest is related to the natural propensity for individuals to automatically engage in active mental processes at rest, which are often of a social, emotional, or introspective nature.

The default network and autism

There are several reasons to suspect dysfunction of the default network in autism. First, the types of tasks that normally engage the default network – namely, social, emotional, and introspective tasks - seem to be particularly impaired in autism (Kanner, 1943; Lord et al., 1994; Lord et al., 2000). Furthermore, performance on goal-directed

cognitive tasks of the sort that do not rely upon or that actually deactivate the default network are often relatively or entirely spared in autism (Eskes et al., 1990; Garretson et al., 1990; Ozonoff and Jensen, 1999; Allen and Courchesne, 2001). Second, several published studies identified functional abnormalities in various default network regions, including the MPFC, RSC/PCC, and PrC. As expected, these abnormalities were observed across a wide variety of socioemotional tasks, including a facial emotion processing task, a gender discrimination task using personally-familiar faces, and a mental state attribution task (Castelli et al., 2002; Pierce et al., 2004; Wang et al., 2004). Third, abnormalities of default network regions have also been observed during performance of non-socioemotional and non-introspective tasks [e.g., a visually-guided motor task (Muller et al., 2001); a spatial working memory task (Luna et al., 2002); a verbal learning test (Haznedar et al., 2000)], suggesting that there may also be functional abnormalities of the default network at rest. In other words, the high resting metabolic activity, which presumably supports typical resting mental activity, may be abnormal in autism.

Aims

The following series of autism fMRI studies examined the functioning of the default network across a number of different experimental contexts, in an attempt to characterize the severity and pervasiveness of default network abnormality in autism.

In Chapter II, default network functionality was investigated with fMRI during a resting condition and an implicit emotion processing task. The aims of this study were 1) to first demonstrate a deficit in default network functionality at rest, 2) to demonstrate

overlap between regions of the default network and regions involved in emotional processing, 3) to test whether an implicit emotion processing task activated these default regions in autism, and 4) to determine whether there were any clinical correlates of default network abnormality in autism.

In Chapter III, default network functionality was further studied at rest using functional connectivity MRI (fcMRI). This technique was used to assess the pattern of spontaneous occurring fluctuations in the BOLD (blood oxygenation level-dependent) signal within the default network, providing a measure of functional organization and functional engagement of this network at rest. The primary aims of this study were 1) to provide converging evidence of abnormality of the default network during an unconstrained resting state, and 2) if found to be abnormal, to determine whether functional connectivity abnormalities are pervasive across the entire network, or regionally-specific to particular nodes of the network.

In Chapter IV, default network functionality was examined with fMRI during performance of a self- and other-reflection task. Specifically, subjects were asked to make judgments about themselves or a close other person, regarding either psychological personality traits or physical, external attributes and behaviors. The aims of this study were 1) to determine if the default network would activate to near normal levels in autism under these specific experimentally-constrained task conditions, and 2) to examine whether there might be abnormal patterns of activation of default network regions during particular task conditions (i.e., self vs. other judgments; psychological vs. physical judgments).

References

Allen, G., Courchesne, E., 2001. Attention function and dysfunction in autism. Front Biosci 6, D105-119.

Andreasen, N.C., O'Leary, D.S., Cizadlo, T., Arndt, S., Rezai, K., Watkins, G.L., Ponto, L.L., Hichwa, R.D., 1995. Remembering the past: two facets of episodic memory explored with positron emission tomography. Am J Psychiatry 152, 1576-1585.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., Rutter, M., 1995. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25, 63-77.

Binder, J.R., Frost, J.A., Hammeke, T.A., Bellgowan, P.S., Rao, S.M., Cox, R.W., 1999. Conceptual processing during the conscious resting state. A functional MRI study. J Cogn Neurosci 11, 80-95.

Buckner, R.L., Carroll, D.C., 2007. Self-projection and the brain. Trends Cogn Sci 11, 49-57.

Castelli, F., Frith, C., Happe, F., Frith, U., 2002. Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. Brain 125, 1839-1849.

Cato, M.A., Crosson, B., Gokcay, D., Soltysik, D., Wierenga, C., Gopinath, K., Himes, N., Belanger, H., Bauer, R.M., Fischler, I.S., Gonzalez-Rothi, L., Briggs, R.W., 2004. Processing words with emotional connotation: an FMRI study of time course and laterality in rostral frontal and retrosplenial cortices. J Cogn Neurosci 16, 167-177.

Cavanna, A.E., Trimble, M.R., 2006. The precuneus: a review of its functional anatomy and behavioural correlates. Brain 129, 564-583.

Critchley, H.D., Melmed, R.N., Featherstone, E., Mathias, C.J., Dolan, R.J., 2001. Brain activity during biofeedback relaxation: a functional neuroimaging investigation. Brain 124, 1003-1012.

D'Argembeau, A., Collette, F., Van der Linden, M., Laureys, S., Del Fiore, G., Degueldre, C., Luxen, A., Salmon, E., 2005. Self-referential reflective activity and its relationship with rest: a PET study. Neuroimage 25, 616-624.

Eskes, G.A., Bryson, S.E., McCormick, T.A., 1990. Comprehension of concrete and abstract words in autistic children. J Autism Dev Disord 20, 61-73.

Fletcher, P.C., Happe, F., Frith, U., Baker, S.C., Dolan, R.J., Frackowiak, R.S., Frith, C.D., 1995. Other minds in the brain: a functional imaging study of "theory of mind" in story comprehension. Cognition 57, 109-128.

Fossati, P., Hevenor, S.J., Graham, S.J., Grady, C., Keightley, M.L., Craik, F., Mayberg, H., 2003. In search of the emotional self: an FMRI study using positive and negative emotional words. Am J Psychiatry 160, 1938-1945.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 102, 9673-9678.

Frith, U., 2004. Emanuel Miller lecture: confusions and controversies about Asperger syndrome. J Child Psychol Psychiatry 45, 672-686.

Gallagher, H.L., Frith, C.D., 2003. Functional imaging of 'theory of mind'. Trends Cogn Sci 7, 77-83.

Gallagher, H.L., Jack, A.I., Roepstorff, A., Frith, C.D., 2002. Imaging the intentional stance in a competitive game. Neuroimage 16, 814-821.

Garretson, H.B., Fein, D., Waterhouse, L., 1990. Sustained attention in children with autism. J Autism Dev Disord 20, 101-114.

Greene, J.D., Sommerville, R.B., Nystrom, L.E., Darley, J.M., Cohen, J.D., 2001. An fMRI investigation of emotional engagement in moral judgment. Science 293, 2105-2108.

Gusnard, D.A., Akbudak, E., Shulman, G.L., Raichle, M.E., 2001. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci U S A 98, 4259-4264.

Haznedar, M.M., Buchsbaum, M.S., Wei, T.C., Hof, P.R., Cartwright, C., Bienstock, C.A., Hollander, E., 2000. Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. Am J Psychiatry 157, 1994-2001.

Iacoboni, M., Lieberman, M.D., Knowlton, B.J., Molnar-Szakacs, I., Moritz, M., Throop, C.J., Fiske, A.P., 2004. Watching social interactions produces dorsomedial prefrontal and medial parietal BOLD fMRI signal increases compared to a resting baseline. Neuroimage 21, 1167-1173.

Johnson, S.C., Baxter, L.C., Wilder, L.S., Pipe, J.G., Heiserman, J.E., Prigatano, G.P., 2002. Neural correlates of self-reflection. Brain 125, 1808-1814.

Kanner, L., 1943. Autistic disturbances of affective contact. Nervous Child 2, 217-250.

Kelley, W.M., Macrae, C.N., Wyland, C.L., Caglar, S., Inati, S., Heatherton, T.F., 2002. Finding the self? An event-related fMRI study. J Cogn Neurosci 14, 785-794.

Kjaer, T.W., Nowak, M., Lou, H.C., 2002. Reflective self-awareness and conscious states: PET evidence for a common midline parietofrontal core. Neuroimage 17, 1080-1086.

Lord, C., Rutter, M., Le Couteur, A., 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord 24, 659-685.

Lord, C., Risi, S., Lambrecht, L., Cook, E.H., Jr., Leventhal, B.L., DiLavore, P.C., Pickles, A., Rutter, M., 2000. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30, 205-223.

Lou, H.C., Luber, B., Crupain, M., Keenan, J.P., Nowak, M., Kjaer, T.W., Sackeim, H.A., Lisanby, S.H., 2004. Parietal cortex and representation of the mental Self. Proc Natl Acad Sci U S A 101, 6827-6832.

Luna, B., Minshew, N.J., Garver, K.E., Lazar, N.A., Thulborn, K.R., Eddy, W.F., Sweeney, J.A., 2002. Neocortical system abnormalities in autism: an fMRI study of spatial working memory. Neurology 59, 834-840.

Macrae, C.N., Moran, J.M., Heatherton, T.F., Banfield, J.F., Kelley, W.M., 2004. Medial prefrontal activity predicts memory for self. Cereb Cortex 14, 647-654.

Maddock, R.J., Garrett, A.S., Buonocore, M.H., 2001. Remembering familiar people: the posterior cingulate cortex and autobiographical memory retrieval. Neuroscience 104, 667-676.

Maddock, R.J., Garrett, A.S., Buonocore, M.H., 2003. Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. Hum Brain Mapp 18, 30-41.

Mason, M.F., Norton, M.I., Van Horn, J.D., Wegner, D.M., Grafton, S.T., Macrae, C.N., 2007. Wandering minds: the default network and stimulus-independent thought. Science 315, 393-395.

Mazoyer, B., Zago, L., Mellet, E., Bricogne, S., Etard, O., Houde, O., Crivello, F., Joliot, M., Petit, L., Tzourio-Mazoyer, N., 2001. Cortical networks for working memory and executive functions sustain the conscious resting state in man. Brain Res Bull 54, 287-298.

McKiernan, K.A., Kaufman, J.N., Kucera-Thompson, J., Binder, J.R., 2003. A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging. J Cogn Neurosci 15, 394-408.

Mitchell, J.P., Banaji, M.R., Macrae, C.N., 2005a. General and specific contributions of the medial prefrontal cortex to knowledge about mental states. Neuroimage 28, 757-762.

Mitchell, J.P., Neil Macrae, C., Banaji, M.R., 2005b. Forming impressions of people versus inanimate objects: social-cognitive processing in the medial prefrontal cortex. Neuroimage 26, 251-257.

Muller, R.A., Pierce, K., Ambrose, J.B., Allen, G., Courchesne, E., 2001. Atypical patterns of cerebral motor activation in autism: a functional magnetic resonance study. Biol Psychiatry 49, 665-676.

Nagai, Y., Critchley, H.D., Featherstone, E., Trimble, M.R., Dolan, R.J., 2004. Activity in ventromedial prefrontal cortex covaries with sympathetic skin conductance level: a physiological account of a "default mode" of brain function. Neuroimage 22, 243-251.

Ochsner, K.N., Beer, J.S., Robertson, E.R., Cooper, J.C., Gabrieli, J.D., Kihsltrom, J.F., D'Esposito, M., 2005. The neural correlates of direct and reflected self-knowledge. Neuroimage 28, 797-814.

Ozonoff, S., Jensen, J., 1999. Brief report: specific executive function profiles in three neurodevelopmental disorders. J Autism Dev Disord 29, 171-177.

Paulus, M.P., Frank, L.R., 2003. Ventromedial prefrontal cortex activation is critical for preference judgments. Neuroreport 14, 1311-1315.

Pierce, K., Haist, F., Sedaghat, F., Courchesne, E., 2004. The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. Brain 127, 2703-2716.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc Natl Acad Sci U S A 98, 676-682.

Shulman, G.L., Fiez, J.A., Corbetta, M., Buckner, R.L., Miezin, F.M., Raichle, M.E., Petersen, S.E., 1997. Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. J Cogn Neurosci 9, 648-663.

Vogeley, K., May, M., Ritzl, A., Falkai, P., Zilles, K., Fink, G.R., 2004. Neural correlates of first-person perspective as one constituent of human self-consciousness. J Cogn Neurosci 16, 817-827.

Vogeley, K., Bussfeld, P., Newen, A., Herrmann, S., Happe, F., Falkai, P., Maier, W., Shah, N.J., Fink, G.R., Zilles, K., 2001. Mind reading: neural mechanisms of theory of mind and self-perspective. Neuroimage 14, 170-181.

Wang, A.T., Dapretto, M., Hariri, A.R., Sigman, M., Bookheimer, S.Y., 2004. Neural correlates of facial affect processing in children and adolescents with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 43, 481-490.

Whalen, P.J., Bush, G., McNally, R.J., Wilhelm, S., McInerney, S.C., Jenike, M.A., Rauch, S.L., 1998. The emotional counting Stroop paradigm: a functional magnetic resonance imaging probe of the anterior cingulate affective division. Biol Psychiatry 44, 1219-1228.

Williams, J.H., Waiter, G.D., Perra, O., Perrett, D.I., Whiten, A., 2005. An fMRI study of joint attention experience. Neuroimage 25, 133-140.

CHAPTER II

Failing to deactivate: Resting functional abnormalities in autism

Abstract

Several regions of the brain (including medial prefrontal cortex, rostral anterior cingulate, posterior cingulate, and precuneus) are known to have high metabolic activity during rest, which is suppressed during cognitively-demanding tasks. With functional magnetic resonance imaging (fMRI), this suppression of activity is observed as "deactivations," which are thought to be indicative of an interruption of the mental activity that persists during rest. Thus, measuring deactivation provides a means by which rest-associated functional activity can be quantitatively examined. Applying this approach to autism, we found that the autism group failed to demonstrate this deactivation effect. Furthermore, there was a high correlation between a clinical measure of social impairment and functional activity within the ventral medial prefrontal cortex. We speculate that the lack of deactivation in the autism group is indicative of abnormal internally-directed processes at rest, which may be an important contribution to the social and emotional deficits of autism.

Introduction

Internally-directed processes, such as self-reflective thought and most higher-order social and emotional processes, consistently activate a medial cortical network involving several brain regions – namely, the medial prefrontal cortex (MPFC) and adjacent rostral anterior cingulate cortex (rACC), posterior cingulate cortex (PCC), and precuneus (PrC) (Maddock, 1999; Gusnard and Raichle, 2001; Northoff and Bermpohl, 2004; Ochsner et al., 2005). Interestingly, this network is active when normal subjects are passively resting (Raichle et al., 2001), leading many to speculate that these internally-directed thoughts dominate the resting state (Gusnard et al., 2001; Mazoyer et al., 2001; Kjaer et al., 2002; Cavanna and Trimble, 2006). Self-reports from subjects while at rest further support this interpretation, wherein they typically describe "autobiographical reminiscences, either recent or ancient, consisting of familiar faces, scenes, dialogues, stories, and melodies" (Mazoyer et al., 2001). Conversely, activity in this midline "resting network" is reduced when subjects perform externally-directed, attention-demanding, goal-oriented tasks (such as the Stroop task or math calculations), and the resulting "deactivation" of this network is thought to be an indicator of an interruption of ongoing internally-directed thought processes (Shulman et al., 1997; Gusnard et al., 2001; Raichle et al., 2001; McKiernan et al., 2003; Cavanna and Trimble, 2006). In this context, the term "deactivation" simply refers to activity that is greater during rest than during task performance (i.e. the opposite of the more typically reported activations). Thus, an objective method for testing the functioning of this midline resting network is to measure whether there is deactivation in these regions during externally-directed tasks as compared to passive rest. Similar approaches of examining this "deactivation effect" have

been utilized in studies of patients with fragile X (Menon et al., 2004), a developmental disorder with some characteristics that overlap with autism, and in patients with dementia of the Alzheimer type (Lustig et al., 2003) and Alzheimer's disease (Greicius et al., 2004).

There are several lines of evidence to suggest that this resting network might be functioning abnormally in autism. First, the functions it subserves (including emotional processing (Whalen et al., 1998; Maddock et al., 2003; Cato et al., 2004), perception of social interactions (Iacoboni et al., 2004), theory of mind (Fletcher et al., 1995; Vogeley et al., 2001; Gallagher et al., 2002; Gallagher and Frith, 2003), experience of joint attention (Williams et al., 2005), and person familiarity (Maddock et al., 2001; Pierce et al., 2004)) overlap remarkably well with the social and emotional deficits that characterize autism. Second, in anterior regions of this network, researchers have documented volumetric, metabolic, cellular, and developmental growth abnormalities in this disorder (reviewed in Courchesne et al., 2004). Third, neuroimaging studies of autism have often observed functional abnormalities in these midline cortical regions during a variety of both socioemotional (Castelli et al., 2002; Pierce et al., 2004; Wang et al., 2004) *and* non-socioemotional tasks (Ring et al., 1999; Muller et al., 2001; Luna et al., 2002). These functional abnormalities in non-socioemotional tasks (e.g. a visually-cued motor task (Muller et al., 2001)) are particularly interesting because they suggest that the results may be due to resting baseline differences between groups, rather than task-related differences.

To objectively determine whether in autism this resting network functions abnormally, we used functional magnetic resonance imaging (fMRI) to measure this

deactivation effect in autistic and normal control subjects. In order to control for task performance across patient and control groups, we used the Stroop task because it is known that autistic patients are unimpaired in this task relative to controls (Eskes et al., 1990; Ozonoff and Jensen, 1999). To demonstrate that the magnitude of the deactivation effect in these regions can be modulated in control but not autistic subjects without changing the specific task demands, three conditions of the counting Stroop task were used; one with incongruent number stimuli (NUMBER), one with emotional stimuli (EMOTIONAL), and one with neutral stimuli (NEUTRAL). Activity in each condition was compared to passive rest. To examine both individual subject and group effects, we used a whole-brain group analysis followed-up with a region of activation approach in which the pattern of activity for each autistic and control individual could be characterized and quantified.

We had two main comparisons of interest: First, we predicted that the greatest level of functional deactivation from a passive resting state in control subjects would be seen in the most cognitively demanding task condition, since this would draw the most attention away from internal thought processes that occur during rest. Based on previous research on the counting Stroop task (Whalen et al., 1998), we chose the NUMBER condition to contrast with rest, since counting the number of words while reading incongruent numbers would create more interference (as reflected in longer reaction times and lower accuracy) than counting the number of emotional or neutral words. We hypothesized that in subjects with autism, while the NUMBER condition will still be the most cognitively demanding condition, this deactivation in the NUMBER vs. REST contrast would be absent, thus implicating differences in functional activity (and,

therefore, functional processes) during rest. Second, based on previous studies using negatively-valenced words (Whalen et al., 1998), we predicted that control subjects would show relatively greater activity in anterior midline regions (MPFC and rACC) in the EMOTIONAL compared to the NEUTRAL condition. However, we hypothesized that autistic subjects would fail to show this activity related to implicit emotional processing of negatively-valenced words. Such a pattern of results would suggest that there is abnormal functioning of the midline resting network in autism during rest and emotion processing, which we speculate may reflect a more general failure to engage in the types of internally-directed thoughts which normally recruit this network.

Methods

Participants:

Fifteen participants with autism spectrum disorder (ASD) (10 high functioning autism, 3 Asperger's syndrome, 2 pervasive developmental disorder) (2 left handed, 1 ambidextrous) and 14 healthy controls (3 left-handed) participated in this experiment. Three participants with ASD (2 autism, 1 PDD-NOS) were excluded from all analyses due to uncorrectable head motion. All participants or their legal guardian gave informed written consent and were monetarily compensated for participation in the experiment. The protocol was approved by the Institutional Review Board of UCSD and Children's Hospital at San Diego. All ASD participants were diagnosed by a clinical psychologist using the Autism Diagnostic Interview – Revised (ADI-R) (Lord et al., 1994) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000), and administered the Wechsler Adult Intelligence Scale (WAIS) or WAIS-R (Revised). The mean ages of

Table 2.1: Clinical information for ASD subjects

				ADI-R			IQ		
Subject	**Diagnosis**	**Age**	**Sex**	**Social (cutoff = 10)**	**Communication (cutoff = 8)**	**Stereotypy (cutoff = 3)**	**Verbal**	**Performance**	**Full-Scale**
A1	autism	39.2	M	21	22	10	98	114	104
A2	autism	27.4	M	45	20	4	80	81	79
A3	autism	19.8	M	27	22	12	86	109	98
A4	autism	16.8	M	27	19	7	76	89	80
A5	autism	15.2	M	26	19	6	108	108	108
A6	autism	44.3	M	22	19	6	89	92	89
A7	autism	23.5	M	35	32*	19	115	116	117
A8	autism	37.1	M	26	20	6	68	92	76
A9	PDD-NOS	16.6	M	30	20	10	70	75	70
A10	Asperger's	21.7	M	7	11	10	116	109	114
A11	Asperger's	23.2	M	22	22	8	106	118	112
A12	Asperger's	21.2	M	21	20	7	111	99	106
Mean (s.d.)		**25.5 (9.61)**		**25.8 (9.1)**	**19.5 (3.0)**	**8.8 (4.0)**	**93.6 (17.7)**	**100.2 (14.3)**	**96.1 (16.5)**

* cutoff = 10

ASD participants (25.49±9.61) and control participants (26.07±7.95) were not significantly different [t(24)=-.169, p=.87]. Clinical data for individual ASD subjects are reported in **Table 2.1**.

Stimuli:

While in the scanner, subjects performed a counting Stroop task with three conditions of interest (Bush et al., 1998). This task was based on the classic color-word Stroop (Stroop, 1935) but adapted to be more suitable for the fMRI environment (Bush et al., 1998). Instead of naming the color of a word, subjects were instructed to count the number of words that appeared on the screen and respond as quickly and accurately as possible by pressing the button on the response device corresponding to either two, three, or four words. Subjects were presented with three different types of words: emotional, neutral, and number words. Number words were always incongruent with the number of words that appeared on the screen (e.g. “two” written 3 times). The emotional words were chosen from a set of negatively-valenced emotional words that had been rated by a separate set of participants for degree of emotional arousal. Ten words with the highest ratings of emotional arousal were included in the study. Examples of these include “murder,” “torture,” and “blood.” The ten neutral words, each naming a household item, included words like “table,” “curtain,” and “desk.” Stimuli were presented for 1.5 seconds each in a block design with each block containing 20 presentations of either EMOTIONAL, NEUTRAL, or NUMBER words, for a total of 30 seconds per block. There were 12 task blocks, interlaced with three 21 second rest periods, wherein subjects were simply instructed to passively view a fixation cross.

Behavioral data acquisition and analysis:

Stimuli were presented using the Presentation software package (Neurobehavioral Systems Inc., Albany, CA), whereby reaction time and number of errors were recorded during scanning. Additionally, immediately after scanning, each participant was asked to complete a surprise word recognition test outside of the scanner. The test consisted of two columns; one with twenty neutral words describing household items and one with twenty negatively-valenced emotional words. Subjects were instructed to identify the words they recognized from the scanning session.

Subject responses were scored for average reaction time (from stimulus onset until subject response) and percent correct for each condition. Data from the word recognition test were scored for hits, misses, and false alarms and then converted into a percent correct score. All behavioral analyses were conducted with SPSS 12.0 statistical software package (SPSS Inc., Chicago, IL). Two repeated-measures ANOVAs were run with group (autism, control) as the between subjects factor and reaction time or percent correct for each of the three conditions (EMOTIONAL, NEUTRAL, NUMBER) as the within subjects factor. A third repeated measures ANOVA was run on the post-test word recognition data with group as the between subjects variable and percent correct for each condition (emotional words or neutral words) as the within subjects factor.

Functional imaging data acquisition and analysis:

Images were acquired on a Siemens Symphony 1.5 Tesla Scanner at the UC San Diego Hillcrest Medical Center. Whole brain axial slices were collected with a gradient-recalled echo-planar imaging (EPI) pulse sequence (TR (repetition time) = 3000 ms; TE (echo time) = 35 ms; flip angle = 90 deg; field of view (FOV) = 256 mm; matrix = 64x64 ($4mm^2$ in-plane resolution); slice thickness = 4 mm; # of slices = 30; # of volumes =

148). A T1-weighted anatomical image using an MPRAGE sequence was collected during each scan session for co-registration with the functional images. Anatomical scans were collected in the sagittal plane (FOV = 256 mm; matrix = 256x256 (1 mm^2 in-plane resolution); slice thickness = 1 mm; # of slices = 180).

All fMRI analyses were conducted with the Analysis of Functional Neuroimages statistical software package (AFNI; version 2.56; http://afni.nimh.nih.gov/afni) (Cox, 1996). Motion correction and three-dimensional registration of each participant's functional images were performed using an automated alignment program (3dvolreg), which co-registered each volume in the time series to the middle volume acquired in that run using an iterative process. Brief periods of motion in nine ASD subjects that were uncorrectable by 3dvolreg were removed from analysis. The percent of the run that was removed ranged from 2.7 % to 14.9 % (mean = 7.13 %). Images were then smoothed with a Gaussian filter (full-width half-maximum = 6 mm).

Individual data were analyzed using the program 3dDeconvolve. This program first estimates an impulse response function (IRF) based on the measured fMRI signal data and input stimulus functions. These 9 functions included the 3 word conditions (NUMBER, NEUTRAL, EMOTIONAL) and 6 motion parameters derived from the output of 3dVolreg (intra-run motion in the x, y, and z, and roll, pitch, and yaw planes). The IRF was then convolved with the input stimulus time series and multiple regressions were run to determine a “goodness of fit” coefficient (or linear contrast weight). The global mean and linear trend were included in the regression to remove their effect from the analysis. For each condition, the linear contrast weight was determined for 0, 3, 6, and 9 seconds after stimulus presentation. These weights were summed for each condition to

give an overall best-fit for each condition compared to the REST condition. Additionally, an a priori contrast of EMOTIONAL versus NEUTRAL was determined for each individual.

Group Analysis: In order to average data across participants, the functional images were then transformed into Talaraich space. T-tests were conducted to determine if linear contrast weights for both the autism and control group were significantly different from zero for each of the four main comparisons of interest (EMOTIONAL vs. REST; NUMBER vs. REST; NEUTRAL vs. REST; EMOTIONAL vs. NEUTRAL). An additional set of t-tests was then run to determine if the contrast weights were significantly different between the autism and control groups for the four comparisons. All data were intensity thresholded at $p < 0.01$ and cluster thresholded at a voxel-wise alpha level of $p < 0.05$. The contrasts of any condition vs. REST reveal both regions of activation and deactivation, and are displayed together in each image shown.

Exploratory Correlation Analysis: Since we predicted that functional activity at rest is related to social behavioral impairment in ASD, we ran a whole brain regression analysis between the NUMBER vs. REST contrast and the social subscale score on the ADI-R. Since results did not survive correction for multiple comparisons based on cluster volume, data was thresholded at $p < 0.005$, uncorrected, and we termed this analysis "exploratory."

Individual Region of Activation Analysis: In order to examine individual differences in fMRI data, values were first normalized to percent signal change from baseline (baseline = mean signal intensity across the entire run). Specifically, the mean baseline MR signal intensity was subtracted from each image on a voxel-by-voxel basis

and the resulting value was divided by the baseline for that voxel and multiplied by 100. Mean percent signal change for each condition was determined by averaging images acquired 3 seconds after the start of the block until 3 seconds after the end of the block.

We chose two ROA masks of interest. First, to examine individual differences in both the deactivation during the NUMBER vs. REST condition and activation during the EMOTIONAL vs. NEUTRAL condition, we created a mask derived from the overlap between functional activity in the control subjects in the NUMBER vs. REST and EMOTIONAL vs. NEUTRAL conditions ($p < 0.01$, cluster corrected). Then, in order to reduce the amount of noise introduced into this individual analysis, only data at the individual level that reached significance at $p < 0.05$ (uncorrected) was extracted from within this mask. Thus, only data from the voxels in the NUMBER vs. REST comparison that survived this individual subject threshold were used to determine the individual NUMBER vs. REST mean percent signal change value. Similarly, data from voxels in the EMOTIONAL vs. NEUTRAL comparison that survived this threshold comprised the individual EMOTIONAL vs. NEUTRAL mean percent signal change value.

Our second ROA mask was created from a cortical region consisting of the activity common to all three task conditions vs. REST in control subjects (thresholded at $p < 0.01$, cluster corrected). This mask was chosen to ensure that the variability we observed in our first ROA was not common to all regions, and that it was not a consequence of our analysis procedure (e.g. selecting the mask based on control subjects). Analysis methods were the same as those described above, such that voxels that fell within this ROA mask were thresholded at the individual subject level at $p < 0.05$

for NUMBER vs. REST and EMOTIONAL vs. REST conditions separately. Then, percent signal change data was extracted from these voxels and averaged, providing a single resulting value for each subject and for each condition.

Results

Behavior:

As predicted, autism spectrum disorder (ASD) and control participants showed similar behavioral responses while performing the counting Stroop task (see **Figure 2.1a, b**). Reaction time did not differ significantly between groups [ASD = 676.1 ms, control = 664.1 ms; $F(1,23)=.097$; $p=.759$]; however, there was a main effect of condition [$F(2,22)=4.811$; $p=.018$]. Contrasts corrected for multiple comparisons revealed that this effect was due to significantly longer reaction times in the NUMBER condition (692.0 ms) as compared to the EMOTIONAL (659.3 ms) and NEUTRAL condition (659.0 ms) [$F(1,23)=10.05$; $p=.004$]. There was no significant interaction between condition and group for reaction time [$F(2,22)=.730$; $p=.493$]. In the accuracy data, the ASD subjects did have a significantly lower overall percent correct score than the control group [ASD = 95.8%, control = 98.8%; $F(1,22)=6.499$; $p=.018$]. However, mean accuracy for both groups was over 95% for all three conditions, indicating that despite group differences, both groups were performing the task with a high level of accuracy. There was also a main effect of condition with number showing a lower percent correct than the other two conditions [NEUTRAL = 97.7%, EMOTIONAL = 97.4%, NUMBER = 96.7%; $F(2,21)=7.08$; $p=.004$] but no significant interaction between condition and group [$F(2,21)=.679$; $p=.518$].

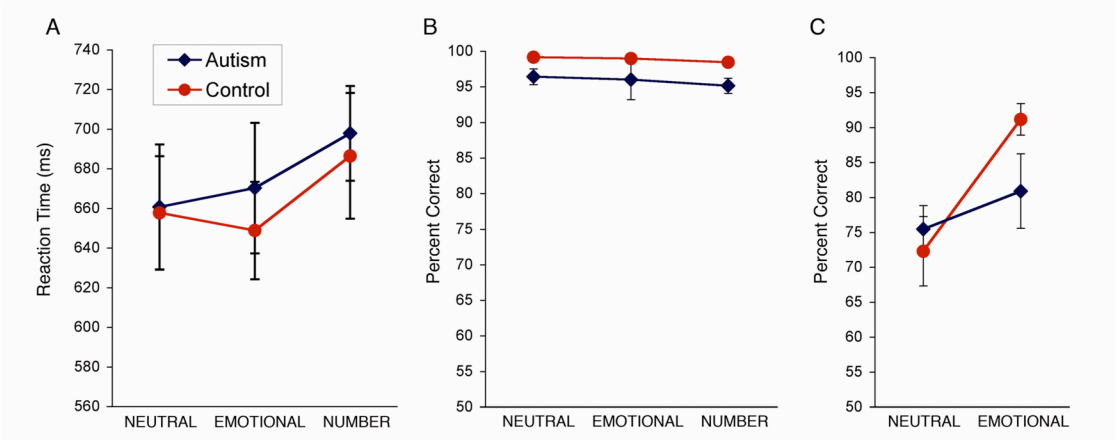


Figure 2.1. Behavioral data for control and autism groups. **a)** Reaction time and **b)** percent correct measures obtained during fMRI acquisition. **c)** Percent correct values from the word recognition test that immediately followed scanning. Autism = blue diamonds, Control = red circles

Immediately after image acquisition, subjects were given a word recognition task in which they were asked to mark all of the words they had seen during scanning. Repeated measures ANOVA revealed a main effect of word type (emotional vs. neutral) [Emotional = 86.0%, Neutral = 73.8%; $F(1,19)=11.43$; $p=.003$] and a trend towards an interaction between group and word type [$F(1,19)=4.24$; $p=.062$] (**Figure 2.1c**). Follow-up within group t-tests revealed a significant effect of word type in control subjects [Neutral = 72.2%, Emotional = 91.1%; $t(1,8)=-4.02$; $p=.004$] but not in ASD subjects [Neutral = 75.4%, Emotional = 80.8%; $t(1,11)=-1.15$; $p=.274$].

Functional Imaging:

Group Analysis: For control subjects, whole brain analysis of the NUMBER vs. REST contrast revealed large regions of deactivation in MPFC/rACC and PCC/PrC ($p < 0.01$, cluster corrected) (**Figure 2.2a**; see also **Table 2.2**). However, this deactivation was entirely absent in ASD subjects. Further, a direct group comparison between control and ASD subjects revealed a significant difference between groups in MPFC/rACC and PrC (**Figure 2.2a**; see also **Table 2.3**). The right superior temporal sulcus (STS) and bilateral angular gyrus also deactivated in control but not ASD subjects, although these regions were not significantly different in the direct group comparison (see **Table 2.2** and **Table 2.3**).

Similar, but weaker effects, were seen in the NEUTRAL vs. REST and EMOTIONAL vs. REST comparisons. In the NEUTRAL vs. REST contrast (**Figure 2.2b**), cluster volume in control subjects was reduced compared to that seen in the NUMBER vs. REST comparison, likely reflecting differences in difficulty between the conditions (McKiernan et al., 2003). Again, the autism group showed no significant

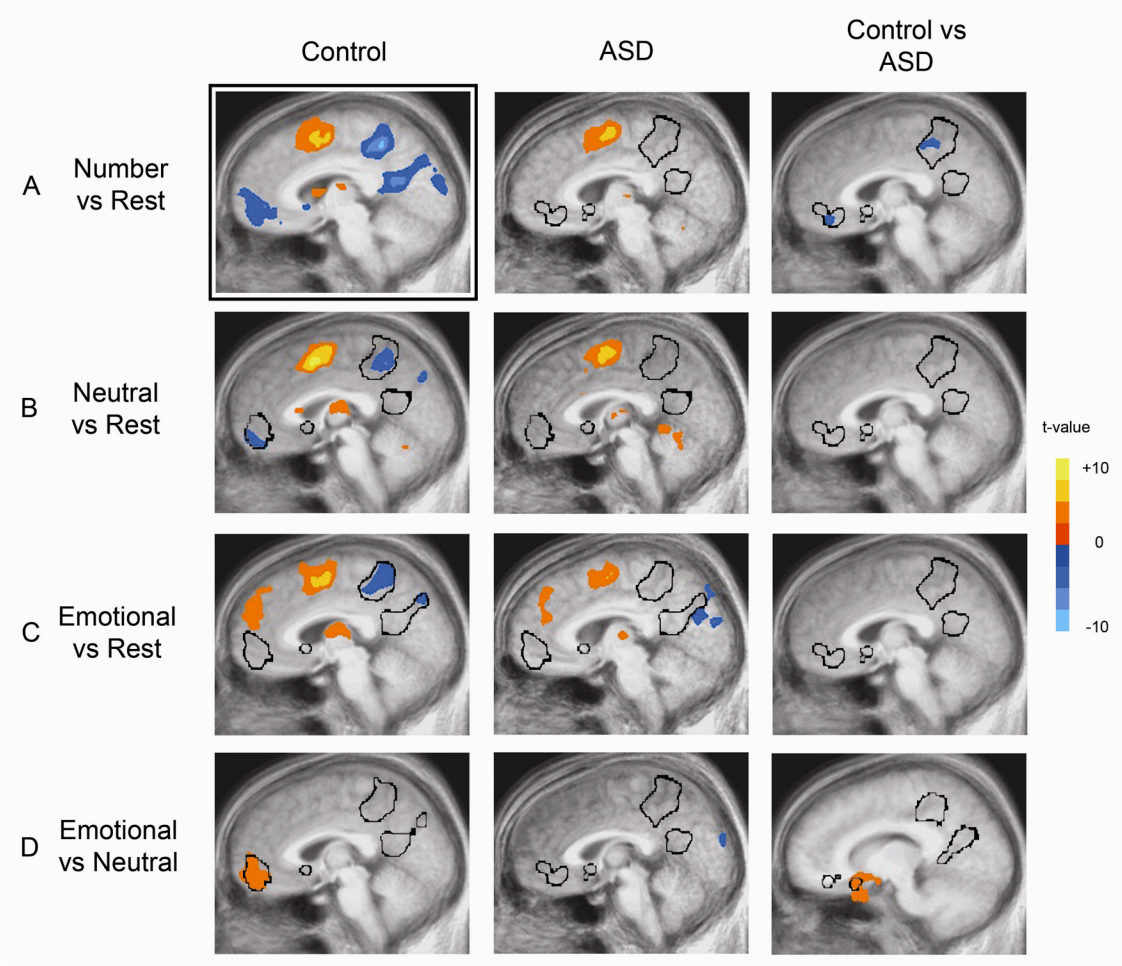


Figure 2.2. Significant functional activity derived from group whole-brain analyses ($p < 0.01$, cluster corrected). T-values are displayed, with negative t-values representing deactivations. The black outlines correspond to the area of deactivation derived from the NUMBER vs. REST condition in controls (**a**, left panel), mapped onto the sagittal slices of the images shown. These outlines, which represent regions active during rest, highlight the presence or absence of activations or deactivations in each image. Each sagittal slice location differs slightly since each image was chosen in order to best represent the midline activity for each group and comparison. The comparisons shown are **a**) NUMBER vs. REST, **b**) NEUTRAL vs. REST, **c**) EMOTIONAL vs. REST, and **d**) EMOTIONAL vs. NEUTRAL.

Table 2.2: Regions of activation and deactivation in control and ASD groups

Control						ASD				
Condition	Region	Talairach Coordinates (x,y,z)	Brodmann Area	t-value	Volume (mm^3)	Region	Talairach Coordinates (x,y,z)	Brodmann Area	t-value	Volume (mm^3)
Number vs rest										
Activations						Activations				
	L Precentral & Postcentral Gyrus, Inferior Parietal Lobule, Bilateral Supplementary Motor Area	(-45, -36, 55)	40/4/9/6/7	3.217	66,878	L & R Precentral & L & R Postcentral Gyrus, L & R Inferior Parietal Lobule, Bilateral Supplementary Motor Area	(-41, -41, 55)	40/4/7/6	5.315	94,002
	R Inferior Parietal Lobule	(39, -53, 52)	40	3.089	5,428	L Inferior Frontal and Precentral gyrus	(-51, 7, 34.)	9/44/6	3.108	9,262
	R Precentral Gyrus	(50, 4, 35)	6	3.747	4,114	R Inferior Occipital Gyrus	(10, -89, -12)	18	3.16	7,484
	L Fusiform Gyrus	(-30, -81, -13)	19/18	3.889	12,647	L Inferior Occipital Gyrus	(-30, -84, -8)	18	6.186	5,244
	R Fusiform Gyrus	(46, -76, -16)	19	3.087	10,562	R Cerebellum	(26, -45, -20)		3.583	2,951
	R Cerebellum	(31, -61, -17)		3.111	3,991	L Insula	(-42, 4, 4)		3.151	1,879
	L Thalamus	(-2, -11, 16)		6.257	3,616	L Thalamus	(-5, -13, 13)		3.116	1,903
	R Caudate	(31, 16, 11)		3.961	3,125	R Thalamus	(7, -12, 17)		3.137	1,412
Deactivations						Deactivations				
	Bilateral Posterior Cingulate & Precuneus	(-1, -49, 16)	30/23/31	4.258	19,169	R Cuneus	(15, -84, 35)	19	3.371	2,042
	Bilateral Precuneus	(1, -41, 67)	7	3.029	15,677					
	L Medial Frontal & Anterior Cingulate	(-1, 51, 3)	10/32/24/25	3.748	6,763					
	R Anterior Cingulate	(-1, 12, -4)	25	3.263	1,335					
	L Angular Gyrus	(-41, -73, 27)	39	5.876	3,705					
	R Angular Gyrus	(50, -64, 19)	39	3.242	3,321					
	R Postcentral Gyrus	(54, -5, 7)	43	3.285	1,834					
	R Superior Temporal Sulcus	(50, -1, -13)	21	4.919	1,315					
Emotional vs Neutral										
Activations						Activations				
	L Medial Frontal & Anterior Cingulate	(-2, 51, -4)	10/32	3.928	5,655	NONE				
	L Temporal pole	(-18, 7, -28)	28/38	3.175	4,635					
	R Temporal pole	(35, 12, -16)	38	3.588	1,943					
Deactivations						Deactivations				
	R Inferior Parietal Lobule	(50, -48, 47)	40	3.613	1,423	R Middle Occipital Gyrus	(38, -73, 12)	19	3.325	3,571
						R Precentral Gyrus	(43, -8, 55)	6	3.904	2,705
						L Postcentral gyrus	(-34, -40, 63)	2	3.113	1,447
						R Postcentral gyrus	(54, -24, 48)	2	3.392	1,036
						R Middle Occipital Gyrus	(10, -81, 40)	19	3.144	1,109
						R Inferior Parietal Lobule	(50, -48, 48)	40	3.116	2,517
						L Inferior Parietal Lobule	(-50, -34, 55)	40	3.114	1,042

Talairach coordinates are listed for the voxel with the maximum intensity in each cluster

Table 2.3: Regions of significant difference between ASD and control groups

Control vs ASD					
Condition	Region	Talairach Coordinates (x,y,z)	Brodmann Area	t-value	Volume (mm^3)
Number vs rest					
Control > ASD					
	NONE				
ASD > Control					
	R Supramarginal Gyrus	(54, -24, 48)	40	2.865	2878
	R Precuneus	(2, -44, 47)	7	3.217	2290
	R Inferior Parietal Lobule	(42, -40, 56)	40	2.832	2097
	L Inferior Parietal Lobule	(-57, -29, 31)	40	2.994	1109
	R Superior Frontal Gyrus	(19, -12, 55)	6	4.831	1477
	L Medial Frontal/Anterior Cingulate	(-5, 39, -9)	32	3.259	1096
Emotional vs Neutral					
Control > ASD					
	R medial Orbitofrontal Cortex	(15, 20, -12)	47	4.045	2714
	R Middle Occipital Gyrus	(38, -72, 12)	19/39	3.15	1096
ASD > Control					
	NONE				

Talairach coordinates are listed for the voxel with the maximum intensity in each cluster

deactivations in this comparison. Similarly, in the EMOTIONAL vs. REST contrast (**Figure 2.2c**), significant deactivations were seen in the PCC and PrC in controls but not in ASD subjects. Furthermore, a significant cluster of positive activity was observed in the dorsal MPFC in both groups, although this activity was of a greater extent in control subjects. In both of these comparisons, there were no significant differences in these midline regions in the direct group comparison.

To determine functional activity specific to emotional processing, we contrasted the EMOTIONAL vs. NEUTRAL conditions (**Figure 2.2d**). As predicted, significantly increased activity was observed in the ventral MPFC/rACC, extending into the medial portion of the orbital frontal cortex, for control subjects, but this activation was absent in ASD subjects (**Table 2.2**). Direct group comparison revealed significantly greater activity in the control subjects in this medial orbital region of the resting network (**Figure 2.2d** and **Table 2.3)**.

Finally, to ensure that there were no between group differences in functional activity relating to the interference aspect of the task (namely, in the dorsal ACC (Bush et al., 1998)), we examined group differences between the NUMBER vs. NEUTRAL contrast. Even at a more relaxed threshold ($p < 0.05$, uncorrected), dorsal ACC activity was still not significantly different between groups (see **Figure 2.3**), although differences can be seen in MPFC and PrC.

Exploratory Correlational Analysis: We found a cluster of voxels in the ventral MPFC ($p < 0.005$, uncorrected) whose activity during the NUMBER vs. REST contrast significantly correlated with the social subscale score on the ADI-R in ASD individuals [$r(11)=.939$; $p < 0.0001$)] (**Figure 2.4**). This region was partially overlapping with the

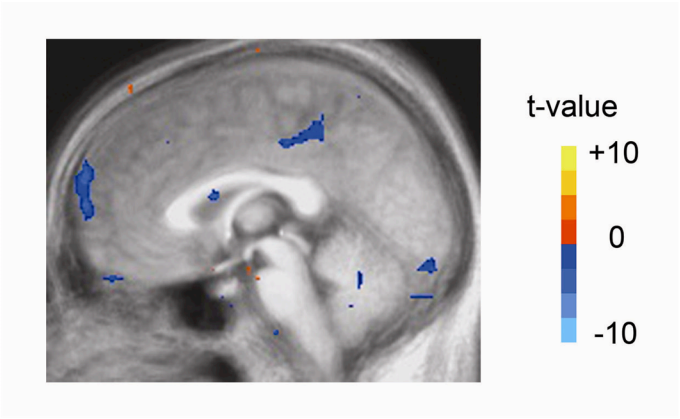


Figure 2.3. A between group comparison for the NUMBER vs. NEUTRAL contrast, shown at $p < 0.05$ (uncorrected). Even at this more relaxed threshold, there is no group difference in dorsal ACC activity.

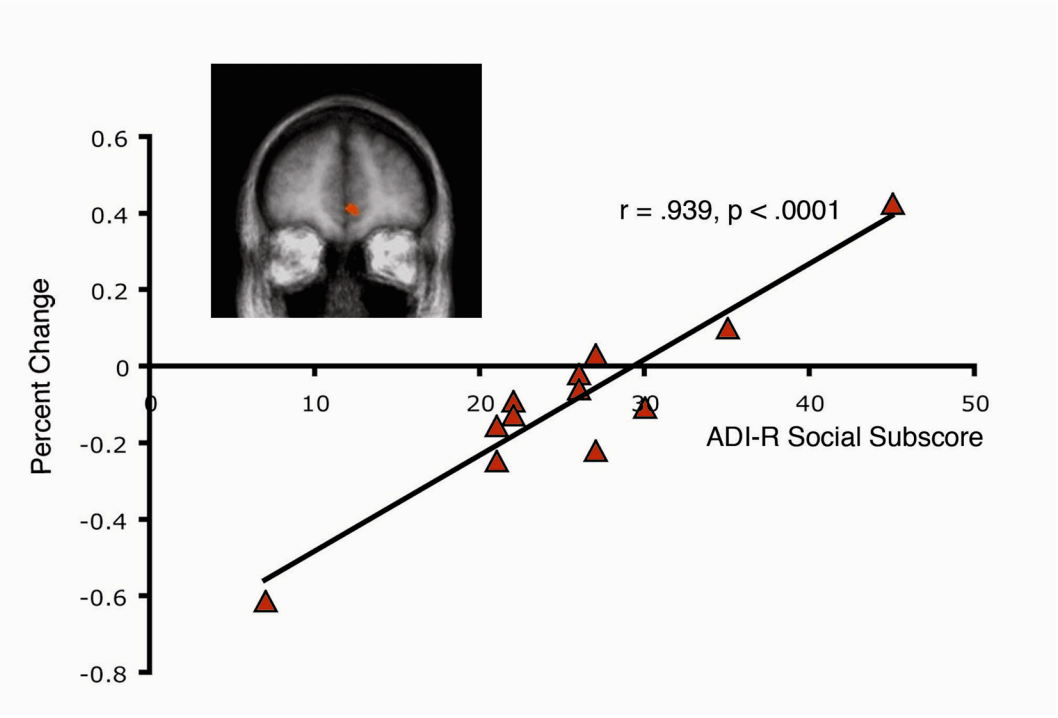


Figure 2.4. Correlation between functional activity in a ventral MPFC cluster (see inset) and score on the social subscale of the ADI-R in the ASD group.

region found to be significantly different between the ASD and control groups in the NUMBER vs. REST contrast (see **Figure 2.4** inset). ASD subjects with greater deactivation had lower social impairment scores, whereas those that showed less deactivation (or, in certain individuals, activation) had greater social impairment scores. Furthermore, this correlation was still significant when the two extreme points (i.e. subjects with the lowest and highest scores on the social subscale) were removed from the analysis [$r(9)=.669$; $p = 0.034$].

Region of Activation-Guided Individual Analysis: Since we noted individual variability in deactivation and activation patterns in the ASD subjects, we chose to examine the data at the individual subject level. To identify regions common to cognitive deactivation and emotional activation, a region-of-activation (ROA) mask was created from the overlap between the NUMBER vs. REST and the EMOTIONAL vs. NEUTRAL conditions in control subjects (both thresholded at $p < 0.01$, cluster corrected). This overlapping functional activity was restricted to the MPFC (see inset in **Figure 2.5a**).

As seen in **Figure 2.5a**, the majority of control subjects (11 of 14) showed the expected pattern of deactivation in the NUMBER condition and activation in the EMOTIONAL vs. NEUTRAL condition. However, this pattern was not consistently seen in ASD subjects. Interestingly, 3 of the 4 PDD and Asperger's subjects who participated in this study showed the typical pattern of activation and deactivation.

To test if the variability seen in the autism group was simply a result of the ROA being selected based on the control subjects' data, we created another control-defined ROA mask in a different region. This mask was based on the functional activity common to the NUMBER vs. REST, NEUTRAL vs. REST, and EMOTIONAL vs. REST

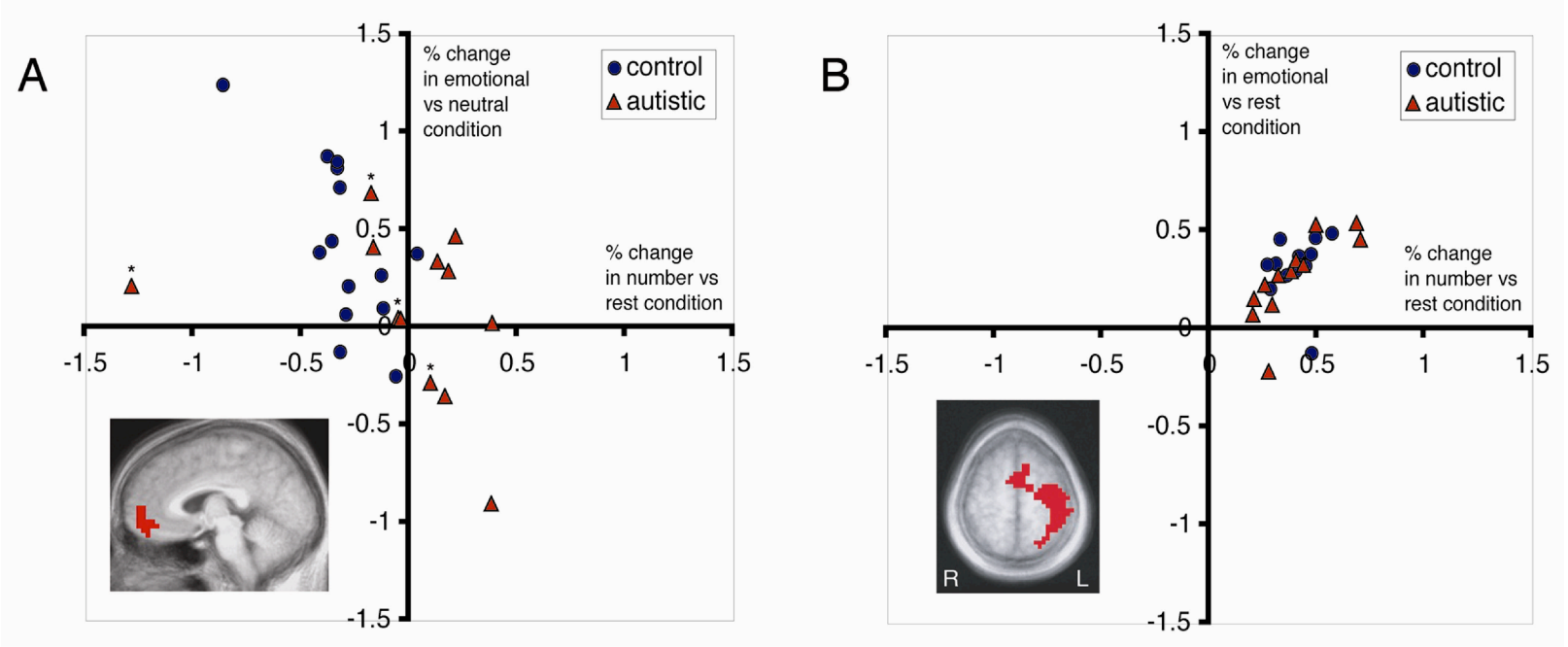


Figure 2.5. Individual subject analyses. **a)** Percent change values in the MPFC region (ROA mask is shown in inset) in the NUMBER vs. REST condition (x-axis) and the EMOTIONAL vs. NEUTRAL condition (y-axis). Results demonstrate that, unlike control participants, the majority of subjects with autism failed to show a consistent pattern of both deactivation in the NUMBER vs. REST condition and activation in the EMOTIONAL vs. NEUTRAL condition. * = Asperger's and PDD-NOS subjects. **b)** Percent change values in sensory and motor cortices (ROA mask shown in the inset) in the NUMBER vs. REST condition (x-axis) and the EMOTIONAL vs. REST condition (y-axis). Subjects with autism and control subjects showed similar activity in these conditions, demonstrating that the abnormality observed in **Figure 2.5a** is not simply due to the analysis methods.

contrasts, and included the left precentral gyrus, left postcentral gyrus, and supplementary motor area/dorsal ACC (see inset in **Figure 2.5b**), likely reflecting a combination of motor, sensory, and Stroop-related inhibitory neurofunctional processes. Results showed that there was an extremely close correspondence in activity between both ASD and control subjects (**Figure 2.5b**). Thus, the observed individual ASD variability in MPFC likely reflects real individual variability, rather than simply a result of the analysis procedure used.

There were no significant correlations between functional activity in the MPFC ROA-mask and behavioral performance (i.e. reaction times, Stroop performance accuracy, word recognition accuracy) in control or ASD groups. There was, however, a correlation between activity in the NUMBER condition and activity in the EMOTIONAL vs. NEUTRAL comparison within the MPFC ROA in control [$r(13)=-.67$; $p = .008$] but not ASD subjects [$r(11)=-.32$; $p=.304$] (**Figure 2.5a**). Furthermore, no significant correlations were found between functional activity in the MPFC ROA-mask and verbal, performance, or full-scale IQ in the ASD group.

Discussion

Our results demonstrate that autism spectrum disorder (ASD) subjects fail to show the deactivation effect in resting network regions (MPFC/rACC and PCC/PrC) in the NUMBER vs. REST comparison (**Figure 2.2a**). These results can not be accounted for merely by differences in task performance since 1) no differences were seen in reaction time between groups, 2) slightly reduced accuracy (and thus, increased difficulty) in the ASD group should lead to greater deactivation (McKiernan et al., 2003),

although we observed the exact opposite result, 3) there were no significant correlations between task performance and functional activity in the anterior ROA-mask, and 4) there were no neurofunctional differences in regions involved in Stroop task performance (namely, the dorsal ACC) (see **Figure 2.2a;** see also **Figure 2.3 and Figure 2.5b**). ASD subjects also failed to show normal behavioral and neurofunctional processing of emotional stimuli. While control subjects showed a recognition bias for emotional words, the autism group did not show an effect of word type on recognition ability, consistent with previous behavioral reports in autism (Beversdorf et al., 1998). Further, we demonstrated that autistic subjects failed to show the normal pattern of functional activation in the medial orbital frontal region of the resting network when processing emotional words compared to neutral words (**Figure 2.2d**), demonstrating a failure of emotional task-induced modulation of this region. Finally, in the ASD group, there was a high correlation ($r = 0.939$) between a clinical measure of social impairment and functional activity in the ventral MPFC (**Figure 2.4**). The subjects with higher social impairment scores had less deactivation in the NUMBER vs. REST contrast (i.e. the greater the behavioral abnormality, the greater the neurofunctional abnormality), and vice versa. We should emphasize, however, that we have only identified a correlative relationship between abnormal social behavior and brain activity, rather than a causative one. Thus, although tantalizing, this result can not be used to argue that neurofunctional abnormality causes social impairment, or that social impairment causes neurofunctional abnormality.

There are two possible reasons why the ASD group failed to show the typical deactivation effect. One possibility is that midline resting network activity during both

rest and task performance is high, and thus, a subtraction between these conditions would reveal no difference in activity levels. We believe, however, that it is unlikely that high midline network activity was maintained during the cognitively-demanding NUMBER task in autism for several reasons. First, as mentioned previously, behavioral performance was similar between control and ASD groups. This, however, would be unexpected if the ASD group were carrying out additional mental processing that control subjects inhibit during cognitively demanding conditions. Second, PET studies of autism, which provide an absolute measure of brain metabolism, have found reduced, as opposed to increased, glucose metabolism in rACC and PCC (Haznedar et al., 2000) during task performance, as compared to controls. Further, one PET study found that lower blood flow in MPFC and rACC at rest was correlated with more severe social and communicative impairments in subjects with autism (Ohnishi et al., 2000), a finding similar to our correlational results. Third, reduced anatomical volumes and neurochemical deficiencies have consistently been observed in MPFC/rACC in adults with autism (reviewed in Courchesne et al., 2004), likely indicative of reduced functioning of these regions. Therefore, an alternative explanation, the one to which we attribute the lack of deactivation, is that midline activity is low during rest. We suggest, then, that the absence of deactivation in this network indicates that the mental processes that normally occur at rest are absent or abnormal in autism.

What are these mental processes that dominate during rest? Evidence in the literature, to date, seems to suggest that tasks that induce certain types of internal processing activate this resting network. Examples of such tasks are self- and other-person judgments (Fletcher et al., 1995; Gusnard et al., 2001; Vogeley et al., 2001;

Gallagher et al., 2002; Johnson et al., 2002; Kelley et al., 2002; Kjaer et al., 2002; Fossati et al., 2003; Gallagher and Frith, 2003; Lou et al., 2004; Macrae et al., 2004; D'Argembeau et al., 2005; Mitchell et al., 2005b; Mitchell et al., 2005a; Ochsner et al., 2005), person familiarity judgments (Maddock et al., 2001; Pierce et al., 2004), emotion processing (Whalen et al., 1998; Adolphs, 1999; Maddock et al., 2003; Cato et al., 2004), perspective-taking (Vogeley et al., 2001; Vogeley et al., 2004), passive observation of social interactions vs. non-social interactions (Iacoboni et al., 2004), relaxation based on interoceptive biofeedback (Critchley et al., 2001; Nagai et al., 2004), conceptual judgments (based on internal knowledge stores) vs. perceptual judgments (Binder et al., 1999), and episodic memory tasks (Andreasen et al., 1995), among others (moral decision making, joint attention experience, pleasantness judgments (Greene et al., 2001; Paulus and Frank, 2003; Williams et al., 2005)). Therefore, the activity in these regions at rest might simply reflect the extent to which these types of internally-directed thoughts are engaged at rest. In fact, a particularly intriguing behavioral study found that individuals with ASD report very different internal thoughts than control subjects (Hurlburt et al., 1994; Frith and Happe, 1999), lending support to our interpretation that an absence of this resting activity in autism may be directly related to abnormal internal thought. Admittedly, this is a speculative hypothesis, but one that can be explicitly tested in future experiments.

The findings in the present study are supported by previous functional imaging studies of autism, which have consistently found abnormalities in these midline regions during a variety of tasks. While these functional abnormalities would be expected in socioemotional tasks (i.e. personally-familiar face processing (Pierce et al., 2004), facial

affect processing (Wang et al., 2004), theory of mind (Castelli et al., 2002)) since these resting network regions support these processes, these abnormalities have also been seen using non-socioemotional tasks (i.e. spatial working memory (Luna et al., 2002), visually-cued motor task (Muller et al., 2001), embedded figures task (Ring et al., 1999)). Further, within both socioemotional and non-socioemotional task domains, the direction of abnormality has not been consistent, in that some studies report greater activity while others report reduced activity as compared to control subjects. Unfortunately, interpretation of these findings is not straight-forward, since different studies report results in different ways (e.g. individual group results or direct group comparisons) and use different control conditions. For instance, in our study, if we only reported results from the direct comparison for control vs. ASD subjects in the NUMBER vs. REST condition, it would seem like ASD subjects had greater activity in resting network regions (**Figure 2.2a,** right panel), although such an oversimplified interpretation would be flawed since it actually reflects an absence of deactivation in the autism group. Thus, although functional abnormality within midline anterior and posterior regions has been repeatedly shown in ASD, the precise nature of and explanation for these abnormalities have been unclear based on past studies. We offer an encompassing explanation for these disparate imaging results by suggesting that the seemingly inconsistent findings can be attributed to a lack of presenting individual group data and to a lack of a common reference to a resting baseline condition.

Although our discussion of resting network regions has been limited to midline resting regions, other regions, including STS, temporal pole, and angular gyrus, are known to show the same deactivation effect (Binder et al., 1999; Raichle et al., 2001). In

fact, we observed deactivation in these regions in control subjects in the NUMBER vs. REST contrast, and, as expected, these regions failed to deactivate in the ASD group (**Table 2.2**). However, functional activity in these regions was not found to be significantly different in our relatively conservative direct group comparison ($p < .01$, whole-brain corrected), likely due to the smaller size and increased anatomic variability of these regions (particularly, the STS) as compared to midline regions. Interestingly, several functional imaging studies have shown abnormalities in these regions (Baron-Cohen et al., 1999; Castelli et al., 2002; Gervais et al., 2004; Pierce et al., 2004; Pelphrey et al., 2005). Follow-up studies using region of interest approaches or sulcal mapping are needed to determine the functionality of these other resting network regions in autism.

It is particularly remarkable that the autism behavioral phenotype can be caused by a number of different factors (including genetic, infectious, and environmental toxins), suggesting these factors are all likely acting on a common neural substrate that must be particularly vulnerable to developmental insult. In fact, Raichle and colleagues (Raichle et al., 2001) speculated that the PrC and PCC might be highly susceptible to damage due to their high metabolic rate. Further, since these highly active midline regions are richly connected with numerous cortical and subcortical regions (Carmichael and Price, 1995; Ongur and Price, 2000; Kobayashi and Amaral, 2003; Parvizi et al., 2006), an early insult affecting any one of these areas may have devastating, widespread consequences on brain connectivity and subsequent functionality. Interestingly, a recent diffusion tensor imaging study on autism identified white matter abnormality in the region underlying the rACC/MPFC (Barnea-Goraly et al., 2004). Furthermore, several fMRI studies have suggested reduced functional connectivity in the autistic brain in a variety of tasks

(Horwitz et al., 1988; Castelli et al., 2002; Just et al., 2004; Koshino et al., 2005). Functional connectivity studies, which allow for observation of brain activity during rest (Greicius et al., 2003; Fox et al., 2005) or naturalistic viewing conditions (Bartels and Zeki, 2005), may be particularly useful in further characterizing resting state abnormalities in autism.

Although purely speculative, it is interesting to hypothesize about the nature of resting thoughts in ASD, if the typical internally-directed thoughts are absent or abnormal. One possibility is that their thoughts are directed toward their obsessive interests and preoccupations (Frith and Happe, 1999), which are often of a concrete, as opposed to abstract and subjective, nature (for instance, calendars, maps, or schedules). Or, perhaps hypersensitivity to their external environment constantly interrupts the full emergence and elaboration of internally-directed thoughts.

In sum, this study demonstrates that neural activity at rest in ASD is abnormal, which suggests that individuals with ASD might fail to engage in typical internally-directed resting thoughts. Furthermore, during an emotion processing task, both behavioral performance and functional activity in a specific region of this resting network (medial orbital frontal cortex) was abnormal in ASD, suggesting that behavioral impairment might persist in tasks which recruit these resting network regions. Finally, the amount of functional abnormality in MPFC correlated with the amount of social impairment in individuals with ASD. Future experiments exploring resting network activity and organization in ASD, along with examining specific aspects of resting functional processes, such as self-reflection, may provide valuable insights into the neurocognitive basis of the disorder.

Acknowledgements

We thank the subjects who graciously gave of their time to participate in this study. This research was supported by NIH RO1 MH36840 awarded to E.C.

This chapter, in full, is a reprint of material as it appears in *PNAS* 2006; Kennedy, DP, Redcay, E, and Courchesne, E; 103(21); 8275-8280. The dissertation author was co-primary author on this paper. The secondary author was the thesis advisor.

References

Adolphs, R., 1999. Social cognition and the human brain. Trends Cogn Sci 3, 469-479.

Andreasen, N.C., O'Leary, D.S., Cizadlo, T., Arndt, S., Rezai, K., Watkins, G.L., Ponto, L.L., Hichwa, R.D., 1995. Remembering the past: two facets of episodic memory explored with positron emission tomography. Am J Psychiatry 152, 1576-1585.

Barnea-Goraly, N., Kwon, H., Menon, V., Eliez, S., Lotspeich, L., Reiss, A.L., 2004. White matter structure in autism: preliminary evidence from diffusion tensor imaging. Biol Psychiatry 55, 323-326.

Baron-Cohen, S., Ring, H., Wheelwright, S., Bullmore, E.T., Brammer, M.J., Simmons, A., Williams, S.C.R., 1999. Social intelligence in the normal and autistic brain: an fMRI study. Eur J Neurosci 11, 1891-1898.

Bartels, A., Zeki, S., 2005. Brain dynamics during natural viewing conditions-A new guide for mapping connectivity in vivo. Neuroimage 24, 339-349.

Beversdorf, D.Q., Anderson, J.M., Manning, S.E., Anderson, S.L., Nordgren, R.E., Felopulos, G.J., Nadeau, S.E., Heilman, K.M., Bauman, M.L., 1998. The effect of semantic and emotional context on written recall for verbal language in high functioning adults with autism spectrum disorder. J Neurol Neurosurg Psychiatry 65, 685-692.

Binder, J.R., Frost, J.A., Hammeke, T.A., Bellgowan, P.S., Rao, S.M., Cox, R.W., 1999. Conceptual processing during the conscious resting state. A functional MRI study. J Cogn Neurosci 11, 80-95.

Bush, G., Whalen, P.J., Rosen, B.R., Jenike, M.A., McInerney, S.C., Rauch, S.L., 1998. The counting Stroop: an interference task specialized for functional neuroimaging--validation study with functional MRI. Hum Brain Mapp 6, 270-282.

Carmichael, S.T., Price, J.L., 1995. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. J Comp Neurol 363, 615-641.

Castelli, F., Frith, C., Happe, F., Frith, U., 2002. Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. Brain 125, 1839-1849.

Cato, M.A., Crosson, B., Gokcay, D., Soltysik, D., Wierenga, C., Gopinath, K., Himes, N., Belanger, H., Bauer, R.M., Fischler, I.S., Gonzalez-Rothi, L., Briggs, R.W., 2004. Processing words with emotional connotation: an FMRI study of time

course and laterality in rostral frontal and retrosplenial cortices. J Cogn Neurosci 16, 167-177.

Cavanna, A.E., Trimble, M.R., 2006. The precuneus: a review of its functional anatomy and behavioural correlates. Brain 129, 564-583.

Courchesne, E., Redcay, E., Kennedy, D.P., 2004. The autistic brain: birth through adulthood. Curr Opin Neurol 17, 489-496.

Cox, R.W., 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 29, 162-173.

Critchley, H.D., Melmed, R.N., Featherstone, E., Mathias, C.J., Dolan, R.J., 2001. Brain activity during biofeedback relaxation: a functional neuroimaging investigation. Brain 124, 1003-1012.

D'Argembeau, A., Collette, F., Van der Linden, M., Laureys, S., Del Fiore, G., Degueldre, C., Luxen, A., Salmon, E., 2005. Self-referential reflective activity and its relationship with rest: a PET study. Neuroimage 25, 616-624.

Eskes, G.A., Bryson, S.E., McCormick, T.A., 1990. Comprehension of concrete and abstract words in autistic children. J Autism Dev Disord 20, 61-73.

Fletcher, P.C., Happe, F., Frith, U., Baker, S.C., Dolan, R.J., Frackowiak, R.S., Frith, C.D., 1995. Other minds in the brain: a functional imaging study of "theory of mind" in story comprehension. Cognition 57, 109-128.

Fossati, P., Hevenor, S.J., Graham, S.J., Grady, C., Keightley, M.L., Craik, F., Mayberg, H., 2003. In search of the emotional self: an FMRI study using positive and negative emotional words. Am J Psychiatry 160, 1938-1945.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 102, 9673-9678.

Frith, U., Happe, F., 1999. Theory of mind and self-consciousness: What is it like to be autistic? Mind Lang 14, 1-22.

Gallagher, H.L., Frith, C.D., 2003. Functional imaging of 'theory of mind'. Trends Cogn Sci 7, 77-83.

Gallagher, H.L., Jack, A.I., Roepstorff, A., Frith, C.D., 2002. Imaging the intentional stance in a competitive game. Neuroimage 16, 814-821.

Gervais, H., Belin, P., Boddaert, N., Leboyer, M., Coez, A., Sfaello, I., Barthelemy, C., Brunelle, F., Samson, Y., Zilbovicius, M., 2004. Abnormal cortical voice processing in autism. Nat Neurosci 7, 801-802.

Greene, J.D., Sommerville, R.B., Nystrom, L.E., Darley, J.M., Cohen, J.D., 2001. An fMRI investigation of emotional engagement in moral judgment. Science 293, 2105-2108.

Greicius, M.D., Krasnow, B., Reiss, A.L., Menon, V., 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 100, 253-258.

Greicius, M.D., Srivastava, G., Reiss, A.L., Menon, V., 2004. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 101, 4637-4642.

Gusnard, D.A., Raichle, M.E., 2001. Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2, 685-694.

Gusnard, D.A., Akbudak, E., Shulman, G.L., Raichle, M.E., 2001. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci U S A 98, 4259-4264.

Haznedar, M.M., Buchsbaum, M.S., Wei, T.C., Hof, P.R., Cartwright, C., Bienstock, C.A., Hollander, E., 2000. Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. Am J Psychiatry 157, 1994-2001.

Horwitz, B., Rumsey, J.M., Grady, C.L., Rapoport, S.I., 1988. The cerebral metabolic landscape in autism. Intercorrelations of regional glucose utilization. Arch Neurol 45, 749-755.

Hurlburt, R.T., Happe, F., Frith, U., 1994. Sampling the form of inner experience in three adults with Asperger syndrome. Psychol Med 24, 385-395.

Iacoboni, M., Lieberman, M.D., Knowlton, B.J., Molnar-Szakacs, I., Moritz, M., Throop, C.J., Fiske, A.P., 2004. Watching social interactions produces dorsomedial prefrontal and medial parietal BOLD fMRI signal increases compared to a resting baseline. Neuroimage 21, 1167-1173.

Johnson, S.C., Baxter, L.C., Wilder, L.S., Pipe, J.G., Heiserman, J.E., Prigatano, G.P., 2002. Neural correlates of self-reflection. Brain 125, 1808-1814.

Just, M.A., Cherkassky, V.L., Keller, T.A., Minshew, N.J., 2004. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 127, 1811-1821.

Kelley, W.M., Macrae, C.N., Wyland, C.L., Caglar, S., Inati, S., Heatherton, T.F., 2002. Finding the self? An event-related fMRI study. J Cogn Neurosci 14, 785-794.

Kjaer, T.W., Nowak, M., Lou, H.C., 2002. Reflective self-awareness and conscious states: PET evidence for a common midline parietofrontal core. Neuroimage 17, 1080-1086.

Kobayashi, Y., Amaral, D.G., 2003. Macaque monkey retrosplenial cortex: II. Cortical afferents. J Comp Neurol 466, 48-79.

Koshino, H., Carpenter, P.A., Minshew, N.J., Cherkassky, V.L., Keller, T.A., Just, M.A., 2005. Functional connectivity in an fMRI working memory task in high-functioning autism. Neuroimage 24, 810-821.

Lord, C., Rutter, M., Le Couteur, A., 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord 24, 659-685.

Lord, C., Risi, S., Lambrecht, L., Cook Jr., E., Leventhal, B., DiLavore, P., Pickles, A., Rutter, M., 2000. The Autism Diagnostic Observation Schedule—Generic: A Standard Measure of Social and Communication Deficits Associated with the Spectrum of Autism. J Autism Dev Disord 30, 205-223.

Lou, H.C., Luber, B., Crupain, M., Keenan, J.P., Nowak, M., Kjaer, T.W., Sackeim, H.A., Lisanby, S.H., 2004. Parietal cortex and representation of the mental Self. Proc Natl Acad Sci U S A 101, 6827-6832.

Luna, B., Minshew, N.J., Garver, K.E., Lazar, N.A., Thulborn, K.R., Eddy, W.F., Sweeney, J.A., 2002. Neocortical system abnormalities in autism: an fMRI study of spatial working memory. Neurology 59, 834-840.

Lustig, C., Snyder, A.Z., Bhakta, M., O'Brien, K.C., McAvoy, M., Raichle, M.E., Morris, J.C., Buckner, R.L., 2003. Functional deactivations: change with age and dementia of the Alzheimer type. Proc Natl Acad Sci U S A 100, 14504-14509.

Macrae, C.N., Moran, J.M., Heatherton, T.F., Banfield, J.F., Kelley, W.M., 2004. Medial prefrontal activity predicts memory for self. Cereb Cortex 14, 647-654.

Maddock, R.J., 1999. The retrosplenial cortex and emotion: new insights from functional neuroimaging of the human brain. Trends Neurosci 22, 310-316.

Maddock, R.J., Garrett, A.S., Buonocore, M.H., 2001. Remembering familiar people: the posterior cingulate cortex and autobiographical memory retrieval. Neuroscience 104, 667-676.

Maddock, R.J., Garrett, A.S., Buonocore, M.H., 2003. Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. Hum Brain Mapp 18, 30-41.

Mazoyer, B., Zago, L., Mellet, E., Bricogne, S., Etard, O., Houde, O., Crivello, F., Joliot, M., Petit, L., Tzourio-Mazoyer, N., 2001. Cortical networks for working memory and executive functions sustain the conscious resting state in man. Brain Res Bull 54, 287-298.

McKiernan, K.A., Kaufman, J.N., Kucera-Thompson, J., Binder, J.R., 2003. A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging. J Cogn Neurosci 15, 394-408.

Menon, V., Leroux, J., White, C.D., Reiss, A.L., 2004. Frontostriatal deficits in fragile X syndrome: relation to FMR1 gene expression. Proc Natl Acad Sci U S A 101, 3615-3620.

Mitchell, J.P., Neil Macrae, C., Banaji, M.R., 2005a. Forming impressions of people versus inanimate objects: social-cognitive processing in the medial prefrontal cortex. Neuroimage 26, 251-257.

Mitchell, J.P., Banaji, M.R., Macrae, C.N., 2005b. General and specific contributions of the medial prefrontal cortex to knowledge about mental states. Neuroimage 28, 757-762.

Muller, R.-A., Pierce, K., Ambrose, J.B., Allen, G., Courchesne, E., 2001. Atypical patterns of cerebral motor activation in autism: a functional magnetic resonance study. Biol Psychiatry 49, 665-676.

Nagai, Y., Critchley, H.D., Featherstone, E., Trimble, M.R., Dolan, R.J., 2004. Activity in ventromedial prefrontal cortex covaries with sympathetic skin conductance level: a physiological account of a "default mode" of brain function. Neuroimage 22, 243-251.

Northoff, G., Bermpohl, F., 2004. Cortical midline structures and the self. Trends Cogn Sci 8, 102-107.

Ochsner, K.N., Beer, J.S., Robertson, E.R., Cooper, J.C., Gabrieli, J.D., Kihsltrom, J.F., D'Esposito, M., 2005. The neural correlates of direct and reflected self-knowledge. Neuroimage 28, 797-814.

Ohnishi, T., Matsuda, H., Hashimoto, T., Kunihiro, T., Nishikawa, M., Uema, T., Sasaki, M., 2000. Abnormal regional cerebral blood flow in childhood autism. Brain 123, 1838-1844.

Ongur, D., Price, J.L., 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb Cortex 10, 206-219.

Ozonoff, S., Jensen, J., 1999. Brief report: specific executive function profiles in three neurodevelopmental disorders. J Autism Dev Disord 29, 171-177.

Parvizi, J., Van Hoesen, G.W., Buckwalter, J., Damasio, A., 2006. Neural connections of the posteromedial cortex in the macaque. Proc Natl Acad Sci U S A 103, 1563-1568.

Paulus, M.P., Frank, L.R., 2003. Ventromedial prefrontal cortex activation is critical for preference judgments. Neuroreport 14, 1311-1315.

Pelphrey, K.A., Morris, J.P., McCarthy, G., 2005. Neural basis of eye gaze processing deficits in autism. Brain 128, 1038-1048.

Pierce, K., Haist, F., Sedaghat, F., Courchesne, E., 2004. The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. Brain 127, 2703-2716.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc Natl Acad Sci U S A 98, 676-682.

Ring, H.A., Baron-Cohen, S., Wheelwright, S., Williams, S.C.R., Brammer, M., Andrew, C., Bullmore, E.T., 1999. Cerebral correlates of preserved cognitive skills in autism. A functional MRI study of embedded figures task performance. Brain 122, 1305-1315.

Shulman, G.L., Fiez, J.A., Corbetta, M., Buckner, R.L., F.M., M., Raichle, M.E., Peterson, S.E., 1997. Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. J Cogn Neurosci 9, 648-663.

Stroop, J.R., 1935. Studies of interference in serial verbal reactions. J Exp Psychol 18, 643-662.

Vogeley, K., May, M., Ritzl, A., Falkai, P., Zilles, K., Fink, G.R., 2004. Neural correlates of first-person perspective as one constituent of human self-consciousness. J Cogn Neurosci 16, 817-827.

Vogeley, K., Bussfeld, P., Newen, A., Herrmann, S., Happe, F., Falkai, P., Maier, W., Shah, N.J., Fink, G.R., Zilles, K., 2001. Mind reading: neural mechanisms of theory of mind and self-perspective. Neuroimage 14, 170-181.

Wang, A.T., Dapretto, M., Hariri, A.R., Sigman, M., Bookheimer, S.Y., 2004. Neural correlates of facial affect processing in children and adolescents with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 43, 481-490.

Whalen, P.J., Bush, G., McNally, R.J., Wilhelm, S., McInerney, S.C., Jenike, M.A., Rauch, S.L., 1998. The emotional counting Stroop paradigm: a functional magnetic resonance imaging probe of the anterior cingulate affective division. Biol Psychiatry 44, 1219-1228.

Williams, J.H., Waiter, G.D., Perra, O., Perrett, D.I., Whiten, A., 2005. An fMRI study of joint attention experience. Neuroimage 25, 133-140.

CHAPTER III

The intrinsic functional organization of the brain is altered in autism

Abstract

In higher-functioning individuals with autism, a striking disparity exists between impaired social and emotional abilities and relatively preserved sustained attention and goal-directed cognitive abilities. As these two functional domains appear to map onto two distinct large-scale brain networks, the Task-Negative Network and the Task-Positive Network, respectively, we examined their intrinsically-defined functional organization in individuals with autism. Using resting functional connectivity MRI (fcMRI), we found altered functional organization of the network underlying social and emotional processing, but entirely spared functional organization of the network underlying sustained attention and goal-directed cognition. We suggest that these findings serve to relate the seemingly disparate strengths and weaknesses of the autistic behavioral, perceptual, and cognitive phenotype into a tractable neurofunctional framework. These results also highlight the usefulness of resting fcMRI for studying the brain in neuropsychiatric and neurodevelopmental disorders.

Introduction

A cardinal characteristic of autism that has puzzled parents, clinicians, and researchers alike for more than half a century is the imbalance between socioemotional incapacity and disinterest on the one hand and often spared or even heightened cognitive capacity and interest in non-social objects on the other. In the first clinical description of a child with autism, Kanner wrote, "when taken into a room, he completely disregarded the people and instantly went for objects, preferably those that could be spun" (Kanner, 1943). While this fascinating behavioral profile has been well-documented anecdotally, clinically, and experimentally, we know little of the neural circuits responsible. Recent findings regarding the functional organization of the typical brain may help to inform the neural basis of this striking characteristic of autism.

In typically-developing subjects, socioemotional and cognitive-attentional processes appear to map onto distinct large-scale brain networks. One of these networks, termed the Task-Positive Network (TPN) or dorsal attention network, includes pre-supplementary motor area, intraparietal sulcus, and superior precentral sulcus, and activates during performance of externally-directed cognitively-demanding tasks (e.g., math calculations, sustained attention, working memory, Stroop task) (Cabeza and Nyberg, 2000; Corbetta and Shulman, 2002). The other network, termed the Task-Negative Network (TNN; also known as the default mode (Raichle et al., 2001)), includes medial prefrontal cortex, posterior cingulate/precuneus, and angular gyrus, and activates during performance of social, emotional, and introspective (i.e., self-reflective) tasks, including theory of mind (Fletcher et al., 1995; Gallagher et al., 2000; Vogeley et al., 2001), social perception (Iacoboni et al., 2004), emotional processing (Maddock et al.,

2003; Cato et al., 2004), experience of joint attention (Williams et al., 2005), episodic memory (Andreasen et al., 1995), viewing personally familiar faces (Gobbini et al., 2004; Pierce et al., 2004), and self and other-person reflection (Gusnard et al., 2001; Johnson et al., 2002; Kelley et al., 2002; Kjaer et al., 2002; Fossati et al., 2003; Lou et al., 2004; Macrae et al., 2004; D'Argembeau et al., 2005; Mitchell et al., 2005a; Mitchell et al., 2005b; Ochsner et al., 2005; Moran et al., 2006) (for a meta-analysis of self studies, see Northoff et al., 2006). The TNN is so-named because it deactivates (i.e., displays negative activation) during performance of externally-directed cognitively-demanding tasks not of a social, emotional, or introspective nature. This deactivation of the TNN coincident with activation of the TPN further underscores the functional separation of these two networks.

Since autism is largely characterized by deficits in TNN-type processes (i.e., social, emotional, self-relevant) but relatively less impaired, entirely spared, or even heightened in TPN-type processes (Garretson et al., 1990; Allen and Courchesne, 2001) (i.e., cognitively-demanding tasks not of a social, emotional, or self related nature), we hypothesized that this might be due to dysfunction of the TNN but normal functioning of the TPN.

To test this hypothesis, we used a methodological approach known as resting functional connectivity MRI (fcMRI). This approach relies on the fact that, even at rest, the brain exhibits coherent patterns of low frequency spontaneous fluctuations of the BOLD (blood oxygenation level-dependent) signal within functionally-related regions (Buckner and Vincent, 2007). This phenomenon, termed “functional connectivity,” was first documented for the somatomotor system (Biswal et al., 1995), but has since been

observed in sensory networks (e.g., visual, auditory (Cordes et al., 2000)) as well as higher-order cognitive networks (e.g., attention networks (Fox et al., 2006b), recollection memory networks (Vincent et al., 2006)), including the TPN (Fox et al., 2005; Fransson, 2005; Fox et al., 2006b) and TNN (Greicius et al., 2003; Greicius and Menon, 2004; Fox et al., 2005; Fransson, 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Fransson, 2006). Furthermore, these spontaneous oscillations persist during sleep (Fukunaga et al., 2006) and under light anesthesia (Kiviniemi et al., 2000), and have recently been documented to occur in anesthetized monkeys (Vincent et al., 2007). Importantly, because many, if not all, brain networks exhibit these low frequency spontaneous fluctuations, one can examine the functional organization of 2 or more intrinsically organized networks simultaneously – that is, within a single subject and single dataset.

Methods

We scanned a total of 15 male Autism Spectrum Disorder (ASD) and 13 male control subjects, whose only instructions were to stare at the plus on the screen, while remaining still, relaxed, and awake. One ASD subject was removed due to problems with data acquisition, and 2 ASD subjects and 1 control subject were removed from the analysis due to excessive movement during scanning, resulting in a final sample size of 12 ASD subjects (6 autism, 6 Asperger's syndrome) and 12 control subjects. Five of the ASD subjects who were included in the final analysis, and none of the control subjects, participated in an earlier study on TNN functionality in autism (Kennedy et al., 2006). These subjects were selected independent of the earlier results. All participants or their legal guardian gave informed written consent and received monetary compensation for

participation in the experiment. The protocol was approved by the Institutional Review Board of UCSD and Children's Hospital at San Diego. All ASD participants were diagnosed by a clinical psychologist using the Autism Diagnostic Interview – Revised (ADI-R) (Lord et al., 1994) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000), and all participants were administered the Wechsler Adult Intelligence Scale (WAIS) or WAIS-R (Revised). The mean ages of the autism participants (26.5 years) and the control participants (27.5 years) was not significantly different [$t(22)=.201$, $p>.80$]. Subject groups did not differ significantly in verbal, performance, or full-scale IQ [verbal: $t(21)=1.486$, $p=.152$; performance: $t(21)=1.793$, $p=.087$; full-scale: $t(21)=1.973$, $p=.062$]. See **Table 3.1** for detailed clinical information.

All images were acquired on a 3 Tesla GE Signa EXCITE scanner. Axial slices covering the entire brain were collected with a gradient-recalled echo-planar imaging (EPI) pulse sequence with the following parameters: TR (repetition time) = 2000 ms; TE (echo time) = 30 ms; flip angle = 90˚; field of view (FOV) = 220 mm; matrix = 64x64 (3.44 mm^2 in-plane resolution); slice thickness = 4 mm; # of axial slices = 32; # of volumes = 215; total scan time = 7 min, 10 sec). T1-weighted anatomical images were collected during each scan session for co-registration with the functional images (FOV = 256 mm; matrix = 256x256 (1 mm^2 in-plane resolution); slice thickness = 1 mm; # of axial slices = 124).

FcMRI analyses were carried out using the Analyses of Functional NeuroImages (AFNI) statistical software package (version 2.56; http://afni.nimh.nih.gov/afni) (Cox, 1996). In order to facilitate cross-study comparisons, we analyzed the data in a manner very similar to Fox et al. (Fox et al., 2005). Briefly, the preprocessing steps were as

Table 3.1: Clinical information for autism and control participants.

				IQ				ADI-R			ADOS		
Subject	**Diagnosis**	**Age**	**Sex**	**Verbal**	**Performance**	**Full-Scale**	**handedness**	**Social (cutoff = 10)**	**Communication (cutoff = 8)**	**Stereotypy (cutoff = 3)**	**Social (cutoff = 4)**	**Communication (cutoff = 2)**	**Stereotypy**
A1	Autism	15.7	M	73	66	67	Right	10	21	11	10	3	3
A2	Asperger's	16.2	M	120	124	125	Right	13	17	3	11	6	1
A3	Asperger's	17.4	M	99	93	96	Right	23	18	9	9	5	1
A4	Autism	17.7	M	101	118	109	Right	26	19	6	7	5	1
A5	Asperger's	18.3	M	108	107	109	Right	14	8	6	5	3	1
A6	Autism	18.8	M	55	109	80	Right	28	20	4	9	5	0
A7	Asperger's	22.9	M	97	105	101	Right	13	12	3	6	3	0
A8	Asperger's	24.0	M	116	109	114	Right	7	11	10	8	2	2
A9	Asperger's	27.7	M	111	99	106	Right	21	20	7	11	6	0
A10	Autism	41.3	M	98	114	104	Left	21	22	10	11	5	2
A11	Autism	46.4	M	86	115	100	Right	22	19	6	7	5	1
A12	Autism	52.0	M	102	105	104	Right	26	17	6	9	4	1
Mean (SD)		**26.5** (12.8)		**97.2** (18.4)	**105.3** (14.9)	**101.6** (15.2)							
C1	control	15.9	M	95	99	97	Left						
C2	control	16.2	M	N/A	N/A	N/A	Right						
C3	control	17.8	M	107	119	114	Right						
C4	control	19.0	M	106	118	113	Right						
C5	control	20.6	M	99	106	103	Left						
C6	control	22.9	M	107	93	100	Right						
C7	control	25.3	M	109	116	114	Right						
C8	control	29.4	M	109	125	118	Right						
C9	control	32.3	M	108	128	119	Right						
C10	control	40.7	M	108	132	121	Right						
C11	control	44.6	M	106	109	108	Right						
C12	control	45.4	M	108	128	119	Right						
Mean (SD)		**27.5** (10.9)		**105.6** (4.5)	**115.7** (12.7)	**111.5** (8.3)							

follows: the first 5 TRs of each scan were removed to allow for T1 equilibration effects, field maps (which were acquired during the scan session) were used to correct for field inhomogeneities, functional volumes were corrected for motion using an automated alignment program (3dvolreg) that co-registers each volume in the time series to a middle volume of the scan using an iterative process, images were corrected for slice acquisition timing and spatially smoothed with a Gaussian filter (full-width half-maximum = 6 mm), then temporally bandpass filtered ($0.01 < f < 0.1$), and converted to percent signal change. Due to the high sensitivity of fcMRI to subject motion, periods of excessive motion were entirely removed from the analysis. These periods were identified using an objective calculation based on the amount of rotational (roll, pitch, yaw) and translational (x, y, z) movement by the participant across time. This was calculated by first determining the square root of the sum of squares of the derivatives of the rotational and translational movements (calculated by 3dvolreg) for each TR. If the sum of these rotational and translational values exceeded a threshold of 0.30, that TR, along with the TR before and after, was removed from analysis. Subjects with more than 20% of the run removed were excluded entirely from the study (2 ASD, 1 control excluded). For the remaining participants, 4.35% and 4.86% of the run from the control and autism groups, respectively, was removed from analysis. This difference was not significant ($p = .84$). Next, multiple linear regression analysis was used to model several types of noise in the functional data, which were then removed as regressors of no interest: the linear trend, the global signal (average intensity of every voxel across the entire brain calculated for each of the 215 time points), 6 motion parameters (3 rotational and 3 translational directions) and their 6 temporal derivatives. Finally, for group analysis, images were

spatially normalized to Talairach space (Talairach and Tournoux, 1988) using AFNI's 12 sub-volume piecewise linear transformation based on manually-defined landmarks.

The locations of the seed regions were identical to those used in Fox et al. (Fox et al., 2005) to facilitate cross-study comparisons. The three task-positive seed regions (defined from a previous independent study of visual attention (Corbetta et al., 2002)) were the left intraparietal sulcus (-25, -57, 46), the right superior precentral sulcus (25, -13, 50), and the left middle temporal region (-45, -69, -2), while the three task-negative seed regions (defined from a previous meta-analysis of task-related deactivations (Shulman et al., 1997)) were the medial prefrontal cortex (MPFC; -1, 47, -4), posterior cingulate/precuneus (-5, -49, 40), and the left angular gyrus (-45, -67, 36). At each of these seed locations, the BOLD signal timecourse was extracted and used to calculate a correlation coefficient between that seed and all other voxels in the brain. Importantly, the resulting correlation maps (created from individual seed voxels at the coordinates listed above) were nearly identical to maps that were generated with a 12-mm diameter seed region centered at those same coordinates (i.e., the procedure used by Fox et al., 2005).

Six one-sample t-tests for both autism and control groups were performed to find regions of significant correlation with the seed region. These correlation coefficient seed maps were then normalized using Fisher's r-to-z' transformation, and converted to Z-scores. For the control subjects and autism groups separately, the 3 TNN-seed maps were averaged to form a single TNN map, and the 3 TPN-seed maps were averaged to form a single TPN map. These TNN and TPN maps were set to an intensity threshold of $Z = 3.88$, $p < .0001$, and a minimum cluster volume of 192 mm^3. The cluster size was

calculated using an iterative monte carlo simulation using AFNI's AlphaSim program with a voxel-wise threshold of $p < .05$. Throughout the text, we refer to this volume threshold as "volume-corrected". The control TNN and TPN maps were then used to extract timeseries data from individual autism and control subjects (see below).

To determine within-network functional connectivity, all voxels within a particular node (i.e., cluster) were extracted and averaged together to yield a single timeseries for each TNN and TPN node for each individual. Then, using the SPSS statistical software package (Chicago, IL; version 12.0), a separate correlation coefficient was calculated between the timeseries of each node of a network (either TPN or TNN) and the timeseries averaged over the remaining nodes of that particular network. Repeated measures ANOVAs were run on the Fisher's r-to-z' normalized correlation coefficients, with z' values as the repeated measure and group (autism or control) as the between subjects factor. We report the multivariate test output of this analysis since, although a more conservative statistical test, it avoids the assumption of sphericity. Follow-up t-tests were run for all significant interactions, and are reported without correction for multiple comparisons.

To determine between-network functional connectivity (i.e., anticorrelations), the timeseries from all positive voxels and all negative voxels in the group mask were extracted and averaged separately, which yielded the average TPN timeseries and the average TNN timeseries for each subject. Correlation coefficients between these average TPN and TNN timeseries were then calculated for each individual, normalized using Fisher's r-to-z' transformation, and compared across groups with a two-sample t-test.

Results

For the control group, the TNN and TPN functional connectivity maps were very similar to that previously reported by Fox et al. (2005) and Frannson (2005). The TNN map included the dorsal and ventral MPFC, the posterior cingulate/precuneus, left and right angular gyrus, right temporal pole, and right superior temporal gyrus/sulcus ($p < .0001$, volume-corrected) (**Figure 3.1a**, **Table 3.2**). At a more liberal threshold of $p < .001$ (volume-corrected), superior temporal gyrus/sulcus activation was seen bilaterally. The TPN map included bilateral inferior parietal lobule, bilateral superior precentral sulcus, right inferior frontal gyrus, bilateral middle occipital gyrus, bilateral middle temporal gyrus, and right fusiform gyrus ($p < .0001$, volume-corrected) (**Figure 3.2a**, **Table 3.2**).

For the autism group, the TNN map only included the posterior cingulate/precuneus and the left angular gyrus (**Figure 3.1a**, **Table 3.2**). At a more liberal threshold of $p < .001$ (volume-corrected), however, the TNN map also included the dorsal and ventral MPFC, right angular gyrus, and bilateral superior temporal gyrus/sulcus. The TPN map included all the regions seen in the control group (**Figure 3.2a**, **Table 3.2**), and also included bilateral pre-supplementary motor area, left fusiform gyrus, and left putamen. **Figure 3.1b** and **Figure 3.2b** show regions of overlap between groups for the TNN and TPN respectively.

To directly test whether there were group differences in *within-network* functional connectivity of the TNN nodes (n=6) or the TPN nodes (n=7), we ran two repeated-measures ANOVAs. As hypothesized, there was a main effect of group on TNN connectivity [$F(1,22)=5.453$, $p=.029$], with reduced connectivity in the autism group, and

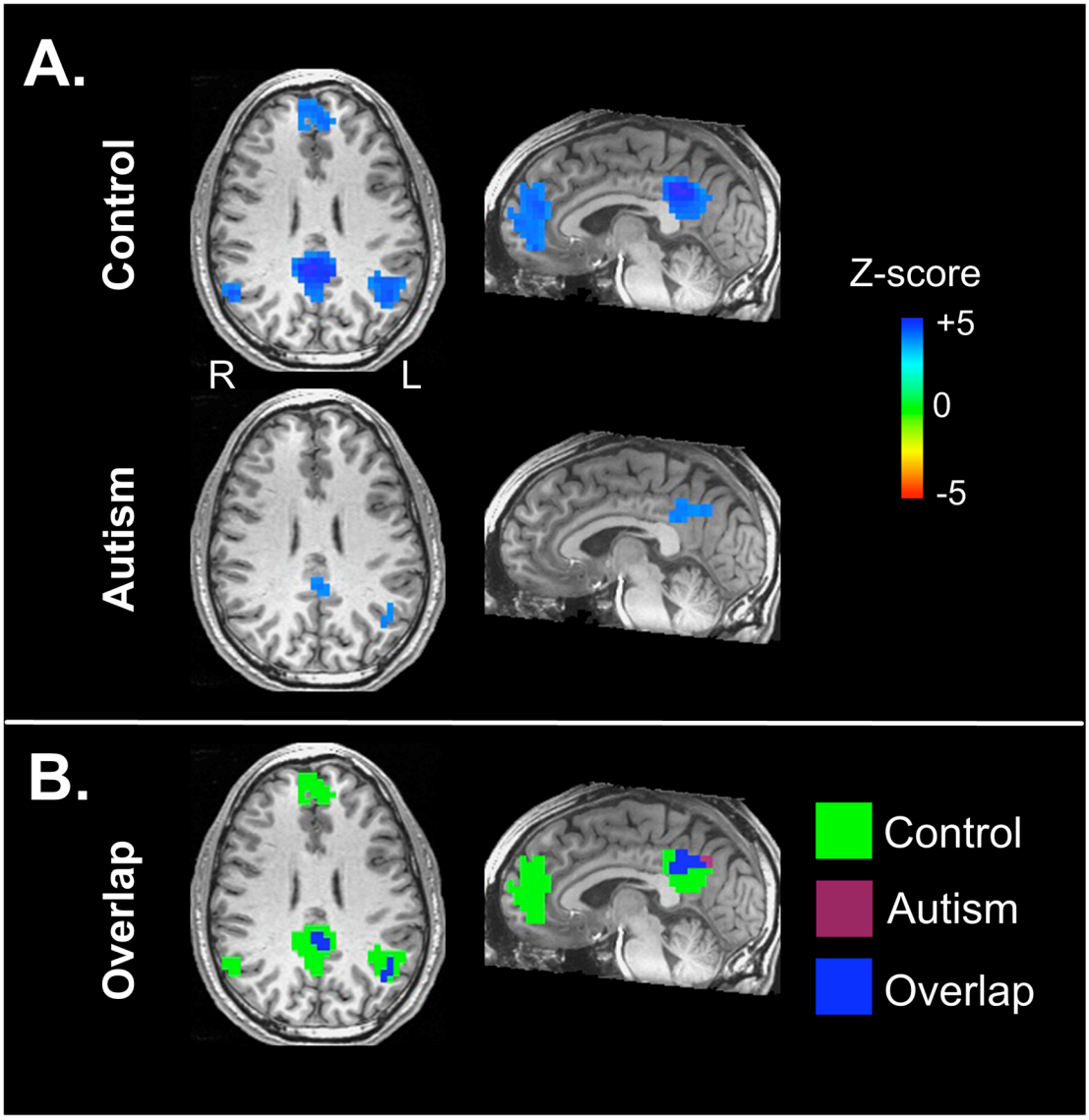


Figure 3.1: Intrinsically-defined functional connectivity maps of the TNN (shown at $p < .0001$, volume-corrected). (a) TNN maps for control and autism groups (Scale bar represent Z-score). (b) Overlap of control and autism TNN maps.

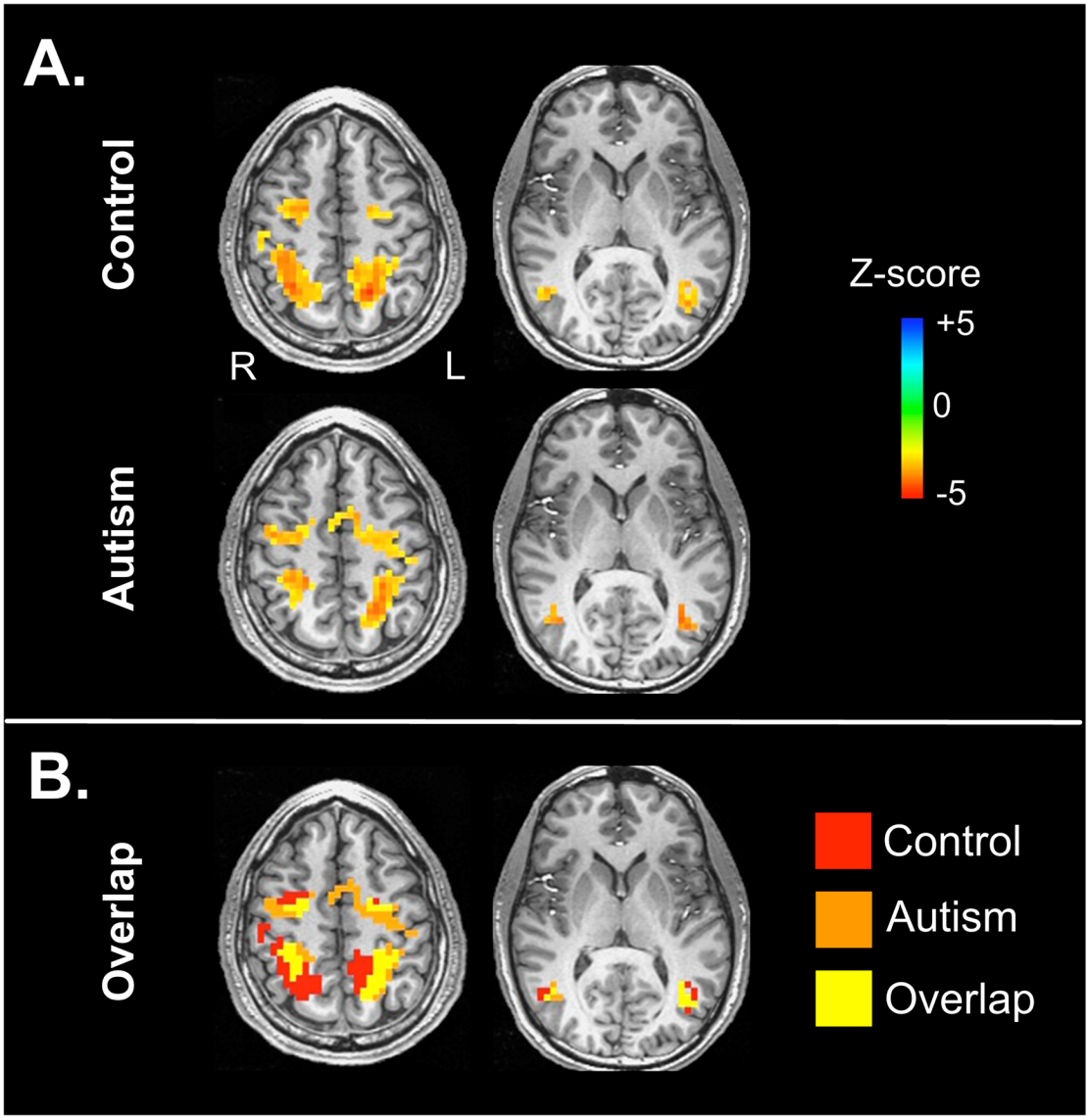


Figure 3.2: Intrinsically-defined functional connectivity maps of the TPN (shown at $p < .0001$, volume-corrected). (a) TPN maps for control and autism groups (Scale bar represents Z-score). (b) Overlap of control and autism TPN maps.

Table 3.2: Talairach locations of peak significance of the intrinsically-defined TNN and TPN functional connectivity maps for control and autism groups.

		CONTROL		AUTISM	
	Region	(X, Y, Z)	Z-value	(X, Y, Z)	Z-value
TNN	ventral MPFC	(2, -47, -4)	4.47		
	dorsal MPFC	(-6, -43, 36)	4.47		
	post. cingulate/precuneus	(-2, 45, 32)	5.16	(6, 45, 36)	4.33
	L angular gyrus	(45, 65, 32)	4.62	(38, 65, 24)	4.14
	R angular gyrus	(-54, 61, 24)	4.68		
	R temporal pole	(-54, 1, -16)	4.17		
	R superior temporal gyrus/sulcus	(-58, 13, -4)	4.27		
	L superior frontal gyrus			(10, -27, 44)	4.03
TPN	L inf. parietal lobule	(26, 45, 56)	4.97	(22, 57, 48)	4.61
	R inf. parietal lobule	(-26, 53, 56)	4.74	(-30, 37, 44)	4.64
	L superior precentral sulcus	(18, 5, 48)	4.28	(22, 9, 56)	4.41
	R superior precentral sulcus	(-22, 5, 52)	4.32	(-34, 13, 44)	4.39
	Bilateral pre-supplementary motor area			(10, 1, 52)	4.43
	R inf. frontal gyrus	(-50, -7, 17)	4.12	(-46, -3, 24)	4.18
	L middle occipital gyrus	(26, 69, 20)	4.42	(26, 69, 24)	3.97
	R middle occipital gyrus	(-38, 69, 16)	4.10		
	L middle temporal gyrus (MT+)	(46, 69, 0)	4.57	(46, 69, 0)	4.58
	R middle temporal gyrus (MT+)	(-46, 57, 0)	4.95	(-38, 65, 8)	4.17
	R fusiform gyrus	(-42, 45, -12)	4.49		

a significant group by node interaction [F(5,18)=6.690, p=.001]. Further, there was no main effect of group on TPN connectivity [F(1,22)=3.659, p=.069] nor a group by node interaction [F(6,17)=.640, p=.698]. Importantly, this pattern of results did not change when verbal IQ, performance IQ, and full-scale IQ were used as covariates in the analysis (TNN: main effect – [F(1,18)=4.890, p=.040], interaction – [F(5,14)=4.797, p=.009]; TPN: main effect – [F(1,18)=1.752, p=.202], interaction – [F(6,13)=.512. p=.798]). Follow-up t-tests revealed that reduced TNN connectivity was regionally specific to the medial prefrontal cortex (MPFC) [t(22)=3.593, p=.002] and left angular gyrus [t(22)=2.732, p=.012; all other regions, p>.10] (see **Figure 3.3**). Finally, there was a negative correlation (i.e., anticorrelation) between the TNN and TPN in the control group [z'= -.281; t(11)=2.379, p=.037], consistent with previous findings by Fox et al. (Fox et al., 2005) and Frannson (Fransson, 2005). However, network anticorrelation was not significantly different from zero in the autism group [z'=-.140; t(11)=1.132, p=.282]. The difference between groups was not significant [t(22)=.822, p=.420].

To further ensure that IQ did not affect our primary results, we re-ran the analyses excluding the two ASD subjects that had the lowest full-scale IQ scores (subjects A1 and A6; see **Table 3.1**). After removing these subjects, there was no significant group difference in full-scale, verbal, and performance IQ (all p > .15). We again obtained a similar pattern of results to those described above for both the TNN (main effect of group – [F(1,20)=5.625, p=.028], interaction – F(5,16)=6.295, p=.002]) and the TPN (main effect of group – [F(1,20)=2.167, p=.157], interaction – [F(5,16)=.763, p=.610]), demonstrating reduced and altered connectivity of the TNN but normal connectivity of the TPN. Follow-up analyses of the TNN again revealed significant regional abnormality

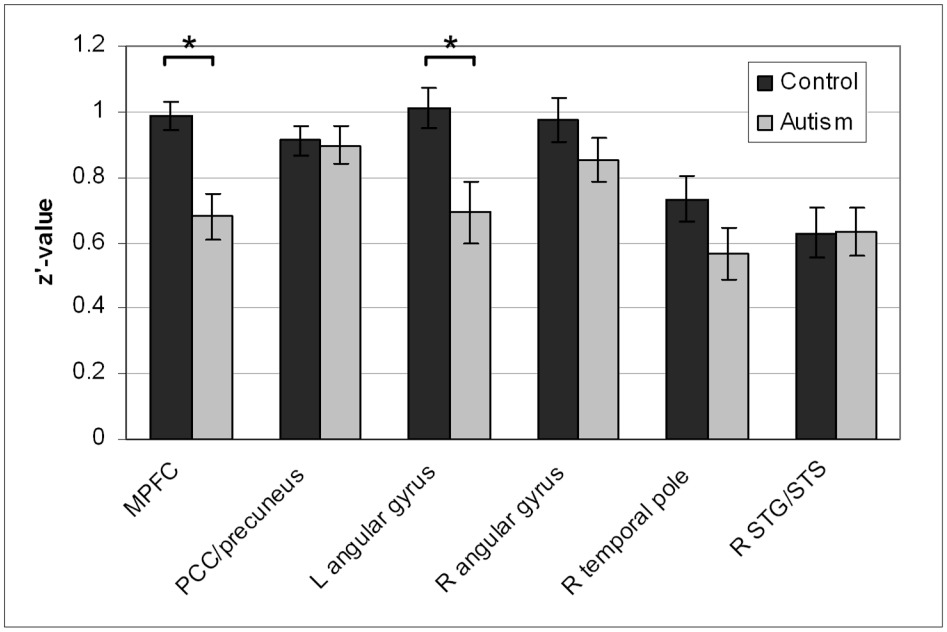


Figure 3.3: Localized abnormalities of the TNN in autism. The reduced functional connectivity within the TNN in the autism group was specific to 2 regions: the MPFC and left angular gyrus (*, $p<.01$). There was no significant difference in any of the other regions ($p > .05$). Brackets show standard error of the mean. (PCC, posterior cingulate; STG, superior temporal gyrus; STS, superior temporal sulcus).

of the MPFC [t(20)=3.245, p=.004] and the left angular gyrus [t(20)=2.780, p=.012], but also included the right temporal pole [t(20)=2.272, p=.034]. The TPN-TNN anticorrelation remained non-significantly different from zero in the autism group [z'=-.126; t(9)=.875, p=.404], but the group difference in network anticorrelation remained non-significant [t(20)=.841, p=.410]. These results suggest that the inclusion of the two lowest full-scale IQ ASD subjects did not account for the above described group functional connectivity differences of the TNN, and provide further evidence that the present results are not accounted for by IQ.

Four of the 12 ASD subjects were taking psychoactive medication at the time of scanning. Reanalysis of the data excluding these 4 subjects slightly altered the results described above. We still found group differences in TNN connectivity (main effect of group – [F(1,18)=6.924, p=.017], group by region interaction – [F(5,14)=5.928, p=.004]). Additionally, follow-up analyses still revealed reduced TNN functional connectivity of the MPFC [t(18)=4.499, p=.0003)] and left angular gyrus [t(18)=2.387, p=.028], but also including the right angular gyrus [t(18)=2.211, p=.04]. There was also a main effect of group on TPN activity [F(1,18)=6.479, p=.020], but again no group by region interaction [F(6,13)=.354, p=.895]. Thus, while medication status did not affect TNN connectivity, it is possible that it might influence TPN connectivity. However, this influence is not robust, as a follow-up non-parametric Wilcoxon rank-sum test revealed that the average TPN connectivity was not significantly different between medicated and non-medicated subjects (p>.35).

Discussion

We found evidence for disrupted intrinsic functional organization of the TNN in autistic patients but, at the same time and within the same patients, intact organization of the TPN. These findings make sense in light of the disrupted and intact abilities of higher-functioning individuals with autism and the putative functional roles of these two networks. The altered organization of the TNN, which normally supports social, emotional, and introspective processes, may underlie these deficits in autism. The entirely normal organization of the TPN, which supports goal-directed cognitive and attentional processes, may underlie the relative sparing and even enhanced abilities in these general domains in individuals with autism. We also failed to observe the normal anticorrelated functional relationship between the TPN and TNN in the autism group, although this relationship was present in the control group. Together, these findings suggest that there is an *unevenness* and *imbalance* in the functioning of these two large-scale brain networks, which we suggest might bias the autistic brain toward a particular non-social and non-emotional cognitive processing style.

It is interesting to speculate on the relationship between the current findings and Baron-Cohen's theory that suggests that the autism cognitive phenotype reflects one extreme end of a continuum of brain types – one that is heavily biased toward "systemizing" rather than "empathizing" (Baron-Cohen, 2002). While systemizers are driven "to analyze the variables in a system, to derive the underlying rules that govern the behaviour of a system," empathizers are driven "to identify another person's emotions and thoughts," thus enabling one to predict another person's behavior (Baron-Cohen,

2002; Baron-Cohen et al., 2003). Interestingly, the TNN seems to be involved in "empathizing"-type functions (e.g., social and emotional interactions, mentalizing), while the TPN seems to underlie functions necessary for "systemizing" (e.g., goal-directed cognitive operations, sustained attention). Although speculative, one could imagine how early dysfunction in the neural circuitry underlying "empathizing" might bias the autistic cognitive style away from such functions, but toward developing functions which rely on the intact neural circuitry supporting "systemizing" functions. Furthermore, the relationship between cognitive style and network functionality is likely to be reciprocally-reinforcing, such that a cognitive bias might, in turn, reinforce the disparity between the functioning of these two networks. The resting fcMRI approach might be particularly well-suited to study both the early emergence and subsequent refinement of network functionality, as this approach is minimally demanding for participants, thus allowing for the inclusion of participants of a wide range in age and cognitive ability.

Interestingly, even the seemingly intact social and emotional abilities of high-functioning autistic individuals (such as the majority of participants in this study) are thought to rely heavily on cognitive decisions and memorized scripts rather than the intuitive and fluid responses that characterize normal social and emotional interactions. This has been exemplified in research on theory of mind, or mentalizing, abilities in high-functioning individuals with autism. Research suggests that they can solve certain mentalizing problems by using an "explicit" or cognitive theory of mind, based on logical reasoning (reviewed in Frith, 2004). However, they fail at tests that rely on "intuitive" mentalizing, such as automatically attributing mental states to moving geometric objects (Klin, 2000; Castelli et al., 2002). We suggest that this pattern of mentalizing ability and

inability in autism may be accounted for by the spared TPN and defective TNN functional organization. Social and emotional cues, normally disambiguated by the TNN, must be evaluated by the unsuited but functionally intact TPN. A cognitive TPN-supported approach to mentalizing (and other social and emotional tasks) may be sufficient in certain circumstances, but such an approach would be more likely to fail during naturalistic tests or real-life situations.

Our results demonstrate that the connectivity abnormalities in autism are specific, both within and across functional networks, rather than reflecting global non-specific reductions in connectivity. We found that the overall reduction of functional connectivity within the TNN was due to specific abnormalities of the MPFC and left angular gyrus alone, while other regions of this same network demonstrated the typical pattern of coherent spontaneous BOLD signal fluctuations (**Figure 3.3**). Furthermore, we found that the functional connectivity of the TPN was entirely normal, thus demonstrating selective, rather than pervasive, network abnormality of the TNN. As previously suggested (Kennedy et al., 2006), this specificity may be related to the uniquely high metabolic activity of the TNN, which has been hypothesized to confer particular susceptibility to damage and dysfunction (Raichle et al., 2001; Buckner et al., 2005). Importantly, although the functional connectivity abnormalities were regionally specific, there are likely widespread and far-reaching consequences of these localized disruptions. Regions of the TNN have rich cortical and subcortical anatomical connectivity (Ongur and Price, 2000; Parvizi et al., 2006), such that an early insult affecting the anatomical or functional development of even one region of the TNN would very likely affect the subsequent anatomy and functionality of the entire network. These functional

abnormalities would be particularly apparent during tasks that typically recruit the dysfunctional region or regions.

Abnormalities of the TNN have also recently been found in a separate autism functional connectivity study (Cherkassky et al., 2006), although their method of reporting the results makes direct comparison with the present findings difficult. They reported that within the TNN, 94% of the pairwise connectivities computed were reduced in the autism group relative to the control group. While this finding might suggest pervasive and widespread reductions of functional connectivity, this is not necessarily the case. In fact, if we analyze the present data in the identical pairwise fashion, we find that 86.7% (13/15) of the pairwise TNN connectivities are lower in the autism group. Importantly, abnormalities of only 2 regions accounts for reductions in 9 of the 15 pairwise comparisons (60%). Assuming the results of the remaining 6 of 15 comparisons are due to chance, we would expect a total of 80% (12/15) of the pairwise connectivities to be reduced in the autism group – a result very close to the observed 86.7% (13/15). Furthermore, this method of data analysis is highly susceptible to outlier subjects, as reduced or increased values in several subjects could affect the group mean. As apparently high percentages can result either from localized connectivity abnormalities, outlier subjects, and non-specific widespread reductions in connectivity, care should be taken in resolving these alternative possibilities.

Importantly, there are at least two independent factors that could have contributed to the findings of reduced functional connectivity of the TNN in autism. First, as previously described, there are spontaneous low frequency BOLD signal fluctuations, which seem to be a pervasive feature of many, if not all, brain networks (Vincent et al.,

2007). Because these fluctuations persist across different levels of consciousness (e.g., sleep, sedation), they are not simply a reflection of spontaneously occurring thoughts and cognitive processes (Biswal et al., 1995; Kiviniemi et al., 2000; Fox et al., 2006a; Fukunaga et al., 2006; Vincent et al., 2007). Further, Fox et al. (Fox et al., 2006a) cleverly demonstrated that spontaneous fluctuations are a unique component of the fMRI BOLD signal, and are separable from task-evoked or cognition-evoked BOLD responses. Thus, these spontaneously-occurring intrinsic oscillations could be disrupted in autism, suggestive of an underlying functional disorganization of this network, and accounting for the present findings. Additionally, however, it should also be recognized that during the time these spontaneous fluctuations are occurring, ongoing spontaneous mental activity is also occurring. Such mental activity can drive coherent activity across co-activated or co-deactivated brain regions, thus affecting the degree of functional connectivity between regions. More specifically, as social, emotional, and introspective tasks are known to modulate activity of the TNN, group differences in the propensity to naturally default to these types of thoughts would affect the measured functional connectivity between regions of the TNN. Consistent with this idea, we previously found that the uniquely high resting metabolic activity of the TNN (or default network) was reduced in autism, providing evidence that this network is doing something different at rest in individuals with autism (Kennedy et al., 2006). One particularly interesting behavioral study lends additional support to this idea (Hurlburt et al., 1994). When individuals with autism or Asperger's syndrome were asked to report on the contents of their minds at random times throughout their day, qualitative differences in the types of ongoing thoughts were found (Hurlburt et al., 1994; Frith and Happe, 1999). The design

of the current experiment cannot resolve which of these two factors – either differences in spontaneous fluctuations or differences in resting cognition – contributed to the reduced functional connectivity of the TNN in autism. However, future experiments may be able to address this issue. For instance, one could examine functional connectivity of the TNN during continuous performance of a cognitively demanding task that is known to reduce stimulus independent thought (e.g., the two-back working memory task (Fransson, 2006)) and TNN activity (McKiernan et al., 2006), thus reducing or removing the influence of differences in spontaneous mental and subsequent functional activity on TNN connectivity.

In sum, we speculate that our findings may provide a neurofunctional framework for understanding one of the most profound differences between the typically-developing child and the autistic child. While the former possesses a strong interest in and capacity for human social and emotional relationships, the child with autism lacks these interests, but instead possesses an equally powerful interest in objects, rules, and regularities. The autistic individual lives in a TNN-decipherable world, but might lack the proper neural machinery to disambiguate the rather complex and subtle cues of social and emotional communication. Therefore, perhaps they develop an alternative cognitive style and alternative approach to interacting with the world – focused on their cognitive and attentional strengths, rather than their social and emotional weaknesses. We are hopeful that further study of the cognitive strengths and weaknesses of individuals with autism, as well as the neural underpinnings of these abilities, will have important implications for developing and measuring the effectiveness of early intervention programs.

Acknowledgements

We thank Dr. Cindy Carter for clinical assessment, Graham Wideman for technical assistance and helpful discussions, Doreen Nguyen for assistance with data collection, Elizabeth Redcay and Jody Buckwalter for helpful discussions, and the researchers and staff at the UCSD Center for Functional MRI. We also thank the participants who graciously gave of their time to participate in this study. This research was supported by NIH RO1 MH36840 awarded to E.C.

This chapter, in full, has been submitted for publication; Kennedy, DP, and Courchesne, E. The dissertation author was primary author on this paper. The secondary author was the thesis advisor.

References

Allen, G., Courchesne, E., 2001. Attention function and dysfunction in autism. Front Biosci 6, D105-119.

Andreasen, N.C., O'Leary, D.S., Cizadlo, T., Arndt, S., Rezai, K., Watkins, G.L., Ponto, L.L., Hichwa, R.D., 1995. Remembering the past: two facets of episodic memory explored with positron emission tomography. Am J Psychiatry 152, 1576-1585.

Baron-Cohen, S., 2002. The extreme male brain theory of autism. Trends Cogn Sci 6, 248-254.

Baron-Cohen, S., Richler, J., Bisarya, D., Gurunathan, N., Wheelwright, S., 2003. The systemizing quotient: an investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences. Philos Trans R Soc Lond B Biol Sci 358, 361-374.

Biswal, B., Yetkin, F.Z., Haughton, V.M., Hyde, J.S., 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med 34, 537-541.

Buckner, R.L., Vincent, J.L., 2007. Unrest at rest: Default activity and spontaneous network correlations. Neuroimage, doi:10.1016/jneuroimage.2007.01.010.

Buckner, R.L., Snyder, A.Z., Shannon, B.J., LaRossa, G., Sachs, R., Fotenos, A.F., Sheline, Y.I., Klunk, W.E., Mathis, C.A., Morris, J.C., Mintun, M.A., 2005. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci 25, 7709-7717.

Cabeza, R., Nyberg, L., 2000. Imaging cognition II: An empirical review of 275 PET and fMRI studies. J Cogn Neurosci 12, 1-47.

Castelli, F., Frith, C., Happe, F., Frith, U., 2002. Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. Brain 125, 1839-1849.

Cato, M.A., Crosson, B., Gokcay, D., Soltysik, D., Wierenga, C., Gopinath, K., Himes, N., Belanger, H., Bauer, R.M., Fischler, I.S., Gonzalez-Rothi, L., Briggs, R.W., 2004. Processing words with emotional connotation: an FMRI study of time course and laterality in rostral frontal and retrosplenial cortices. J Cogn Neurosci 16, 167-177.

Cherkassky, V.L., Kana, R.K., Keller, T.A., Just, M.A., 2006. Functional connectivity in a baseline resting-state network in autism. Neuroreport 17, 1687-1690.

Corbetta, M., Shulman, G.L., 2002. Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci 3, 201-215.

Corbetta, M., Kincade, J.M., Shulman, G.L., 2002. Neural systems for visual orienting and their relationships to spatial working memory. J Cogn Neurosci 14, 508-523.

Cordes, D., Haughton, V.M., Arfanakis, K., Wendt, G.J., Turski, P.A., Moritz, C.H., Quigley, M.A., Meyerand, M.E., 2000. Mapping functionally related regions of brain with functional connectivity MR imaging. AJNR Am J Neuroradiol 21, 1636-1644.

Cox, R.W., 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 29, 162-173.

Damoiseaux, J.S., Rombouts, S.A., Barkhof, F., Scheltens, P., Stam, C.J., Smith, S.M., Beckmann, C.F., 2006. Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci U S A 103, 13848-13853.

D'Argembeau, A., Collette, F., Van der Linden, M., Laureys, S., Del Fiore, G., Degueldre, C., Luxen, A., Salmon, E., 2005. Self-referential reflective activity and its relationship with rest: a PET study. Neuroimage 25, 616-624.

De Luca, M., Beckmann, C.F., De Stefano, N., Matthews, P.M., Smith, S.M., 2006. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. Neuroimage 29, 1359-1367.

Fletcher, P.C., Happe, F., Frith, U., Baker, S.C., Dolan, R.J., Frackowiak, R.S., Frith, C.D., 1995. Other minds in the brain: a functional imaging study of "theory of mind" in story comprehension. Cognition 57, 109-128.

Fossati, P., Hevenor, S.J., Graham, S.J., Grady, C., Keightley, M.L., Craik, F., Mayberg, H., 2003. In search of the emotional self: an FMRI study using positive and negative emotional words. Am J Psychiatry 160, 1938-1945.

Fox, M.D., Snyder, A.Z., Zacks, J.M., Raichle, M.E., 2006a. Coherent spontaneous activity accounts for trial-to-trial variability in human evoked brain responses. Nat Neurosci 9, 23-25.

Fox, M.D., Corbetta, M., Snyder, A.Z., Vincent, J.L., Raichle, M.E., 2006b. Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. Proc Natl Acad Sci U S A 103, 10046-10051.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 102, 9673-9678.

Fransson, P., 2005. Spontaneous low-frequency BOLD signal fluctuations: an fMRI investigation of the resting-state default mode of brain function hypothesis. Hum Brain Mapp 26, 15-29.

Fransson, P., 2006. How default is the default mode of brain function? Further evidence from intrinsic BOLD signal fluctuations. Neuropsychologia 44, 2836-2845.

Frith, U., 2004. Emanuel Miller lecture: confusions and controversies about Asperger syndrome. J Child Psychol Psychiatry 45, 672-686.

Frith, U., Happe, F., 1999. Theory of mind and self-consciousness: What is it like to be autistic? Mind Lang 14, 1-22.

Fukunaga, M., Horovitz, S.G., van Gelderen, P., de Zwart, J.A., Jansma, J.M., Ikonomidou, V.N., Chu, R., Deckers, R.H., Leopold, D.A., Duyn, J.H., 2006. Large-amplitude, spatially correlated fluctuations in BOLD fMRI signals during extended rest and early sleep stages. Magn Reson Imaging 24, 979-992.

Gallagher, H.L., Happe, F., Brunswick, N., Fletcher, P.C., Frith, U., Frith, C.D., 2000. Reading the mind in cartoons and stories: an fMRI study of 'theory of mind' in verbal and nonverbal tasks. Neuropsychologia 38, 11-21.

Garretson, H.B., Fein, D., Waterhouse, L., 1990. Sustained attention in children with autism. J Autism Dev Disord 20, 101-114.

Gobbini, M.I., Leibenluft, E., Santiago, N., Haxby, J.V., 2004. Social and emotional attachment in the neural representation of faces. Neuroimage 22, 1628-1635.

Greicius, M.D., Menon, V., 2004. Default-mode activity during a passive sensory task: uncoupled from deactivation but impacting activation. J Cogn Neurosci 16, 1484-1492.

Greicius, M.D., Krasnow, B., Reiss, A.L., Menon, V., 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 100, 253-258.

Gusnard, D.A., Akbudak, E., Shulman, G.L., Raichle, M.E., 2001. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci U S A 98, 4259-4264.

Hurlburt, R.T., Happe, F., Frith, U., 1994. Sampling the form of inner experience in three adults with Asperger syndrome. Psychol Med 24, 385-395.

Iacoboni, M., Lieberman, M.D., Knowlton, B.J., Molnar-Szakacs, I., Moritz, M., Throop, C.J., Fiske, A.P., 2004. Watching social interactions produces dorsomedial prefrontal and medial parietal BOLD fMRI signal increases compared to a resting baseline. Neuroimage 21, 1167-1173.

Johnson, S.C., Baxter, L.C., Wilder, L.S., Pipe, J.G., Heiserman, J.E., Prigatano, G.P., 2002. Neural correlates of self-reflection. Brain 125, 1808-1814.

Kanner, L., 1943. Autistic disturbances of affective contact. Nervous Child 2, 217-250.

Kelley, W.M., Macrae, C.N., Wyland, C.L., Caglar, S., Inati, S., Heatherton, T.F., 2002. Finding the self? An event-related fMRI study. J Cogn Neurosci 14, 785-794.

Kennedy, D.P., Redcay, E., Courchesne, E., 2006. Failing to deactivate: resting functional abnormalities in autism. Proc Natl Acad Sci U S A 103, 8275-8280.

Kiviniemi, V., Jauhiainen, J., Tervonen, O., Paakko, E., Oikarinen, J., Vainionpaa, V., Rantala, H., Biswal, B., 2000. Slow vasomotor fluctuation in fMRI of anesthetized child brain. Magn Reson Med 44, 373-378.

Kjaer, T.W., Nowak, M., Lou, H.C., 2002. Reflective self-awareness and conscious states: PET evidence for a common midline parietofrontal core. Neuroimage 17, 1080-1086.

Klin, A., 2000. Attributing social meaning to ambiguous visual stimuli in higher-functioning autism and Asperger syndrome: The Social Attribution Task. J Child Psychol Psychiatry 41, 831-846.

Lord, C., Rutter, M., Le Couteur, A., 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord 24, 659-685.

Lord, C., Risi, S., Lambrecht, L., Cook, E.H., Jr., Leventhal, B.L., DiLavore, P.C., Pickles, A., Rutter, M., 2000. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30, 205-223.

Lou, H.C., Luber, B., Crupain, M., Keenan, J.P., Nowak, M., Kjaer, T.W., Sackeim, H.A., Lisanby, S.H., 2004. Parietal cortex and representation of the mental Self. Proc Natl Acad Sci U S A 101, 6827-6832.

Macrae, C.N., Moran, J.M., Heatherton, T.F., Banfield, J.F., Kelley, W.M., 2004. Medial prefrontal activity predicts memory for self. Cereb Cortex 14, 647-654.

Maddock, R.J., Garrett, A.S., Buonocore, M.H., 2003. Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. Hum Brain Mapp 18, 30-41.

McKiernan, K.A., D'Angelo, B.R., Kaufman, J.N., Binder, J.R., 2006. Interrupting the "stream of consciousness": an fMRI investigation. Neuroimage 29, 1185-1191.

Mitchell, J.P., Banaji, M.R., Macrae, C.N., 2005a. General and specific contributions of the medial prefrontal cortex to knowledge about mental states. Neuroimage 28, 757-762.

Mitchell, J.P., Macrae, C.N., Banaji, M.R., 2005b. Forming impressions of people versus inanimate objects: social-cognitive processing in the medial prefrontal cortex. Neuroimage 26, 251-257.

Moran, J.M., Macrae, C.N., Heatherton, T.F., Wyland, C.L., Kelley, W.M., 2006. Neuroanatomical evidence for distinct cognitive and affective components of self. J Cogn Neurosci 18, 1586-1594.

Northoff, G., Heinzel, A., de Greck, M., Bermpohl, F., Dobrowolny, H., Panksepp, J., 2006. Self-referential processing in our brain--a meta-analysis of imaging studies on the self. Neuroimage 31, 440-457.

Ochsner, K.N., Beer, J.S., Robertson, E.R., Cooper, J.C., Gabrieli, J.D., Kihsltrom, J.F., D'Esposito, M., 2005. The neural correlates of direct and reflected self-knowledge. Neuroimage 28, 797-814.

Ongur, D., Price, J.L., 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb Cortex 10, 206-219.

Parvizi, J., Van Hoesen, G.W., Buckwalter, J., Damasio, A., 2006. Neural connections of the posteromedial cortex in the macaque. Proc Natl Acad Sci U S A 103, 1563-1568.

Pierce, K., Haist, F., Sedaghat, F., Courchesne, E., 2004. The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. Brain 127, 2703-2716.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc Natl Acad Sci U S A 98, 676-682.

Shulman, G.L., Fiez, J.A., Corbetta, M., Buckner, R.L., Miezin, F.M., Raichle, M.E., Petersen, S.E., 1997. Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. J Cogn Neurosci 9, 648-663.

Talairach, J., Tournoux, P. (1988) Co-planar Stereotaxic Atlas of the Human Brain. 3-Dimensional Proportional System: An Appraoch to Cerebral Imaging. New York: Thieme.

Vincent, J.L., Snyder, A.Z., Fox, M.D., Shannon, B.J., Andrews, J.R., Raichle, M.E., Buckner, R.L., 2006. Coherent spontaneous activity identifies a hippocampal-parietal memory network. J Neurophysiol 96, 3517-3531.

Vincent, J.L., Patel, G.H., Fox, M.D., Snyder, A.Z., Baker, J.T., Van Essen, D.C., Zempel, J.M., Snyder, L.H., Corbetta, M., Raichle, M.E., 2007. Intrinsic functional architecture in the anaesthetized monkey brain. Nature 447, 83-86.

Vogeley, K., Bussfeld, P., Newen, A., Herrmann, S., Happe, F., Falkai, P., Maier, W., Shah, N.J., Fink, G.R., Zilles, K., 2001. Mind reading: neural mechanisms of theory of mind and self-perspective. Neuroimage 14, 170-181.

Williams, J.H., Waiter, G.D., Perra, O., Perrett, D.I., Whiten, A., 2005. An fMRI study of joint attention experience. Neuroimage 25, 133-140.

CHAPTER IV

Default network activity during self- and other-reflection in autism

Abstract

Previous studies on autism have identified abnormalities in regions of the default network across a wide range of social, emotional, mentalizing, and resting conditions. Nonetheless, it is still unclear whether these abnormalities reflect pervasive dysfunction of default regions, or, alternatively, a failure to engage these regions at the appropriate times and in the appropriate circumstances. This latter explanation leaves open the possibility that these default regions are still capable of responding like normal, given particular experimental contexts. In the present fMRI study, 13 autistic and 12 control participants were scanned while making true/false judgments for various statements about themselves (SELF) or a close other person (OTHER), and pertaining to either psychological personality traits (PSYC) or physical attributes and behaviors (PHYS) (e.g., "I am a friendly person"; "My mother reads the newspaper"). Although some group differences were observed, we found that both groups displayed largely similar overall levels of activity in default regions. Taken together with previous research, these findings suggest that although default regions do not activate in the normal manner across many different experimental tasks in autism, they are nonetheless capable of activating, given an explicitly defined and carefully controlled experimental task.

Introduction

Recent findings on autism have identified functional abnormalities of several brain regions, including the medial prefrontal cortex (MPFC), retrosplenial cortex/posterior cingulate cortex (RSC/PCC), and angular gyrus (ANG) (Haznedar et al., 2000; Muller et al., 2001; Castelli et al., 2002; Luna et al., 2002; Pierce et al., 2004; Wang et al., 2004; Cherkassky et al., 2006; Kennedy et al., 2006; Gaffrey et al., 2007; Kennedy and Courchesne, under review). These regions belong to a brain network known as the "default network" (Raichle et al., 2001), so named because it maintains a high level of metabolic activity at rest, in the absence of a explicitly-defined cognitively-demanding task. In other words, the brain *defaults* to this pattern of activity when allowed to rest. Interestingly, similarly high activity of this network is also seen when subjects engage in tasks of a social, emotional, or introspective nature, suggesting that socioemotional and introspective processes may be the default mental state of the brain.

We recently found that, in autism, this normally high default network activity during rest is attenuated (Kennedy et al., 2006). Additionally, we and others have also demonstrated abnormally reduced resting state functional connectivity between particular regions of this network in autism (Cherkassky et al., 2006; Kennedy and Courchesne, under review). Importantly, there are at least two alternative explanations for these results. One possibility is that abnormal resting activity and functional connectivity of the default network might reflect a pervasive dysfunction of this network, and that this abnormality would be found across all task contexts that normally activate this network (e.g., rest, social, emotional, introspective). A second possibility is that these findings might reflect the selective inability to engage this network in particular task contexts

(e.g., rest), although it might be capable of responding more like normal in other contexts (e.g., social, emotional, introspective).

In fact, there is substantial evidence for abnormalities in regions of the default network during resting *and* various non-resting type tasks in autism, including social, emotional, and mentalizing tasks. For instance, abnormalities of default network regions have been noted during viewing of personally-familiar faces (Pierce et al., 2004), reading of negatively-valenced emotional words (Kennedy et al., 2006), and in a mentalizing task where subjects observed geometric objects moving in particular ways to imply intentionality (Castelli et al., 2002).

However, none of the three studies provide conclusive evidence of pervasive dysfunction of this network, as none of these tasks actually *required* social, emotional, or mentalizing processes for successful performance. For instance, in Pierce et al. (2004), subjects were required simply to identify female faces, regardless of whether they were familiar or not. In Kennedy et al. (2006), subjects were asked only to count the number of words displayed on the screen, rather than explicitly process the meaning of the words. Lastly, in Castelli et al. (2002), subjects were asked to describe what they observed, and were free to interpret the meaning of the movements in any context they wanted (i.e., social or physical). Thus, in each of these studies, the social, emotional, or mentalizing component of the experiment was neither required for the subject to perform nor was it the explicit focus of the task in which the subject was engaged.

Thus, whether or not default regions are dysfunctional or simply not automatically engaged in particular task contexts remains largely an open question. In the current experiment, we used an experimentally-constrained task to examine whether default

regions can function normally in autism during self- and other-reflection, which is known to robustly activate default regions including MPFC, RSC/PCC, and ANG (Fletcher et al., 1995; Gusnard et al., 2001; Johnson et al., 2002; Kelley et al., 2002; Gallagher and Frith, 2003; Ochsner et al., 2005). While being scanned, subjects were required to read particular statements about themselves or about a close other person (i.e., their mother), and make judgments as to whether the statements were true or false. Thus, in contrast to the above described studies, the subjects' task (i.e., making true/false judgments about themselves or others) was directly relevant to the experimental conditions of interest (i.e., reflection of oneself and others), reducing the likelihood of non-engagement in the mental processes of interest. We also included two types of self- and other-reflection statement conditions – 1) those regarding psychological personality traits (PSYC) and 2) those regarding physical, external attributes and behaviors (PHYS). This allowed us to examine whether there may be a selective impairment in one or the other type of judgment.

Methods

Participants:

Fourteen male Autism Spectrum Disorder (ASD) and 13 male control subjects were scanned. Due to excessive movement during scanning, 1 ASD subject and 1 control subject were removed from the analysis, resulting in a final sample size of 13 ASD (6 autism, 6 Asperger's, 1 PDD-NOS) and 12 control subjects. Informed written consent was obtained from all participants or, when appropriate, their legal guardians, and all participants received monetary compensation for their time. The protocol was approved

by the Institutional Review Board of UCSD and Children's Hospital at San Diego. ASD participants were diagnosed by a clinical psychologist using the Autism Diagnostic Interview – Revised (ADI-R) (Lord et al., 1994) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000), and, with the exception of 1 control subject, IQ scores were obtained from all participants using the Wechsler Adult Intelligence Scale (WAIS) or WAIS-R (Revised). The mean age of the autism participants (26.9 years) and the control participants (27.5 years) was not significantly different [$t(23)=.129$, $p>.85$]. Subject groups did not differ significantly in verbal, performance, or full-scale IQ [verbal: $t(22)=1.641$, $p=.115$; performance: $t(22)=1.512$, $p=.145$; full-scale: $t(22)=1.959$, $p=.063$]. See **Table 4.1** for detailed clinical information.

Stimuli:

While in the scanner, subjects made true/false judgments for various statements about themselves (SELF condition) or a close other person (OTHER condition). These SELF and OTHER statements either referred to psychological personality traits (PSYC condition) or to physical, external attributes and behaviors (PHYS condition). In all cases, the close other was their mother, with the exception of 1 control subject who read statements about a close friend rather than his mother, as his parents were deceased. Thus, there were 4 statement conditions in total: PSYC-SELF, PSYC-OTHER, PHYS-SELF, and PHYS-OTHER. Additionally, subjects were shown math equations (MATH condition) in the form of a two-digit number plus a one-digit number equaling either a correct or incorrect answer, and again instructed to respond via button presses as to whether the equation was true or false. This MATH condition served as an experimental

Table 4.1: Clinical information for autism and control participants.

				IQ				ADI-R			ADOS		
Subject	**Diagnosis**	**Age**	**Sex**	**Verbal**	**Performance**	**Full-Scale**	**handedness**	**Social (cutoff = 10)**	**Communication (cutoff = 8)**	**Stereotypy (cutoff = 3)**	**Social (cutoff = 4)**	**Communication (cutoff = 2)**	**Stereotypy**
A1	Autism	15.7	M	73	66	67	Right	10	21	11	10	3	3
A2	Asperger's	16.2	M	120	124	125	Right	13	17	3	11	6	1
A3	Asperger's	17.4	M	99	93	96	Right	23	18	9	9	5	1
A4	Autism	17.7	M	101	118	109	Right	26	19	6	7	5	1
A5	Asperger's	18.3	M	108	107	109	Right	14	8	6	5	3	1
A6	Autism	18.8	M	55	109	80	Right	28	20	4	9	5	0
A7	Asperger's	22.9	M	97	105	101	Right	13	12	3	6	3	0
A8	Asperger's	24.0	M	116	109	114	Right	7	11	10	8	2	2
A9	Asperger's	27.7	M	111	99	106	Right	21	20	7	11	6	0
A10	PDD-NOS	31.4	M	90	126	107	Right	14	14	3	6	3	2
A11	Autism	41.3	M	98	114	104	Left	21	22	10	11	5	2
A12	Autism	46.4	M	86	115	100	Right	22	19	6	7	5	1
A13	Autism	52.0	M	102	105	104	Right	26	17	6	9	4	1
Mean (SD)		**26.9** (12.3)		**96.6** (17.7)	**106.9** (15.4)	**101.7** (14.6)							
C1	control	15.9	M	95	99	97	Left						
C2	control	16.2	M	N/A	N/A	N/A	Right						
C3	control	17.8	M	107	119	114	Right						
C4	control	19.0	M	106	118	113	Right						
C5	control	20.6	M	99	106	103	Left						
C6	control	22.9	M	107	93	100	Right						
C7	control	25.3	M	109	116	114	Right						
C8	control	29.4	M	109	125	118	Right						
C9	control	32.3	M	108	128	119	Right						
C10	control	40.7	M	108	132	121	Right						
C11	control	44.6	M	106	109	108	Right						
C12	control	45.4	M	108	128	119	Right						
Mean (SD)		**27.5** (10.9)		**105.6** (4.5)	**115.7** (12.7)	**111.5** (8.3)							

baseline condition. The functional scans also included 2 additional conditions, but these were not included in the current analysis.

Each trial consisted of a statement or equation shown for 2500 msec, followed by a blank screen for 500 msec. Conditions were presented in a counterbalanced block design manner, with 6 trials per block, 8 blocks per condition, and each block lasting 18 seconds. The specific statements that appeared within each block were randomized for each subject. The total time of the experiment was 17 min, 28 sec, which was divided into two shorter functional runs lasting 8 min, 44 sec each.

***Behavioral Data Acquisition and Analysis*:**

Stimuli were presented using the Presentation software package (Neurobehavioral Systems, Albany, CA). Subject response (true/false) and reaction time (from stimuli onset until subject response) were recorded during scanning. Responses that occurred any time within the 3000 msec trial were recorded.

After scanning was complete (approximately 1 hour later), subjects were asked to again provide true/false judgments for each statement. This procedure allowed us to calculate the reliability of each participant's responses, ensuring they made deliberate choices, rather than simply guessing while in the scanner. Due to a computer problem, true/false responses were not recorded from 1 ASD subject and 1 control subject.

All behavioral analyses were conducted with SPSS 12.0 statistical software package (SPSS, Chicago, IL). Independent samples t-tests were run for RT and percent concordance (or accuracy) for SELF, OTHER, PSYC, PHYS, and MATH conditions. To determine if there were any condition by group interactions, repeated measures ANOVAs were run with task condition (i.e., SELF and OTHER; PSYC and PHYS) as the repeated

measure, and group (i.e., autism or control) as the between-subjects factor. Follow-up t-tests were run for all significant main effects of group or group by condition interactions.

***Functional Imaging Data Acquisition and Analysis*:**

Functional and anatomical images were acquired using a 3 Tesla GE Signa EXCITE scanner. Whole brain axial slices were collected with a gradient-recalled echo-planar imaging pulse sequence with the following parameters: TR (repetition time) = 2000 ms; TE (echo time) = 30 ms; flip angle = 90˚; field of view (FOV) = 220 mm; matrix = 64x64 (3.44 mm^2 in-plane resolution); slice thickness = 4 mm; # of axial slices = 32; # of volumes = 262 (for each of the 2 runs). T1-weighted anatomical images were collected for co-registration with the functional images (FOV = 256 mm; matrix = 256x256 (1 mm^2 in-plane resolution); slice thickness = 1 mm; # of axial slices = 124).

Functional analyses were carried out using the Analyses of Functional NeuroImages (AFNI) statistical software package (version 2.56; http://afni.nimh.nih.gov/afni) (Cox, 1996). First, field maps, which were acquired during the scan sessions, were used to correct for field inhomogeneities. Next, the first 10 TRs (which consisted of 20 seconds of fixation) were removed from the beginning of each functional run. Motion correction and three-dimensional registration of each participant's functional images were performed with AFNI's automated alignment program (3dVolReg), which co-registers each individual functional volume with a manually specified middle reference volume. Brief periods of subject movement, which were objectively defined from the output of this volume registration procedure, were removed from the analysis (for details, see Kennedy and Courchesne, under review). Subjects with greater than 20% of the entire run removed were excluded entirely from the

study (1 ASD, 1 control subject). There was no difference in the percent of the scans removed from the remaining participants (control = 2.46%; autism = 1.93%; t(23)=.492, p =.628). Images were corrected for slice acquisition timing, spatially smoothed with a Gaussian filter (full-width half-maximum = 6 mm), and linear trend was removed from the timeseries. Next, the data were converted into percent signal change and the two separate functional runs were then concatenated, producing a single timeseries.

Functional data were analyzed using 3dDeconvolve. First, an impulse response function (IRF) was estimated based on the measured fMRI signal for each voxel and the input stimulus functions. These input functions included 6 experimental conditions (only 4 of which were examined in the present paper – PSYC SELF, PSYC OTHER, PHYS SELF, PHYS OTHER) and 6 motion parameters (i.e., rotational movement (roll, pitch, yaw) and translational movement (x, y, z)). The MATH condition served as the baseline state. The estimated IRF was then convolved with the input stimulus timeseries, and multiple regressions were run to determine a goodness-of-fit coefficient (i.e., linear contrast weight) for 0, 2, 4, and 6 seconds after stimulus presentation. These 4 linear contrast weights were summed for each condition separately, yielding a single linear contrast weight for each of the 4 conditions at each voxel. Next, several *a priori* contrasts were carried out (PSYC vs. MATH, PHYS vs. MATH, SELF vs. MATH, OTHER vs. MATH, PSYC vs. PHYS, SELF vs. OTHER, and MENTAL (all 4 conditions) vs. MATH) at every voxel.

For group analysis, images were spatially normalized to Talairach space (Talairach and Tournoux, 1988) using AFNI's 12 sub-volume piecewise linear transformation based on manually-defined landmarks. T-tests were run for each group

separately to determine if each of the above contrasts were significantly different from zero. Whole-brain functional maps are shown at a voxel threshold of p<.001, and a minimum cluster volume of 320 mm^3. This cluster volume was calculated using an iterative monte carlo simulation using AFNI's AlphaSim program with a voxel-wise threshold of $p < .05$. Throughout the text, we refer to this volume threshold as "volume-corrected".

Finally, all group comparisons were carried out using a region-of-activation (ROA) approach, with ROAs defined as regions active in the MENTAL (all 4 conditions) vs. MATH contrast ($p < .001$, volume-corrected) that overlapped between the two groups. For the purpose of this paper, we limit our ROA analysis to regions known to be part of the default network (Raichle et al., 2001). For within-group analyses, we ran 2 separate paired t-tests using SPSS for each region of interest (1 for PSYC and PHYS; 1 for SELF and OTHER). Similarly, for between-group analyses, repeated measures ANOVAs were run separately for each ROA, with condition type (PSYC and PHYS; or SELF and OTHER) as the repeated measure, and group (control or ASD) as the between subjects factor.

Results

***Behavioral Results*:**

There was no main effect of group for reliability of judgments (across SELF, OTHER, PSYC, and PHYS conditions) [$F(1,21)=.289$, $p=.596$]. However, there were group by condition interactions in reliability for both PSYC vs. PHYS statements [$t(21)=3.134$, $p=.005$] and SELF vs. OTHER statements [$t(21)=2.930$, $p=.008$]. Follow-

up t-tests revealed that the control group had greater reliability of responses for SELF compared to OTHER judgments [89.4% vs. 84.1%, t(10)=2.384, p=.0384] and PHYS compared to PSYC judgments [88.8% vs. 84.6%, t(10)=2.701, p=.0223], while reliability between these conditions was not significantly different in the autism group [84.4% vs. 86.1% and 83.4% vs. 87.1%, respectively; both p values > .05].

In terms of reaction time, there was also no main effect of group (across SELF, OTHER, PSYC, and PHYS conditions) [F(1,21)=3.211, p=.086]. Further, there were no group by condition interactions for either SELF vs. OTHER or PSYC vs. PHYS judgments [t(23)=.524, p=.605; t(23)=1.379, p=.181]. Both groups responded faster to SELF compared to OTHER statements [control: 1341.9 msec vs. 1513.4 msec, t(11)=8.519, p<.00001; autism: 1547.3 msec vs. 1703.6 msec, t(12)=11.223, p<.00001], and were faster to PSYC compared to PHYS statements [control: 1401.3 msec vs. 1487.8 msec, t(11)=4.588, p=.0008; autism: 1543.3 msec vs. 1673.8 msec, t(12)=5.183, p=.0002]. Although there was neither a main effect of group nor an interaction, the control group did respond significantly faster than the autism group for PHYS judgments [t(23)=2.140, p=.043, all other conditions, p > .05].

Finally, for the MATH baseline condition, there was no group difference in accuracy (control: 93.1 %, autism: 91.8%, t(23)=.536, p=.60) or reaction time (control: 1573.7 msec, autism: 1703.5 msec, t(23)=1.639, p=.12).

***Functional Imaging Results*:**

Whole-brain Analyses: Both groups recruited largely similar and partially overlapping brain regions in the MENTAL vs. MATH contrast (**Figure 4.1**; **Table 4.2**). These overlapping regions included dorsal medial prefrontal cortex (dMPFC),

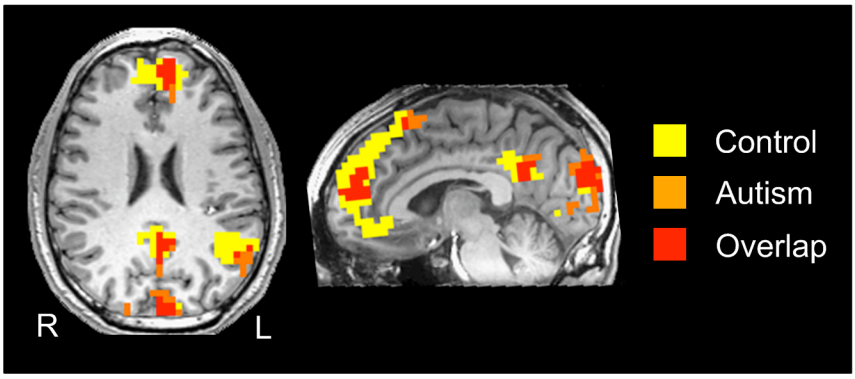


Figure 4.1: Areas of functional overlap between control and autism groups in the MENTAL (all 4 conditions) vs. MATH contrast ($p<.001$, volume-corrected).

Table 4.2: Talairach locations of peak significance for the MENTAL (all 4 conditions) vs. MATH contrast in control and autism groups.

	CONTROL		AUTISM	
Region	**(X, Y, Z)**	**t-value**	**(X, Y, Z)**	**t-value**
medial prefrontal cortex*	(2, -51, 28)	11.94	(6, -51, 24)	5.82
L superior frontal gyrus	(6, -39, 48)	12.24	(6,-7,60)	6.85
L middle frontal gyrus	(42, -3, 48)	8.25	(42, -3, 44)	5.06
L inferior frontal gyrus	(42, -19, 16)	8.53	(38, -19, 8)	5.99
subgenual anterior cingulate cortex	(2, -31, 0)	7.07		
L temporal pole	(42, -3, -28)	6.97		
L superior temporal gyrus	(50, 33, 4)	4.80	(50, 21, -4)	5.65
R angular gyrus	(-58, 65, 28)	6.26		
L angular gyrus*	(50, 65, 36)	8.06	(58, 61, 24)	6.67
posterior cingulate/retrosplenial cortex*	(-2, 49, 28)	8.80	(6, 61, 32)	7.51
striate cortex	(3, 97, 24)	8.31	(6, 97, 24)	7.62

* indicates regions that are included in intensity analysis

retrosplenial cortex/posterior cingulate cortex (RSC/PCC), left angular gyrus (lANG), bilateral striate and extrastriate cortices, left inferior frontal gyrus, and left middle and superior frontal gyrus (thresholded at p>.001, volume-corrected). Additionally, however, the control group had unique activation of the left superior temporal sulcus (STS), left temporal pole, the right ANG, and bilateral subgenual anterior cingulate, while the autism group had unique activation of a more anterior portion of the left STS.

Although the two groups had overlapping activity in numerous regions, the spatial extent of activation was reduced in the autism group, particularly noticeable in the MPFC (**Figure 4.1**, **Figure 4.2**). However, these differences were less apparent at lower thresholds. Furthermore, there was not simply a non-specific, global reduction in extent of activation, as the extent of striate and extrastriate activity in the autism group was equal to, if not greater than, that of the control group (**Figure 4.1**, **Figure 4.2**).

ROA Analyses: Only three regions met criteria for inclusion in the ROA analysis: MPFC, RSC/PCC, and lANG. Percent signal change values from these 3 regions were extracted from each individual and analyzed using SPSS. For all ROAs, there was no main effect of group across PSYC, PHYS, SELF, and OTHER conditions {dMPFC: [$F(1,23)<.001$, $p=.999$]; RSC/PCC: [$F(1,23)=.434$, $p=.517$]; lANG: [$F(1,23)=.080$, $p=.780$]}, suggesting that the overall level of activity within these regions was equivalent between autism and control groups when collapsed across conditions. There were no significant 3-way interactions (PSYC/PHYS x SELF/OTHER x group) in any of the 3 regions of interest {dMPFC: [$F(1,23)=3.318$, $p=.082$]; RSC/PCC: [$F(1,23)=1.608$, $p=.217$]; lANG [$F(1,23)=1.106$, $p=.304$]}. We report the results for PSYC/PHYS judgments and SELF/OTHER judgments separately below (see also **Figure 4.3**).

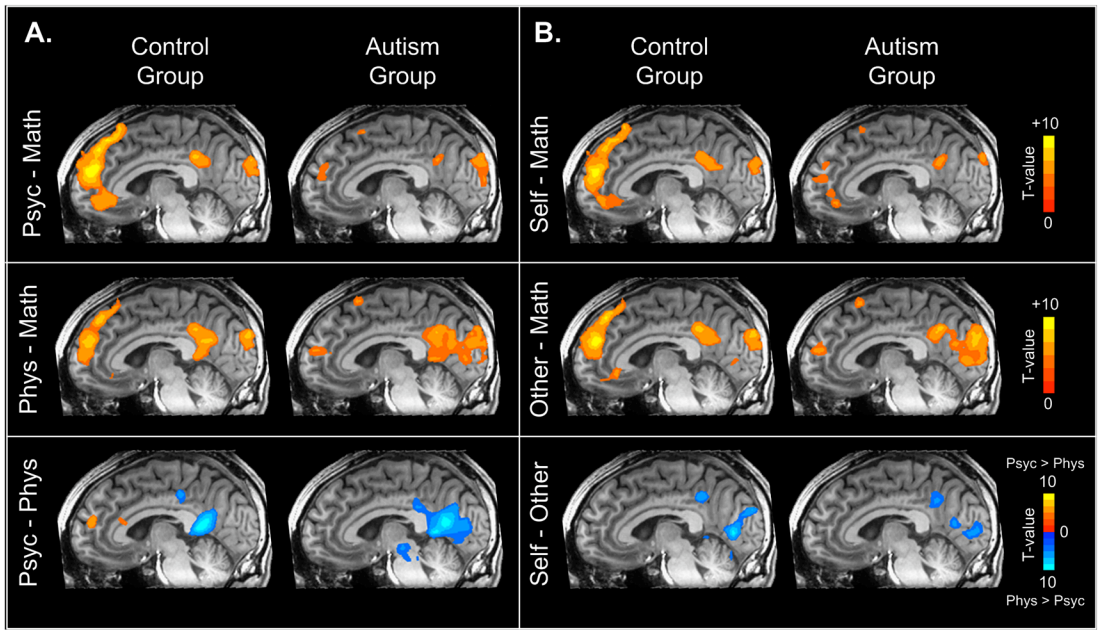


Figure 4.2: Functional activity for control and autism groups for **a)** PSYC vs. MATH, PHYS vs. MATH, and PSYC vs. PHYS contrasts, and **b)** SELF vs. MATH, OTHER VS. MATH, and SELF vs. OTHER contrasts. The same mid-sagittal slice location was chosen for each image, and displayed at $p<.001$ (volume-corrected).

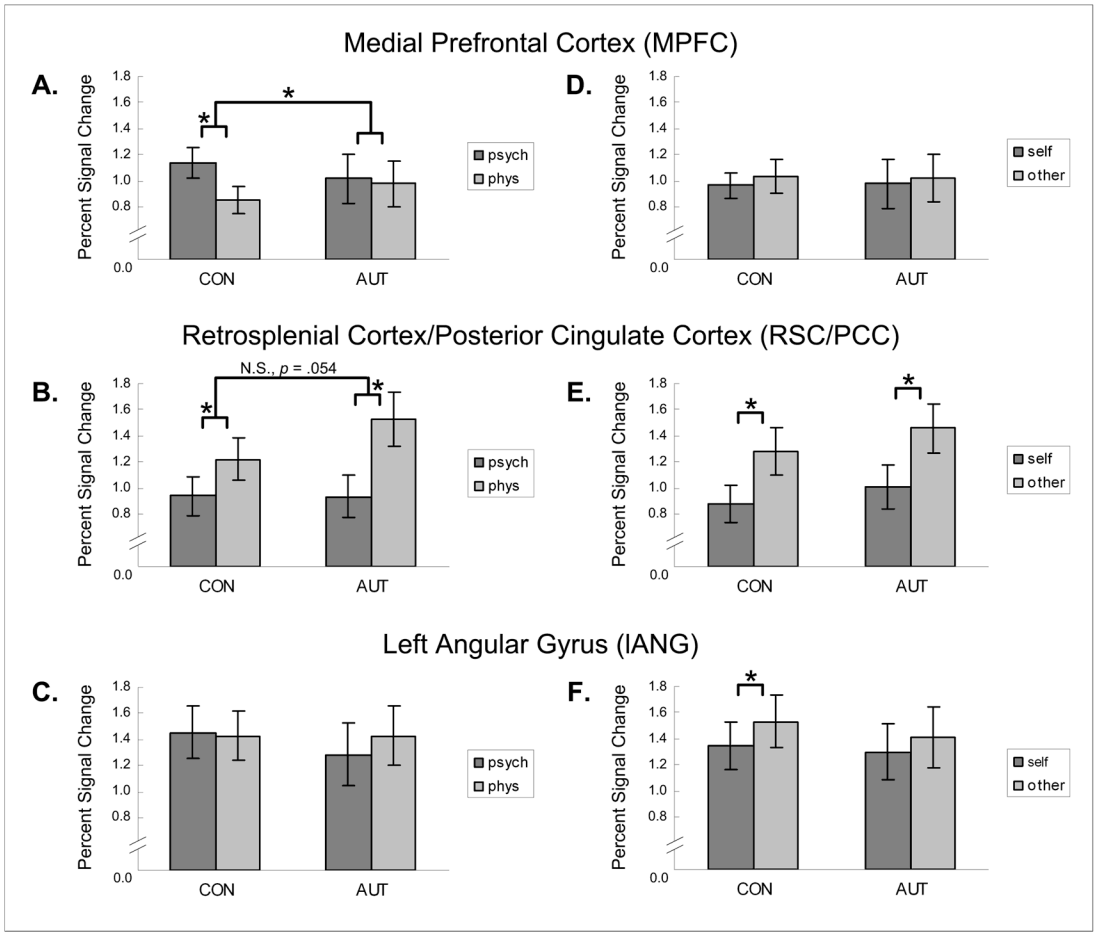


Figure 4.3: Bar graphs depicting percent signal change in control and autism groups during PSYC and PHYS judgments (**a, b, c**), and SELF and OTHER judgments (**d, e, f**), each relative to the baseline MATH condition. Individual graphs are shown for each of the three default regions of interest – medial prefrontal cortex (**a, d**), retrosplenial cortex/posterior cingulate cortex (**b, e**), and left angular gyrus (**c, f**).

PSYC/PHYS judgments: In the dMPFC, although there was no main effect of group, there was a significant group by condition interaction [F(1,23)= 4.434, p=.046]. Follow-up t-tests revealed that the control group had greater activity during PSYC judgments compared to PHYS judgments [t(11)=4.521, p=.001], but that this effect was absent in the autism group [t(12)=.390, p=.704] (**Figure 4.3a**).

In the RSC/PCC, there was also no main effect of group, but there was a trend toward a significant group by condition interaction [F(1,23)=4.137, p=.054]. While follow-up t-tests revealed that both the control group and the autism group had greater activity during PHYS compared to PSYC judgments [control: t(11)=4.131, p=.002; autism: t(12)=4.397, p=.001], the autism group demonstrated greater than normal RSC/PCC activation during PHYS judgments (**Figure 4.3b**).

In the lANG, there was neither a main effect of group nor a group by condition interaction [F(1,23)=.936, p=.343], and no difference between PSYC and PHYS conditions in either the control group [t(11)=.225, p=.826] or autism group [t(12)=1.046, p=.316] (**Figure 4.3c**).

All between-group analyses were re-run using RT and response reliability for PSYC and PHYS conditions as covariates. The results of these analyses remained unchanged, except that the above described non-significant trend toward a group by condition interaction within the RSC/PCC became significant [F(1,19)=4.945, p=.038]. Finally, for the autism group, there were no significant correlations between functional activity during PSYC vs. PHYS and ADI-R and ADOS social subscores (all p-values > .15).

SELF/OTHER judgments: In the dMPFC, there was neither a main effect of group nor a group by condition interaction [F(1,23)=.021, p=.885], and no difference between SELF and OTHER conditions in either the control group [t(11)=.657, p=.525] or the autism group [t(12)=.713, p=.489] (**Figure 4.3d**).

In the RSC/PCC, there was neither a main effect of group nor a group by condition interaction [F(1,23)=.130, p=.722]. Follow-up t-tests revealed that both the control group and the autism group had greater activity during the OTHER compared to SELF judgments [control: t(11)=3.962, p=.002; autism: t(12)=5.016, p<.001] (**Figure 4.3e**).

In the lANG, there was neither a main effect of group nor a group by condition interaction [F(1,23)=.424, p=.521]. Follow-up t-tests revealed that the control group had greater activity during OTHER judgment compared to SELF judgments [t(11)=2.586, p=.025], while this effect was absent in the autism group [t(12)=1.467, p=.168] (**Figure 4.3f**).

All between-group analyses were re-run using RT and response reliability for SELF and OTHER conditions as covariates. The results of these analyses remained unchanged. Finally, for the autism group, there were no significant correlations between functional activity during SELF vs. OTHER and ADI-R and ADOS social subscores (all p-values > .25).

Discussion

We found that both autism and control groups activated default network brain regions (namely, the MPFC, RSC/PCC, and lANG) when reflecting upon various aspects

of themselves or a close other person. However, the measured BOLD responses differed between the groups in two respects. First, the autism group had reduced spatial extent of activation in all three regions of interest (MPFC, RSC/PCC, and lANG), with this reduction being particularly pronounced in the MPFC (**Figure 4.1**, **Figure 4.2**). Importantly, these extent abnormalities were not pervasive across all brain regions, as extent of activity in early visual cortices was entirely normal, if not greater than normal, in the autism group (**Figure 4.1**, **Figure 4.2**). Second, for particular conditions, the autism group had abnormal magnitudes of activation in the MPFC and RSC/PCC. In the MPFC, the control group had greater activity for PSYC compared to PHYS judgments, while there was no differential activation between these conditions in the autism group (**Figure 4.3a**). In the RSC/PCC, both control and autism groups had greater activity for PHYS compared to PSYC judgments, but there was a trend (p=.054) for this effect being exaggerated in the autism group (**Figure 4.3b**). There were no group differences for SELF and OTHER judgments within the MPFC, RSC/PCC, or lANG (**Figure 4.3d,e,f**).

Abnormalities in the differential responsiveness of the MPFC and RSC/PCC for PSYC and PHYS judgments cannot be explained by a general, persistent dysfunction of these regions, as the differential and overall level of functional activity was normal across SELF and OTHER contrasts. Furthermore, differences cannot be accounted for simply by differences in reaction time or reliability of judgments, because using these performance measures as covariates did not change the results. However, a number of other plausible cognitive and behavioral explanations may account for these abnormalities. One possibility is that individuals with autism may have a specific deficit in making judgments that rely on inference (e.g., PSYC judgments), but an intact or even

enhanced ability in making judgments that rely on observation (e.g., PHYS judgments). This suggestion is consistent with previous behavioral findings on autism. When asked to describe a scene composed of geometric shapes moving in such a way to imply intentionality, subjects with autism often describe the physical, observable features of the stimuli, rather than the non-observable, but readily inferable, intentions of the stimuli (Klin, 2000; Castelli et al., 2002). Second, and potentially relating to a bias toward the observable over the inferable, individuals with autism may have less experience and expertise in making PSYC judgments, but more experience and expertise in making PHYS judgments. Lastly, there may be uncontrolled group differences in the depth of processing (e.g., the richness, detail, and completeness of person representations) during PSYC and PHYS judgments. For instance, the lack of differential MPFC activity in the autism group might be a consequence of less elaborate person representations during judgments of personality traits (i.e., PSYC), while the exaggerated differential RSC/PCC activity might relate to more elaborate person representations during judgments of physical attributes and behaviors (i.e., PHYS).

Although the above-described abnormalities of default regions were observed, it is perhaps equally interesting that, in general, the magnitude and loci of maximum activity were rather similar between autism and control groups. In fact, the magnitude of activation for each region, when collapsed across all 4 judgment conditions, was not different between groups (i.e., no main effect of group). Thus, when faced with a person-reflection task (either self-relevant or other-relevant, and regarding either psychological traits or concrete, physical attributes and behaviors), the autism group recruited the appropriate default regions largely to the same degree as the control group.

A possible explanation for the relatively normal level of functional activity of default regions in the present experiment as compared to previous findings of abnormalities in these regions may have to do with the level of experimental control over the participants' cognitive processes. In the present study, we ensured that subjects engaged in specific self- and other-directed cognitive processing by requiring true/false responses to self- and other-relevant statements. In contrast, during previous studies that used a resting baseline state (Cherkassky et al., 2006; Kennedy et al., 2006; Kennedy and Courchesne, under review) or other socioemotional or mentalizing tasks (Castelli et al., 2002; Pierce et al., 2004; Kennedy et al., 2006), subjects were largely free to engage in their choice of cognitive processes. Thus, differences in functional activity or functional connectivity during such mentally-unconstrained or underspecified circumstances may be due to differences in autistic individuals' default mental processes, rather than reflecting a more pervasive dysfunction of the brain regions (Kennedy and Courchesne, under review).

Importantly, this is not to say that measuring brain activity during an unconstrained or underspecified task is any less important than during an experimentally-constrained task, as each provides a unique perspective on brain functioning. On the one hand, functional imaging studies examining a non-task resting state or an underspecified experimental task can reveal what the autistic brain naturally does (i.e., what it defaults to) when unconstrained by somewhat artificial, rigidly-defined, and externally-imposed task demands. On the other hand, studies using experimentally-constrained tasks with explicit instructions and requirements can reveal what the autistic brain is capable of doing when challenged with a particular task or situation. Thus, although we and others

previously found that, across various rest and non-resting conditions, individuals with autism do not automatically engage default regions to the same extent as controls, the current experiment finds that they are nonetheless capable of engaging these regions, given the proper set of experimental circumstances.

In studies of patient populations, manipulating, constraining, and controlling seemingly subtle cognitive and behavioral factors can have a large impact on functional activity and/or behavioral performance. For instance, Wang and colleagues (Wang et al., 2007) directly examined the effect of explicit task instructions on functional activity in high-functioning individuals with autism. After viewing and listening to short cartoon vignettes, subjects were asked to determine whether or not a story character's utterance was sincere or sarcastic. When given vague instructions (i.e., "pay attention"), the autism group did not activate several brain regions that were active in control subjects (including the MPFC). Remarkably, however, when given explicit directions to attend to faces or prosody of voices, the autism group had more normal levels of activity in the MPFC. In a separate study on face processing in autism, Dalton and colleagues (Dalton et al., 2005) found that gaze fixation, often an uninstructed and uncontrolled behavior, correlated with the amount of brain activity in the fusiform gyrus and amygdala. Along the same lines, two additional studies (Hadjikhani et al., 2004; Pierce et al., 2004) found that modifying certain aspects of a face processing task, including making the task more engaging by including familiar faces and requiring subjects to maintain gaze fixation near the eye region, resulted in more typical levels of fusiform activity in autism. Thus, as previously hypothesized (Hadjikhani et al., 2004; Pierce et al., 2004; Dalton et al., 2005), lack of control over gaze fixation and task engagement or attention may have contributed to

earlier findings of hypoactivation of these regions (Schultz et al., 2000; Pierce et al., 2001). Lastly, Adolphs and colleagues (Adolphs et al., 2005) found that, in a patient with bilateral lesions of the amygdala, behavioral impairment in recognizing fearful facial expressions could be corrected simply by instructing the subject to look at the eye region of the face. Interestingly, this patient reverted back to not looking at the eyes in subsequent trials, further highlighting the importance of explicit task instructions and the need for high levels of experimental control and monitoring when testing patients.

Although not directly addressed in the current experiment, it is interesting to speculate on possible explanations as to why default regions fail to engage automatically in individuals with autism. One possibility is that regions or networks which modulate activity of the default network are not functioning properly. For instance, abnormal input from brain regions involved in the detection of salient social and emotional stimuli might lead to a reduced tendency for the default network to be engaged in underspecified situations. A second possibility is that there may be an abnormally high threshold for activation of the default network in individuals with autism, perhaps due to hypothesized increases in neural noise (Courchesne et al., 2003; Rubenstein and Merzenich, 2003; Belmonte et al., 2004b) or altered patterns of connectivity (Belmonte et al., 2004a; Just et al., 2004; Courchesne and Pierce, 2005). As a consequence, carefully constructed and largely artificial experimental situations would then be necessary to surpass this exceedingly high threshold and activate the default network. As more is learned regarding the automatic and effortless engagement of the default network in control subjects, we will be able to develop more informed explanations for the lack of automatic default network engagement in autism.

In sum, the current experiment provides evidence that default network regions can activate at near normal levels in high-functioning autistic subjects during a self- and other-reflection task. More generally, our findings suggest that default regions, previously shown to be dysfunctional, are capable of being engaged within a more rigidly and explicitly specified experimental context. Of particular interest is determining why such carefully controlled experimental conditions are necessary to engage default regions in autistic individuals, whereas high activity of these regions is the natural, default state of brain functioning in control subjects (Raichle et al., 2001). The answers to this question may be important in developing or further refining effective early intervention programs.

Acknowledgements

We thank Dr. Cindy Carter for clinical assessment, Graham Wideman and Stephanie Carapetian for assistance with programming the stimuli, Doreen Nguyen for assistance with data collection, Elizabeth Redcay and Graham Wideman for helpful discussions, and the researchers and staff at the UCSD Center for Functional MRI. We also thank the participants and their families for graciously giving their time to take part in this study. This research was supported by National Institutes of Health (RO1 MH36840 to E.C.).

References

Adolphs, R., Gosselin, F., Buchanan, T.W., Tranel, D., Schyns, P., Damasio, A.R., 2005. A mechanism for impaired fear recognition after amygdala damage. Nature 433, 68-72.

Belmonte, M.K., Allen, G., Beckel-Mitchener, A., Boulanger, L.M., Carper, R.A., Webb, S.J., 2004a. Autism and abnormal development of brain connectivity. J Neurosci 24, 9228-9231.

Belmonte, M.K., Cook, E.H., Jr., Anderson, G.M., Rubenstein, J.L., Greenough, W.T., Beckel-Mitchener, A., Courchesne, E., Boulanger, L.M., Powell, S.B., Levitt, P.R., Perry, E.K., Jiang, Y.H., DeLorey, T.M., Tierney, E., 2004b. Autism as a disorder of neural information processing: directions for research and targets for therapy. Mol Psychiatry 9, 646-663.

Castelli, F., Frith, C., Happe, F., Frith, U., 2002. Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. Brain 125, 1839-1849.

Cherkassky, V.L., Kana, R.K., Keller, T.A., Just, M.A., 2006. Functional connectivity in a baseline resting-state network in autism. Neuroreport 17, 1687-1690.

Courchesne, E., Pierce, K., 2005. Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. Curr Opin Neurobiol 15, 225-230.

Courchesne, E., Carper, R., Akshoomoff, N., 2003. Evidence of brain overgrowth in the first year of life in autism. Jama 290, 337-344.

Cox, R.W., 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 29, 162-173.

Dalton, K.M., Nacewicz, B.M., Johnstone, T., Schaefer, H.S., Gernsbacher, M.A., Goldsmith, H.H., Alexander, A.L., Davidson, R.J., 2005. Gaze fixation and the neural circuitry of face processing in autism. Nat Neurosci 8, 519-526.

Fletcher, P.C., Happe, F., Frith, U., Baker, S.C., Dolan, R.J., Frackowiak, R.S., Frith, C.D., 1995. Other minds in the brain: a functional imaging study of "theory of mind" in story comprehension. Cognition 57, 109-128.

Gaffrey, M.S., Kleinhans, N.M., Haist, F., Akshoomoff, N., Campbell, A., Courchesne, E., Muller, R.A., 2007. A typical participation of visual cortex during word processing in autism: an fMRI study of semantic decision. Neuropsychologia 45, 1672-1684.

Gallagher, H.L., Frith, C.D., 2003. Functional imaging of 'theory of mind'. Trends Cogn Sci 7, 77-83.

Gusnard, D.A., Akbudak, E., Shulman, G.L., Raichle, M.E., 2001. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci U S A 98, 4259-4264.

Hadjikhani, N., Joseph, R.M., Snyder, J., Chabris, C.F., Clark, J., Steele, S., McGrath, L., Vangel, M., Aharon, I., Feczko, E., Harris, G.J., Tager-Flusberg, H., 2004. Activation of the fusiform gyrus when individuals with autism spectrum disorder view faces. Neuroimage 22, 1141-1150.

Haznedar, M.M., Buchsbaum, M.S., Wei, T.C., Hof, P.R., Cartwright, C., Bienstock, C.A., Hollander, E., 2000. Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. Am J Psychiatry 157, 1994-2001.

Johnson, S.C., Baxter, L.C., Wilder, L.S., Pipe, J.G., Heiserman, J.E., Prigatano, G.P., 2002. Neural correlates of self-reflection. Brain 125, 1808-1814.

Just, M.A., Cherkassky, V.L., Keller, T.A., Minshew, N.J., 2004. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 127, 1811-1821.

Kelley, W.M., Macrae, C.N., Wyland, C.L., Caglar, S., Inati, S., Heatherton, T.F., 2002. Finding the self? An event-related fMRI study. J Cogn Neurosci 14, 785-794.

Kennedy, D.P., Courchesne, E., under review. The intrinsic functional organization of the brain is altered in autism.

Kennedy, D.P., Redcay, E., Courchesne, E., 2006. Failing to deactivate: resting functional abnormalities in autism. Proc Natl Acad Sci U S A 103, 8275-8280.

Klin, A., 2000. Attributing social meaning to ambiguous visual stimuli in higher-functioning autism and Asperger syndrome: The Social Attribution Task. J Child Psychol Psychiatry 41, 831-846.

Lord, C., Rutter, M., Le Couteur, A., 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord 24, 659-685.

Lord, C., Risi, S., Lambrecht, L., Cook, E.H., Jr., Leventhal, B.L., DiLavore, P.C., Pickles, A., Rutter, M., 2000. The autism diagnostic observation schedule-

generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30, 205-223.

Luna, B., Minshew, N.J., Garver, K.E., Lazar, N.A., Thulborn, K.R., Eddy, W.F., Sweeney, J.A., 2002. Neocortical system abnormalities in autism: an fMRI study of spatial working memory. Neurology 59, 834-840.

Muller, R.A., Pierce, K., Ambrose, J.B., Allen, G., Courchesne, E., 2001. Atypical patterns of cerebral motor activation in autism: a functional magnetic resonance study. Biol Psychiatry 49, 665-676.

Ochsner, K.N., Beer, J.S., Robertson, E.R., Cooper, J.C., Gabrieli, J.D., Kihsltrom, J.F., D'Esposito, M., 2005. The neural correlates of direct and reflected self-knowledge. Neuroimage 28, 797-814.

Pierce, K., Haist, F., Sedaghat, F., Courchesne, E., 2004. The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. Brain 127, 2703-2716.

Pierce, K., Muller, R.A., Ambrose, J., Allen, G., Courchesne, E., 2001. Face processing occurs outside the fusiform 'face area' in autism: evidence from functional MRI. Brain 124, 2059-2073.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc Natl Acad Sci U S A 98, 676-682.

Rubenstein, J.L., Merzenich, M.M., 2003. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2, 255-267.

Schultz, R.T., Gauthier, I., Klin, A., Fulbright, R.K., Anderson, A.W., Volkmar, F., Skudlarski, P., Lacadie, C., Cohen, D.J., Gore, J.C., 2000. Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. Arch Gen Psychiatry 57, 331-340.

Talairach, J., Tournoux, P. (1988) Co-planar Stereotaxic Atlas of the Human Brain. 3-Dimensional Proportional System: An Approach to Cerebral Imaging. New York: Thieme.

Wang, A.T., Lee, S.S., Sigman, M., Dapretto, M., 2007. Reading affect in the face and voice: neural correlates of interpreting communicative intent in children and adolescents with autism spectrum disorders. Arch Gen Psychiatry 64, 698-708.

Wang, A.T., Dapretto, M., Hariri, A.R., Sigman, M., Bookheimer, S.Y., 2004. Neural correlates of facial affect processing in children and adolescents with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 43, 481-490.

CHAPTER V

Discussion

Summary

The functioning of the default network in autism was examined across several different tasks and using several different methodological approaches. In Chapter II, functional abnormalities in regions of the default network were revealed both at rest and during an implicit emotion processing task. Furthermore, the degree of resting functional abnormality in the MPFC correlated to the severity of social impairment in individuals with autism. In Chapter III, we used resting functional connectivity MRI to provide further evidence of default network abnormality at rest. We found reduced functional connectivity within the default network, particularly pronounced for the MPFC and lANG. Importantly, reduced network functional connectivity was not a pervasive feature of the resting brain, since abnormality was not found in the dorsal attention network (i.e., the TPN). In Chapter IV, we used an explicitly-defined self- and other-reflection task to further examine default network functionality in autism. While we did observe several subtle abnormalities of default network regions (e.g., reduced MPFC activity during judgments pertaining to psychological personality traits; increased RSC/PCC activity during judgments pertaining to physical attributes and behaviors), we also found that there was a rather similar overall level of default network activity between the groups.

Conclusions

In sum, this series of studies has identified task- and context-specific abnormalities of the default network in autism. During loosely constrained experimental tasks (i.e., resting fixation; an *implicit* emotion processing task), functional abnormalities were readily apparent. However, during a more constrained and specified task (i.e., an

explicit self-other reflection task), seemingly normal levels of activity were observed. This suggests the default network is actually functional – in other words, it can activate, given particular experimental conditions. However, it also suggests that the default network does not engage as readily and automatically in individuals with autism compared to control subjects. This conclusion is also supported by a recent study by Wang and colleagues (Wang et al., 2007), which directly manipulated the implicit/explicit nature of a irony comprehension task via specific task instructions. The autism group only activated the MPFC during the explicit instruction condition, while control subjects utilized this region during both the implicit and explicit instruction conditions. Thus, what is automatic and effortless for control subjects seems to be non-automatic and effortful for individuals with autism.

During the time these experiments were being carried out, an independent line of research on the brain basis of social perception in autism reached largely similar conclusions (Hadjikhani et al., 2004; Pierce et al., 2004; Dalton et al., 2005). Early on, researchers first reported hypoactivation of the face responsive region of the fusiform cortex (i.e., the fusiform face area) in autism during performance of standard face processing tasks (Schultz et al., 2000; Pierce et al., 2001). However, modification of these tasks to control for eye gaze (Hadjikhani et al., 2004; Dalton et al., 2005) and to presumably increase subject interest and attention (Pierce et al., 2004) resulted in no difference in levels of fusiform activity between autism and control groups. Thus, this research also identified brain abnormalities in autism that are context- and task-specific, rather than reflecting a more generalized (i.e., task- and context-independent) dysfunction.

Together, these findings on the functionality of the default network and the fusiform face area may provide an important clue as to the neural basis of autism. Since these neural systems share a similar pattern of context-specific dysfunction, it is likely that they might also share a common neural substrate underlying their dysfunction – perhaps stemming from disrupted modulatory input to these and other social- and emotional-responsive brain regions and brain networks. One possibility could be that a brain system underlying reward feedback for social and emotional stimuli is disrupted, thus reducing the likelihood that the autistic individual will seek out social and emotional information from the environment. Over time, this behavioral abnormality might become exaggerated, as the child attends less and less to social and emotional aspects of the world, and more toward non-social, non-emotional concrete features of the world (e.g., calendars, maps, computers, facts, schedules, etc.). As a result, the autistic individual might only perform a social or emotional task in a highly artificial environment, such as when an extrinsic reward (e.g., money) is directly linked to the explicitly-defined social and emotional demands of the task. In fact, such an approach (i.e., linking extrinsic reward to performance of social and communicative behaviors of interest) forms the basis of several types of effective behavioral treatments for autism.

A second possibility is that there could be a disruption in the modulatory input from a brain system involved in the detection of salient social and emotional stimuli in the environment. With a dysfunctional salience detector, social and emotional stimuli would not be any more attention-getting than non-social and non-emotional stimuli in the environment, of which the latter is much more pervasive in the environment (e.g., look around, and note the number of socially- and emotionally-relevant objects compared to

objects that are not socially- nor emotionally-relevant). Over time, the autistic individual would spend less time focusing on social and emotional aspects of the world, defaulting instead toward non-social and non-emotional features of the environment. As a consequence, only artificial experimental contexts that well-isolate social and emotional aspects of a task would bypass such a faulty salience detector, and engage the appropriate brain networks.

In terms of the implications of this research for treatment of autism, I believe that the results are highly encouraging. Although we did find abnormality of a brain network that is critically involved in social, emotional, and introspective processing, we and others have shown that this network is actually capable of responding at a normal level, given particular experimental circumstances. This suggests that the neural substrates of social and emotional processing might be relatively intact, though underutilized, in higher functioning individuals with autism. If this is confirmed, the challenge lies in figuring out how to get the default network to automatically engage during naturalistic, real-world situations in individuals with autism.

References

Dalton, K.M., Nacewicz, B.M., Johnstone, T., Schaefer, H.S., Gernsbacher, M.A., Goldsmith, H.H., Alexander, A.L., Davidson, R.J., 2005. Gaze fixation and the neural circuitry of face processing in autism. Nat Neurosci 8, 519-526.

Hadjikhani, N., Joseph, R.M., Snyder, J., Chabris, C.F., Clark, J., Steele, S., McGrath, L., Vangel, M., Aharon, I., Feczko, E., Harris, G.J., Tager-Flusberg, H., 2004. Activation of the fusiform gyrus when individuals with autism spectrum disorder view faces. Neuroimage 22, 1141-1150.

Pierce, K., Haist, F., Sedaghat, F., Courchesne, E., 2004. The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. Brain 127, 2703-2716.

Pierce, K., Muller, R.A., Ambrose, J., Allen, G., Courchesne, E., 2001. Face processing occurs outside the fusiform 'face area' in autism: evidence from functional MRI. Brain 124, 2059-2073.

Schultz, R.T., Gauthier, I., Klin, A., Fulbright, R.K., Anderson, A.W., Volkmar, F., Skudlarski, P., Lacadie, C., Cohen, D.J., Gore, J.C., 2000. Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. Arch Gen Psychiatry 57, 331-340.

Wang, A.T., Lee, S.S., Sigman, M., Dapretto, M., 2007. Reading affect in the face and voice: neural correlates of interpreting communicative intent in children and adolescents with autism spectrum disorders. Arch Gen Psychiatry 64, 698-708.