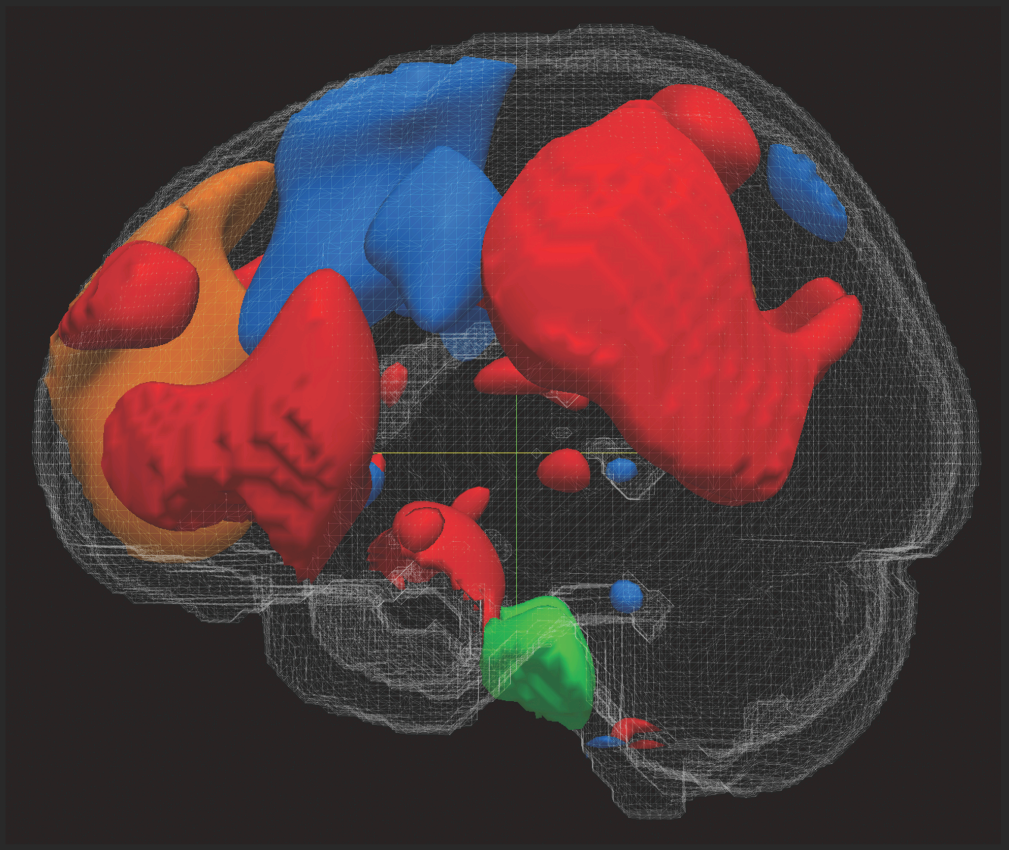
The Developmental Neurobiology of Brain Connectivity in Schizophrenia



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ISBN

Layout: Tonya White
Cover: Marcus Schmidt (in collaboration with Tonya White)
Printing: Optima Grafische Communicatie, Rotterdam

This research was funded by the National Institutes of Mental Health (K08-MH068540), National Alliance for Research in Schizophrenia and Affective Disorders (through the Essel and Blowitz-Ridgeway Foundations), the Mental Illness and Neuroscience Discovery (MIND) Research Network, and the Center for Neurobehavioral Development (CNBD) at the University of Minnesota. Additional finanacial support was provided by the Department of Psychiatry at the University of Minnesota, and through a KEMPF Award by the American Psychiatric Association. I would also like to acknowledge grants from the National Institutes of Mental Health that support the infrastructure at the University of Minnesota: P30 NS057091 & P41 RR00807.

Financial support for this thesis and partial support for publication of this thesis was also provided by the Department of Child and Adolescent Psychiatry at the Erasmus Medical Centre, Rotterdam, Netherlands.

The Developmental Neurobiology of Brain Connectivity in Schizophrenia

Het Ontwikkelings Neurobiologie van Brain-Verbinding in Schizofrenie

Proefschrift
ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden
op woensdag 1 september 2010 om 9:30 uur.

door

Tonya White
Geboren te Moline, Illinois, in de Verenigde Staten van Amerika

Promotiecommissie

Promotoren:	Prof.dr. F.C. Verhulst Prof.dr. N. C. Andreasen, M.D., Ph.D.
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Connectivity, whether in the brain or in the world around us, is the sustenance of life.

This Work is dedicated to those with whom I am most connected: My Mother and Father and my three sons, Kristof, Andreas, and Jon Erik

Table of Contents

"I have always enjoyed a good story."

Prologue

I admire researchers who weave beautiful stories of science. Stories not necessarily woven in the form of a biography, but stories that emerge as the history of their life work is pieced together to become a whole. I listen for these stories during conference presentations and keynote lectures where I hear phrases such as "We landed upon this result and it puzzled us. It's not what we expected and we looked at it from different angles…. and it led us off in this new direction." I have enjoyed these stories from those who have contributed much to our knowledge of neuroscience and developmental neurobiology.

Such stories have emerged from my mentors and remain with me as examples as I piece together the genesis of my own story. One of my early research mentors was William McMahon, who described the story of seeing a ten-year-old boy in the outpatient clinic who had Tourette. In obtaining the family history he learned that the boy was from a fundamentalist Mormon family and his grandfather had ten wives. This interaction led him into a career in studying the genetics of Tourette. Nancy Andreasen at the University of Iowa was surprised to continually find activations in the cerebellum while subjects were performing cognitive tasks. This finding led her to evaluate more closely the role of the cerebellum in cognition and developing the concept of cognitive dysmetria. Irving Gottesman in attempting to find a link between genes and behavior, introduced the concept of an intermediate phenotype, or endophenotypes. Kelvin Lim found white matter differences using nuclear magnetic spectroscopy, which led him to use diffusion tensor imaging to look more closely at the white matter microstructure in schizophrenia. Chuck Nelson and others' work in examining development, cognition, and behavior in Romanian children in orphanages resulted in the government closing all the Romanian orphanages and replacing the entire orphanage system with foster care. These are wonderful stories of science from my mentors.

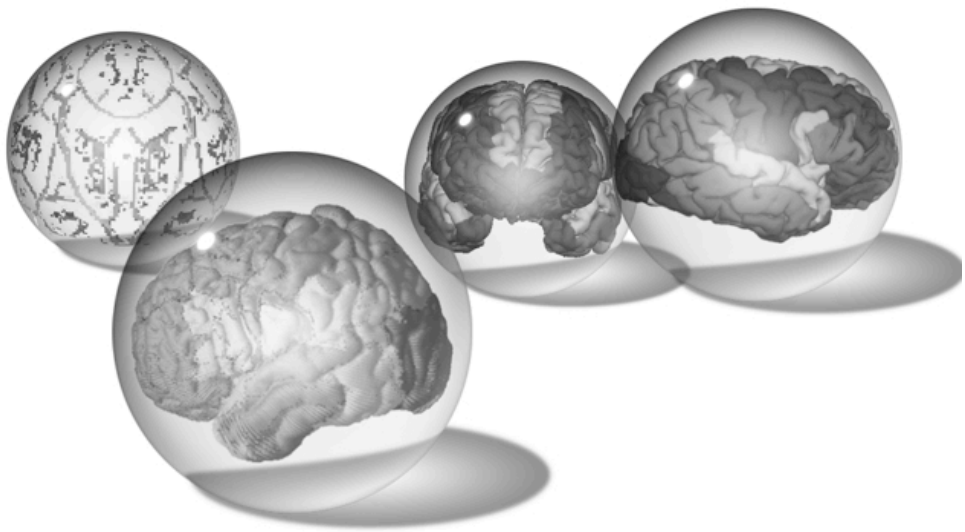
Since I am writing this PhD thesis not as a junior researcher, but rather in the middle of my research career, it provides the opportunity to present a partial version of my own story and how each of the papers within this collection are linked to this story. The story is not one of seeing the picture and searching for the puzzle pieces to form the picture, but rather a story of an unfocused camera gradually coming into focus. The story leads me from Utah to Illinois, back to Utah, to short stints in Nebraska and Iowa, then on to eight years in Minnesota, and now to the Netherlands, where I currently am sitting in a small dorm room as I write this prologue, my story to date.

But perhaps my story should start earlier, perhaps the story should start in elementary school, while living in Frankfurt, Germany, where my father ordered science kits that came once a month in a small blue box. Some were interesting, some less so, but they would plant a seed of science which has grown and become deeply rooted. Since my undergraduate years, I have been involved continuously in research. My introduction into research was while I was an undergraduate student in electrical engineering at the University of Utah where I worked at the Center for Biomedical Design on the Utah Artificial Arm. I would leave the beautiful Wasatch mountains that surround Salt Lake City to return to my home state of Illinois, with its acres and acres of corn and soy beans, to attend the University of Illinois in Champaign-Urbana. It's a bit ironic for an MRI researcher, that while I was working towards my Masters degree in electrical engineering at the University of Illinois, that I sat in the lab of Nobel laureate Paul Lauterbur. My focus at the time was in bioinstrumentation and not in MRI research. My submersion into applied MRI research would occur over ten years later, after medical school and residency, when I started my postdoctoral research program at the University of Iowa under Professor Nancy Andreasen. This marks the start of this thesis, as two of the papers within this book originate from my postdoctoral research fellowship.

Scientists are writers, both figuratively and literally. A large part of science involves the ability to convey the work in an intelligent and thoughtful manner. Thus, I must also acknowledge the role of my mother, who as a teacher would interrupt dinner to bring a dictionary or World Book Encyclopedia to the dining room table to look up those unknown things relating to our conversation. She also corrected my grammar, broadened my vocabulary, and helped form the writer that I am today.

There are some of us who it seems are destined to follow the path of research, to forgo the lucrative call of the private practice physician because the unanswered questions that we face as clinicians are legion, and we feel drawn, compelled, perhaps even forced by our own nature to address these questions. I must admit that I must be counted among these. I hope that you enjoy the content of this thesis, as I weave together the beginnings of new editions into the book of science.

1 Introduction



Tonya White

Introduction

Much work over the past two decades supports the concept that schizophrenia involves a disruption in the orchestration of the multiple neural networks that participate in higher cognitive functions (1-4). The connectivity between neural networks arising during normal development is potentially disrupted, leading to the recruitment of either inappropriate regions for task execution, or alternatively, alters the processing requirements in expected regions. For example, studies utilizing both glucose metabolism (FDG-PET), SPECT, and H_2O^{15} PET have demonstrated hypofrontality in the dorslolateral prefrontal cortex (DLPFC) on tasks of executive function (5-9). Additionally, studies utilizing diffusion tensor imaging (DTI), which measures the coherence of neuronal fiber tracts, have shown a decrease in the coherence of white matter tracts in schizophrenia (10, 11). This is presumed to be additional evidence for disrupted connectivity in schizophrenia.

The clinical phenotype that is associated with schizophrenia also provides support for disrupted connectivity. The constellation of symptoms, which can include hallucinations, delusions, disorganized thought, disorganized speech, and the negative symptoms of avolition, apathy, alogia, and flattened affect cannot be localized to one specific brain region. The constellation of these diverse symptoms provides support for more global processes in schizophrenia. In addition, the nature of the cognitive deficits, deficits that cross multiple cognitive domains (Chapter 2.1), also provide support for more global processes in schizophrenia. Deficits in motor function, memory, problem solving, and language are present at approximately one standard deviation below the mean.

Since working memory is thought to be a building block for higher-order cognitive function, it has received considerable attention in studies of schizophrenia. Recent studies have shown that patients with schizophrenia show impairments in working memory that is associated with an increased blood flow to working memory circuits in the prefrontal cortex (12, 13). Interestingly, typically developing children and adolescents perform less well than adults on working memory tasks (14) and also show increased prefrontal activation during working memory paradigms (15, 16). As there have been several studies supporting a different developmental trajectory in adolescents with schizophrenia, I set out to study the parallel between the functional brain activity in schizophrenia to that of healthy children and adolescents whose prefrontal cortex is continuing to develop? Perhaps schizophrenia involves a disruption in brain maturation that prevents the optimization of cognition for efficient task execution. Alternatively, schizophrenia could involve diffuse brain alterations, leading to completely different neuronal connectivity and an associated decrease in performance different than that seen in children and adolescents.

There is little known about the functional neurodevelopmental trajectory of the prefrontal cortex and its connections during the period when higher order cognitive functions are coming on line. An understanding of the typical neurodevelopment trajectories of these higher order functions will greatly enhance

the ability to assess where youth in the process of developing schizophrenia reside within (or outside of) the typical neurodevelopmental trajectory.

In recent years, magnetic resonance imaging techniques have allowed the study of brain structure, function, and structure/function relationships. MRI utilizes non-ionizing radiation, which makes it well suited to study neurodevelopmental trajectories in both typically developing children and in children and adolescents with neuropsychiatric disorders. In addition, a number of imaging techniques (i.e., diffusion tensor imaging) and image analysis techniques (i.e., independent components analyses) that allow for quantitative measures of different facets of brain connectivity. Brain connectivity is the thread that weaves together the papers in this thesis.

The major questions that I sought to address involved the developmental neurobiology of brain connectivity in children and adolescents with schizophrenia. More specifically, I was very interested in studying connections with the prefrontal cortex (PFC), as there is a temporal relationship between the protracted development of the PFC and the peak age of onset for schizophrenia. I hypothesized that there was an inability to optimize the functional architecture of the PFC during this period of protracted development. Furthermore, I hypothesized that aberrations in myelination in the prefrontal cortex would contribute to this dysregulation of optimized neural connectivity. The connectivity of the PFC with other brain regions is an area of considerable research.

Anatomical Basis of Connectivity

One historical approach of defining the anatomic boundaries of the prefrontal cortex is through criteria that are based on connectivity with the medialdorsal nucleus of the thalamus (MDN) (19). Although other thalamic nuclei have direct connections with regions of the PFC (20), architecture alone is not sufficient to define the boundaries of the PFC and has been coupled with connectivity (19). Many of the studies that define connectivity between brain regions involve the use of tracer compounds injected into one region of a primate brain and allowed to migrate along the neuronal tracts. A web-based database exists to identify published studies of neuronal connectivity and brain mapping studies in the macaque brain and can be found at www.cocomac.org (21).

PFC – MDN Connectivity: The afferent projections from the medialdorsal nucleus are topographically organized and consist of two primary cytoarchitectural components, the magnocellular and parvocellular components (20, 22). These projections course through anterior thalamic radiations and the inferior thalamic peduncle to the PFC. The projections that connect to the dorsolateral PFC (DLPFC) developed later in phylogeny and emanate primarily from the parvocellular cytoarchitectural compenent of the MDN (19, 20, 23).

PFC – Posterior Parietal Lobe Connectivity: Projections from the primary sensory areas to the PFC occur along sequential pathways that include feedback loops from the PFC (19). Area 7 in the parietal lobe is the third link in the pathway from

primary sensory modalities to the PFC. There is a direct connection between area 7a (posterior parietal lobe in the rhesus monkey) with the DLPFC (24), which is considered a component of a distributed network involved in spatiotemporal cognitive processes (25).

Cerebellum – MDN Connectivity: The cerebellum, once thought to be associated primarily with motor function, has been demonstrated to play a significant role in higher cognitive processes (26, 27). The evidence of direct connectivity between the DLPFC and the cerebellum stems from fluorescent dye and retrograde transneuronal tracers placed in areas of the PFC and cerebellum (27-29). The pathway involves the dentate nucleus to the MDN and the MDN to the DLPFC.

Basal Ganglia – MDN Connectivity: One of the non-motor circuits connecting the basal ganglia has been theorized to include the DLPFC via the thalamus (30). Retrograde transneuronal tracers placed in area 46 along the principle sulcus of the DLPFC identified regions in the internal segment of the globus pallidus in addition to three thalamic nuclei, including the MDN (27). After spatially localizing the basal ganglia output regions in the thalamus using electrophysiologic techniques, McFarland & Haber (2002) demonstrated neuronal connectivity between the DLPFC and MDN (23). The target layer (I-VI) of the cortex differed with evidence of both reciprocal and nonreciprocal pathways.

PFC Connectivity with other Brain Regions: In addition to the reciprocal connections with the MDN, connections have also been described linking the DLPFC with the ventral anterior thalamus (23), brainstem tegmentum (31, 32), locus coeruleus and raphe nuclei (33), hypothalamus (34), amygdala (35), and the claustrum (36). and from the primary somatosensory cortices via corresponding association cortices (19).

Summarizing the neuroanatomic basis for connectivity, direct neuronal connections exist between the PFC and MDN, the PFC and the Posterior Parietal Lobe, and between the MDN and basal ganglia. The connectivity between the cerebellum and basal ganglia to the DLPFC is modulated by the thalamus. The posterior parietal lobe has direct connections with the thalamus, primarily the (VL) and VA nuclei. Studies have demonstrated pathohistological abnormalities in both the MDN and DLPFC in schizophrenia (37-43). Neuronal cell loss in the MDN has been replicated by several investigators (37-40), although not all (44). Popken et al. (2000) reported greater cell loss in the parvocellular and dorsocellular subnuclei within the MDN. Reductions in cell count in the parvocellular subnuclei would be expected to have the greatest impact on working memory secondary to its connectivity with the DLPFC. Additional evidence of aberrant disconnection between the MDN and the PFC comes from Lewis et al (45) who reported a decrease in the projections of MDN axons to the middle cortical layers of the PFC.

Neurophysiological Basis of Connectivity

Tasks that require an internal representation of the location of an object in space have been widely used in primate single-unit recording studies (46-53). These

studies have provided information on the neural circuits involved in observing the location of an object in space (cue), maintaining this location in working memory (delay), and identifying the location of the object after a set delay period (i.e., saccade). The critical nodes within the circuit that have been well delineated to date include the DLPFC, posterior parietal cortex, and the thalamus.

Studies utilizing multiple site, single-cell recordings in both the DLPFC and posterior parietal cortex have demonstrated similar spatial tuning between assorted cells during a delayed response task (DRT) (48, 52). Although the posterior parietal lobe may demonstrate more specificity to the cue, and the DLPFC to the delay (48), both regions are active during each phase of the task and their similarity supported a reciprocal projection linking these two brain regions (52). Both posterior parietal and DLPFC cells are active with both memory-guided saccades and memory-guided arm responses (48, 51), although studies have demonstrated that a circuit involving the posterior parietal, premotor, and motor cortices are involved in motor movements (54, 55), including those that involve memory tasks (56).

A single cell study of the thalamus that included the firing rate of MDN cells to a DRT task demonstrated similar associations as that seen in the DLPFC (57). The authors report a high degree of task-related specificity among the neurons tested. In addition, recordings in the lateral dorsal and lateral posterior nuclei of the thalamus demonstrated similar coding properties as those in the posterior parietal cortex. Finally, single cell studies support connectivity between the cerebellum and motor associated nuclei within the thalamus (58, 59).

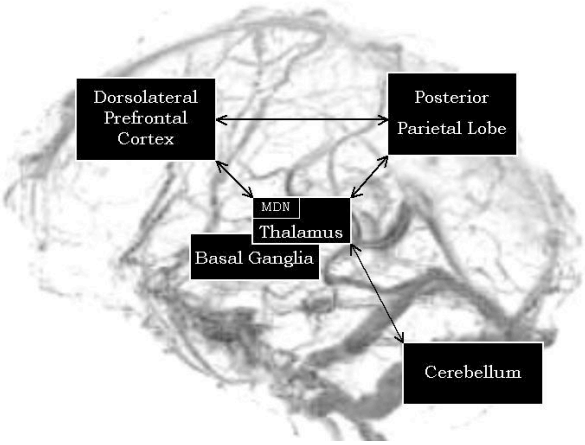
Functional Magnetic Resonance Imaging Basis for Connectivity

Whereas neuroanatomic studies of connectivity define actual neural pathways, and neurophysiologic studies describe the coupling neuronal activity with a specific sensorimotor or cognitive task, fMRI measures of connectivity are a step removed from the actual measurement of neuronal activity. Considerable effort is directed towards defining the relationship between the underlying neuronal activity and the fMRI signal (60). The fMRI signal is based on the paramagnetic properties of deoxygenated hemoglobin (61, 62) and is a result of changes in the equilibrium between blood flow and oxygen uptake. There is emerging evidence supporting a coupling between a spatially specific increase in neuronal activity and increased blood flow, termed the neurovascular response (60). The time course of these spatially specific hemodynamic changes has been shown to approximate a low-pass filter of the total neuronal activity for a specific activated region (63).

A comparison of multiple site, single unit recordings and the T_2^* or gradient echo (GRE) blood-oxygen-level-dependent (BOLD) fMRI technique in the occipital cortex of a cat have found that the T_2^* BOLD signal to be correlated with neuronal activity when the spatial resolution of the acquisition is on the order of 4 to 5 mm (64). Studies that involve group comparisons of the brains within a defined stereotactic space often account for individual anatomic variability by applying spatial filters (65), often on the order of 8 mm. Although this spatial resolution is reasonable for many cognitive neuroimaging studies, higher spatial resolutions are

likely associated with a higher correlation between the fMRI signal and the underlying neuronal activity (64). For studies of functional connectivity, it is optimal to have a high correlation between the fMRI signal and the underlying neuronal activity.

Figure 1 – *Brain Regions thought to be Abnormal in Schizophrenia during Higher-Order Cognitive Tasks*



One factor limiting the spatial resolution of the GRE-BOLD technique is the significant contribution of signal from the draining arterioles and veins. A study evaluating the connectivity of adjacent gyri by combining DTI and GRE BOLD fMRI (66) was likely confounded by these flow artifacts. Several different image acquisition sequences have been developed to reduce the signal from the larger vessels and to increase the spatial sensitivity of the signal. One such technique, the spin-echo (SE) BOLD sequence, causes a rephasing of the field gradient surrounding larger vessels and at high fields reduces the signal (T_2) from venous blood (67). Although SE sequences have been demonstrated to be more spatially selective at 1.5 Tesla (68), the spatial resolution is localized almost completely to the extravascular space with higher field strengths (67, 69). SE BOLD has been performed in an fMRI task of cognition with greater spatial localization and detection of BOLD changes in the ventromedial cortex, which is often difficult to detect in standard GE BOLD secondary to susceptibility gradients (70).

The methodologies for analyzing patterns of functional activity in the brain utilizing fMRI have been a source of considerable research and discussion over the past decade. The appearance of multiple techniques highlight that no one approach to fMRI data analysis applies to all conditions. The earliest approaches to analyze fMRI data utilized a block fMRI experiment and correlated the temporal waveform for each voxel with a phase-shifted waveform at the same frequency as the block (71). This approach defines brain regions with hemodynamic properties that directly correlate with the task presentation, and thus the hypothesis is that those regions form a network that is 'functionally' related to the task. This approach has been used in a number of fMRI studies utilizing spatial working memory tasks in both adults (72, 73) and children (15, 16). The brain regions that demonstrate hemodynamic correlations with the spatial working memory task show an excellent overlap with the nodes presented in Figure 1. Although these studies are able to

define brain regions that are involved in the network, they cannot ascertain the functional interactions between brain regions in the network that have different hemodynamic patterns. Thus paradigms that measure the functional connectivity between brain regions have been developed.

Functional connectivity is defined as the temporal correlation of the hemodynamic waveforms (i.e., BOLD signal for a specific voxel or regional clusters of voxels over time), between different brain regions (74). The theory is that a distributed neural network within the brain mediates the performance on a specific task, and different tasks tap into different distributed networks. Examining the covariance patterns of the fMRI signal between different brain regions can provide information regarding the nodes that are involved for each task (75). Another form of connectivity, effective connectivity, is defined by the causal influence that one brain region has on another (76). Structural equation modeling was adopted as a technique to investigate effective connectivity (77).

In the analysis of functional connectivity of raw fMRI data, a continuum exists that is dependent upon constraints or inferences that are placed on the data (78). Strategies utilizing exploratory multivariate methodologies, such as canonical variates analysis (CVA) or principle components analysis-MANCOVA-PCA, lie on one end of the continuum (79, 80). By searching for covariance patterns, these methods approach the data without preconceived ideas or constraints regarding connectivity between different brain regions. Although this approach is less adept at testing specifically defined models, it can provide important clues to areas of connectivity that were not initially included in the model. On the opposite side of the continuum of exploratory multivariate analyses are path analytic models (77, 81-83). These models place constraints on the data that are based on various factors, such as knowledge that two specific regions are anatomically connected. Structural equation modeling is one path analytic model that calculates strengths between different nodes (or predefined brain regions) within a network. (77). The most common approach of SEM utilizes linear equation modeling of the covariance patterns between the different nodes. However, non-linear techniques have evolved and may provide a better approach for some models (84), especially those that utilize cognitive tasks (85).

McIntosh et al. (83) demonstrated changes in the effective connectivity between nodes associated with the delay period on a delayed match to sample (DMS) task to faces using different delay periods. They described an interaction between nodes in the temporal and occipitotemporal association region with the PFC (BA 47) and anterior cingulate that strengthens with increasing delay periods. In addition, alterations in the interactions have been shown to be associated with aging (86, 87). To date there are no published studies utilizing SEM to study neurodevelopment in children and adolescents. One intriguing aspect of SEM in this population is its ability to describe task-dependent changes in the effective connectivity in children and adolescents compared to adults. For example, it is known that children perform less well on tasks of working memory compared to adults (88). It is also known that the PFC has continued development through adolescence shown by studies of synaptic pruning (89), GM changes (90, 91), and

myelination (92-95). Since a decrease in the BOLD 'activation' region in the brain may actually indicate an increased strength of association between brain regions (96), SEM provides a mechanism to determine whether the changes in the BOLD signal are related to more efficient processing or a decrease in communication between regions. With the developmental changes in the PFC, a prediction would be an age-associated increase in communication with circuits connecting the PFC through adolescence. Identifying the differences in the effective connectivity in children and adolescents with schizophrenia can provide a window into the neurodevelopmental course of the illness.

Structural and Diffusion Tensor Imaging Basis for Connectivity

Currently, structural MRI can provide only indirect and theoretical measures of connectivity in brains that have no distinct lesions. Studies that demonstrate correlations between specific volumes of brain regions that are known to be connected and follow similar developmental trajectories may provide evidence for connectivity between the regions. For example, in monozygotic twins, the correlation between the thalamic volume and cortical GM was found to be 0.76 ($p<0.0001$), a finding that remained significant when controlling for total brain tissue (97).

A number of structural neuroimaging studies in patients with schizphrenia have demonstrated abnormalities in the putative nodes of the DRT circuit in both adults (98-102) and in children and adolescents (103-110). Focusing on the cerebral nodes that make up the DRT circuit, these studies demonstrate a decrease in frontal gray matter (98, 99, 103, 106, 107), abnormalities in the thalamus (109, 111-113), including a specific reduction in the size of the MDN (114), and a decrease in the size of the vermis in the cerebellum (102, 110). Interestingly, Thompson et al. (2001) demonstrated in a sample of childhood-onset schizophrenia subjects recruited at the NIMH, that the GM changes started in the parietal lobe and progressed to include the frontal area during adolescence. Since the children were ill on average for 3 years prior to entry into the study, the GM changes may reflect a downstream effect of the illness. Finally, a study of surface morphology in childhood and adolescent-onset schizophrenia demonstrated altered patterns of curvature in the sulci and a decrease in cortical thickness (Chapter 5-1). These changes may reflect aberrant connectivity between regions based on Van Essen's theory of the 'tension based morphogenesis' of the cerebral cortex (115).

Diffusion tensor imaging (DTI) has properties that may allow for high-resolution in vivo measurements of connectivity between brain regions. DTI is based on the diffusion properties of water (116). When water molecules are able to freely diffuse, unrestricted, in any direction, this is termed isotropic diffusion. However, when there are boundaries preventing the diffusion of water molecules in certain directions, the diffusion properties of water change to what is termed anisotropic diffusion. With the ability to measure water diffusion properties by applying strong magnetic fields and modulating the hydrogen molecules attached to the water, it is possible to measure the microstructure of the regions that

surround water molecules (117). Utilizing at least six non-collinear directions, it is possible to calculate both the average magnitude and direction of water diffusion within a voxel (116). This allows for the observation of regions in the brain that demonstrate high anisotropy, such as the visualization of white matter tracts (118).

The ability to utilize DTI to define specific fiber tracts, or DTI tractography, is still a relatively new phenomenon (119-122), although new algorithms to map white matter tracts are developing rapidly. The approaches to reconstruct the WM pathways can be subsumed under two major categories (121). The first set of techniques use line propagation algorithms that utilize information from one voxel to determine the most probable next step in the fiber pathway (119, 120, 122). The second set of techniques is based on algorithms that produce a global minimum energy for the system or the minimum energy to connect two brain regions (66, 121, 123). Although the fiber pathways identified have restrictions in the detail of the anatomy, applied algorithms are able to show reasonable anatomic consistency with WM tracts such as the geniculo-calcarine visual pathway (119), corticocortico connections within the parietal lobe (119), and branches in the corona radiata and optic radiations (123).

One limitation of fiber tracking utilizing DTI is the inability, at least at present, to define connectivity at the cellular level. Typical voxel dimensions used in DTI are on the order of millimeters, whereas a single axon is on the scale of micrometers. Intersections of neuronal tracks, for example, result in a decrease in the level of anisotropy and a reduction in the directional sensitivity of the vector. Reducing the volume of voxel size will produce greater directional information, but with a decrease in the signal to noise ratio. Increasing the signal to noise ratio can be accomplished by using higher field strengths, longer acquisition times, optimized sequences and state of the art MRI hardware (123).

There are to date a number of studies that utilize DTI to study either typical neurodevelopment (124-126) or schizophrenia (10, 11, 127-130). DTI was recently used to demonstrate differences in anisotropy in a cross-sectional study of a group of 10-year-old children and adults (125). Klingberg et al.'s work supported the early pioneering work on postmortem brains demonstrating continued white matter development into adulthood by Yakovlev and Lecours (95).

Numerous studies have demonstrated either regional or global abnormalities in white matter in schizophrenia (10, 11, 128, 130, 131), although exceptions do exist (129, 132). The differences in findings may relate to methodological issues or differences in the regions of interest studies. The alteration in white matter pathways may be associated with disruptions in the distributed networks of brain activity and result in the diversity of symptoms that are associated with schizophrenia.

Cognitive Developmental Neuroscience and Connectivity

Children acquire specific cognitive abilities during specific points in development (133). Gross and fine motor function, language, and the various aspects of social cognition all develop within specific windows of

neurodevelopment. Higher order cognitive processes, complex problem solving skills and the ability to engage in abstract reasoning show the most robust development after the first decade of life (88). Functional neuroimaging of both sensorimotor and higher-order cognitive processes demonstrate that increasing performance on a specific task results in a change in the functional architecture of altered blood flow within the brain. There tends to be increased activity (alteration in regional blood flow) that is correlated with poorer task performance.

While automaticity may be associated with an alteration in the functional architecture (134), especially in adults, this is likely not a large influence with children. Irrespective of how children may practice a higher-order cognitive task, there is a ceiling effect that is dependent on their stage of neurodevelopment. For example; relative to adults, children on average are able to store less information in working memory and retain that information for a briefer period of time (88). Children also have a greater spatial distribution of their activated regions during functional imaging studies in comparison with adults (15). Thus the neurodevelopment of specific sensitive periods allows for an optimization of cortical neuronal activity that may be related to continued structural and neurochemical development.

Schizophrenia can also be considered as developing within a sensitive period. The age-of-onset of schizophrenia has a peak during the late teens and early twenties and has a skewed distribution (135). Prepubertal onset of schizophrenia is rare and thus the etiologic mechanism either directly interacts with the developing brain, or requires a sensitive state of neurodevelopment prior to the onset of symptoms. Since typically developing children and patients with schizophrenia have a greater spatial distribution of their functional brain activity on working memory tasks (12, 13, 15, 16), and since both perform less well than adults on these tasks, it is possible that differences in functional connectivity emerge in children as they approach adulthood, compared to patients with schizophrenia as they approach adulthood.

Summary of Connectivity

There is considerable evidence supporting the prefrontal cortex, and notably the dorsolateral prefrontal cortex (DLPFC) as a node affected by the underlying pathophysiology of schizophrenia (5, 7-9, 12, 18, 136-138). Additionally, a decrease in performance in working memory tasks has been shown to be a genetically determined endophenotype of schizophrenia (138), present in relatives of patients with schizophrenia (139). In *approaching the puzzling etiologic question of schizophrenia*, it is intriguing consider the integration that: (1) both patients with schizophrenia and children perform less well on tasks of working memory; (2) one prime neurodevelopmental event during adolescent to adult development is continued myelination of the prefrontal cortex (93-95, 140, 141); (3) aberrations in white matter have been identified in subjects with schizophrenia in both DTI studies (10, 11) and in studies of postmortem gene expression (142); and (4) and

both children and patients with schizophrenia have greater heterogeneity in patterns of activation in the DLPFC (12, 13, 15, 16).

This thesis explores the developmental neurobiology of schizophrenia across several domains and using different modalities. Chapter 2 focuses on cognition in schizophrenia. Chapter 2-1 demonstrates that children and adolescents with schizophrenia present with global cognitive deficits. While they do show more pronounced differences in the cognitive domains of language, working memory, and motor function compared to adults compared to adults with schizophrenia, both the working memory and language domains were not different when controlling for the typical developmental trajectory of the control adolescents (i.e., the adolescent controls performed significantly worse on working memory and language than the adult controls). Chapter 2-2 describes a study using the oculomotor delayed response task (oDRT) in children and adolescents with schizophrenia, attention deficit hyperactivity disorder (ADHD), and healthy controls. In this visuospatial working memory task, we found evidence encoding problems in children and adolescents with schizophrenia. We also found specific differences in cognitive function between ADHD and schizophrenia, although both have working memory deficits. There are relatively few studies that compare different psychiatric disorders, and these findings highlight the importance of carefully designed tasks that tap into specific neural networks that could potentially be used to parse out the different disorders.

Chapters 3 through 5 provides results from studies of structural (Chapter 3), DTI (Chapter 4), and fMRI (Chapter 5) experiments. These modalities were used to evaluate gyrification, white matter, and functional connectivity, respectively. While the results clearly support aberrant connectivity in schizophrenia, the results were somewhat different than expected. Such differences often occurs in the stories of science. One potential ramification of this work is that findings commonly reported in adults with schizophrenia may actually be downstream effects of early insults, possibly in the limbic system, and more specifically, the hippocampus.

References

1. Andreasen NC, Paradiso S, O'Leary DS (1998): "Cognitive dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical-subcortical-cerebellar circuitry? *Schizophr Bull*. 24:203-218.
2. Friston KJ, Frith CD (1995): Schizophrenia: a disconnection syndrome? *Clin Neurosci*. 3:89-97.
3. Dolan RJ, Fletcher PC, McKenna P, Friston KJ, Frith CD (1999): Abnormal neural integration related to cognition in schizophrenia. *Acta Psychiatr Scand Suppl*. 395:58-67.
4. McGuire PK, Frith CD (1996): Disordered functional connectivity in schizophrenia. *Psychological Medicine*. 26:663-667.
5. Andreasen NC, Rezai K, Alliger R, Swayze VWd, Flaum M, Kirchner P, et al. (1992): Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia. Assessment with xenon 133 single-photon emission computed tomography and the Tower of London. *Arch Gen Psychiatry*. 49:943-958.
6. Weinberger D, Berman KF (1988): Speculation on the meaning of cerebral metabolic hypofrontality in schizophrenia. *Schizophrenia Bulletin*. 14:157-168.
7. Weinberger DR, Berman KF, Suddath R, Torrey F (1992): Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: A magnetic resonance and regional cerebral blood flow study of discordant monozygotic twins. *American Journal Psychiatry*. 149:890-897.

8. Andreasen NC, O'Leary DS, Flaum M, Nopoulos P, Watkins GL, Boles Ponto LL, et al. (1997): Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. *Lancet*. 349:1730-1734.
9. Buchsbaum MS, Wu JC (1987): Hypofrontality in schizophrenia as assessed by PET. Am J Psychiatry.
10. Buchsbaum MS, Tang CY, Peled S, Gudbjartsson H, Lu D, Hazlett EA, et al. (1998): MRI white matter diffusion anisotropy and PET metabolic rate in schizophrenia. *Neuroreport*. 9:425-430.
11. Lim KO, Hedehus M, Moseley M, de Crespigny A, Sullivan EV, Pfefferbaum A (1999): Compromised white matter tract integrity in schizophrenia inferred from diffusion tensor imaging. *Arch Gen Psychiatry*. 56:367-374.
12. Manoach DS, Gollub RL, Benson ES, Searl MM, Goff DC, Halpern E, et al. (2000): Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. *Biol Psychiatry*. 48:99-109.
13. Manoach DS, Press DZ, Thangaraj V, Searl MM, Goff DC, Halpern E, et al. (1999): Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. *Biol Psychiatry*. 45:1128-1137.
14. Luciana M, Nelson CA (1998): The functional emergence of prefrontally-guided working memory systems in four- to eight-year-old children. *Neuropsychologia*. 36:273-293.
15. Nelson CA, Monk CS, Lin J, Carver LJ, Thomas KM, Truwit CL (2000): Functional neuroanatomy of spatial working memory in children. *Dev Psychol*. 36:109-116.
16. Thomas KM, King SW, Franzen PL, Welsh TF, Berkowitz AL, Noll DC, et al. (1999): A developmental functional MRI study of spatial working memory. *Neuroimage*. 10:327-338.
17. Friston KJ (1998): The disconnection hypothesis. *Schizophr Res*. 30:115-125.
18. Goldman-Rakic PS (1999): The physiological approach: functional architecture of working memory and disordered cognition in schizophrenia. *Biol Psychiatry*. 46:650-661.
19. Fuster JM (1997): *The prefrontal cortex : anatomy, physiology, and neuropsychology of the frontal lobe*. 3rd ed. Philadelphia: Lippincott-Raven.
20. Goldman-Rakic PS, Porrino LJ (1985): The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. *J Comp Neurol*. 242:535-560.
21. Stephan KE, Kamper L, Bozkurt A, Burns GA, Young MP, Kotter R (2001): Advanced database methodology for the Collation of Connectivity data on the Macaque brain (CoCoMac). *Philos Trans R Soc Lond B Biol Sci*. 356:1159-1186.
22. Kievit J, Kuypers HG (1977): Organization of the thalamo-cortical connexions to the frontal lobe in the rhesus monkey. *Exp Brain Res*. 29:299-322.
23. McFarland NR, Haber SN (2002): Thalamic relay nuclei of the basal ganglia form both reciprocal and nonreciprocal cortical connections, linking multiple frontal cortical areas. *J Neurosci*. 22:8117-8132.
24. Cavada C, Goldman-Rakic PS (1989): Posterior parietal cortex in rhesus monkey: II. Evidence for segregated corticocortical networks linking sensory and limbic areas with the frontal lobe. *J Comp Neurol*. 287:422-445.
25. Schwartz ML, Goldman-Rakic PS (1984): Callosal and intrahemispheric connectivity of the prefrontal association cortex in rhesus monkey: relation between intraparietal and principal sulcal cortex. *J Comp Neurol*. 226:403-420.
26. Leiner HC, Leiner AL, Dow RS (1991): The human cerebro-cerebellar system: its computing, cognitive, and language skills. *Behav Brain Res*. 44:113-128.
27. Middleton FA, Strick PL (1994): Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science*. 266:458-461.
28. Yamamoto T, Yoshida K, Yoshikawa H, Kishimoto Y, Oka H (1992): The medial dorsal nucleus is one of the thalamic relays of the cerebellocerebral responses to the frontal association cortex in the monkey: horseradish peroxidase and fluorescent dye double staining study. *Brain Res*. 579:315-320.
29. Middleton FA, Strick PL (2001): Cerebellar projections to the prefrontal cortex of the primate. *J Neurosci*. 21:700-712.
30. Alexander GE, DeLong MR, Strick PL (1986): Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*. 9:357-381.
31. Llamas A, Reinoso-Suarez F, Martinez-Moreno E (1975): Projections to the gyrus proreus from the brain stem tegmentum (locus coeruleus, raphe nuclei) in the cat, demonstrated by retrograde transport of horseradish peroxidase. *Brain Res*. 89:331-336.
32. Porrino LJ, Goldman-Rakic PS (1982): Brainstem innervation of prefrontal and anterior cingulate cortex in the rhesus monkey revealed by retrograde transport of HRP. *J Comp Neurol*. 205:63-76.

33. Arnsten AF, Goldman-Rakic PS (1984): Selective prefrontal cortical projections to the region of the locus coeruleus and raphe nuclei in the rhesus monkey. *Brain Res*. 306:9-18.
34. Kievit J, Kuypers HG (1975): Basal forebrain and hypothalamic connection to frontal and parietal cortex in the Rhesus monkey. *Science*. 187:660-662.
35. Krettek JE, Price JL (1974): A direct input from the amygdala to the thalamus and the cerebral cortex. *Brain Res*. 67:169-174.
36. Tanne-Gariepy J, Boussaoud D, Rouiller EM (2002): Projections of the claustrum to the primary motor, premotor, and prefrontal cortices in the macaque monkey. *J Comp Neurol*. 454:140-157.
37. Pakkenberg B (1990): Pronounced reduction of total neuron number in mediodorsal thalamic nucleus and nucleus accumbens in schizophrenics. *Arch Gen Psychiatry*. 47:1023-1028.
38. Pakkenberg B (1992): The volume of the mediodorsal thalamic nucleus in treated and untreated schizophrenics. *Schizophr Res*. 7:95-100.
39. Heckers S (1997): Neuropathology of schizophrenia: cortex, thalamus, basal ganglia, and neurotransmitter-specific projection systems. *Schizophr Bull*. 23:403-421.
40. Popken GJ, Bunney WE, Jr., Potkin SG, Jones EG (2000): Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. *Proc Natl Acad Sci U S A*. 97:9276-9280.
41. Young KA, Manaye KF, Liang C, Hicks PB, German DC (2000): Reduced number of mediodorsal and anterior thalamic neurons in schizophrenia. *Biol Psychiatry*. 47:944-953.
42. Selemon LD, Rajkowska G, Goldman-Rakic PS (1998): Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. *J Comp Neurol*. 392:402-412.
43. Selemon LD, Mrzljak J, Kleinman JE, Herman MM, Goldman-Rakic PS (2003): Regional specificity in the neuropathologic substrates of schizophrenia: a morphometric analysis of Broca's area 44 and area 9. *Arch Gen Psychiatry*. 60:69-77.
44. Cullen TJ, Walker MA, Parkinson N, Craven R, Crow TJ, Esiri MM, et al. (2003): A postmortem study of the mediodorsal nucleus of the thalamus in schizophrenia. *Schizophr Res*. 60:157-166.
45. Lewis DA, Cruz DA, Melchitzky DS, Pierri JN (2001): Lamina-specific deficits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: evidence for fewer projections from the thalamus. *Am J Psychiatry*. 158:1411-1422.
46. Funahashi S, Bruce CJ, Goldman-Rakic PS (1989): Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J Neurophysiol*. 61:331-349.
47. Gnadt JW, Andersen RA (1988): Memory related motor planning activity in posterior parietal cortex of macaque. *Exp Brain Res*. 70:216-220.
48. Batuev AS, Shaefer VI, Orlov AA (1985): Comparative characteristics of unit activity in the prefrontal and parietal areas during delayed performance in monkeys. *Behav Brain Res*. 16:57-70.
49. Niki H (1974): Prefrontal unit activity during delayed alternation in the monkey. I. Relation to direction of response. *Brain Res*. 68:185-196.
50. Kojima S, Goldman-Rakic PS (1982): Delay-related activity of prefrontal neurons in rhesus monkeys performing delayed response. *Brain Res*. 248:43-49.
51. Quintana J, Fuster JM (1992): Mnemonic and predictive functions of cortical neurons in a memory task. *Neuroreport*. 3:721-724.
52. Chafee MV, Goldman-Rakic PS (1998): Matching patterns of activity in primate prefrontal area 8a and parietal area 7ip neurons during a spatial working memory task. *J Neurophysiol*. 79:2919-2940.
53. Rao SC, Rainer G, Miller EK (1997): Integration of what and where in the primate prefrontal cortex. *Science*. 276:821-824.
54. Ashe J, Georgopoulos AP (1994): Movement parameters and neural activity in motor cortex and area 5. *Cereb Cortex*. 4:590-600.
55. Kalaska JF, Caminiti R, Georgopoulos AP (1983): Cortical mechanisms related to the direction of two-dimensional arm movements: relations in parietal area 5 and comparison with motor cortex. *Exp Brain Res*. 51:247-260.
56. Smyrnis N, Taira M, Ashe J, Georgopoulos AP (1992): Motor cortical activity in a memorized delay task. *Exp Brain Res*. 92:139-151.
57. Tanibuchi I, Goldman-Rakic PS (2003): Dissociation of spatial-, object-, and sound-coding neurons in the mediodorsal nucleus of the primate thalamus. *J Neurophysiol*. 89:1067-1077.
58. Jinnai K, Nambu A, Tanibuchi I, Yoshida S (1993): Cerebello- and pallido-thalamic pathways to areas 6 and 4 in the monkey. *Stereotact Funct Neurosurg*. 60:70-79.
59. Butler EG, Harvey MC, Finkelstein DI, Horne MK (1998): Neuronal activity in the monkey ventrolateral thalamus following perturbations of voluntary wrist movements. *Exp Brain Res*. 118:393-407.

60. Arthurs OJ, Boniface S (2002): How well do we understand the neural origins of the fMRI BOLD signal? *Trends Neurosci*. 25:27-31.
61. Ogawa S, Tank DW, Menon R, Ellermann JM, Kim SG, Merkle H, et al. (1992): Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci U S A*. 89:5951-5955.
62. Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, et al. (1992): Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A*. 89:5675-5679.
63. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A (2001): Neurophysiological investigation of the basis of the fMRI signal. *Nature*. 412:150-157.
64. Ugurbil K, Toth L, Kim DS (2003): How accurate is magnetic resonance imaging of brain function? *Trends Neurosci*. 26:108-114.
65. White T, O'Leary D, Magnotta V, Arndt S, Flaum M, Andreasen NC (2001): Anatomic and functional variability: the effects of filter size in group fMRI data analysis. *Neuroimage*. 13:577-588.
66. Koch MA, Norris DG, Hund-Georgiadis M (2002): An investigation of functional and anatomical connectivity using magnetic resonance imaging. *Neuroimage*. 16:241-250.
67. Duong TQ, Yacoub E, Adriany G, Hu X, Ugurbil K, Kim SG (2003): Microvascular BOLD contribution at 4 and 7 T in the human brain: Gradient-echo and spin-echo fMRI with suppression of blood effects. *Magn Reson Med*. 49:1019-1027.
68. Constable RT, Kennan RP, Puce A, McCarthy G, Gore JC (1994): Functional NMR imaging using fast spin echo at 1.5 T. *Magn Reson Med*. 31:686-690.
69. Yacoub E, Duong TQ, Van De Moortele PF, Lindquist M, Adriany G, Kim SG, et al. (2003): Spin-echo fMRI in humans using high spatial resolutions and high magnetic fields. *Magn Reson Med*. 49:655-664.
70. Norris DG, Zysset S, Mildner T, Wiggins CJ (2002): An investigation of the value of spin-echo-based fMRI using a Stroop color-word matching task and EPI at 3 T. *Neuroimage*. 15:719-726.
71. Bandettini PA, Jesmanowicz A, Wong EC, Hyde JS (1993): Processing strategies for time-course data sets in functional MRI of the human brain. *Magn Reson Med*. 30:161-173.
72. McCarthy G, Blamire AM, Puce A, Nobre AC, Bloch G, Hyder F, et al. (1994): Functional magnetic resonance imaging of human prefrontal cortex activation during a spatial working memory task. *Proc Natl Acad Sci U S A*. 91:8690-8694.
73. Cohen JD, Perlstein WM, Braver TS, Nystrom LE, Noll DC, Jonides J, et al. (1997): Temporal dynamics of brain activation during a working memory task. *Nature*. 386:604-608.
74. Friston KJ, Frith CD, Liddle PF, Frackowiak RS (1993): Functional connectivity: the principal-component analysis of large (PET) data sets. *J Cereb Blood Flow Metab*. 13:5-14.
75. Horwitz B, Friston KJ, Taylor JG (2000): Neural modeling and functional brain imaging: an overview. *Neural Netw*. 13:829-846.
76. Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ (1997): Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage*. 6:218-229.
77. McIntosh AR, Grady CL, Ungerleider LG, Haxby JV, Rapoport SI, Horwitz B (1994): Network analysis of cortical visual pathways mapped with PET. *J Neurosci*. 14:655-666.
78. Petersson KM, Nichols TE, Poline JB, Holmes AP (1999): Statistical limitations in functional neuroimaging. I. Non-inferential methods and statistical models. *Philos Trans R Soc Lond B Biol Sci*. 354:1239-1260.
79. Worsley KJ, Poline JB, Friston KJ, Evans AC (1997): Characterizing the response of PET and fMRI data using multivariate linear models. *Neuroimage*. 6:305-319.
80. Friston KJ, Frith CD, Frackowiak RS, Turner R (1995): Characterizing dynamic brain responses with fMRI: a multivariate approach. *Neuroimage*. 2:166-172.
81. Horwitz B, Duara R, Rapoport SI (1984): Intercorrelations of glucose metabolic rates between brain regions: application to healthy males in a state of reduced sensory input. *J Cereb Blood Flow Metab*. 4:484-499.
82. Horwitz B, McIntosh AR, Haxby JV, Grady CL (1995): Network analysis of brain cognitive function using metabolic and blood flow data. *Behav Brain Res*. 66:187-193.
83. McIntosh AR, Grady CL, Haxby JV, Ungerleider LG, Horwitz B (1996): Changes in limbic and prefrontal functional interactions in a working memory task for faces. *Cereb Cortex*. 6:571-584.
84. Friston KJ (1996): Statistical parametric mapping and other analyses of functional imaging data. In: Toga AW, Mazziota JC, editors. *Brain Mapping*: Academic Press.
85. Buchel C, Friston KJ (1997): Modulation of connectivity in visual pathways by attention: Cortical interactions evaluated with structural equation modelling and fMRI. *Cerebral Cortex*. 7:768-778.

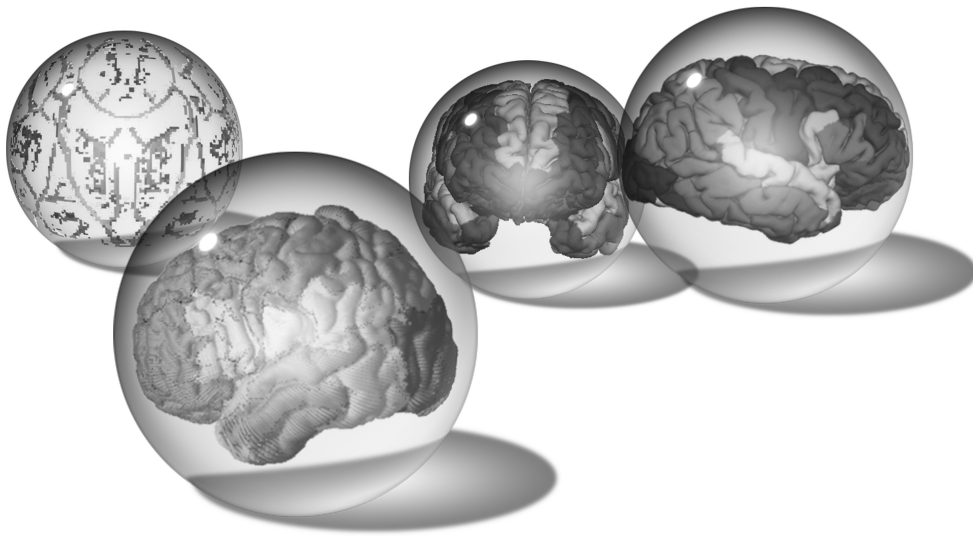
86. Cabeza R, McIntosh AR, Tulving E, Nyberg L, Grady CL (1997): Age-related differences in effective neural connectivity during encoding and recall. *Neuroreport*. 8:3479-3483.
87. Grady CL, McIntosh AR, Bookstein F, Horwitz B, Rapoport SI, Haxby JV (1998): Age-related changes in regional cerebral blood flow during working memory for faces. *Neuroimage*. 8:409-425.
88. Luciana M, Nelson CA (2000): Neurodevelopmental assessment of cognitive function using CANTAB: Validation and future goals. In: Ernst, Rumsey, editors. *Functional Neuroimaging in Child Psychiatry*. Cambridge: Cambridge University Press, pp 379-397.
89. Huttenlocher PR (1979): Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Res*. 163:195-205.
90. Sowell ER, Thompson PM, Holmes CJ, Batth R, Jernigan TL, Toga AW (1999): Localizing age-related changes in brain structure between childhood and adolescence using statistical parametric mapping. *Neuroimage*. 9:587-597.
91. Sowell ER, Thompson PM, Holmes CJ, Jernigan TL, Toga AW (1999): In vivo evidence for post-adolescent brain maturation in frontal and striatal regions. *Nat Neurosci*. 2:859-861.
92. De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, et al. (2001): Sex differences in brain maturation during childhood and adolescence. *Cereb Cortex*. 11:552-557.
93. Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, et al. (1999): Structural maturation of neural pathways in children and adolescents: in vivo study. *Science*. 283:1908-1911.
94. Bartzokis G, Beckson M, Lu PH, Nuechterlein KH, Edwards N, Mintz J (2001): Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study. *Arch Gen Psychiatry*. 58:461-465.
95. Yakovlev PI, Lecours AR (1967): The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, editor. *Regional Development of the Brain in Early Life*. Oxford: Blackwell, pp 3-70.
96. Buchel C, Coull JT, Friston KJ (1999): The predictive value of changes in effective connectivity for human learning. *Science*. 283:1538-1541.
97. White T, Andreasen NC, Nopoulos P (2002): Brain Volumes and Surface Morphology in Monozygotic Twins. *Cereb Cortex*. 12:486-493.
98. Pearlson GD, Marsh L (1999): Structural brain imaging in schizophrenia: a selective review. *Biol Psychiatry*. 46:627-649.
99. Shenton ME, Dickey CC, Frumin M, McCarley RW (2001): A review of MRI findings in schizophrenia. *Schizophr Res*. 49:1-52.
100. Sitskoorn MM, Appels MC, Hulshoff Pol HE, Kahn RS (2000): Evidence of fronto-thalamic involvement in schizophrenia. *Prog Brain Res*. 126:343-355.
101. Gilbert AR, Rosenberg DR, Harenski K, Spencer S, Sweeney JA, Keshavan MS (2001): Thalamic volumes in patients with first-episode schizophrenia. *Am J Psychiatry*. 158:618-624.
102. Nopoulos PC, Ceilley JW, Gailis EA, Andreasen NC (1999): An MRI study of cerebellar vermis morphology in patients with schizophrenia: evidence in support of the cognitive dysmetria concept. *Biol Psychiatry*. 46:703-711.
103. Sowell ER, Toga AW, Asarnow R (2000): Brain abnormalities observed in childhood-onset schizophrenia: a review of the structural magnetic resonance imaging literature. *Ment Retard Dev Disabil Res Rev*. 6:180-185.
104. Sowell ER, Levitt J, Thompson PM, Holmes CJ, Blanton RE, Kornsand DS, et al. (2000): Brain abnormalities in early-onset schizophrenia spectrum disorder observed with statistical parametric mapping of structural magnetic resonance images. *Am J Psychiatry*. 157:1475-1484.
105. Keller A, Castellanos FX, Vaituzis AC, Jeffries NO, Giedd JN, Rapoport JL (2003): Progressive loss of cerebellar volume in childhood-onset schizophrenia. *Am J Psychiatry*. 160:128-133.
106. Thompson PM, Vidal C, Giedd JN, Gochman P, Blumenthal J, Nicolson R, et al. (2001): Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. *Proc Natl Acad Sci U S A*. 98:11650-11655.
107. Rapoport JL, Giedd JN, Blumenthal J, Hamburger S, Jeffries N, Fernandez T, et al. (1999): Progressive cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. *Arch Gen Psychiatry*. 56:649-654.
108. Kumra S, Giedd JN, Vaituzis AC, Jacobsen LK, McKenna K, Bedwell J, et al. (2000): Childhood-onset psychotic disorders: magnetic resonance imaging of volumetric differences in brain structure. *Am J Psychiatry*. 157:1467-1474.
109. Dasari M, Friedman L, Jesberger J, Stuve TA, Findling RL, Swales TP, et al. (1999): A magnetic resonance imaging study of thalamic area in adolescent patients with either schizophrenia or bipolar disorder as compared to healthy controls. *Psychiatry Res*. 91:155-162.

110. Jacobsen LK, Giedd JN, Berquin PC, Krain AL, Hamburger SD, Kumra S, et al. (1997): Quantitative morphology of the cerebellum and fourth ventricle in childhood-onset schizophrenia. *Am J Psychiatry*. 154:1663-1669.
111. Andreasen NC, Arndt S, Swayze V, 2nd, Cizadlo T, Flaum M, O'Leary D, et al. (1994): Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. *Science*. 266:294-298.
112. Buchsbaum MS, Someya T, Teng CY, Abel L, Chin S, Najafi A, et al. (1996): PET and MRI of the thalamus in never-medicated patients with schizophrenia. *Am J Psychiatry*. 153:191-199.
113. Hazlett EA, Buchsbaum MS, Byne W, Wei TC, Spiegel-Cohen J, Geneve C, et al. (1999): Three-dimensional analysis with MRI and PET of the size, shape, and function of the thalamus in the schizophrenia spectrum. *Am J Psychiatry*. 156:1190-1199.
114. Byne W, Buchsbaum MS, Kemether E, Hazlett EA, Shinwari A, Mitropoulou V, et al. (2001): Magnetic resonance imaging of the thalamic mediodorsal nucleus and pulvinar in schizophrenia and schizotypal personality disorder. *Arch Gen Psychiatry*. 58:133-140.
115. Van Essen DC (1997): A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature*. 385:313-318.
116. Basser PJ, Mattiello J, LeBihan D (1994): MR diffusion tensor spectroscopy and imaging. *Biophys J*. 66:259-267.
117. Pierpaoli C, Basser PJ (1996): Toward a quantitative assessment of diffusion anisotropy. *Magn Reson Med*. 36:893-906.
118. Pajevic S, Pierpaoli C (1999): Color schemes to represent the orientation of anisotropic tissues from diffusion tensor data: application to white matter fiber tract mapping in the human brain. *Magn Reson Med*. 42:526-540.
119. Conturo TE, Lori NF, Cull TS, Akbudak E, Snyder AZ, Shimony JS, et al. (1999): Tracking neuronal fiber pathways in the living human brain. *Proc Natl Acad Sci U S A*. 96:10422-10427.
120. Jones DK, Simmons A, Williams SC, Horsfield MA (1999): Non-invasive assessment of axonal fiber connectivity in the human brain via diffusion tensor MRI. *Magn Reson Med*. 42:37-41.
121. Mori S, Van Zijl PC (2002): Fiber tracking: principles and strategies - a technical review. *NMR Biomed*. 15:468-480.
122. Basser PJ, Pajevic S, Pierpaoli C, Duda J, Aldroubi A (2000): In vivo fiber tractography using DT-MRI data. *Magn Reson Med*. 44:625-632.
123. Parker GJ, Stephan KE, Barker GJ, Rowe JB, MacManus DG, Wheeler-Kingshott CA, et al. (2002): Initial demonstration of in vivo tracing of axonal projections in the macaque brain and comparison with the human brain using diffusion tensor imaging and fast marching tractography. *Neuroimage*. 15:797-809.
124. Schmithorst VJ, Wilke M, Dardzinski BJ, Holland SK (2002): Correlation of white matter diffusivity and anisotropy with age during childhood and adolescence: a cross-sectional diffusion-tensor MR imaging study. *Radiology*. 222:212-218.
125. Klingberg T, Vaidya CJ, Gabrieli JD, Moseley ME, Hedehus M (1999): Myelination and organization of the frontal white matter in children: a diffusion tensor MRI study. *Neuroreport*. 10:2817-2821.
126. Neil J, Miller J, Mukherjee P, Huppi PS (2002): Diffusion tensor imaging of normal and injured developing human brain - a technical review. *NMR Biomed*. 15:543-552.
127. Hoptman MJ, Volavka J, Johnson G, Weiss E, Bilder RM, Lim KO (2002): Frontal white matter microstructure, aggression, and impulsivity in men with schizophrenia: a preliminary study. *Biol Psychiatry*. 52:9-14.
128. Agartz I, Andersson JL, Skare S (2001): Abnormal brain white matter in schizophrenia: a diffusion tensor imaging study. *Neuroreport*. 12:2251-2254.
129. Steel RM, Bastin ME, McConnell S, Marshall I, Cunningham-Owens DG, Lawrie SM, et al. (2001): Diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (1H MRS) in schizophrenic subjects and normal controls. *Psychiatry Res*. 106:161-170.
130. Burns J, Job D, Bastin ME, Whalley H, Macgillivray T, Johnstone EC, et al. (2003): Structural disconnectivity in schizophrenia: a diffusion tensor magnetic resonance imaging study. *Br J Psychiatry*. 182:439-443.
131. Foong J, Maier M, Clark CA, Barker GJ, Miller DH, Ron MA (2000): Neuropathological abnormalities of the corpus callosum in schizophrenia: a diffusion tensor imaging study. *J Neurol Neurosurg Psychiatry*. 68:242-244.
132. Foong J, Symms MR, Barker GJ, Maier M, Miller DH, Ron MA (2002): Investigating regional white matter in schizophrenia using diffusion tensor imaging. *Neuroreport*. 13:333-336.

133. Thompson RA, Nelson CA (2001): Developmental science and the media. Early brain development. *Am Psychol*. 56:5-15.
134. Jansma JM, Ramsey NF, Slagter HA, Kahn RS (2001): Functional anatomical correlates of controlled and automatic processing. *J Cogn Neurosci*. 13:730-743.
135. Hafner H, Nowotny B (1995): Epidemiology of early-onset schizophrenia. *Eur Arch Psychiatry Clin Neurosci*. 245:80-92.
136. Callicott JH, Bertolino A, Mattay VS, Langheim FJ, Duyn J, Coppola R, et al. (2000): Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. *Cereb Cortex*. 10:1078-1092.
137. Karatekin C, Asarnow RF (1998): Working memory in childhood-onset schizophrenia and attention- deficit/hyperactivity disorder. *Psychiatry Res*. 80:165-176.
138. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al. (2001): Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A*. 98:6917-6922.
139. Conklin HM, Curtis CE, Katsanis J, Iacono WG (2000): Verbal working memory impairment in schizophrenia patients and their first-degree relatives: evidence from the digit span task. *Am J Psychiatry*. 157:275-277.
140. Thompson PM, Giedd JN, Woods RP, MacDonald D, Evans AC, Toga AW (2000): Growth patterns in the developing brain detected by using continuum mechanical tensor maps. *Nature*. 404:190-193.
141. Paus T, Collins DL, Evans AC, Leonard G, Pike B, Zijdenbos A (2001): Maturation of white matter in the human brain: a review of magnetic resonance studies. *Brain Res Bull*. 54:255-266.
142. Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, et al. (2001): Genome-wide expression analysis reveals dysregulation of myelination- related genes in chronic schizophrenia. *Proc Natl Acad Sci U S A*. 98:4746-4751.

2.1 Neuropsychological Performance in First-Episode Adolescents with Schizophrenia

A comparison with first-episode adults and adolescent controls



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Biol Psychiatry (2006) 60: 463-47

Abstract

Background: The goal of this study was to compare the extent of cognitive deficits between adolescents and adults early in the course of schizophrenia.

Method: A comprehensive neuropsychological battery was performed on 49 adolescents with childhood or adolescent-onset schizophrenia, 139 adults with adult-onset schizophrenia, 32 healthy adolescent volunteers, and 240 healthy adult volunteers. Both patient groups were assessed early in the course of their illness and were matched to their respective control groups on age and parental education.

Results: The adolescent patients performed significantly worse than the adult patients on tasks of working memory, language, and motor function. The healthy adolescents also performed significantly worse than the healthy adults in working memory and language tasks, but were significantly better than the adults in motor function. When accounting for developmental differences in the control group, only motor performance was worse in the adolescent patients compared to the adult patients.

Conclusions: These findings, when coupled with published retrospective studies reporting greater cognitive deficits in earlier onset schizophrenia, implicate a cessation in development in specific cognitive domains following the onset of schizophrenia in adolescent patients.

CHAPTER 2

INTRODUCTION

There is considerable evidence to support substantial brain maturation between adolescence and adulthood. Structural brain changes during this time include a decline in gray matter volume that shifts from parietal to prefrontal areas (1-3), a decrease in gray matter (GM) density in the subcortical regions (1), and an increase in cerebral spinal fluid (CSF) volume (4). A number of studies have also shown abnormalities in these same structures in children and adolescents with schizophrenia (5-10). For example, longitudinal studies, which provide a mechanism to evaluate changes over time compared to typical neurodevelopmental trajectories, have demonstrated a more pronounced decline in GM volume (8) that follows the same parietal to frontal progression (9, 10).

Functional changes have also been identified during typical neurodevelopment from adolescence to adulthood. For example, whereas children activate similar neural circuitry as adults during working memory tasks (11, 12), working memory performance continues to improve into early adulthood (13, 14). A comprehensive neuropsychological evaluation allows for the functional assessment of multiple brain regions. The neuropsychological tasks can be grouped into different domains (15, 16), with each task involving an orchestration of neural processes necessary to accomplish the task. By performing multiple tasks that access distributed but overlapping neural networks, it is possible to better hone in on the source of specific cognitive deficits (17). In addition, neuropsychological measures provide an opportunity to assess specific neural networks as they 'come on line' or are optimized during development.

Studying patients with differing ages of onset can address the impact of the illness on the downstream development of these cognitive functions. For example, one question that has not been addressed in the literature is whether adolescents in the early stages of schizophrenia show a greater developmental deviation in specific neuropsychological tasks than a matched early-onset adult group. Since a number of studies have demonstrated greater cognitive deficits in adult patients who developed schizophrenia during adolescence or early adulthood (18-25), it would be beneficial to know if the magnitude of these deficits was present early or was associated with a decline or cessation in post-illness neurodevelopment. In addition, since specific academic deficits have been identified even prior to the prodromal phase (26-28), the evaluation of early-onset adolescent patients provides an additional marker of the developmental trajectory of these deficits.

Studies that directly compare adolescent patients with age-matched controls consistently demonstrate poorer performance in the schizophrenia group across a number of neuropsychological domains (17, 25, 29-32). Whereas some studies demonstrate fairly broad deficits in neuropsychological performance (29, 32), others show more specific deficits in the areas of working memory, attention, problem solving, and abstraction (18, 30, 31). In a study of neuroleptic naïve adolescents, significant deficits were found in attention, verbal memory, and executive functions (25). Compared to a heterogeneous clinical control group,

adolescents with schizophrenia have been shown to have greater deficits in performance IQ (33), abstraction (34), and auditory perception (34).

Since the studies comparing adolescent and adult patient groups evaluated the subjects after reaching adulthood, the developmental trajectory of the cognitive deficits are poorly understood. The purpose of this study is to evaluate a wide range of cognitive domains between a group of first-episode adolescents and adults with schizophrenia in order to better understand the development of cognitive deficits in adolescents with schizophrenia.

METHODS AND MATERIALS

Subjects

The patient sample included 188 subjects with a schizophrenia spectrum diagnosis who were recruited for a first-episode longitudinal study of the phenomenology of schizophrenia (35). The participants were all evaluated at the time of entry and all gave written informed consent/assent for their participation. The study protocol was approved by the University of Iowa Institutional Review Board. Diagnoses were based on a structured diagnostic interview, the Comprehensive Assessment of Symptoms and History (CASH), (36) coupled with a consensus diagnosis of at least two research psychiatrists. Patients were excluded if there was a history of substance dependence or current abuse, central nervous system pathology or trauma (loss of consciousness for greater than 30 minutes or any neurological sequelae), or a full-scale IQ less than 70.

The patients were stratified into two groups based on their age-of-onset and chronological age at the time of neuropsychological testing (Table 1). Age-of-onset was derived from the CASH and defined as the age that the subject first experienced psychotic symptoms (36). The inter-rater reliability for the age-of-onset measure was performed on 56 patients with a kappa of 0.681 and intraclass correlation coefficient (r) equal to 0.995. Children and adolescents who developed psychotic symptoms and were evaluated during the adolescent years (ages 12 to 19 years) were considered the adolescent group. The adult group consisted of individuals between 20 and 39 years of age who developed psychosis after the age of 20. There were 49 individuals in the adolescent group and 139 individuals in the adult group. The patterns of diagnoses within the two groups were similar. The adolescent group had 78% with schizophrenia, 2% with schizoaffective disorder, and 20% with schizophreniform disorder (Table 2). The adult group consisted of 79% with schizophrenia, 2% with schizoaffective disorder, and 19% with schizophreniform disorder.

Table 1 – Demographic information.

	Adolescent Patients N=49	Adolescent Controls N=32	Adult Patients N=139	Adult Controls N=240
Age	18.1 (1.2)	18.9 (0.3)	27.6 (4.4)	26.3 (5.2)
Sex – (Male / Female)	37 / 12	21 / 11	94 / 45	119 / 121
Educational Level	11.0 (1.3)	12.6 (0.7)	13.5 (2.4)	15.1 (1.8)
Paternal Educational Level	13.7 (3.5)	13.6 (3.0)	13.3 (3.7)	12.8 (2.8)
Maternal Educational Level	13.8 (2.4)	13.0 (1.6)	13.1 (2.5)	12.8 (1.8)
Intelligence Testing (WAIS-R)				
- Full Scale IQ	91 (13)	114 (13)	89 (12)	112 (12)
- Verbal IQ	93 (12)	113 (14)	90 (12)	109 (12)
- Performance IQ	89 (15)	113 (11)	89 (15)	112 (12)

Table 2 – Clinical Characteristics of the Adolescent and Adult Patient Groups

	Adolescent Patients	Adult Patients	p value
Symptoms – mean (SD)			
Age of onset of psychotic symptoms	16.3 (2.3) yrs.	24.6 (3.6) yrs.	p <0.001
Duration of Untreated Psychosis (Naïve Subjects)*	2.4 (3.4) yrs.	1.3 (1.2) yrs.	p = 0.03
Negative symptom score	2.90 (0.80)	2.81 (0.94)	n.s.
Psychotic symptom score	2.60 (1.30)	2.70 (1.34)	n.s.
Disorganized symptom score	1.62 (0.90)	1.67 (1.03)	n.s.
Diagnoses (number / percent)			
- schizophrenia	38 (78%)	109 (79%)	n.s.
- schizoaffective disorder	1 (2%)	3 (2%)	n.s.
- schizophreniform disorder	10 (20%)	27 (19%)	n.s.
Neuroleptic Status			
- neuroleptic naïve or near naïve	22 (45%)	69 (50%)	n.s.
- duration of neuroleptic treatment (months)	6.7 (9.8)	7.6 (12.9)	n.s.

- T-test log normalized due to non-linearity.

Clinical symptoms of schizophrenia were assessed using the Scale for the Assessment of Positive Symptoms (SAPS) (37) and the Scale for the Assessment of Negative Symptoms (SANS) (38). These instruments reflect the worst level of both positive and negative symptoms of schizophrenia during the month predating the evaluation.

The control group consisted of 272 healthy volunteers, with 32 subjects age 19 and younger and 240 between the ages of 20 and 39 years (Table 1). Exclusion criteria for the control group included a history of substance abuse, history of a neurological disorder, head trauma with a loss of consciousness greater than a few minutes or associated with neurological sequelae, and IQ < 70, and a history of an Axis I psychiatric disorder or antisocial personality disorder in the control or a primary family member (i.e., parents or siblings).

Cognitive Assessment

All study subjects were administered a comprehensive neuropsychological battery by psychometrists who were trained in standardized assessment and scoring procedures. Testing generally took 4 hours to complete and, when necessary, occurred over several sessions.

Verbal memory. The verbal memory domain was comprised of variables from the Rey Auditory Verbal Learning Test (RAVLT) and the Logical Memory subtest of the Wechsler Memory Scale – Revised (WMS-R) (39-42). Variables from the RAVLT included the summary score of trials 1-5, the total correct score for trial 7, and the total correct score for the delayed recall trial (40, 42). Similarly, the free and delayed recall trials of the Logical Memory subtest from the WMS-R (43) were also included.

Nonverbal memory. Variables from tests of nonverbal memory included the immediate and delayed recall trials of the Rey-Osterrieth Complex Figure Test (ROCFT) (44, 45) and the total number correct from the Benton Visual Retention Test - Revised (BVRT-R) (46-48).

Working memory. The best measures of working memory in the current battery used at our research center included the Arithmetic and Digit Span subtests from the Wechsler Adult Intelligence Scale – Revised (WAIS-R) (49).

Language skills. The language skills domain was composed of the Vocabulary subtests from both the WAIS-R (49) and the Shipley Institute of Living Scale (50).

Visuospatial skills. The copy portion from the Rey-Osterrieth Complex Figure Test (ROCFT) (44, 45) was included as a measure of visuospatial ability. Other measures comprising this domain included the WAIS-R Block Design and Object Assembly subtests (49), and the Judgment of Line Orientation (JLO). Lezak (41) stated that "(i)nclusion of both assembling and drawing tests in the test battery will help the examiner to discriminate between the spatial and visual aspects of a constructional disability and estimate the relative contributions of each" (p. 587).

Initiation and speed. Initiation and speed were assessed with variables from six different test measures. These measures included the Digit Symbol subtest from the WAIS-R (49), Trail Making Test A (51), trials 1 & 2 from the Stroop Color

and Word Test (52), and the time to complete and number correct from the Alpha Tree and Alpha Curved tests. The Oral Word Association subtest (FAS) from the Multilingual Aphasia Examination (MAE) (53, 54) was also included. While verbally loaded, tests of verbal fluency are also important measures of frontal executive abilities such as initiation and maintenance of a desired behavior. In this case, the measure involved the initiation of a search for words beginning with a particular letter and speeded verbal or written production of these words by the test subject.

Sustained and selective attention. A measure of sustained and selective attention was derived from four tests. The proportion of hits (from possible "targets") from a computerized version of the Continuous Performance Test (CPT) (55), the time to complete the Circle A Letter-cancellation task (56), trial 3 from the Stroop Color and Word Test (52), and the Trail Making Test B (51) comprised this functional category.

Problem solving. The problem solving domain is similar to what many researchers have termed executive functions. We chose to use the term "problem solving," however, as we found it to be more descriptive of the process being undertaken by a test subject in successfully navigating through the various tests comprising this domain. The number of categories attained and number of perseverative errors from the Wisconsin Card Sorting Test (WCST) (57) were used to assess this ability. Accordingly, the Abstractions subtest from the Shipley Institute of Living Scale (50) was included, as were the Comprehension, Similarities, Picture Completion, and Picture Arrangement subtests from the WAIS-R (49).

Motor skills. Motor functions were assessed using the Finger Tapping test from the Halstead-Reitan Neuropsychological battery (51) and the Purdue Pegboard test (58, 59). Variables from the Finger Tapping test included an average of the five 10-second trials for each hand. The three measures drawn from the Purdue Pegboard included the total number of pegs inserted with the right hand, the left hand, and with both hands.

Statistical Analysis

The demographic and clinical characteristics were assessed using t tests for the continuous data and chi-square analyses for the nominal data. Duration of neuroleptic treatment was significantly skewed and thus was log normalized prior to analysis.

In order to efficiently analyze the cognitive functioning of study subjects, 36 neuropsychological test variables were grouped into 9 cognitive domains, described above. These theoretical groupings were then tested for internal reliability using Cronbach's Alpha analyses. Prior to summing scores from the various tests comprising each domain, raw test scores were converted to z scores (mean = 0, standard deviation = 1). When necessary, scores were reversed so that for all test values, a larger score indicated better performance. Once standardized, the tests were averaged within the nine cognitive domains.

In order to reduce the number of tests performed, a MANCOVA was utilized combining all nine cognitive domains to assess the differences between the four groups. Age and the combination of age and sex were used as covariates. Similarly, MANCOVA approaches were utilized to assess differences between the adolescent patient and control groups, the adult patient and control groups, and between the adolescent and adult patient groups. When group differences were identified, t-tests were used to identify the individual neuropsychological domain contributing to the group differences. ANCOVA's were performed on the adolescent and adult patient groups that adjusted for the difference between the healthy adolescents and adults on each domain. Effect sizes between each of the groups were also calculated. Finally, as a separate analysis, a linear mixed effects model was run to evaluated each neuropsychological domain using age of onset as a continuous variable (23). The fixed effects used for each analysis included; age of onset, sex, medication status (neuroleptic naïve versus medicated), highest education level of the father, and highest education level of the mother.

RESULTS

Since schizophrenia is associated with a decline in cognitive function, the groups were matched by level of parental education. The differences in the level of paternal and maternal education level were not statistically different between the four groups. In fact, the parents of the adolescent patient group tended toward a slightly higher level of education compared to the three other groups (Table 1). The ratio of males and females were also well matched, except for the adult control group, which had a greater proportion of females. The overall results did not change significantly when sex was used as a covariate.

The mean age-of-onset of schizophrenia in the adolescent group was 16.5 years, compared to 24.4 years in the adult-onset group (Table 2). There were no differences between the adolescent and adult groups on measures of negative, disorganized, or psychotic symptoms. Subtypes of schizophrenia included two adults with catatonic subtype, compared to none of the adolescents; 10% of adults had disorganized subtype, compared to 11% of the adolescents; 45% of the adults had a paranoid subtype, compared to 22% of the adolescents; and 46% of the adults had an undifferentiated subtype, compared to 67% of the adolescents. A Fisher's Exact Test comparing the two domains of paranoid and undifferentiated schizophrenia showed a significant difference between the adolescent and adult groups ($p = 0.02$) After the onset of psychotic symptoms, the adolescents came to clinical attention in nearly half the time as the adults. Approximately 50% of both the adolescent and adult patients were neuroleptic naïve or near naïve at the time of testing.

Cronbach Alpha Coefficients for the Cognitive Domains

Cronbach Alpha coefficients provide a measure of internal consistency of the specific neuropsychological tests within a specific domain. The Cronbach Alpha's for the patients with schizophrenia and control subjects respectively are as follows: Verbal Memory; 0.88 and 0.88, Nonverbal Memory; 0.79 and 0.81, Working Memory; 0.63 and 0.49, Language Skills; 0.88 and 0.83, Visuospatial Skills; 0.81 and 0.59, Initiation and Speed; 0.85 and 0.76, Sustained and Selective Attention; 0.70 and 0.62, Problem Solving; 0.85 and 0.69, and Motor Skills; 0.78 and 0.65. Not only do these cognitive domains have good internal consistency, these groupings are also similar to those proposed by others in the field (15, 60-62).

Adolescent Patients versus Adolescent Controls

A MANCOVA of all nine neuropsychological domains found that the adolescent patient group performed significantly worse than the control group ($F=7.41$, $df=9,74$, $p<0.0001$). Results of the t-tests showed that in each of the nine cognitive domains the patient group performed significantly worse than the control group ($t's > 4.09$, $df > 92$, $p < 0.001$ for all nine domains). The adolescent patients performed approximately one standard deviation below the control group across the nine domains (Figure 1). Although some variability was seen in performance across the different domains, these were not statistically significant. There were no significant differences between the neuroleptic naïve and medicated patients.

Adolescent versus Adult Patient Group

A MANCOVA found no differences comparing the adolescent and adult patient groups ($F=1.48$, $df=199$, $p=0.16$). While the overall difference was not statistically significant, we chose to evaluate the specific domains using t-tests, and found that the adolescent patients performed worse than the adult patients on three specific neuropsychological domains (Figure 2). The adolescent patients performed worse on language skills ($t=2.99$, $df=227$, $p=0.003$), working memory ($t=2.10$, $df=229$, $p=0.037$), and motor function ($t=2.45$, $df=219$, $p=0.015$). Interestingly, the adolescent control group performed statistically worse than the adult control group in both language skills ($t=3.07$, $df=270$, $p=0.002$) and working memory ($t=1.96$, $df=269$, $p=0.05$). This finding was similar to the adolescent and adult patient group. However, unlike the patient groups, the adolescent controls performed better than the adult controls on motor function. When co-varying for the difference between healthy adolescents and healthy adults, no differences were found between the adolescent and adult patient groups in language skills and working memory. Motor function, however, had a wider separation between the adolescent and adult patients ($t=4.44$, $df=219$, $p<0.0001$). The effect sizes between adolescent and adult patients were in the moderate range for working memory, language, and motor function (Table 3).

Table 3 – *Cohen's D for the comparisons between the adolescent and adult patient groups and between these two groups and the control groups. Small and medium effect sizes are of the order of 0.2 and 0.5, respectively. Large effect sizes are greater than 0.8.*

	Adolescent versus Adult Patients		**Adolescent Patients versus Adolescent Controls**		**Adult Patients versus Adult Controls**	
Neuropsychological domains	Non-naïve	Naïve	Non-naïve	Naïve	Non-naïve	Naïve
- Verbal memory	0.22	0.13	1.51	1.48	1.69	1.87
- Non-verbal memory	0.04	0.28	0.79	1.04	1.05	1.02
- Working memory	0.31	0.25	1.44	1.34	1.41	1.47
- Language skills	0.48	0.43	1.13	1.10	1.28	1.56
- Visuospatial skills	0.32	0.64	1.66	1.77	1.58	1.55
- Initiation / speed	0.13	0.30	1.51	1.82	1.59	1.97
- Sustained and selective attention	-0.04	0.01	0.98	1.02	1.26	1.41
- Problem solving	0.11	0.22	1.52	1.65	1.71	2.13
- Motor	0.37	-0.10	1.18	0.82	0.57	0.68

There was no statistical difference in duration of typical versus atypical medications used in these two groups. The medicated adolescents were treated with atypical neuroleptics an average of 45 days, versus 44 days for the adult group. The duration of typical antipsychotic use was 192 days for the adult group (n=30), versus 111 days for the adolescent group (n=14). Due to the skew in the data for typical neuroleptics, the data was log normalized prior to analyzing the data using a t-test. No significant differences were found between the duration of both typical and atypical neuroleptic use between the adolescent and the adult patient groups.

In addition, there were no differences between the neuroleptic naïve adolescent and adult patients (Table 3). Since the sample of neuroleptic naïve adolescents included only 22 subjects, the study would be able to detect only differences with large effect sizes. The effect sizes for the neuroleptic naïve youth compared to the neuroleptic naive adults were moderate for initiation/speed, language and visuospatial skills (Table 3). Motor function was nearly identical between the neuroleptic naïve adolescent and adult groups. There was a statistically significant difference (p=0.03) in the duration of untreated psychosis (DUP) between the neuroleptic naïve adolescents and adults (Table 2). The adolescents had a shorter DUP than the adults. An ANCOVA, with age and DUP as the covariates, was consistent in demonstrating no cognitive differences between the adolescent and adult groups. Finally, DUP was not associated with cognitive function in the first-onset neuroleptic naïve group.

Mixed Effects Analyses

The linear mixed effects model demonstrated poorer performance for those with an earlier age of onset in three of the cognitive domains. These included working memory ($F=6.02$, DF 165, $p = 0.01$); language ($F=10.33$, DF 165, $p < 0.002$); and motor function ($F=8.39$, DF 164, $p < 0.005$). The contributions from the fixed-effects included sex to the non-verbal memory domain ($p < 0.02$); and combined parental education level to verbal memory ($p=0.03$), non-verbal memory ($p < 0.03$), working memory ($p<0.001$), language ($p<0.001$), visual spatial skills domain ($p < 0.001$), visual spatial skills ($p<0.005$), problem solving ($p<0.001$).

DISCUSSION

The goal of this study was to assess a broad array of cognitive measures between a large group of first-episode adolescent and adult patients with schizophrenia. In order to account for the typical developmental changes associated with each of the neuropsychological domains, both the adolescent and adult patient groups were matched to adolescent and adult control groups, respectively. Comparing the adolescent and adult patient groups, we found that the adolescents with schizophrenia performed significantly worse than the adult patients on tasks that measure working memory, language, and motor function. Interestingly, when the healthy adolescent controls were compared to the adult controls, the healthy adolescents had lower performance than the healthy adults in the working memory and language domains. When the developmental trajectory for language and working memory were controlled for, no significant differences were found between the adolescent and adult patient groups in any domains except motor function. A separate analysis using a mixed effects analysis with age-of-onset as a continuous variable demonstrated identical results, with those patients with a younger age-of-onset having poorer performance in language, working memory, and motor domains.

The healthy adolescents performed significantly better than the healthy adults in motor function, whereas the adolescent patients functioned significantly worse than the adult patients. Thus, when the developmental trajectory of the healthy volunteers was accounted for, the gap between the adolescent and adult patient groups in motor performance was even greater. Thus, in contrast to the working memory and language domains, the difference in motor function between the adolescent and adult patients cannot be accounted for by the typical developmental trajectory during adolescence. Similar differences in developmental trajectories have been demonstrated in a longitudinal study of neurological abnormalities in childhood-onset schizophrenia (63). In addition, Manschreck et al. (2004) have shown that an earlier age of is associated with greater motor deficits.

Although the number of neuroleptic naïve patients in the adolescent group is small (n=22), there were no significant differences in motor performance between the neuroleptic naïve adolescent and adult patients (Figure 2). Since the healthy

Figure 1 – *Z-scores of Adolescent Patients Compared to Adolescent Controls (Controls Set to Zero)*

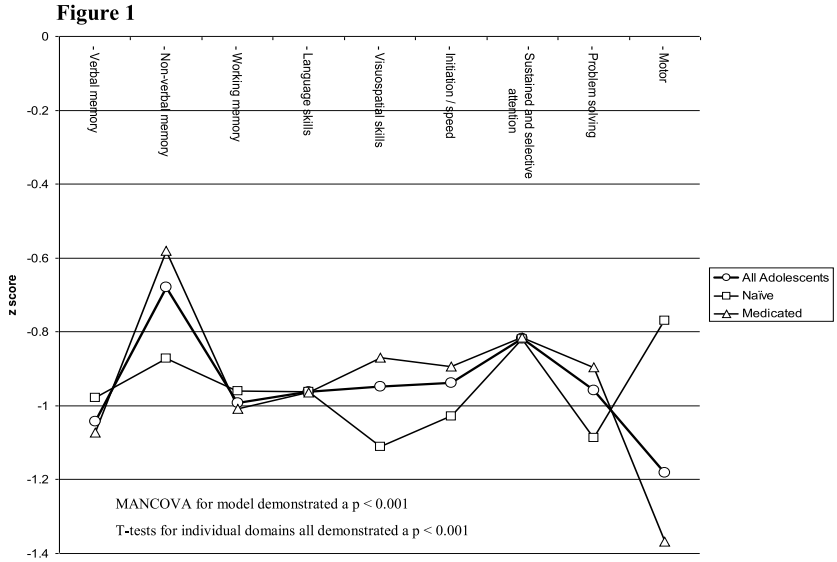
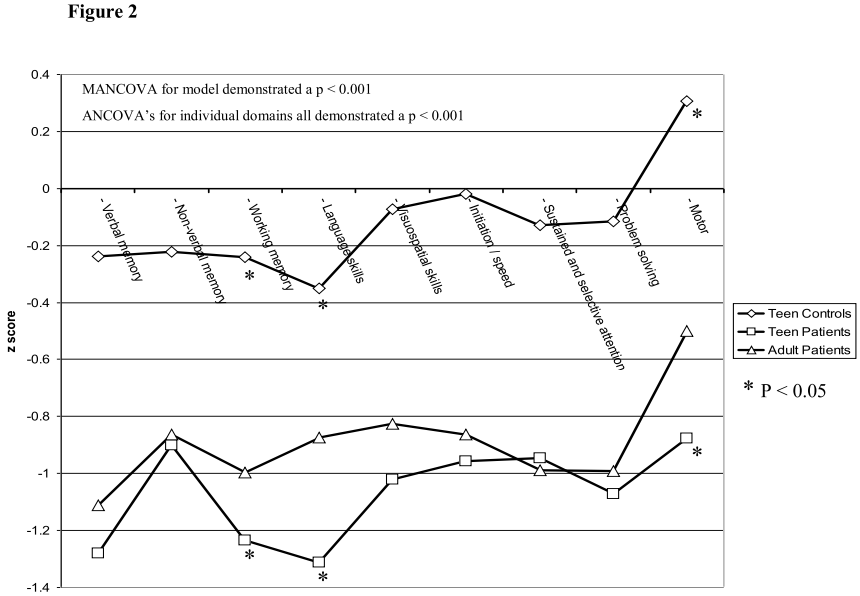


Figure 2 – *Z-scores of Adolescent Patients, Adolescent Controls, and Adult Patients Compared to Adult Controls (Adult Controls Set to Zero).*



adolescents had significantly better fine motor performance than the healthy adult volunteers, the equal performance between naïve adolescents and adults may equate with poorer fine motor performance in the naïve adolescents. Assessing a larger neuroleptic naïve or high-risk population with a larger number of motor tests will be beneficial in assessing illness versus medication effects. In addition, the reports of early, pre-illness aberrations in motor development in patients who later develop schizophrenia (26, 63-65) support an earlier insult to the motor system.

The poorer fine motor performance in the adolescent patients may also be secondary to the adolescent's greater susceptibility to psychotropic-induced alterations in fine motor control. Younger patients have been shown to be more prone to developing extrapyramidal symptoms (EPS) with both typical and atypical neuroleptics (66, 67). Since fine motor control has been shown to correlate with the amount of striatal dopamine (68), and since studies of typical neurodevelopment have shown greater subcortical GM density in adolescents compared to adults (1), the larger basal ganglia in adolescents may be more sensitive to medication. In addition, studies of children and adolescents with schizophrenia have shown enlargements of the caudate, putamen, and the globus pallidus (69). The enlargement of the caudate is associated with an earlier age of onset (21). The volume of the basal ganglia is sensitive to both typical and atypical neuroleptic medication, shrinking with the use of atypical agents (70, 71) and increasing in volume with the typical neuroleptics (71). Thus, the poorer performance of the adolescent patient group may reflect an interplay between illness factors and medication on an enlarged and not yet optimally pruned basal ganglia.

Since this study evaluated patients with first-episode schizophrenia, the impairments in both working memory and language appear to take place within the developmental trajectory of these neuropsychological functions. If the illness process impeded the continued development of these functions, the adolescents would show significantly worse performance in these two measures as adults. Studies that assess age of onset, either as continuous or discrete measures, have found that adolescent-onset illness is marked by poorer performance across a number of domains (18-20). Since the adolescent patients in this study are no different than the adult patients when assessed within a developmental framework, this implicates that the onset of schizophrenia may prevent the further development of these specific cognitive domains. Since there have been no studies that prospectively compare adolescent and adult cognitive trajectories, it is not known whether adolescents cease to develop cognitive functions with illness onset, or whether there is an actual loss of cognitive functions during this time period. This important question would be best verified via a longitudinal study. Addressing this question will help tease apart whether the cognitive deficits are a primary deficit directly linked to the pathogenesis of schizophrenia, or are affected via downstream mechanisms.

There were several limitations to the present study. First and foremost, as noted above, a longitudinal study is the best approach to tease apart whether the cognitive deficits are a primary deficit of the illness, or alternatively, are linked via downstream mechanisms to the pathogenesis of schizophrenia. Second, since the initial MANCOVA comparing all cognitive domains between the two patient groups was negative, it could be argued that no differences exist between the two groups. The MANCOVA is a conservative approach to limit the number of tests and reduce false positives, but may also increase false negatives. Thus, not only were ANCOVA's run on each domain, but a mixed effects model was also used to further validate the results. Nevertheless, the negative MANCOVA is a limitation to the present study and these findings should be replicated. Furthermore, since this was not a population-based study, the presence of an ascertainment bias may limit the generalization of the findings.

The adult control sample had a more evenly weighted distribution of males and females compared to the adolescent control and the two patient groups. Comparing the adult males and females across the nine neuropsychological domains, the females performed significantly better in verbal memory ($t=-3.14$, $df=215$, $p=0.002$) and initiation and speed ($t=-2.61$, $df=238$, $p=0.002$). The males performed better on motor function ($t=4.78$, $df=226$, $p<0.001$), working memory ($t=2.20$, $df=237$, $p=0.03$), and problem solving ($t=1.99$, $df=238$, $p=0.05$). Covarying for sex in the patient and control analyses yielded the same results.

The timing of the neuropsychological testing differed between the neuroleptic naïve and medicated groups. Subjects who had never received neuroleptic medications were tested prior to the administration of these medications in order to obtain a clearer assessment of the effects of illness on cognition. One weakness of this approach is that the clinical symptoms may interfere with an accurate assessment of cognition. The neuropsychological testing was carefully administered to these subjects and done over several days if possible. There were no significant differences between the neuroleptic naïve and medicated groups when assessed globally with a MANCOVA using all domains. Nor was there any differences using an ANCOVA to test the individual domains. Motor function appeared to worsen with neuroleptic exposure in the adolescent group and although not statistically significant overall, within the motor domain, it was significant for the grooved pegboard (right hand, $p = 0.03$; both hands, $p = 0.05$), and not for finger tapping. Although differences existed in the clinical subtypes of schizophrenia between the two patient groups, the adolescent and adult groups were matched on the rate of positive, negative, and disorganized symptoms. Since adolescents have been shown to have greater rates of negative symptoms, and since negative symptoms have been associated with greater cognitive deficits, direct comparisons with prior studies should note the potential differences in study populations. Finally, not all the neuropsychological tasks were designed for pediatric populations. However, since our adolescent sample was weighted toward older teenagers, this poses less of a problem than when studying younger children.

Both the adolescent and adult patient groups have global deficits of approximately one standard deviation below their respective control groups. The

extent of this deviation is similar to studies comparing adolescent patients to either healthy controls (30, 31) or to test norms (29). The presence of these global cognitive deficits are likely the basis for the decline in academic performance during the prodromal and early illness phases of schizophrenia. Although some degree of variability existed in the performance across the specific domains in our sample, none of the neuropsychological domains stood out as being significantly preserved. Had specific cognitive domains been preserved, these could potentially tapped to augment academic performance (i.e., individuals with preservation of nonverbal memory could utilize nonverbal educational material in the classroom). Understanding that there are global deficits in cognitive function will be useful in planning educational strategies within the school setting.

One of the perplexing questions regarding the etiology of schizophrenia is the global disruption in the orchestration of cognitive processes. The neuropsychological tasks utilize a number of different distributed neural networks communicating within and among brain regions. Thus, a global dysregulation of cognition that becomes manifest during adolescence and early adulthood and that appears to be at least partially nested within typical brain development, may involve a more general disruption of connectivity or communication between brain regions. Since white matter continues to develop throughout adolescence and early adulthood (72), is involved in global connections between brain regions, has been shown to be associated with increased cognitive performance (73), and has been implicated in recent genetic studies of schizophrenia (74), it is a potential suspect in the search for the etiology of the cognitive disruptions in schizophrenia. Adolescents with schizophrenia perform worse than adults with schizophrenia in the working memory and language domains and this disruption appears to be disrupted within a developmental trajectory, this is an important clue in the search for the etiology.

References

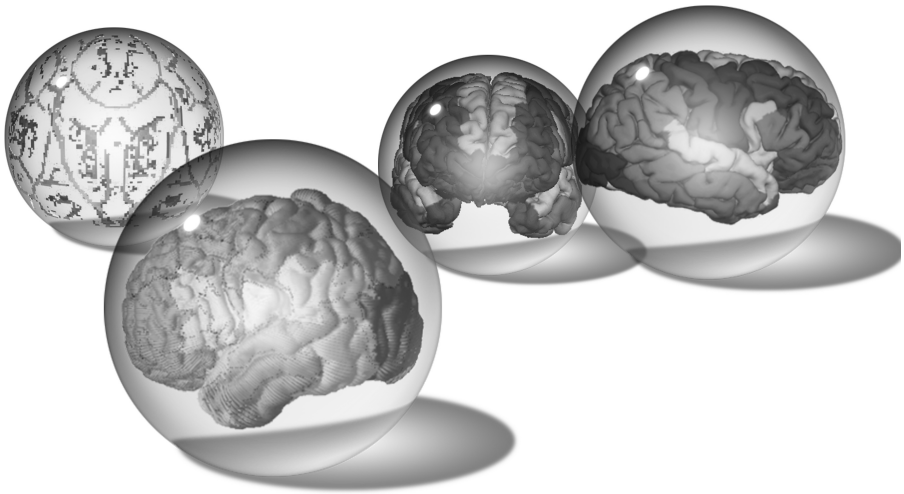
1. Sowell ER, Thompson PM, Holmes CJ, Jernigan TL, Toga AW (1999): In vivo evidence for post-adolescent brain maturation in frontal and striatal regions. Nat Neurosci. 2:859-861.
2. Sowell ER, Thompson PM, Holmes CJ, Batth R, Jernigan TL, Toga AW (1999): Localizing age-related changes in brain structure between childhood and adolescence using statistical parametric mapping. Neuroimage. 9:587-597.
3. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. (2004): Dynamic mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci U S A. 101:8174-8179.
4. Giedd JN, Snell JW, Lange N, Rajapakse JC, Casey BJ, Kozuch PL, et al. (1996): Quantitative magnetic resonance imaging of human brain development: ages 4-18. Cereb Cortex. 6:551-560.
5. Yeo RA, Hodde-Vargas J, Hendren RL, Vargas LA, Brooks WM, Ford CC, et al. (1997): Brain abnormalities in schizophrenia-spectrum children: Implications for a neurodevelopmental perspective. Psychiatry Research: Neuroimaging. 76:1-13.
6. White (2003): Gyrification abnormalities in childhood- and adolescent schizophrenia. Society of Biological Psychiatry. 54:418-426.
7. Sowell ER, Levitt J, Thompson PM, Holmes CJ, Blanton RE, Kornsand DS, et al. (2000): Brain abnormalities in early-onset schizophrenia spectrum disorder observed with statistical parametric mapping of structural magnetic resonance images. Am J Psychiatry. 157:1475-1484.

8. Rapoport JL, Giedd JN, Blumenthal J, Hamburger S, Jeffries N, Fernandez T, et al. (1999): Progressive cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. Arch Gen Psychiatry. 56:649-654.
9. Thompson PM, Vidal C, Giedd JN, Gochman P, Blumenthal J, Nicolson R, et al. (2001): Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proc Natl Acad Sci U S A. 98:11650-11655.
10. Gogtay N, Sporn A, Clasen LS, Nugent TF, 3rd, Greenstein D, Nicolson R, et al. (2004): Comparison of progressive cortical gray matter loss in childhood-onset schizophrenia with that in childhood-onset atypical psychoses. Arch Gen Psychiatry. 61:17-22.
11. Casey BJ, Giedd JN, Thomas KM (2000): Structural and functional brain development and its relation to cognitive development. Biol Psychol. 54:241-257.
12. Nelson CA, Monk CS, Lin J, Carver LJ, Thomas KM, Truwit CL (2000): Functional neuroanatomy of spatial working memory in children. Dev Psychol. 36:109-116.
13. Luciana M, Nelson CA (2000): Neurodevelopmental assessment of cognitive function using CANTAB: Validation and future goals. In: Ernst, Rumsey, editors. Functional Neuroimaging in Child Psychiatry. Cambridge: Cambridge University Press, pp 379-397.
14. Karatekin C (2004): Development of attentional allocation in the dual task paradigm. Int J Psychophysiol. 52:7-21.
15. Green MF, Nuechterlein KH, Gold JM, Barch DM, Cohen J, Essock S, et al. (2004): Approaching a consensus cognitive battery for clinical trials in schizophrenia: the NIMH-MATRICS conference to select cognitive domains and test criteria. Biol Psychiatry. 56:301-307.
16. Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK (2004): Identification of separable cognitive factors in schizophrenia. Schizophr Res. 72:29-39.
17. Asarnow RF, Asamen J, Granholm E, Sherman T, Watkins JM, Williams JE (1994): Cognitive/ neuropsychological studies of children with a schizophrenic disorder. Schizophrenia Bulletin. 20:647-669.
18. Basso MR, Nasrallah HA, Olson SC, Bornstein RA (1997): Cognitive deficits distinguish patients with adolescent- and adult-onset schizophrenia. Neuropsychiatry Neuropsychol Behav Neurol. 10:107-112.
19. Johnstone EC, Owens DG, Bydder GM, Colter N, Crow TJ, Frith CD (1989): The spectrum of structural brain changes in schizophrenia: age of onset as a predictor of cognitive and clinical impairments and their cerebral correlates. Psychol Med. 19:91-103.
20. Yang PC, Liu CY, Chiang SQ, Chen JY, Lin TS (1995): Comparison of adult manifestations of schizophrenia with onset before and after 15 years of age. Acta Psychiatr Scand. 91:209-212.
21. Jeste DV, McAdams LA, Palmer BW, Braff D, Jernigan TL, Paulsen JS, et al. (1998): Relationship of neuropsychological and MRI measures to age of onset of schizophrenia. Acta Psychiatr Scand. 98:156-164.
22. Cuesta MJ, Peralta V, Zarzuela A (1998): Illness duration and neuropsychological impairments in schizophrenia. Schizophr Res. 33:141-150.
23. Tuulio-Henriksson A, Partonen T, Suvisaari J, Haukka J, Lonnqvist J (2004): Age at onset and cognitive functioning in schizophrenia. Br J Psychiatry. 185:215-219.
24. Manschreck TC, Maher BA, Candela SF (2004): Earlier age of first diagnosis in schizophrenia is related to impaired motor control. Schizophr Bull. 30:351-360.
25. Brickman AM, Buchsbaum MS, Bloom R, Bokhoven P, Paul-Odouard R, Haznedar MM, et al. (2004): Neuropsychological functioning in first-break, never-medicated adolescents with psychosis. J Nerv Ment Dis. 192:615-622.
26. Cannon M, Jones P, Huttunen MO, Tanskanen A, Huttunen T, Rabe-Hesketh S, et al. (1999): School performance in Finnish children and later development of schizophrenia: a population-based longitudinal study. Arch Gen Psychiatry. 56:457-463.
27. Cannon TD, Bearden CE, Hollister JM, Rosso IM, Sanchez LE, Hadley T (2000): Childhood cognitive functioning in schizophrenia patients and their unaffected siblings: a prospective cohort study. Schizophr Bull. 26:379-393.
28. Fuller R, Nopoulos P, Arndt S, O'Leary D, Ho BC, Andreasen NC (2002): Longitudinal assessment of premorbid cognitive functioning in patients with schizophrenia through examination of standardized scholastic test performance. Am J Psychiatry. 159:1183-1189.
29. Kumra S, Wiggs E, Bedwell J, Smith AK, Arling E, Albus K, et al. (2000): Neuropsychological deficits in pediatric patients with childhood-onset schizophrenia and psychotic disorder not otherwise specified. Schizophrenia Research. 42:135-144.
30. Kenny JT, Friedman L, Findling RL, Swales TP, Strauss ME, Jesberger JA, et al. (1997): Cognitive impairment in adolescents with schizophrenia. Am J Psychiatry. 154:1613-1615.

31. Oie M, Rund BR (1999): Neuropsychological deficits in adolescent-onset schizophrenia compared with attention deficit hyperactivity disorder. Am J Psychiatry. 156:1216-1222.
32. Kravariti E, Morris RG, Rabe-Hesketh S, Murray RM, Frangou S (2003): The Maudsley early onset schizophrenia study: Cognitive function in adolescents with recent onset schizophrenia. Schizophrenia Research. 61:137-148.
33. Goldberg TE, Karson CN, Leleszi JP, Weinberger DR (1988): Intellectual impairment in adolescent psychosis. A controlled psychometric study. Schizophr Res. 1:261-266.
34. Gottschalk LA, Selin C (1991): Comparative neurobiological and neuropsychological deficits in adolescent and adult schizophrenic and nonschizophrenic patients. Psychother Psychosom. 55:32-41.
35. Flaum MA, Andreasen NC, Arndt S (1992): The Iowa prospective longitudinal study of recent-onset psychoses. Schizophr Bull. 18:481-490.
36. Andreasen NC, Flaum M, Arndt S (1992): The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. Arch Gen Psychiatry. 49:615-623.
37. Andreasen NC (1984): The Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, IA: The University of Iowa.
38. Andreasen NC (1983): The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, IA: The University of Iowa.
39. Spreen O, Strauss E (1998): A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary. 2nd edition ed. New York: Oxford University PRess.
40. Rey A (1958): L'examen clinique en psychologie. Paris: Presse Universitaire de France.
41. Lezak MD (1995): Neuropsychological assessment (3rd ed.). New York, NY, USA: Oxford University Press.
42. Lezak MD (1983): Neuropsychological assessment. 2nd ed. New York, NY: Oxford U Press.
43. Wechsler D (1987): Wechsler Memory Scale - Revised. New York: Psychological Corporation.
44. Rey A (1941): L'examen psychologique dans les cas d'encephalopathie traumatique. Archives de Psychologie. 28:286-340.
45. Osterrieth PA (1944): Le test de copie d'une figure complex: Contribution a l'etude de la perception et de la memoire. Archives de Psychologie. 30:286-356.
46. Benton AL (1946): A Visual Retention Test for Clinical Use. New York: Psychological Corporation.
47. Benton AL (1974): The Revised Visual Retention Test: Clinical and Experimental Applications. 4th edition ed. New York, NY: Psychological Corporation.
48. Sivan AB (1992): Benton Visual Retention Test. 5th ed. San Antonio, TX: The Psychological Corporation.
49. Wechsler D (1981): Wechsler Adult Intelligence Scale - Revised. Cleveland, Ohio: The Psychological Corporation.
50. Shipley WC (1946): Institute of Living Scale. Los Angeles: Western Psychological Services.
51. Reitan RM, Wolfson D (1985): The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation. Tucson, AZ: Neuropsychology Press.
52. Golden CJ (1978): Stroop Color and Word Test: A Manual for Clinical and Experimental Users. Chicago, IL: Stoelting Company.
53. Benton AL, Hamsher K (1976): Multilingual Aphasia Examination. Iowa City, IA: University of Iowa.
54. Benton AL, Hamsher K, Rey GJ, Sivan AB (1994): Multilingual Aphasia Examination. 3rd ed. ed. Iowa City, IA: AJA Associates.
55. Conners CK, Staff M-HS (1995): Conners' Continuous Performance Test. Toronto: MHS.
56. Talland GA, Schwab RS (1964): Performance with multiple sets in Parkinson's disease. Neuropsychologia. 2:45-53.
57. Heaton RK (1981): Wisconsin Card Sorting Test, Manual. Odessa, FL: Psychological Assessment Resources.
58. Tiffin J, Asher EJ (1948): The Purdue Pegboard: Norms and studies of reliability and validity. Journal of Applied Psychology. 32:234-247.
59. Tiffin J (1968): Purdue Pegboard: Examiner Manual. Chicago: Science Research Associates.
60. Hill SK, Beers SR, Kmiec JA, Keshavan MS, Sweeney JA (2004): Impairment of verbal memory and learning in antipsychotic-naive patients with first-episode schizophrenia. Schizophr Res. 68:127-136.
61. Kareken DA, Gur RC, Mozley D, Mozley LH, Saykin AJ, Shtasel DL, et al. (1995): Cognitive functioning and neuroanatomic volume measures in schizophrenia. Neuropsychology. 9:211-219.

62. Saykin AJ, Shtasel DL, Gur RE, Kester DB, Mozley LH, Stafiniak P, et al. (1994): Neuropsychological deficits in neuroleptic naive patients with first-episode schizophrenia. Arch Gen Psychiatry. 51:124-131.
63. Karp BI, Garvey M, Jacobsen LK, Frazier JA, Hamburger SD, Bedwell JS, et al. (2001): Abnormal neurologic maturation in adolescents with early-onset schizophrenia. American Journal of Psychiatry. 158:118-122.
64. Walker E, Lewis N, Loewy R, Palyo S (1999): Motor dysfunction and risk for schizophrenia. Dev Psychopathol. 11:509-523.
65. Walker EF, Savoie T, Davis D (1994): Neuromotor precursors of schizophrenia. Schizophr Bull. 20:441-451.
66. Lewis R (1998): Typical and atypical antipsychotics in adolescent schizophrenia: efficacy, tolerability, and differential sensitivity to extrapyramidal symptoms. Can J Psychiatry. 43:596-604.
67. Keepers GA, Clappison VJ, Casey DE (1983): Initial anticholinergic prophylaxis for neuroleptic-induced extrapyramidal syndromes. Arch Gen Psychiatry. 40:1113-1117.
68. Yang YK, Chiu NT, Chen CC, Chen M, Yeh TL, Lee IH (2003): Correlation between fine motor activity and striatal dopamine D2 receptor density in patients with schizophrenia and healthy controls. Psychiatry Res. 123:191-197.
69. Frazier JA, Giedd JN, Hamburger SD, Albus KE, Kaysen D, Vaituzis AC, et al. (1996): Brain anatomic magnetic resonance imaging in childhood-onset schizophrenia. Arch Gen Psychiatry. 53:617-624.
70. Frazier JA, Giedd JN, Kaysen D, Albus K, Hamburger S, Alaghband-Rad J, et al. (1996): Childhood-onset schizophrenia: brain MRI rescan after 2 years of clozapine maintenance treatment. Am J Psychiatry. 153:564-566.
71. Corson PW, Nopoulos P, Miller DD, Arndt S, Andreasen NC (1999): Change in basal ganglia volume over 2 years in patients with schizophrenia: typical versus atypical neuroleptics. Am J Psychiatry. 156:1200-1204.
72. Yakovlev PI, Lecours AR (1967): The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, editor. Regional Development of the Brain in Early Life. Oxford: Blackwell, pp 3-70.
73. Fuster JM (2002): Frontal lobe and cognitive development. J Neurocytol. 31:373-385.
74. Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al. (2003): White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry. 60:443-456.

2.2 Memory-Guided Saccades in Youth-Onset Psychosis and Attention Deficit Hyperactivity Disorder



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Submitted

Abstract

Background: The oculomotor delayed-response paradigm has been widely used in primate studies to evaluate networks involving the prefrontal cortex, posterior parietal lobe, frontal eye fields, and subcortical regions. The goal of this study was to utilize the oculomotor delay response paradigm to examine spatial working memory performance in 8- to 20-year-olds with psychosis, ADHD and controls.

Methods: Children and adolescents with either a non-affective psychotic disorder (n=25), ADHD (n=33), and controls (n=58) were administered an eye-tracking task with a recall condition and a control condition, the latter which made minimal demands on memory. Memory guided saccades were measured during delay periods were 2, 8 and 10 seconds. In addition, a verbal distracter task was utilized during the delay to constrain the use of verbal strategies during the visuospatial working memory task.

Results: Although both clinical groups had larger distance errors than controls, there was no evidence of a disproportionate impairment in recall compared to controls, nor greater vulnerability to distraction during maintenance in either clinical group. There was no evidence of a delay-dependent impairment in psychosis, however, we did find evidence for a delay-dependent impairment in ADHD when corrective saccades were included. Speed of information processing was correlated with distance errors in psychosis, suggesting that speed of encoding stimulus location may have constrained the accuracy of the saccades.

Conclusions: Our findings suggest impairments during the encoding and/or retrieval stages of both the control and recall conditions in the psychosis group and possible delay dependent deficits in the ADHD group.

Chapter 2

Introduction

The oculomotor delayed response task (DRT) has been widely used to study the neurobiology of working memory (WkM) in humans and non-human primates (1, 2). Two key nodes within the distributed neural networks that are tapped by the DRT include the dorsolateral prefrontal cortex (DLPF) (2) and the inferior parietal lobe (3). In addition, a subset of neurons in the DLPFC have a burst of neuronal firing for the first several seconds after the presentation of a new item in a memory set (4), which may be attributed to differences in encoding and maintenance of WkM. Thus, tasks that assess different delay periods may assist in parsing out which WkM networks are disrupted in different psychiatric disorders.
WkM impairments are present in both schizophrenia (5) and attention deficit hyperactivity disorder (ADHD) (6). In a large meta-analysis of WkM studies in schizophrenia, Lee & Park (7) found that increasing WkM delays beyond 1 second did not result in incremental worsening of the deficit, supporting deficits in encoding and/or early maintenance (less than 1 second) in adults with schizophrenia. Studies using the DRT in adults with schizophrenia consistently show decreased spatial accuracy of memory-guided saccades at delays from 800 msec to 30 sec (8-17). However, several studies have found no impairment in spatial accuracy of memory-guided saccades after corrective saccades were taken into account (11, 18).

The only study to investigate memory-guided saccades in youth-onset schizophrenia, found decreased spatial accuracy in 5 to 16 year olds with psychosis at both 1- and 3-second delays (19). There was no group by delay interaction. There have been only a few studies of memory-guided saccades in ADHD (20). Findings or premature saccades in ADHD were found with delays of 800 msec (21) and 5 seconds (22), but no impairment in the spatial accuracy of saccades. Castellanos et al. (23) found an elevated rate of premature saccades in a larger sample of girls with ADHD, as well as a trend toward lower spatial accuracy of memory-guided saccades after 1.2-sec delays. There is one study to date comparing memory-guided saccades between schizophrenia and ADHD. In 10 adults with schizophrenia, 10 with ADHD, and 10 controls, Ross et al. (24) found an elevated rate of premature saccades in both clinical groups, but decreased spatial accuracy of memory-guided saccades only in the schizophrenia group.

Since WkM continues to develop into early adulthood (25-29), it was our goal to assess developmental differences in the DRT in children and adolescents with ADHD and non-affective psychotic disorders. Directly comparing ADHD and youth onset psychosis using different delay periods may help parse differences in the aberrant neural networks associated with each of the two disorders.

Materials and Methods

Participants

The participants included 25 children and adolescents with a non-affective psychotic disorder, 33 children with ADHD, and 58 controls (Table 1). Participants were excluded if they were not fluent in English, were color blind, if they were premature by more than four weeks, had a history of significant neurological conditions, or an IQ of lower than 70. Participants were excluded from the ADHD group if they were taking psychoactive medications other than psychostimulants, if their parents were not willing to discontinue psychostimulants for 24 hours prior to cognitive testing, if they had been diagnosed with or suspected of having a pervasive developmental disorder, or if they had never met criteria for the combined subtype. Controls were excluded if they had ever taken psychoactive medications, been diagnosed with a major psychiatric disorder, had attention problems for which they had sought help, or had first-degree biological relatives with ADHD or schizophrenia.

Diagnoses were made based on the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version, K-SADS-PL (30). Diagnoses are shown in Table 1. In the psychosis group, average age of onset of psychotic symptoms was 12.8 years (SD = 3.1, range = 7-17). Participants with Psychosis NOS were included only if they had biological relatives with schizophrenia. Participants were asked to refrain from taking psychostimulants for at least 24 hours prior to testing. Informed consent from a parent and assent from the child was obtained on all participants and the study was approved by the institutional review board at the University of Minnesota.

Procedure

Figure 1 depicts the events during the course of a trial. Each trial started with a black fixation cross (0.8° x 0.8°) presented in the center against a white background on a monitor (53 cm diagonal; resolution: 640 x 480 pixels). A small black target dot (1.2° in diameter) was presented 300 msec after the onset of the cross and remained on the screen for 200 msec. Participants were instructed not to look at the target dot, but to maintain fixation at the center. The cross remained on the screen for 400 msec after the disappearance of the target. There were 16 possible locations at which the target could appear. These locations were placed on an imaginary rectangular grid. Each target was at an angle of 6.4°, 9.9°, 12.3° or 14.4° from the center of the screen. The target was assigned randomly to 16 locations, with the constraints that it (1) appeared at least once at each location across the 24 trials in each condition, and (2) did not appear in the same location on consecutive trials.

For the 2, 8 or 20 sec after the offset of the cross, participants performed a verbal distractor task (31), designed to prevent verbal rehearsal and prevent

Table 1 - The Demographic and Clinical Characteristics of the Participants

	Control	Psychosis	ADHD	Diagnosis Effects	Post-Hoc Tests
N	58	25	33		
M:F, %	45:55	56:44	76:24	$X_2^2 = 8.17, p = .017$	ADHD ≠ C
Age in years (*SD*)	12.7 (2.5)	15.1 (3.5)	12.8 (2.7)	$F_{2,113} = 6.96, p = .001$	Psychosis > (C = ADHD)
Range (in months)	106-226	101-241	107-225		
Socioeconomic status (Hollingshead, 1975)	53 (9)	43 (14)	50 (10)	$F_{2,102} = 6.82, p = .002$	Psychosis < (ADHD = C)
[a]Estimated IQ (*SD*)	114 (13)	98 (17)	109 (13)	$F_{2,108} = 11.07, p < .001$	Psychosis < (ADHD = C)
Range	77-144	74-132	85-141		
Vocabulary	13.2 (2.3)	9.3 (2.8)	11.1 (2.7)	$F_{2,110} = 21.92, p < .001$	Psychosis < ADHD < C
Block Design	11.7 (3.0)	9.6 (4.3)	11.9 (2.8)	$F_{2,108} = 3.96, p = .022$	Psychosis < (ADHD = C)
SANS/SAPS					
Negative symptoms		2.9 (0.8)			
Positive symptoms		1.9 (0.9)			
Psychotic symptoms		2.4 (1.2)			
Diagnoses					
Schizophrenia		56%			
Schizophreniform		8%			
Schizoaffective		20%			
Psychosis Not Otherwise Specified		16%			
ADHD					
Combined			94%		
Inattentive (with history of Combined)			6%		

Notes. C = Control. ns = not significant. SANS/SAPS = Scales for the Assessment of Negative/Positive Symptoms (Andreasen, 1983, 1984).
[a]IQ was estimated from the Vocabulary and Block Design subtests from the Wechsler Intelligence Scale, 3rd ed.

participants from looking at the location of the target dot during the delay period. In this task, participants were presented with words belonging to one of several semantic categories flashed in the middle of the screen for 800 msec at a time in random order. One of the semantic categories was always "foods" (target category).

The other words on each trial were chosen randomly from nine other categories (colors, animals, body parts, school supplies, sports, professions, weather terms, articles of clothing, and types of transportation). On all trials, participants pressed a button every time they saw a word that did not belong to the target category. There were 2, 8, and 20 words on each 2-, 8-, and 20-sec trial, respectively. Across the eight 2-sec trials, 10 of the 16 words belonged to the target category. On each 8-sec trial, 5 of the 8 words belonged to the target category, and on each 20-sec trial, 12 of the words belonged to the target category. A 200-msec

blank screen was presented between each pair of words. A green cross appeared 400 msec after the disappearance of the last word on the trial to cue participants to make a saccade to the remembered location of the target. Only the cross remained on the screen for 3 sec after the cue to make the memory-guided saccade.

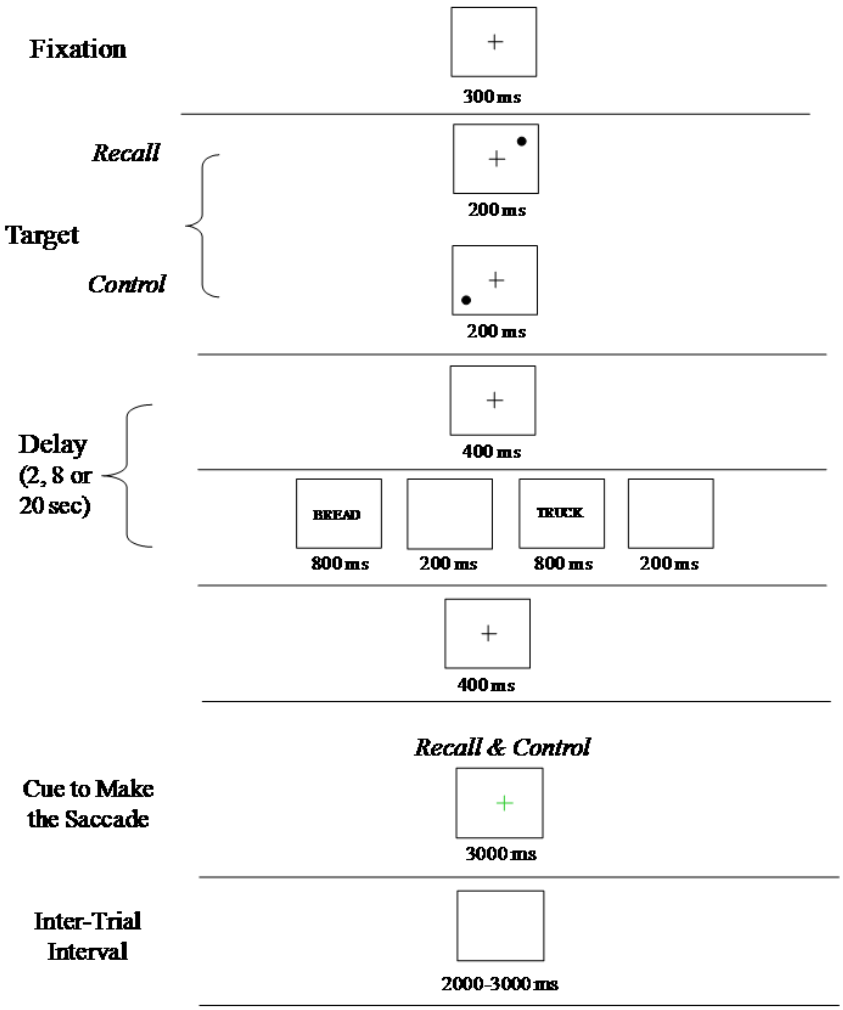


Figure 1. *The course of events during a trial.*

Some participants had difficulty with reading. Therefore, we instructed 1 control and 2 ADHD participants to press one button if the word had the letter "A" and the button if not. The distractor performance of these three participants were excluded from the analyses. We also replaced words with pictures depicting the same objects for 1 ADHD, and 6 psychosis participants. The data of these participants were included in the analyses.

After the recall condition, participants were administered a control condition to assess their performance on visually-guided tasks that made minimal demands on working memory. In this condition, the initial dot always appeared in the same location (lower left quadrant), but did not appear again after the cue to make the saccade.

In both conditions, participants were instructed to look at the center from the beginning of the trial until the cue to make the saccade, and then to look at the target location as quickly and accurately as possible. A blank screen was presented during the inter-trial interval, which varied randomly between 2 and 3 sec to reduce expectancy effects. There were 24 trials in each condition: 3 (delay length) x 8 (replications). Each condition included 1 practice trial for each delay period. The instructions and practice trials were repeated if participants appeared to have difficulty following directions.

Recording of Eye Movements and Behavioral Data

Horizontal and vertical coordinates of the center of gaze and pupillary diameter were recorded with a video-based eye monitor (ISCAN Eye Tracking Laboratory, Model ETL-400), which has a temporal resolution of 60 Hz and a spatial resolution of 1° over the range of visual angles used in the present study. Participants sat 69 cm from the monitor on which the stimuli were displayed. A camera with an attached infrared light source to illuminate the pupil were positioned in front of the computer screen on which the stimuli were presented, below eye level and 40 cm from the participants' eye.

Gaze position was calibrated for each participant at the beginning of the session by focusing the camera on his/her left eye and having him/her look at small visual stimuli in the center and four corners of the screen. A custom-built 2-button button box was used to record manual responses to the distractor tasks. Custom software was used to present the stimuli and to record manual response times (RTs). The eye movement data were merged offline with stimulus presentation and manual RT data. Custom software was used to remove eye-blinks and extract the primary and secondary saccades (28).

Dependent Variables

Spatial Accuracy of the Saccades. Distance error was defined as the distance between the target's center and the landing position of the participant's saccade after the cue to make the saccade. To reduce the impact of outliers, we used median

distance errors. To determine if larger distance errors in the children were due to response factors rather than spatial working memory, we calculated a secondary measure of distance error based on the smaller distance error of the first two saccades after the cue to go. Thus, this measure took into account corrective saccades that may have occurred after the first saccade.

Speed and Accuracy of Manual Responses on the Distractor Task. Error rates were based on the total number of commission errors on the category words and omission errors on the non-category words, divided by the total number of words. Manual RTs were based on median RTs for correct responses to the non-target words. Manual RTs occurring less than 100 msec after the onset of the words were excluded as premature responses.

Statistical Analyses

Statistical analyses were conducted with SPSS 14.0 and MacAnova 5.06 (an open-source cross-platform statistics program (http://www.stat.umn.edu/macanova/). Appropriate transformations of responses to achieve normality and constant variance were sought among the Box-Cox family of distributions (32). Continuous demographic variables were analyzed with univariate ANOVAs, and significant findings were followed up with Tukey tests. Categorical demographic variables were analyzed with $\chi 2$ tests. Correlations between distance errors and RT were calculated using Pearson correlation coefficients, partialing age and IQ.
Repeated-measures Type III ANCOVAs, with age as the covariate, were used to examine effects of subject variables. Each ANCOVA tested linear and quadratic trends for age. Models were selected by backward elimination of non-significant terms involving age, starting with the highest order interactions. When the quadratic trend on age was significant, the linear trend was not reported. Huynh-Feldt adjustments to dfs were used to compute F-statistic p values, and Huynh-Feldt-adjusted dfs were reported where applicable.

Post-hoc analyses of ANCOVA results were conducted using MacAnova. Main effects or interactions were generally not followed up when there were higher-order interactions involving the same variables. Tests of between- and within-subject contrasts and slopes were based on appropriate t statistics. To protect against multiple testing, p values were Bonferroni corrected. When the contrast involved a between-subjects contrast, Tukey-Kramer p values based on the Studentized range were computed and then, where appropriate, Bonferroni corrected by the number of intra-subject contrasts being considered simultaneously.

To examine delay effects on the spatial accuracy of saccades, we compared linear and quadratic contrasts for 2-, 8-, and 20-sec delays.
To calculate effect size, we used a measure similar to Cohen's d but that took into account the age differences among groups. Specifically, we divided the difference of the group means (age adjusted as appropriate) by the square root of the MSe term for the between-subjects analysis section of the ANCOVA. In cases where there was an interaction between group and age, the value reflects the size of the effect at the average age for the whole sample.

Table 2 – Performance as a Function of Condition and Diagnosis and Effects Sizes of Pairwise Group Differences

	Control	Psychosis	ADHD	Effect Size of Pairwise Group Differences		
				C vs. Psychosis	C vs. ADHD	Psychosis vs. ADHD
Distance errors in Control-Recall (degrees)						
2 sec	1.3 (0.6)	1.8 (0.9)	1.8 (1.1)	-0.39	-0.38	-0.01
8 sec	1.4 (0.7)	1.8 (1.1)	2.1 (1.7)	-0.41	-0.30	-0.11
20 sec	1.4 (0.6)	1.9 (1.2)	1.9 (1.0)	-0.55	-0.55	-0.00
Distance errors in Recall (degrees)						
2 sec	1.3 (0.5)	2.0 (1.8)	1.8 (1.4)	-0.89	-0.33	-0.56
8 sec	1.7 (0.6)	2.8 (1.8)	2.0 (1.9)	-1.20	-0.02	-1.17
20 sec	2.1 (0.9)	3.0 (1.9)	3.0 (1.8)	-0.82	-0.60	-0.21

Notes. Numbers in parentheses refer to standard deviations. C = Control.

Results

Proportion of Trials with Recorded Distance Errors

Across conditions, recorded trials of distance error ranged from 70-78% in controls, 50-57% in the ADHD group, and 57-64% in the psychosis group. A 3 (diagnosis) x 4 (condition) ANCOVA showed a linear increase with age, $F(1, 111) = 13.71$, $p < .001$ and more recorded trials in the control than in the recall condition, $F(1, 111) = 11.79$, $p = .001$. There was also a diagnosis effect, $F(2, 111) = 19.77$, $p < .001$. The control group had more recorded trials than either clinical group. Importantly, however, there was no diagnosis by condition interaction, $p = .89$.

Spatial Accuracy of Saccades

Table 2 presents the data and age-adjusted effect sizes of the pairwise group comparisons for the key oculomotor variables. A 3 (diagnosis) x 2 (condition) x 3 (delay) ANCOVA on saccadic distance errors showed a linear decrease in distance errors with age, $F(1, 104) = 28.85$, $p < .001$. Distance errors were larger in recall than in the control condition, $F(1, 104) = 17.17$, $p < .001$.

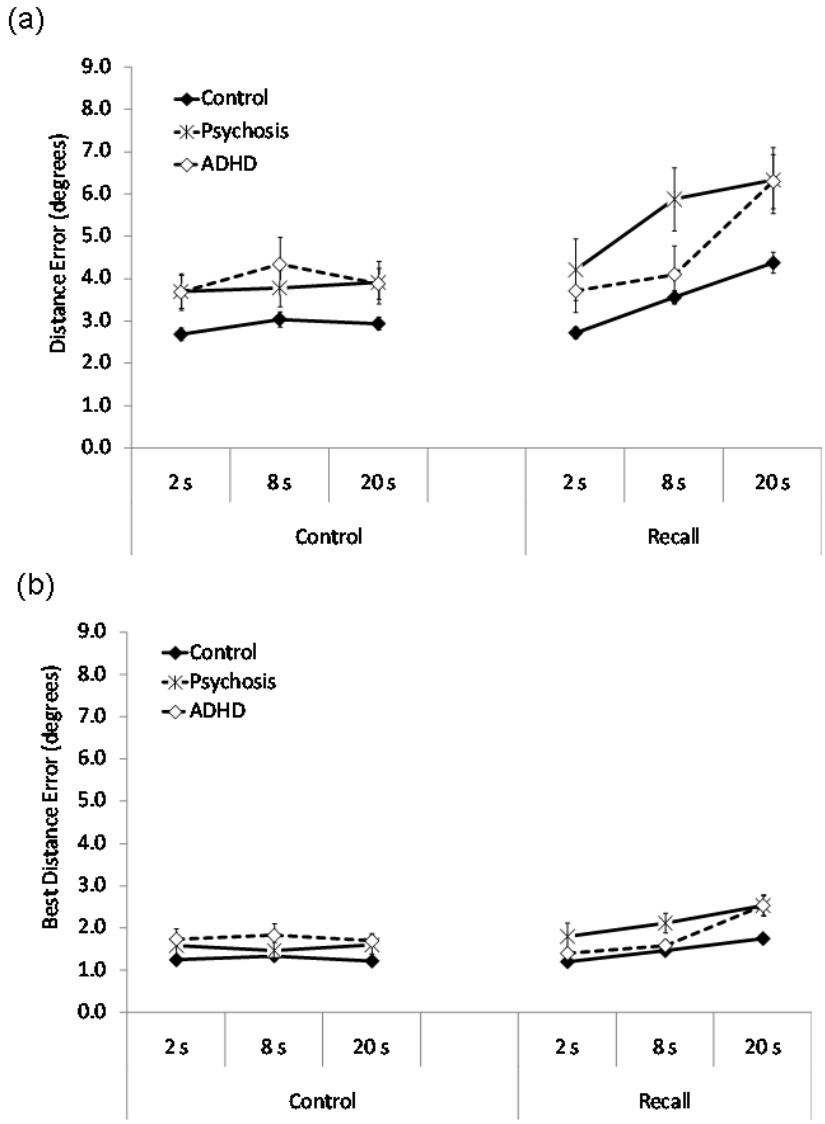


Figure 2. *Distance errors (in degrees) in control-recall and recall as a function of diagnosis and delay. (a) Initial distance errors, (b) better distance error of the first two saccades.*

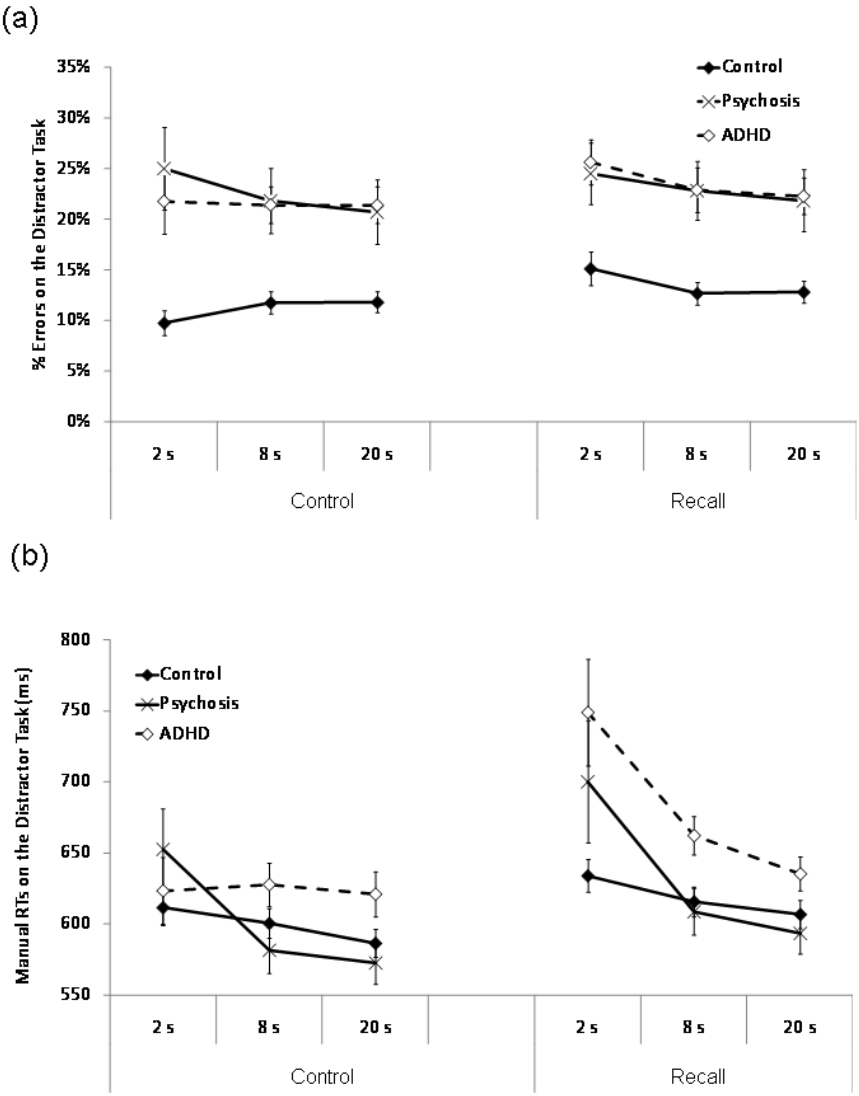


Figure 3. *(a) Error rates and (b) manual RTs (msec) on the distractor task as a function of diagnosis and delay.*

There was a delay effect, $F(2, 208) = 34.20$, $p < .001$, and an interaction between condition and delay, $F(2, 208) = 13.57$, $p < .001$. To explicate the interaction, we tested whether distance errors would increase linearly or

quadratically with delay in the two conditions. As shown in Figure 2, distance errors remained flat across delays in the control condition in all groups, and neither the linear nor the quadratic term was significant. In recall, however, distance errors increased linearly with delay, $F(1, 108) = 93.72$, $p < .001$.

There was also a diagnosis effect on distance errors, $F(2, 104) = 12.81$, $p < .001$. However, the interaction between condition and diagnosis was not significant, $p = .160$. As shown in Figure 2 (a) and Table 2, distance errors were larger in the clinical groups than in the control group.

We tested if the results regarding diagnosis would change if corrective saccades were taken into account. The main effect of diagnosis was significant, $F(2, 103) = 10.02$, $p < .001$, with both clinical groups showing larger distance errors than the controls. Of all the interactions involving diagnosis, only one reached significance: delay by diagnosis, $F(4, 206) = 2.43$, $p = .049$. As shown in Figure 2, the psychosis group had larger distance errors than the control group at all delays, and larger distance errors than the ADHD group at 8 sec. The ADHD group had larger distance errors than controls only at 20 sec.

Behavioral Performance on the Distractor Task

Performance on the distractor task was analyzed to determine if the groups were differentially impacted by the requirement to hold the location of an item in working memory. Error rates and manual RTs on the distractor task are displayed in Figure 3. A 3 (diagnosis) x 2 (condition) x 3 (delay) ANCOVA showed a linear decrease in errors with age, $F(1, 109) = 73.36$, $p < .001$, and more errors in recall than in control, $F(1, 109) = 10.19$, $p = .002$. There was an interaction between condition and the linear trend for age, $F(1, 109) = 7.79$, $p = .006$. Although errors decreased linearly with age in both conditions, the decrease was steeper in the control than in the recall condition.

There was also a diagnosis effect, $F(2, 111) = 30.46$, $p < .001$: both clinical groups made more errors than the control group. Importantly, however, the differences in error rates between the control and recall conditions or between delay periods did not differ across groups. Thus, error rates in all groups appeared to have been affected similarly by the working memory load and by delay.

Analyses of manual RTs showed a linear decrease with age, $F(1, 104) = 30.24$, $p < .001$, a condition effect, $F(1, 104) = 33.34$, $p < .001$, a delay effect, $F(1.8, 122.7) = 26.20$, $p < .001$, and an interaction between condition and delay, $F(1.2, 122.6) = 4.42$, $p = .032$. With increasing delay, manual RTs decreased linearly in the control condition but quadratically in the recall condition.

There was also a diagnosis effect, $F(2, 104) = 4.23$, $p = .017$, and an interaction between diagnosis and the linear trend for age, $F(2, 104) = 6.22$, $p = .003$. Overall, the ADHD group had longer RTs than the psychosis group. In addition, RTs decreased linearly with age in the control and ADHD groups, but not in the psychosis group.

Correlations Between Processing Speed and Distance Error

To test if processing speed was related to spatial accuracy of saccades, we correlated RTs on the distractor task with distance errors (both measures were averaged across the control and recall conditions). Longer RTs were associated with larger distance errors in the psychosis group, $r(19) = -.44$, $p = .047$, but not in the ADHD, $r(25) = .24$, $p = .226$, or control groups, $r(52) = -.10$, $p = .4635$.

Discussion

We found that the DRT provided a valid measure of working memory in all groups. Distance errors were larger in the recall than in the control condition; and distance errors did not vary as a function of delay in the control condition but increased linearly in recall. When the better distance error of the first two saccades was taken into account, there was an improvement in spatial accuracy of saccades in all groups but no interaction between group and extent of improvement. Thus, the clinical groups were as motivated as controls to improve their performance with corrective saccades. Nevertheless, both clinical groups had larger distance errors than the control group in both conditions, whether corrective saccades were taken into account or not.

Interestingly, we found that both clinical groups were impaired on the control condition and they were not disproportionately impaired on the recall compared to the control condition. The impairment in the control condition could be due to difficulties in oculomotor control. However, the fact that both groups were still impaired on the control condition even after corrective saccades were taken into account suggests that basic difficulties in oculomotor control are unlikely to completely account for their impairments in the control condition. Instead, this condition, which was designed to make minimal demands on working memory, may still have taxed the working memory in the clinical groups. Thus, even when working memory demands are minimized by showing the target stimulus in the same location on every trial, the memory trace of the target location may still degrade more rapidly in the clinical groups than in the control group.

The psychosis group did not show worsening performance with increasing delay, whether corrective saccades were taken into account or not. Results for the ADHD group were somewhat more ambiguous. They showed no evidence of delay-dependent deficits for the first saccade, but disproportionately worse performance at 20 sec compared to controls when corrective saccades were taken into account. This may reflect that patients with ADHD were better able to encode the object into WkM, however, the longer delay in which attention was devoted on the distracter task may have interfered with the precision of the recall.

The significant correlation between higher accuracy of memory-guided saccades and faster speed of information processing in youth-onset psychosis is consistent with a growing body of research pointing to slower or inefficient encoding in both verbal and visual-spatial working memory in schizophrenia. In a

meta-analysis of working memory deficits in adults with schizophrenia, Lee and Park (7) concluded that increasing the delay period beyond 1 sec did not lead to disproportionate impairments working memory. Several studies have specifically examined the encoding stage in verbal (33-35), visual (36-40), spatial (41), and visual and spatial (40) working memory in schizophrenia, using behavioral, electrophysiological and neuroimaging methods. All of these studies have concluded that encoding and early maintenance processes are slower and/or less efficient in schizophrenia than in control samples, leading to deficiencies in the nature of the internal representation to be maintained during the delay period. Reduced P1 amplitude during the encoding phase of a visual delayed discrimination task has also been found in adolescents with schizophrenia (40, 42). Since neurons in the DLPFC have a burst of neuronal firing after the presentation of a new item in a memory set (4), one possibility is that these networks are aberrant in schizophrenia more so than in ADHD.

We did find a correlation between speed of processing (as measured through manual RTs) and distance errors (averaged across conditions) in the psychosis group. Thus, speed of encoding the stimulus location may have constrained the spatial accuracy of memory-guided saccades in this group.

There were several limitations to the study. The participants in the ADHD group were limited to the Combined subtype. Thus, we had few girls in the ADHD group as it was difficult to find girls who met criteria for the Combined subtype. Sixteen percent of the non-affective psychosis sample had a diagnosis of Psychosis NOS, and it is possible that not all of them will develop schizophrenia. However, we only included patients with Psychosis NOS if they had a first degree relative with a diagnosis of schizophrenia. Finally, the participants with psychosis were on a variety of medications and while we did not find significant correlations between anticholinergic and chlorpromazine equivalents and performance measures, medication effects in the psychosis group cannot be ruled out. In their meta-analysis of working memory in schizophrenia, Lee and Park (2005) suggest that working memory deficits in this population are not attributable to medications. In addition, impairments in memory-guided saccades are also observed in neuroleptic-naïve patients with schizophrenia and in unaffected relatives of individuals with schizophrenia (7, 12), further suggesting that these impairments may reflect state factors unrelated to pharmacological treatment.

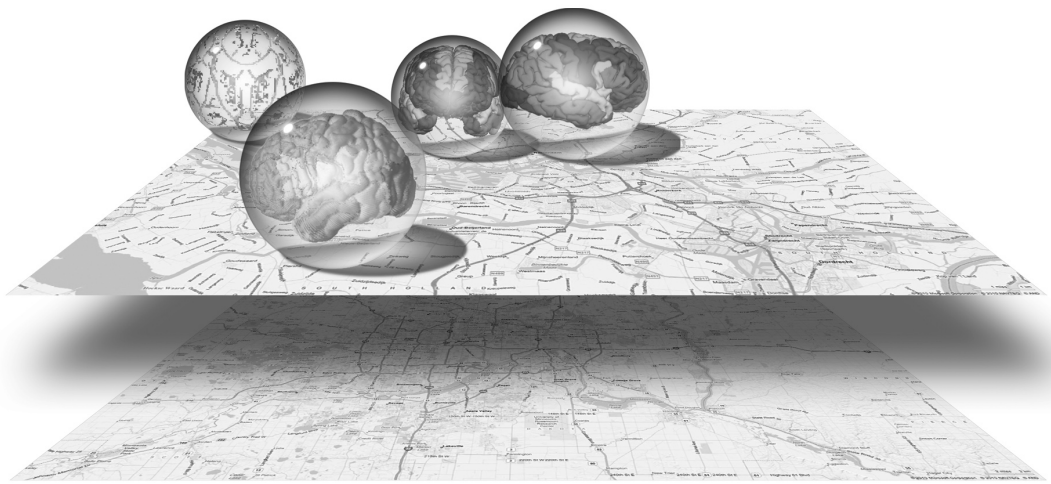
In summary, we did not find evidence for a delay-dependent impairment in spatial working memory in psychosis. A delay-dependent deficit was apparent in ADHD when corrective saccades were taken into account. There was also no evidence for greater vulnerability to distraction during maintenance in either clinical group. The findings are consistent with impairments during the encoding or retrieval stages in patients with psychosis, and possible delay dependent deficits in ADHD. Finally, there were no interactions between diagnosis and age on the spatial accuracy of the saccades. With the caveat that this was not a longitudinal study, results suggest that the groups did not differ in terms of the maturation of the cognitive processes assessed by the task in this study between 8 and 20 years of age.

References

1. Friedman HR, Goldman-Rakic PS (1994): Coactivation of prefrontal cortex and inferior parietal cortex in working memory tasks revealed by 2DG functional mapping in the rhesus monkey. J Neurosci. 14:2775-2788.
2. Funahashi S, Bruce CJ, Goldman-Rakic PS (1989): Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol. 61:331-349.
3. Chafee MV, Goldman-Rakic PS (1998): Matching patterns of activity in primate prefrontal area 8a and parietal area 7ip neurons during a spatial working memory task. J Neurophysiol. 79:2919-2940.
4. Kojima S, Goldman-Rakic PS (1982): Delay-related activity of prefrontal neurons in rhesus monkeys performing delayed response. Brain Res. 248:43-49.
5. Lee J, Park S (2006): The role of stimulus salience in CPT-AX performance of schizophrenia patients. Schizophr Res. 81:191-197.
6. Westerberg H, Hirvikoski T, Forssberg H, Klingberg T (2004): Visuo-spatial working memory span: a sensitive measure of cognitive deficits in children with ADHD. Child Neuropsychol. 10:155-161.
7. Lee J, Park S (2005): Working memory impairments in schizophrenia: a meta-analysis. J Abnorm Psychol. 114:599-611.
8. Camchong J, Dyckman KA, Chapman CE, Yanasak NE, McDowell JE (2006): Basal ganglia thalamocortical circuitry disruptions in schizophrenia during delayed response tasks. Biol Psychiatry. 60:235-241.
9. Karoumi B, Ventre-Dominey J, Vighetto A, Dalery J, d'Amato T (1998): Saccadic eye movements in schizophrenic patients. Psychiatry Res. 77:9-19.
10. Brenner CA, McDowell JE, Cadenhead KS, Clementz BA (2001): Saccadic inhibition among schizotypal personality disorder subjects. Psychophysiology. 38:399-403.
11. Krappmann P, Everling S (1998): Spatial accuracy of primary and secondary memory-guided saccades in schizophrenic patients. Schizophr Res. 30:183-185.
12. McDowell JE, Brenner CA, Myles-Worsley M, Coon H, Byerley W, Clementz BA (2001): Ocular motor delayed-response task performance among patients with schizophrenia and their biological relatives. Psychophysiology. 38:153-156.
13. McDowell JE, Clementz BA (1996): Ocular-motor delayed-response task performance among schizophrenia patients. Neuropsychobiology. 34:67-71.
14. Minor K, Park S (1999): Spatial working memory: absence of gender differences in schizophrenia patients and healthy control subjects. Biol Psychiatry. 46:1003-1005.
15. Muller N, Riedel M, Eggert T, Straube A (1999): Internally and externally guided voluntary saccades in unmedicated and medicated schizophrenic patients. Part II. Saccadic latency, gain, and fixation suppression errors. Eur Arch Psychiatry Clin Neurosci. 249:7-14.
16. Radant AD, Claypoole K, Wingerson DK, Cowley DS, Roy-Byrne PP (1997): Relationships between neuropsychological and oculomotor measures in schizophrenia patients and normal controls. Biol Psychiatry. 42:797-805.
17. Ross RG, Harris JG, Olincy A, Radant A, Adler LE, Freedman R (1998): Familial transmission of two independent saccadic abnormalities in schizophrenia. Schizophr Res. 30:59-70.
18. Everling S, Krappmann P, Preuss S, Brand A, Flohr H (1996): Hypometric primary saccades of schizophrenics in a delayed-response task. Exp Brain Res. 111:289-295.
19. Ross RG, Heinlein S, Zerbe GO, Radant A (2005): Saccadic eye movement task identifies cognitive deficits in children with schizophrenia, but not in unaffected child relatives. J Child Psychol Psychiatry. 46:1354-1362.
20. Karatekin C (2007): Eye tracking studies of normative and atypical development. Developmental Rev. 27:283-348.
21. Ross RG, Hommer D, Breiger D, Varley C, Radant A (1994): Eye movement task related to frontal lobe functioning in children with attention deficit disorder. J Am Acad Child Adolesc Psychiatry. 33:869-874.
22. Mostofsky SH, Lasker AG, Cutting LE, Denckla MB, Zee DS (2001): Oculomotor abnormalities in attention deficit hyperactivity disorder: a preliminary study. Neurology. 57:423-430.
23. Castellanos FX, Marvasti FF, Ducharme JL, Walter JM, Israel ME, Krain A, et al. (2000): Executive function oculomotor tasks in girls with ADHD. J Am Acad Child Adolesc Psychiatry. 39:644-650.

24. Ross RG, Harris JG, Olincy A, Radant A (2000): Eye movement task measures inhibition and spatial working memory in adults with schizophrenia, ADHD, and a normal comparison group. Psychiatry Res. 95:35-42.
25. Luciana M, Conklin HM, Hooper CJ, Yarger RS (2005): The development of nonverbal working memory and executive control processes in adolescents. Child Dev. 76:697-712.
26. Conklin HM, Luciana M, Hooper CJ, Yarger RS (2007): Working memory performance in typically developing children and adolescents: behavioral evidence of protracted frontal lobe development. Dev Neuropsychol. 31:103-128.
27. Luciana M, Nelson CA (2000): Neurodevelopmental assessment of cognitive function using CANTAB: Validation and future goals. In: Ernst, Rumsey, editors. Functional Neuroimaging in Child Psychiatry. Cambridge: Cambridge University Press, pp 379-397.
28. Karatekin C, Marcus DJ, White T (2007): Oculomotor and manual indexes of incidental and intentional spatial sequence learning during middle childhood and adolescence. J Exp Child Psychol. 96:107-130.
29. De Luca CR, Wood SJ, Anderson V, Buchanan JA, Proffitt TM, Mahony K, et al. (2003): Normative data from the CANTAB. I: development of executive function over the lifespan. J Clin Exp Neuropsychol. 25:242-254.
30. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. (1997): Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 36:980-988.
31. Park S, Holzman PS (1992): Schizophrenics show spatial working memory deficits. Arch Gen Psychiatry. 49:975-982.
32. Box GEP, Cox DR (1964): An analysis of transformations. J Royal Statistical Soc, Series B. 26:211-252.
33. Cairo TA, Woodward TS, Ngan ET (2006): Decreased encoding efficiency in schizophrenia. Biol Psychiatry. 59:740-746.
34. Javitt DC, Rabinowicz E, Silipo G, Dias EC (2007): Encoding vs. retention: differential effects of cue manipulation on working memory performance in schizophrenia. Schizophr Res. 91:159-168.
35. Kayser J, Tenke CE, Gates NA, Kroppmann CJ, Gil RB, Bruder GE (2006): ERP/CSD indices of impaired verbal working memory subprocesses in schizophrenia. Psychophysiology. 43:237-252.
36. Fuller RL, Luck SJ, McMahon RP, Gold JM (2005): Working memory consolidation is abnormally slow in schizophrenia. J Abnorm Psychol. 114:279-290.
37. Gold JM, Fuller RL, Robinson BM, McMahon RP, Braun EL, Luck SJ (2006): Intact attentional control of working memory encoding in schizophrenia. J Abnorm Psychol. 115:658-673.
38. Hartman M, Steketee MC, Silva S, Lanning K, McCann H (2003): Working memory and schizophrenia: evidence for slowed encoding. Schizophr Res. 59:99-113.
39. Lencz T, Bilder RM, Turkel E, Goldman RS, Robinson D, Kane JM, et al. (2003): Impairments in perceptual competency and maintenance on a visual delayed match-to-sample test in first-episode schizophrenia. Arch Gen Psychiatry. 60:238-243.
40. Tek C, Gold J, Blaxton T, Wilk C, McMahon RP, Buchanan RW (2002): Visual perceptual and working memory impairments in schizophrenia. Arch Gen Psychiatry. 59:146-153.
41. Badcock JC, Badcock DR, Read C, Jablensky A (2008): Examining encoding imprecision in spatial working memory in schizophrenia. Schizophr Res. 100:144-152.
42. Haenschel C, Bittner RA, Haertling F, Rotarska-Jagiela A, Maurer K, Singer W, et al. (2007): Contribution of impaired early-stage visual processing to working memory dysfunction in adolescents with schizophrenia: a study with event-related potentials and functional magnetic resonance imaging. Arch Gen Psychiatry. 64:1229-1240.

3.1 The Development of Gyrification in Childhood and Adolescence



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Brain & Cognition (In Press)

Abstract

Gyrification is the process by which the brain undergoes changes in surface morphology to create sulcal and gyral regions. The period of greatest development of brain gyrification is during the third trimester of pregnancy, a period of time in which the brain undergoes considerable growth. Little is known about changes in gyrification during childhood and adolescence, although considering the changes in gray matter volume and thickness during this time period, it is conceivable that alterations in the brain surface morphology could also occur during this period of development. The formation of gyri and sulci in the brain allows for compact wiring that promotes and enhances efficient neural processing. If cerebral function and form are linked through the organization of neural connectivity, then alterations in neural connectivity, i.e., synaptic pruning, may also alter the gyral and sulcal patterns of the brain. This paper reviews developmental theories of gyrification, computational techniques for measuring gyrification, and the potential interaction between gyrification and neuronal connectivity. We also present recent findings involving alterations in gyrification during childhood and adolescence.

Introduction

Gyrification is a fascinating and poorly understood developmental process that refers to the development of the folding surface patterns on the brain (1, 2), many of which readily distinguish the human brain from that of other organisms. Since gray matter (GM) forms an external layer around the brain, gyrification results in a dramatic increase in the cortical surface area and thus, in the volume of cortical GM. The ratio of brain cortical GM to body size is the highest in humans compared to all animals, with dolphins and porpoises being relatively close (3). However, unlike dolphins and porpoises, humans utilize their entire brains, rather than alternately putting one of their hemispheres to sleep while the other remains active (4).

The development of gyrification begins prior to birth (see Figure 1), with the early stages of gyral and sulcal formation taking place between 10 to 15 weeks of human fetal life (5, 6). During the third trimester of fetal life, when the brain is undergoing considerable growth (5), the brain develops from a relatively smooth, lissencephalic structure to a brain that more closely resembles the morphology of the adult brain (1, 7-9). In 1988 Zilles et al. (1988) described a quantitive approach to measure gyrification, known as the 'gyrification index' (GI). Brains that have a higher degree of cortical folding yield larger values of the GI. This measure was applied to quantify the comparative anatomy of gyrification (2, 10) as well as the developmental trajectory of gyrification in humans (7). They found that the GI, which is defined as the ratio between the lengths of coronal outlines for the brain including and excluding the sulcal regions, increases dramatically during the third trimester, and then remains relatively constant throughout development (7, 11). Since the brain nearly triples its volume from birth to adulthood, the process of gyrification continues through this developmental period, maintaining this constant ratio.

This constant GI ratio through birth is interesting and may allow for dating specific events that affect gyrification in the late prenatal period (12). However, this constant GI ratio finding was based on 97 brains, which although a substantial number of postmortem brains, is a relatively small number considering they range from 11 weeks of gestational life to 95 years of age. Considering the pronounced decrease in GM that occurs during adolescent development (13, 14), it is somewhat surprising that the GI would remain constant. There have been no MRI studies to date that have specifically addressed the constancy of the GI during typical development.

Recently, new methods have been developed to study cortical foldings without computing gyrification indexes, e.g., shape analysis based on high dimensional spherical basis such as SPHAM (Shen et al., 2004) or spherical wavelets (Yu et al., 2007). These methods provide different ways to characterize shape features. One motive for studying gyrification is to better understand the neurobiology associated with the development of cortical folding patters. However, an alternate motive for studying gyrification is to provide a better understanding of the structural variability between brains (6, 15, 16). For example, the central sulcus

can vary in location by up to 2 cm between individuals (17). This variability results in significant challenges for spatial normalization, which is a common practice in evaluating structural and functional brain images in neuroimaging studies (15).

The goal of this paper is to provide a review of the development of gyrification with a focus on changes that lead up to and through adolescence. We will include a review of the developmental precursors that contribute to gyrification, primary theories of gyrification, and also a description of current computational algorithms used to numerically measure gyrification. We will discuss genetic and environmental contributions to gyrification. Finally, we will present work in-progress on age-related differences in gyrification in typically developing adolescents. Our findings support a changing surface morphology that is associated with underlying neurodevelopmental changes during adolescence.

Precursors to Gyrification

The early antecedents of gyrification occur during the first months of fetal life, when neurons that have formed via mitosis at the ventricular zone migrate outward along radial glial guide cells to the outer layers of the brain. This migration begins at approximately six weeks of fetal life and its description is known as the radial unit hypothesis of cortical formation (18-20). This migration of neurons outward from the ventricular zone forms the basis for the cortical gray matter (GM).

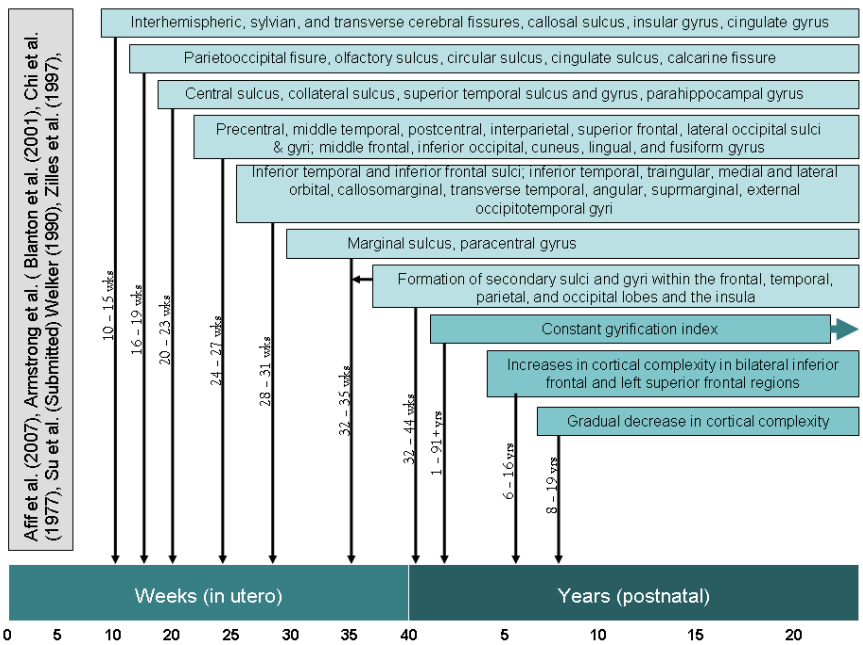


Figure 1 – *Developmental Timeline for Gyrification.*

Prior to six weeks of fetal life, the neural progenitor cells located in the ventricular zone begin symmetric cell division, with each stem cell producing two identical stem cells with each mitotic cycle (21). Thus, this period results in an exponential growth in neuronal progenitor cells. Then, at approximately six weeks of gestational age, the progenitor cells make a gradual shift to asymmetric division. During asymmetric cell division, one daughter cell remains as an undifferentiated stem cell which undergoes further replication, while the other daughter cell matures into a neuron that migrates outward to the cortex. The migration forms an inside-out pattern, with later generations passing through the previously developed cells before reaching their ultimate positions in the cortex (22). The completed cortical GM consists of six layers of cells that have migrated in this inside-out pattern.

During these two phases of symmetric (before six weeks) and asymmetric (six weeks to 24 weeks) cell division, small perturbations can influence either the thickness or surface area of the cortex. In turn, these events can influence gyrification. For example, before six weeks of age, one additional mitotic cycle could potentially double the number of neural progenitor cells, which could have a profound influence on the number of migrating cells.

The surface area of the brain is closely associated with the number of radial units formed by symmetrical division along the ventricular zone (18, 19). A larger number of radial units implies that there will be a larger number of lined projections to the cortical plate, and thus a greater surface area of the brain (22, 23). Since each round of mitosis results in an exponential increase in the number of progenitor cells, small changes affecting the duration of symmetric growth will have a dramatic impact on surface area (24). This developmental principle has been called 'late equals large', as neurons migrating into late-developing brain structures undergo a longer period of symmetrical division, resulting in a larger size of these structures. This principle has been verified for the developmental time table and corresponding size of brain structures in many different species (25, 26).

When the embryos of monkeys are irradiated during the symmetric phase of progenitor cell division, there is a decrease in the total surface area of the brain. However, when radiation is applied after six weeks, during the phase of asymmetric cell division, it results in a deletion of cortical cell layers, and in turn, a decrement in cortical thickness (24). It also disrupts the development of gyrification (27). Thus, the thickness of the six-layered cortex is influenced by events that occur during the asymmetric period of cell division.

Recent studies have found that the migration of neurons into the cortex is not as straightforward as initially thought, with several neuronal subpopulations showing different migratory patterns, e.g., (28). In particular, the radial migration pattern described above is characteristic of pyramidal cells, while different types of cortical interneurons pursue a more tangential migratory path to their target layers (29). The effect of the tangential mode of migration on cortical morphology is likely small or has only a local influence on the development of the cortical layers. The vast majority of cortical neurons are pyramidal cells that follow the predominant radial path; interneurons form connections only in their immediate vicinity.

An additional factor that may affect the surface morphology of the brain is apoptosis, or programmed cell death (30). Apoptosis can result in the elimination of up to 50% of the neurons that are generated early in development (31, 32). Since apoptosis contributes to total neuronal number, and thus to both total brain volume and neuronal connectivity, apoptosis also likely contributes to brain surface morphology in ways that are as yet poorly understood.

To summarize, these prenatal and early postnatal events provide a critical foundation for subsequent changes in gyrification that may occur during childhood, adolescence, and into adulthood.

Phylogeny of Gyrification

A fascinating feature of the human brain is the disproportionally large surface area of the cerebral cortex in relation to its volume, which is due to an extension of its developmental period as compared to other brain regions (25, 33). Greater surface area implies a larger amount of cortical gray matter and thus, a greater potential for computational abilities. The phylogenetic increase in the surface area of the human brain has far exceeded the growth in cortical thickness (1). For example, in humans the surface area of the brain is 1,700 times larger than in shrews, yet the thickness of the cortex is only six times greater (34). Compared to macaque monkeys, the surface area of the human brain is approximately ten times greater, whereas the thickness of the human cortex is only two fold greater (24). This patterning implies that during evolution, the cortex expanded laterally rather than vertically (35), resulting in a convoluted human cortical sheet that is about three times as large as the inner surface of the skull (1, 36-40).

In theory, a greater number of neurons in the cortex could also be obtained by increasing the cortical thickness, rather than increasing cortical surface area. Tripling cortical thickness, from about 5 to 15mm, would allow for a lissencephalic human cortex with only a minor increase in brain volume. However, computer modeling studies (41, 42) suggest that this mechanism would be ill fated. Given the formidable degree of connectivity among cortical neurons (each forming, on average, a thousand or more connections with other neurons (43)), the volume of connections grows exponentially with the number of neurons. Thus, the extra projections required to link neurons in the additional cortical layers would lead to a situation of highly inefficient wiring in the thickened cortex. In order to fit all the connections, neurons within the cortex would need to take detours, resulting in highly inefficient processing (44). These theoretical studies support the idea that the segregation of brain tissue into components of cell bodies within the GM and connections within the WM, in concert with the volume-saving folding of the cortical sheet, reflects an efficiently designed wiring and volume arrangement for the very dense connectivity found in the cerebral cortex (41, 42).

Theories of Gyrification

A number of theories have surfaced that describe the developmental processes underlying the gyrification of the cerebral cortex. The first theory emerged over a century ago and postulated that higher and lower growth rates of different brain regions separate gyral regions as a result of tension and local tissue deformation (45). This theory can be referred to as a "mechanical theory" because it advocates that tensile forces promote gyral development. Several additional mechanical theories emerged over the course of the subsequent 50 years. These theories were similar and postulated that there was differential growth between the gyri and sulci and that regions destined to be become gyri are established by active, localized growth (8) in combination with friction of the different brain structures against each other and with the surrounding skull (46). Subsequent analyses, however, have suggested that gyrification is dependent on mechanisms within the cortical regions, as opposed to restraints of the skull or connections with subcortical regions (47). During the period of rapid brain growth, differential expansion of the individual cortical layers may lead to a buckling of the laminar cortical sheet (1, 36-40). However, theories of cortical morphogenesis need to explain why convolutions are modified even after the destruction of connections (48, 49).

Thus, a third theory emerged a decade ago postulating that viscoelastic tension exerted by cortical fibers contributes to the shaping of cortical convolutions (39). This theory proposes that neuronal connections that develop during the second trimester produce localized fiber tension which draws densely interconnected regions closer together. As regions of greater connectivity move closer together in an enclosed and rapidly growing brain, they form outward bulging gyri. Alternatively, more sparsely connected regions drift apart and form the sulci. The tension, although very small for an individual axon (50), is summed by the very large number of neurons, thus creating differential forces that interact within the rapidly growing brain to form the gyri and sulci. Accordingly, the characteristic pattern of the convolutions is explained by the highly specific organization of the underlying connectivity (51). In drawing regions of greater connectivity closer together, the transit time of the action potentials is decreased, thus enhancing the overall efficiency of brain function. If tension produced by the neuronal connections is involved in the mechanisms of gyrification, then changes in the patterning of the gyri and sulci are expected outcomes of synaptic pruning. Such a theory could link brain surface morphology with regional neuronal connectivity within a developmental framework (33).

Meshing the age-related differences in the morphology of the cerebral cortex to changes in neural connectivity is intriguing. Age-related synaptic and dendritic arborization may result in decreasing the tensile forces that form the gyral and sulcal regions (33, 39). Histological studies of the neuronal pathways have found that the neural fibers tend to course horizontal to the surface in the sulci, whereas in the gyri fiber pathways tend to be oriented tangential to the cortical surface (Welker, 1990). A release of tension would occur along the line horizontal to the predominance of pruned connections. Theoretically, this would result in a

widening of the sulci and a greater, or more peaked, curvature of the gyri (52). These features have been found in a group of healthy adults (53) using techniques to measure the Gaussian-weighted average curvature, or the concavity and convexity of the gyri and sulci.

Measures of Gyrification

Utilizing coronal sections of postmortem brains, Zilles et al. (1988) (10, 54) manually measured the ratio between the length of the outer folded surface of the brain (including sulci) and the length of the outer surface excluding sulci (see Figure 2-left). While the two-dimensional approach was applied to coronal brain sections, sections obtained at 45o oblique angles obtained GI's that were within 8% of the coronal GI measures. GI has been applied to study both the phylogeny (10, 54) and ontogeny (7) of cortical gyrification. Brains that have a higher degree of cortical folding yield larger values of the GI. Anterior-to-posterior maps of human GI measures have shown greater gyrification in the prefrontal and temporal/ parietal association regions of the brain as compared to other regions (10). During development, the GI begins to increase prior to the third trimester and plateaus at birth.

Interestingly, this plateau appears to remain constant across the lifespan, in spite of the rapid brain development and changes associated with aging (7). However, since developmental studies typically require large samples, and since postmortem samples in younger individuals are difficult to obtain, it would be beneficial for the study of Armstrong et al. (7), which had an sample size of 97 individuals, to be replicated/validated with a larger sample size to confirm this finding.

Alternative, computational methods for measuring the surface morphology of the brain involve regional measurements of curvature (i.e., convexity and concavity) (Van Essen & Drury, 1997) and sulcal depth of the cortical surface from magnetic resonance (MR) images (53, 55, 56). For measurements of curvature, these methods first define a triangular surface covering a layer within the cortical rim. Once the surface is constructed, (for example) the angles between vectors normal to the triangular isosurfaces are used to calculate the regional curvature of the cortex. These regional measures can be utilized to study regional curvature between groups (55, 57), or averaged to study global differences (53, 58).

For depth measurements of the sulci, approaches have been utilized that are based on either Euclidean distance (59), geodesic surface distance (60), or geodesic distance marked by the path within the cerebrospinal fluid (CSF) (61). Different distance measurements provide different measures of sulcal depth that may also influence measures of cortical complexity. Gyrification is one measure of cortical complexity. Among these depth measurements, geodesic distance (the path length through the sulcus traveling only through the sulcal CSF and not through tissue) provides a better understanding of the true cortical depth, as it takes into account the circuitous nature of the sulci (see in Figure 2-left).

Since the original gyrification index was defined on coronal sections, unlike curvature and sulcal depth measurements, it does not take into account the 3-D nature of the cortical surface. The GI may be altered if the slice orientation is different, thus it is important to introduce a 3D gyrification index to eliminate the shortcomings of the coronal 2D GI. A number of neuroimaging software packages are currently available to generate 3D reconstructions of human brains, e.g., Freesurfer (Dale et al. 1999)1, SurfRelax (Larsson, 2001)2, and BrainVisa (Mangin et al. 2004). These software packages provide the early preprocessing stages, which are the essential first steps in deriving a 3D gyrification index.

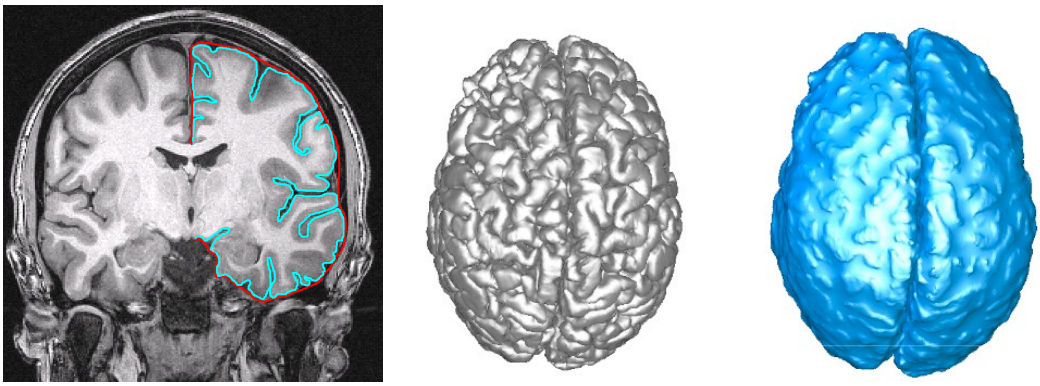
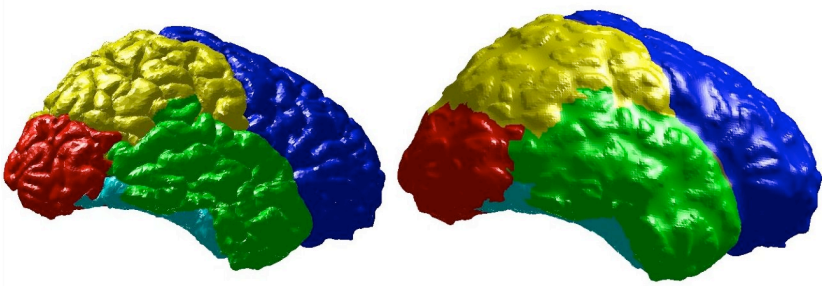


Figure 2 – *(Top left) The Standard Gyrification Index (GI) is a Ratio of the External Brain Surface (Turquoise Line) with the outer Surface excluding the Sulci (Red Line) (Figure 1, Back Cover). The local 3D Gyrification Index (LGI) is the Area Ratio of the Selected Region on the External Brain Surface with the Corresponding Region on the Outer Surface The top middle and right figures show whole brain and the bottom images show regional 3D GI measures (Figure 2 on the Back Cover).*



[1] FreeSurfer, see http://surfer.nmr.mgh.harvard.edu/

[2] SurRelax, see http://www.cns.nyu.edu/~jonas/software.html

A simple extension of the 2D gyrification index to 3D is to use the area ratio between the outer hull surface, which is a surface wrapped around the brain, and the cortical surface. A localized 3-D gyrification index has been developed and applied to a group of children affected by 22q11 Deletion Syndrome (62). This approach uses 3D triangulated mesh reconstructions of both cortical surfaces and outer hull surfaces and measures the amount of cortical surface buried in the sulci (region of interest) by constructing a non-intrinsic sphere with different radii. The index defined for each point on the cortical surface is obtained through a depth-weighted sum of neighboring points on the outer hull surface. Compared to previous measures of the sulcus index (Dubois et al., 2007), this approach is fully localized and thus helps to better define the characteristics of any region of interest in the brain, not restricted to any one sulcus or gyrus.

Another new approach uses the ratio between the surface area of the cortical surface included within a small sphere placed over the cortex with the surface area of a disc corresponding to the radius of the sphere (Toro et al., 2008). This method turns out to be less sensitive, since it is dependent on choosing a specific radius for the sphere, and different radii may give different results. This technique has been applied to a sample of 314 subjects, 164 females and 150 males, and shows a disproportionate ratio of cortical surface area to brain size, similar to the earlier observations across species (Prothero & Sundsten, 1984). In addition, there is an increase in cortical folding in the prefrontal cortex for larger brains. Since this approach does not require the construction of an outer hull surface, it results in a simple and efficient algorithm in comparison to alternative methods (62). Meanwhile, these new methodologies raise important methodological questions in comparision to the previously defined measures (Van Essen & Drury, 1997; Zilles et al., 1988), namely, that the computation should be independent of brain size, since altered brain surface morphology does not necessarily follow the same pattern as alterations in brain size.

Recently, we proposed several new intrinsic and geometric techniques to compute global and local gyrification indices. The simple extension from 3D global GI (see Figure 2) to 3D local GI is to find the corresponding regions on the outer hull surface for any selected region of interest on the external brain surface (see Figure 2-c). Furthermore, 3D GI can also be weighted by local quantities, i.e., curvature and sulcal depth, and is fully intrinsic and different than the method proposed by Schaer et al. (Schaer et al. 2008; Toro et al., 2008) in that it does not depend on a chosen radius nor on a corresponding non-intrinsic sphere to determine the region used to calculate the local GI. The incorporation of the robust sulcal depth computation developed in (Kao et al. 2007) as part of the GI measurement is important to characterize different levels of convolutions in human brains. By applying our method to a population of typically developing children, the proposed measurement of depth-weighted local gyrification turns out to be more robust in finding the developmental differences between children and adolescents, e.g., we observe significantly increased gyrification in the right parietal lobe and right cingulate cortex, as well as age-related differences in the left frontal, right parietal and the right cingulate cortex between the ages of 8 to 19 years. These

findings provide some references for future study of the relationship between gyrification and neurological and psychiatric conditions, in addition to the development of other more advanced techniques to quantify the gyrification of the human brain.

Heritability in Gyrification

Even though the development of the sulcal and gyral patterns in the brain is strongly influenced by genetic processes (63), studies of monozygotic twins (MZ), who share the same genetic complement, show considerable differences in their surface morphology (58, 64). For example, correlations in volume measurements in MZ twins are on average greater than 0.95, whereas measures of gyral and sulcal curvature are significantly less correlated and on the order of 0.5 (65). It is plausible that the greater non-shared environmental influences that are present postnatally for twins, coupled with the pronounced cortical plasticity of early development, bring about differences in MZ twins in the patterns of cortical surface morphology.

In a study of both MZ and dizygotic twins (DZ), Bartley et al. demonstrated that the development of cortical patterns is determined primarily by random environmental factors (66). In addition, evaluating the gyral patterns in monozygotic twins, Lohmann et al. (67) found that the deeper and developmentally earlier sulci of the brain (i.e., the central sulcus or the sylvian fissure) are more highly correlated than the superficial, or tertiary sulci (i.e., the caudomedial lobule). The tertiary sulci, which develop mainly after birth, appear to be more affected by non-genetic influences. To summarize the genetic contribution to gyrification, it is thought that while genetic processes play a large role in gyrification, especially early in development, non-shared environmental factors have a major contribution to the surface morphology of the brain.

Gyrification in Development

If tension produced by neuronal connections is involved in the mechanisms of gyrification, then synaptic pruning or dendritic arborization later in development could conceivably alter brain surface morphology. Indeed, local and remote changes in gyrification have been observed after experimental white-matter lesions in the developing primate brain (48, 49). The link between gyrification and axonal tension has also been supported by recent experimental findings in the primate brain showing a strong correlation between the densities and trajectories of fiber projections (36, 37).

It is known from postmortem studies that while there is little loss of neurons between childhood and adulthood (68), there is a considerable amount of synaptic pruning that occurs during childhood and adolescence (69-71). In fact, synaptic pruning is thought to underlie the developmental changes (14, 72) and differences (13, 73) seen in GM volume between children, adolescents, and adults. After the age of 18, it has been demonstrated that changes in surface curvature

occur, affecting both the gyri and the sulci (53). The sulci tend to develop less curvature, becoming broader, while the gyri develop greater curvature, becoming more peaked. In addition, there is thinning of the cortical mantel, and these differences are consistent with an increased amount of CSF associated with aging.

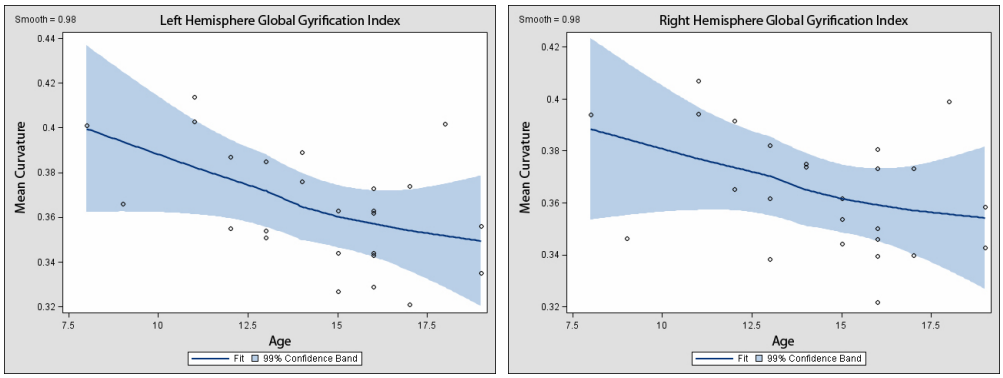


Figure 3 – *Localized Regression Curves Demonstrating Age-Related Differences in Global Gyrification Indices in the Left and Right Hemispheres during Adolescence. Images acquired using SAS (Cary, NC).*

While there have been a number of studies that evaluate gyrification in developmental and psychiatric disorders in children and adolescents (52, 74-76), the only study to date that has evaluated surface complexity associated with typical development found increased cortical complexity in prefrontal regions between the ages of six to sixteen years (77). Thus, there appears to be increased cortical molding as children progress into mid-adolescence in regions that have been both functionally and structurally associated with more protracted brain maturation (70, 73, 78-81).

We have preliminary work (Su et al., submitted) that demonstrates a gradual decrease in the amount of cortical complexity during adolescence in both the right and left hemispheres (Figure 3). We speculate that as synaptic pruning and dendritic arborization proceeds, there are resulting alterations in the surface morphology of the brain. We are currently exploring how these differences in global GI relate to the underlying curvature, thickness, and depth of the sulci (Su et al., in progress) as these will likely reflect age-related differences in underlying brain connectivity.

Alterations in brain connectivity associated with development would expect to have functional outcomes. Thus, exploring the relationship between the developmental trajectories of regional brain gyrification and performance on specific neuropsychological tasks may shed light on the functional consequences associated with gyrification. In addition, coupling functional connectivity measures using high-resolution functional MRI (i.e. spin-echo techniques at high field) with measures of gyrification will also provide information on this relationship. Finally, little is known if changes in the surface morphology take place associated with

other neurodevelopmental changes during this age range, such as myelination. With higher resolution structural and diffusion tensor imaging techniques, it may be possible to assess these relationships, keeping in mind that although items may have similar developmental trajectories, this does not necessarily mean that they directly influence each other.

Gender Differences in Gyrification

There are mixed studies regarding gender differences in gyrification, with a postmortem study of GI finding no differences (10), and an MR study showing that females have greater cortical complexity (57). Since females have on average smaller brain volumes (82), the greater cortical complexity, or gyrification, may produce a brain with equal functional abilities (57). More work in this area is needed.

Discussion

Phylogeny of the human nervous system has resulted in a highly complex brain that is associated with a high degree of cortical folding. Humans, dolphins, and porpoises stand apart from other species in having a disproportionately large cerebral cortex-to-body size ratio (83). The cortical folding increases the surface area of the cortical gray matter and enhances the overall compactness of the brain. While an increase in cortical gray matter could also be achieved by increasing cortical thickness, it turns out that such morphology is ill fated. As an example, a slightly larger lisencephalic, or smoothed surfaced human brain is theoretically possible by increasing the cortical thickness three fold, from approximately 5 to 15 mm. This increase in cortical thickness would preserve the total volume of cortical gray matter, however, computational models have shown that the necessary packing of connections within the cortex would preclude such an evolutionary change (41, 42).

Since each neuron has, on average, more than a thousand connections with other neurons (43), the volume of space required to allow for these connections grows exponentially with the number of neurons. The result would be highly congested and inefficient neuronal pathways, with some neurons taking circuitous paths in order to reach their final destinations (44). Thus, the development of gyral and sulcal folds allows for an optimized compaction of neuronal fibers with an efficient transit time for neuronal signaling. The parcellation of brain tissue into the computationally powerful cortical layers and efficient signal transmission through myelinated fibers, coupled with a compacted gyrification pattern, have resulted in an efficient wiring and volume arrangement for the very dense connectivity that exists within the human brain (41, 42, 84).

The 'form fitting function' design raises the question as to whether alterations in function also result in alterations of form, mediated by neural connectivity. Neurons within the sulci tend to orient horizontal to the cortical surface, due partially to U-fibers that connect gyri via the sulci (1). Thus, synaptic pruning, or a release of tension that occurs along these neuronal fibers could

potentially create a broadening or widening of the sulci (52). Alternatively, the neurons within the gyri tend to be more numerous and lie on average more tangential to the cortical surface (1, 36-40). Alternatively, releasing tension in these fibers could cause the gyri to develop greater curvature, or to become more peaked. Interestingly, the sulcal and gyral brain regions that demonstrated the greatest differences in surface morphology in adolescents with schizophrenia, also were those regions that had the greatest decrease in cortical thickness (52). These gyrification abnormalities may be related to the pronounced cognitive deficits seen in adolescents with schizophrenia (85).

Finally, there is a direct relationship between disorders of neuronal migration (i.e., lissencephaly and polymicrogyri) and aberrant neuronal connectivity (27). These disorders of neuronal migration have profound effects on the gyral and sulcal patterns in the brain and are associated with significant cognitive deficits. The timing of the pathology for these developmental disorders occurs before 24 weeks gestational age, a time when neuronal migration is laying the foundation for gyrification (86). These disorders support the connection between altered form and altered function in the human brain.

While the tension based morphogenesis theory has been gaining recent support (37, 39), other theories also exist. Alternate theories include those that involve differential growth or gyrogenesis (46), mechanical factors, such as abutting cortical plates (40), or a combination of the two (87). There are several important aspects to consider when evaluating the different theories of gyrification. Primarily, while there is variability in the spatial location between gyri and sucli between individuals, there are actually more similarities than differences. The primary gyri and sulci are readily identified in different brains, and although they may have some differences in shape and contours, they have a common pattern (88). In addition, the primary and secondary sulci are under greater genetic control than the tertiary sulci (67), and twin brains have significantly greater similarities in their surface morphology (58, 66). Thus early stages of gyrification involve genetically-mediated processes intrinsic to growth (86), not dependent on external forces of the skull, (47), and are intrinsic to the development of the cortex and thus not dependent on connections with subcortical structures (1, 47).

In summary, during the third trimester of fetal life, the brain evolves from a relatively smooth surfaced structure to a morphology of 'fissures and folds' that resembles the adult human brain (1). The mechanisms behind the process of gyrification are largely unknown, although one recent hypothesis links brain connectivity with gyrification (39). This hypothesis postulates that regions with greater neural connectivity are associated with greater tension that allows these brain regions to remain in closer proximity during brain growth, thus forming gyri. Alterations in connections, such as that which occurs during synaptic pruning and dendritic arborization, could conceivably also alter the morphology of the gyri and sulci.

Our current work demonstrates evidence that such differences in brain surface morphology are occurring during adolescence and we suspect that these morphological differences relate to the underlying connections in the developing

brain. Adolescence is a time of particular interest, as higher-order cognitive functions are continuing to develop (79, 80, 89) and brain structure continues to mature into early adulthood (13, 90). As these changes take place, it is possible that measures of gyrification may provide more localized measures of changes in the underlying connectivity. Identifying local changes or differences in connectivity will assist in pinpointing structure/function relationships associated with typical adolescents development as well as changes associated with emerging psychiatric disorders. The latter is of particular interest, since adolescence and early adulthood is a period where the incidence of several major psychiatric disorders, including schizophrenia, major depressive disorder, and bipolar affective disorder, dramatically increases.

Future work should include longitudinal studies of children, adolescents, and young adults to measure the trajectory of gyrification patterns associated with development. Coupling these studies with trajectories of neuropsychological development may help us understand the functional relationship with these tasks and the regional changes in gyrification. In addition, longitudinal studies in high-risk populations will allow for the detection of the timing and location of regional changes in gyrification. Coupling high-resolution structural imaging with other imaging techniques, such as diffusion tensor imaging, functional MRI, or optical imaging will provide important information on local and regional connectivity associated with the developmental trajectory of surface morphology. While to date, the study of gyrification has received less attention than other aspects of neuroscience, it is quite likely that interesting secrets of neurodevelopment are hidden within the processes involved in the 'fissuring and folding' of the human brain.

References

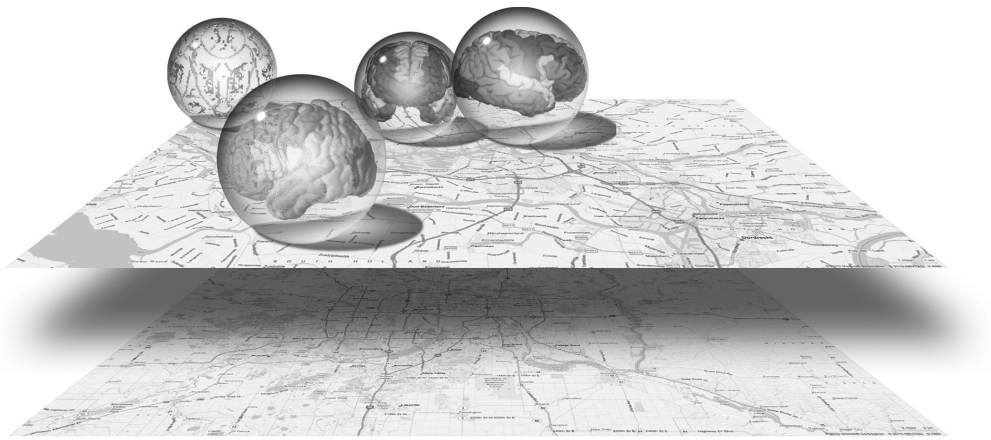
1. Welker W (1990): Why does cerebral cortex fissure and fold. In: Jones EG, Peters A, editors. Cerebral Cortex. New York: Plenum Press, pp 3-136.
2. Zilles K, Armstrong E, Moser KH, Schleicher A, Stephan H (1989): Gyrification in the cerebral cortex of primates. Brain Behav Evol. 34:143-150.
3. Macphail E (1982): Brain and Intelligence in Vertebrates. Oxford, England: Clarendon Press.
4. Ridgway S, Houser D, Finneran J, Carder D, Keogh M, Van Bonn W, et al. (2006): Functional imaging of dolphin brain metabolism and blood flow. J Exp Biol. 209:2902-2910.
5. Chi JG, Dooling EC, Gilles FH (1977): Gyral development of the human brain. Ann Neurol. 1:86-93.
6. Zilles K, Schleicher A, Langemann C, Amunts K, Morosan P, Palomero-Gallagher N, et al. (1997): Quantitative analysis of sulci in the human cerebral cortex: Development, regional heterogeneity, gender difference, asymmetry, intersubject variability, and cortical architecture. Hum Brain Mapp. 5:218-221.
7. Armstrong E, Schleicher A, Omran H, Curtis M, Zilles K (1995): The ontogeny of human gyrification. Cereb Cortex. 5:56-63.
8. Retzius A (1891): Ueber den Bau der Oberflachenschicht der Grosshirnrinde beim Menschen und bei den Saugethieren. Verh Biol Ver. 3:90-103.
9. Naidich TP, Grant JL, Altman N, Zimmerman RA, Birchansky SB, Braffman B, et al. (1994): The developing cerebral surface. Preliminary report on the patterns of sulcal and gyral maturation--anatomy, ultrasound, and magnetic resonance imaging. Neuroimaging Clin N Am. 4:201-240.
10. Zilles K, Armstrong E, Schleicher A, Kretschmann HJ (1988): The human pattern of gyrification in the cerebral cortex. Anat Embryol. 179:173-179.

11. Dareste MC (1862): Sur les rapports de la masse encéphalique avec le développment de l'intelligence. Bull Soc Anthropol. 3:26-54.
12. Dubois J, Benders M, Borradori-Tolsa C, Cachia A, Lazeyras F, Ha-Vinh Leuchter R, et al. (2008): Primary cortical folding in the human newborn: an early marker of later functional development. Brain. 131:2028-2041.
13. Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW (2003): Mapping cortical change across the human life span. Nat Neurosci. 6:309-315.
14. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. (2004): Dynamic mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci U S A. 101:8174-8179.
15. Mangin JF, Riviere D, Cachia A, Duchesnay E, Cointepas Y, Papadopoulos-Orfanos D, et al. (2004): A framework to study the cortical folding patterns. Neuroimage. 23 Suppl 1:S129-138.
16. White (2001): Anatomic and functional variability: The effects of filter size in group fMRI data analysis. NeuroImage. 13:577-588.
17. Talairach J, Tournoux P (1988): Co-planar Stereotactic Atlas of the Human Brain: 3-Dimensional Proportional System: An Approach to Cerebral Imaging. Stuttgart/New York: Thieme Verlag.
18. Rakic P (1988): Specification of cerebral cortical areas. Science. 241:170-176.
19. Rakic P (1995): A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. Trends Neurosci. 18:383-388.
20. Rakic P (2000): Radial unit hypothesis of neocortical expansion. Novartis Found Symp. 228:30-42.
21. Rajkowska G, Goldman-Rakic PS (1995): Cytoarchitectonic definition of prefrontal areas in the normal human cortex: I. Remapping of areas 9 and 46 using quantitative criteria. Cereb Cortex. 5:307-322.
22. Sidman RL, Rakic P (1973): Neuronal migration, with special reference to developing human brain: a review. Brain Res. 62:1-35.
23. Rakic P (1974): Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. Science. 183:425-427.
24. Barondes SH, Alberts BM, Andreasen NC, Bargmann C, Benes F, Goldman-Rakic P, et al. (1997): Workshop on schizophrenia. Proc Natl Acad Sci U S A. 94:1612-1614.
25. Finlay BL, Darlington RB (1995): Linked Regularities in the Development and Evolution of Mammalian Brains. Science. 268:1578-1584.
26. Striedter G (2005): Principles of Brain Evolution. Sinauer.
27. Stewart RM, Richman DP, Caviness VS, Jr. (1975): Lissencephaly and Pachygyria: an architectonic and topographical analysis. Acta Neuropathol (Berl). 31:1-12.
28. Nadarajah B, Alifragis P, Wong R, Parnavelas J (2003): Neuronal migration in the developing cerebral cortex: observations based on real-time imaging. Cereb Cortex. 13:607-611.
29. Kriegstein AR, Noctor SC (2004): Patterns of neuronal migration in the embryonic cortex. Trends Neurosci. 27:392-399.
30. Haydar TF, Kuan CY, Flavell RA, Rakic P (1999): The role of cell death in regulating the size and shape of the mammalian forebrain. Cereb Cortex. 9:621-626.
31. Cowan WM, Fawcett JW, O'Leary DD, Stanfield BB (1984): Regressive events in neurogenesis. Science. 225:1258-1265.
32. Levitt P (2003): Structural and functional maturation of the developing primate brain. J Pediatr. 143:S35-45.
33. White T, Hilgetag CC (2008): Gyrification of the Human Brain. In: Nelson CA, Luciana M, editors. Developmental Cognitive Neuroscience. Cambridge, MA: MIT Press.
34. Hofman MA (1989): On the evolution and geometry of the brain in mammals. Prog Neurobiol. 32:137-158.
35. Chenn A, Walsh CA (2002): Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science. 297:365-369.
36. Toro R, Burnod Y (2005): A morphogenetic model for the development of cortical convolutions. Cereb Cortex. 15:1900-1913.
37. Hilgetag CC, Barbas H (2006): Role of mechanical factors in the morphology of the primate cerebral cortex. PLoS Comput Biol. 2:e22.
38. Hilgetag CC, Barbas H (2005): Developmental mechanics of the primate cerebral cortex. Anat Embryol (Berl). 210:411-417.
39. Van Essen DC (1997): A tension-based theory of morphogenesis and compact wiring in the central nervous system. Nature. 385:313-318.
40. Richman DP, Stewart RM, Hutchinson JW, Caviness VS, Jr. (1975): Mechanical model of brain convolutional development. Science. 189:18-21.

41. Ruppin E, Schwartz EL, Yeshurun Y (1993): Examining the volume efficiency of the cortical architecture in a multi-processor network model. Biol Cybern. 70:89-94.
42. Murre JM, Sturdy DP (1995): The connectivity of the brain: multi-level quantitative analysis. Biol Cybern. 73:529-545.
43. Braitenberg V, Schüz A (1998): Cortex: statistics and geometry of neuronal connectivity. 2nd. ed. Berlin: Springer.
44. Chklovskii DB, Schikorski T, Stevens CF (2002): Wiring optimization in cortical circuits. Neuron. 34:341-347.
45. His W (1874): Unsere Körperform und das physiologische Problem ihrer Entstehung. Leipzig: F. C. W. Vogel.
46. Le Gross Clark WE (1945): Deformation patterns on the cerebral cortex. In: LeGross Clark WE, Medawar PB, editors. Essays on Growth and Form. Oxford: Oxford University PRess, pp 1-23.
47. Barron DH (1950): An experimental analysis of some factors involved in the development of the fissure pattern of the cerebral cortex. J Exp Zool. 113:553-581.
48. Goldman-Rakic PS (1980): Morphological consequences of prenatal injury to the primate brain. Prog Brain Res. 53:1-19.
49. Goldman-Rakic PS, Rakic P (1984): Experimental modification of gyral patterns. In: Geschwind N, Galaburda A, editors. Cerebral dominance. Cambridge, MA: Harvard University Press, pp 179-192.
50. Heidemann SR, Lamoureux P, Buxbaum RE (1995): Cytomechanics of axonal development. Cell Biochem Biophys. 27:135-155.
51. Sporns O, Chialvo DR, Kaiser M, Hilgetag CC (2004): Organization, development and function of complex brain networks. Trends Cogn Sci. 8:418-425.
52. White T, Andreasen NC, Nopoulos P, Magnotta V (2003): Gyrification abnormalities in childhood- and adolescent-onset schizophrenia. Biol Psychiatry. 54:418-426.
53. Magnotta VA, Andreasen NC, Schultz SK, Harris G, Cizadlo T, Heckel D, et al. (1999): Quantitative in vivo measurement of gyrification in the human brain: changes associated with aging. Cereb Cortex. 9:151-160.
54. Armstrong E, Curtis M, Buxhoeveden DP, Fregoe C, Zilles K, Casanova MF, et al. (1991): Cortical gyrification in the rhesus monkey: a test of the mechanical folding hypothesis. Cereb Cortex. 1:426-432.
55. Luders E, Thompson PM, Narr KL, Toga AW, Jancke L, Gaser C (2006): A curvature-based approach to estimate local gyrification on the cortical surface. Neuroimage. 29:1224-1230.
56. Koenderink JJ, van Doorn AJ (1992): Surface shape and curvature scales. Image and Vision Computing. 10:557-564.
57. Luders E, Narr KL, Thompson PM, Rex DE, Jancke L, Steinmetz H, et al. (2004): Gender differences in cortical complexity. Nat Neurosci. 7:799-800.
58. White T, Andreasen NC, Nopoulos P (2002): Brain Volumes and Surface Morphology in Monozygotic Twins. Cereb Cortex. 12:486-493.
59. Lohmann G (1998): Extracting line representations of sulcal and gyral patterns in MR images of the human brain. IEEE Trans Med Imaging. 17:1040-1048.
60. Rettmann ME, Han X, Xu C, Prince JL (2002): Automated sulcal segmentation using watersheds on the cortical surface. Neuroimage. 15:329-344.
61. Kao CY, Hofer M, Sapiro G, Stem J, Rehm K, Rottenberg DA (2007): A geometric method for automatic extraction of sulcal fundi. IEEE Trans Med Imaging. 26:530-540.
62. Schaer M, Cuadra MB, Tamarit L, Lazeyras F, Eliez S, Thiran JP (2008): A surface-based approach to quantify local cortical gyrification. IEEE Trans Med Imaging. 27:161-170.
63. Piao X, Hill RS, Bodell A, Chang BS, Basel-Vanagaite L, Straussberg R, et al. (2004): G protein-coupled receptor-dependent development of human frontal cortex. Science. 303:2033-2036.
64. Thompson PM, Cannon TD, Narr KL, van Erp T, Poutanen VP, Huttunen M, et al. (2001): Genetic influences on brain structure. Nat Neurosci. 4:1253-1258.
65. Chow EW, Zipursky RB, Mikulis DJ, Bassett AS (2002): Structural brain abnormalities in patients with schizophrenia and 22q11 deletion syndrome. Biol Psychiatry. 51:208-215.
66. Bartley AJ, Jones DW, Weinberger DR (1997): Genetic variability of human brain size and cortical gyral patterns. Brain. 120:257-269.
67. Lohmann G, von Cramon DY, Steinmetz H (1999): Sulcal variability of twins. Cereb Cortex. 9:754-763.
68. Peters A, Morrison JH, Rosene DL, Hyman BT (1998): Feature article: are neurons lost from the primate cerebral cortex during normal aging? Cereb Cortex. 8:295-300.

69. Huttenlocher PR (1979): Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res. 163:195-205.
70. Huttenlocher PR, Dabholkar AS (1997): Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol. 387:167-178.
71. Huttenlocher PR, de Courten C (1987): The development of synapses in striate cortex of man. Hum Neurobiol. 6:1-9.
72. Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, Toga AW (2004): Longitudinal mapping of cortical thickness and brain growth in normal children. J Neurosci. 24:8223-8231.
73. Sowell ER, Trauner DA, Gamst A, Jernigan TL (2002): Development of cortical and subcortical brain structures in childhood and adolescence: a structural MRI study. Dev Med Child Neurol. 44:4-16.
74. Thompson PM, Lee AD, Dutton RA, Geaga JA, Hayashi KM, Eckert MA, et al. (2005): Abnormal cortical complexity and thickness profiles mapped in Williams syndrome. J Neurosci. 25:4146-4158.
75. Bearden CE, van Erp TG, Dutton RA, Lee AD, Simon TJ, Cannon TD, et al. (2009): Alterations in midline cortical thickness and gyrification patterns mapped in children with 22q11.2 deletions. Cereb Cortex. 19:115-126.
76. Kesler SR, Vohr B, Schneider KC, Katz KH, Makuch RW, Reiss AL, et al. (2006): Increased temporal lobe gyrification in preterm children. Neuropsychologia. 44:445-453.
77. Blanton RE, Levitt JG, Thompson PM, Narr KL, Capetillo-Cunliffe L, Nobel A, et al. (2001): Mapping cortical asymmetry and complexity patterns in normal children. Psychiatry Res. 107:29-43.
78. Conklin HM, Curtis CE, Katsanis J, Iacono WG (2000): Verbal working memory impairment in schizophrenia patients and their first-degree relatives: evidence from the digit span task. Am J Psychiatry. 157:275-277.
79. Karatekin C, Marcus DJ, White T (2007): Oculomotor and manual indexes of incidental and intentional spatial sequence learning during middle childhood and adolescence. J Exp Child Psychol. 96:107-130.
80. Luciana M, Nelson CA (2000): Neurodevelopmental assessment of cognitive function using CANTAB: Validation and future goals. In: Ernst, Rumsey, editors. Functional Neuroimaging in Child Psychiatry. Cambridge: Cambridge University Press, pp 379-397.
81. Huttenlocher PR, De Courten C, Garey LJ, van der Loos H (1982): Synaptic development in human cerebral cortex. Int J Neurol. 17:144-154.
82. Nopoulos P, Flaum M, O'Leary D, Andreasen NC (2000): Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. Psychiatry Res. 98:1-13.
83. Allmann J (2000): Evolving brains. W H Freeman & Co.
84. Wen Q, Chklovskii DB (2005): Segregation of the brain into gray and white matter: a design minimizing conduction delays. PLoS Comput Biol. 1:e78.
85. White T, Ho BC, Ward J, O'Leary D, Andreasen NC (2006): Neuropsychological performance in first-episode adolescents with schizophrenia: a comparison with first-episode adults and adolescent control subjects. Biol Psychiatry. 60:463-471.
86. Neal J, Takahashi M, Silva M, Tiao G, Walsh CA, Sheen VL (2007): Insights into the gyrification of developing ferret brain by magnetic resonance imaging. J Anat. 210:66-77.
87. Todd PH (1982): A geometric model for the cortical folding pattern of simple folded brains. J Theor Biol. 97:529-538.
88. Thompson PM, Schwartz C, Lin RT, Khan AA, Toga AW (1996): Three-dimensional statistical analysis of sulcal variability in the human brain. J Neurosci. 16:4261-4274.
89. Conklin HM, Luciana M, Hooper CJ, Yarger RS (2007): Working memory performance in typically developing children and adolescents: behavioral evidence of protracted frontal lobe development. Dev Neuropsychol. 31:103-128.
90. Giedd JN (2004): Structural magnetic resonance imaging of the adolescent brain. Ann N Y Acad Sci. 1021:77-85.

3.2 Gyrification Abnormalities in Childhood- and Adolescent-onset Schizophrenia



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Biological Psychiatry (2003) 54: 418-426

Abstract

Background: Gyrification is an important index of brain development. We used magnetic resonance scanning technology to compare brain surface morphology and measures of gyrification in children and adolescents with a schizophrenia spectrum disorder and in age-equivalent healthy controls.

Methods: Magnetic resonance scans were obtained from 42 patients and 24 healthy controls, mean age 17.7 years for both groups. We employed novel quantitative measures of brain morphology, including cortical thickness and a variety of indices of sulcal and gyral curvature. We examined these measures in the whole brain and in the frontal, temporal, parietal, and occipital lobes.

Results: There were significant decreases in cortical thickness in the patients. This was most pronounced in the cortical tissue that underlies the sulci. The patient group had significantly more flattened curvature in the sulci and more steeped or peaked curvature in the gyri.

Conclusions: This study quantitatively examines cortical thickness and surface morphology in children and adolescents with schizophrenia. Patients with schizophrenia demonstrated patterns of brain morphology that were distinctly different from healthy controls. In light of current theories of the formation of gyri and sulci, these changes may reflect aberrations in cerebral and subcortical connectivity.

Introduction

The fact that the typical age of onset for schizophrenia is in the late teens and early 20s may provide important clues as to its pathophysiology and etiology. A relatively broad consensus has emerged that schizophrenia arises at least in part due to abnormalities in brain development [Lewis and Levitt 2002 and Woods 1998]. Brain development and maturation occur as a consequence of orderly processes that begin in utero and continue into the early 20s or later: neuronal differentiation, neuronal migration, axon formation and dendritic proliferation, synaptogenesis, myelination, pruning, apoptosis, and activity-dependent changes [Huttenlocher 1979, Huttenlocher and de Courten 1987, Huttenlocher et al 1982, Lombroso 1998, Naegele and Lombroso 1998, Sidman and Rakic 1973 and Yakovlev and Lecours 1967]. The consequences of these processes can be partially tracked in vivo through structural imaging, using measures of cortical thickness, gray matter (GM) and white matter (WM) volume, and gyrification patterns and indices [Carman et al 1995, Magnotta et al 1999b, Sisodiya et al 1996. S. Sisodiya, S. Free, D. Fish and S. Shorvon, MRI-based surface area estimates in the normal adult human brain: Evidence for structural organisation. J Anat 188 (1996), pp. 425-438. View Record in Scopus (13)Sisodiya et al 1996, Sisodiya and Free 1997, Van Essen 1997 and Van Essen and Drury 1997]. Disease-related abnormalities may also be tracked using such measures, as well as via the observation of an increased rate of neurodevelopmental anomalies such as ectopic gray matter or dysgenesis or agenesis of structures or regions [Nopoulos et al 1995, Nopoulos et al 1996, Swayze et al 1990 and Swayze et al 1997].

Evidence suggesting that schizophrenia is primarily a neurodevelopmental disorder comes from multiple sources. An increased rate of neurodevelopmental abnormalities (e.g., gray matter heterotopias) provides relatively direct evidence. More indirect evidence includes studies of the time window before onset that have identified the presence of premorbid indicators of dysfunction before the onset of the full syndrome [Albee et al 1964, Done et al 1994, Friedman et al 1999, Fuller et al 2002, Goldfarb 1967, Jones et al 1994, Murray et al 1992, Walker and Lewine 1990 and Walker et al 1994]. The presence of structural and functional brain abnormalities in first episode patients at the time of index evaluation also supports a primary neurodevelopmental disorder in schizophrenia [Andreasen 1997, Bogerts et al 1990, DeLisi et al 1990, DeLisi et al 1991, DeLisi et al 1994, James et al 2002, Lieberman et al 1992, Lieberman et al 1993, Nopoulos et al 1995, Schulz et al 1982 and Schulz et al 1983a]. This indirect evidence is based on the assumption that the measurable abnormalities have probably been present for some time before onset of the illness.

Imaging tools have been used to study brain structure and function since the 1980s, beginning with a report of increased ventricular size in schizophrenia using computerized tomography (CT) scanning [Johnstone et al 1976], a finding that has subsequently been consistently replicated with both CT and magnetic resonance (MR) [Andreasen et al 1982a, Andreasen et al 1982b, Andreasen et al 1990, DeLisi et al 1983, Flaum et al 1990, James et al 1999, Nasrallah et al 1986,

Pfefferbaum et al 1988, Rapoport et al 1997, Reiss et al 1983, Reveley 1985, Schulz et al 1982 and Schulz et al 1983b]. Several years after the initial description of ventricular enlargement in adults, the finding was replicated in an adolescent population using CT [Schulz et al 1982 and Schulz et al 1983a] and was also relatively recently replicated in a childhood-onset schizophrenia (COS) population [Frazier et al 1996 and Rapoport et al 1997] using MR; however, the flexibility and power of MR imaging now permits scientists to address many additional questions about the types of brain abnormalities that occur in schizophrenia, which can potentially illuminate the mechanisms of the illness. For example, studies have also reported a decrease in total brain tissue volume [Frazier et al 1996 and Rapoport et al 1997] and GM in the frontal, temporal, parietal, and occipital lobes [Rapoport et al 1999], decreased size of the cerebellar vermis, and a decrease in thalamic size [Dasari et al 1999, Frazier et al 1996 and Rapoport et al 1997]. A recent longitudinal study of a small sample of COS patients suggests that the cortical abnormalities may shift and progress over time, with a movement from parietal to frontal regions [Thompson et al 2001]. Integrating these varied findings in the context of a changing and developing child and adolescent brain presents conceptual challenges.

To address some of these challenges, we have recently developed a group of automated and well-validated quantitative measures that permit tracking of cortical development. In particular, we have developed measures of cortical depth and surface features such as indices of sulcal and gyral curvature [Magnotta et al 1999a and Magnotta et al 2000]. Such gyrification measures are promising for yielding information about neurodevelopmental mechanisms. These methods can be used to explore the mechanisms that drive brain maturation and specifically gyrification, raising the hope that they may eventually be used to more precisely identify the time of onset of neural abnormalities. In this report, using these novel measures of gyrification, we evaluate brain morphology in a large group of children and adolescents with schizophrenia and a sample of healthy controls.

Methods and materials

Subjects

The patient group consisted of 42 subjects between the ages of 12 and 19 with a schizophrenia spectrum disorder. Exclusion criteria for the patient group consisted of severe head injury resulting in loss of consciousness; neurologic disorders such as epilepsy, tuberous sclerosis, or cerebral palsy; and intelligence quotient (IQ) less than 70. Intelligence quotient was screened using the National Adult Reading Test (NART) for both the patient and control groups. Each subject was evaluated using a structured interview, the Comprehensive Assessment of Symptoms and History (CASH) [Andreasen et al 1992b], which has well-documented reliability. In addition, each patient participated in an interview that yielded a consensus diagnosis. This interview was performed or observed by at least two clinicians with experience with children and adolescents. Thirty-three

subjects had a DSM-IV diagnosis of schizophrenia; seven had a diagnosis of schizophreniform disorder; and two had a diagnosis of schizoaffective disorder. Severity of clinical symptoms was assessed using the Scales for the Assessment of Negative (SANS) [Andreasen 1983] and Positive Symptoms (SAPS) [Andreasen 1984]. Global ratings of negative, psychotic, and disorganized symptoms were calculated from the SANS/SAPS [Andreasen et al 1995a]. Twelve patients were neuroleptic naive at intake into the study. Handedness was assessed using the Edinburgh Handedness Inventory [Oldfield 1971]. Their clinical characteristics are summarized in Table 1. The mean age of onset of the illness was 15.7 years with a range spanning from 8 to 19 years.

The control population consisted of 26 healthy controls between the ages of 13 and 19 who were recruited from the community. Exclusion criteria included a positive history of medical, neurologic, or psychiatric illness and IQ less than 70. Individuals with a history of alcohol and substance abuse were also excluded. Demographic information for both the patient and control groups are presented in Table 2. There were no statistical differences between the patient and control groups in age, height, handedness, or either paternal or maternal education level. The patient group had a greater representation of males ($p = .02$), and the control group had attained a higher level of education ($p = .007$).

Table 1. *Clinical Characteristics of the Patients*

Clinical Dimension	Mean (SD)
Age of Onset	15.7 (2.6)
Duration of Illness Prior to Treatment (Months)	24 (28)
SANS / SAPS Measures	
Negative Symptoms	2.83 (0.84)
Positive Symptoms	2.42 (1.42)
Disorganized Symptoms	1.74 (1.01)
Pharmacotherapy	
Duration of Neuroleptic Exposure (Months)	8.6 (12.1)
Number of Neuroleptic Naive Patients	12

SANS/SAPS, Scales for the Assessment of Negative and Positive Symptoms.

Table 2. *Demographics for the Schizophrenia and Healthy Control Subjects*

	Patients n = 42	Controls n = 26
Age (years)	17.7 (1.7)	17.7 (2.0)
Age Range (years)	12-19	13-19
Gender (male / female)	36 / 9	13 / 13
Handedness (right / left / mixed)	41 / 2 / 2	26 / 0 / 0
Subject Education Level (years)	10.7 (1.6)	11.8 (1.7)
Paternal Educational Level (years)	14.0 (3.7)	14.1 (3.0)
Maternal Educational Level (years)	14.0 (2.4)	13.3 (1.8)
Parental Socioeconomic Status	2.96 (0.85)	2.46 (0.51)

Values given as mean (SD).

All subjects were enrolled after the nature of the study was fully explained and informed consent was obtained. In the case of minors, both parental consent and subject assent were obtained. The study was approved by the Institutional Review Board at the University of Iowa.

MR data acquisition and processing

Images were obtained on a 1.5 Tesla GE Signa MR scanner (General Electric Medical Systems, Milwaukee, WI). Three different MR sequences were used for each subject. The longitudinal relaxation time (T1)-weighted spoiled grass sequence was acquired with the following parameters: slice THICKNESS = 1.5 mm, slice NUMBER = 124, echo time (TE) 5 ms, repetition time (TR) 24 ms, flip angle 40°, number of excitations (NEX) 2, field of view (FOV) 26 cm, matrix 256 × 192. The proton density (PD)- and transverse relaxation time (T2)-weighted images were obtained with the following parameters: slice thickness 3.0 or 4.0 mm, TE 36 ms for PD or 96 ms for T2, TR 3000 ms, NEX 1, FOV 26 cm, matrix 256 × 192 with an echo train length = 8. A 4.0-mm T2/PD sequence was used in five patients and six controls in order for the scan to cover the entire brain.

The image processing was performed on a Silicon Graphics workstation (Silicon Graphics, Inc., Mountain View, CA) using the locally developed Brain Research: Analysis of Images, Networks, and Systems (BRAINS; University of Iowa, Iowa City, Iowa) software package [Andreasen et al 1992a, Andreasen et al 1993 and Cohen et al 1992]. The images were initially realigned, resampled, and a

Talairach Atlas was warped to fit the image [Talairach and Tournoux 1988]. Extracranial tissue was removed utilizing edge detection techniques and manual tracing.

Segmentation

Following this step, the pixels representing the GM, WM, and cerebrospinal fluid (CSF) were identified utilizing a multispectral discriminant analysis based segmentation algorithm applied to the three image sequences described above (T1, T2, PD) [Harris et al 1999]. The segmentation algorithm produces both a sharp (discrete) classification and a fuzzy (continuous) classified image. The discrete image set produces only three classes (GM, WM, and CSF), whereas the continuous classifier corrects for partial voluming by assigning an 8-bit number to each voxel (10-70 for CSF, 70-190 for GM, and 190-250 for WM). The continuous classifier identifies voxels that contain "pure" CSF (10), GM (130), and WM (250).

Measures of surface anatomy

A thorough description of the algorithm used to quantify the surface morphology has been described by [Magnotta et al 1999b], and an overview of these methods are provided. The continuous segmented image is used to identify the region of "pure" cortical GM (i.e., the GM corrected for partial voluming). The parametric center of this "pure" cortical GM was calculated, determined by a value of 130, and a triangle-based isosurface was created. The resulting three-dimensional isosurface spanned the brain at the approximate spatial center of the cortical GM. The surface area was calculated as the sum of triangular areas covering this surface of the brain.
Both a sulcal and gyral curvature index is calculated by determining the vector angle normal to each triangle surface compared to neighboring vector angles up to four triangle surfaces away. Convex values (i.e., positive) represent gyri and concave values (i.e., negative) represent sulci. Higher absolute values of curvature reflect "tighter" curvature, whereas lower values represent a more "broader" curve.
Cortical thickness is calculated from vectors that are normal to each triangular surface. Each triangle in the surface is assigned four surface normals, one on each corner and one in the center. The cortical thickness for each triangle is defined as the vector with the minimum distance to the 50% GM and 50% WM regions. Since the triangle isosurface lies at the parametric center of the GM, this cortical thickness is approximately half of the actual cortical depth. Values were therefore doubled to obtain a measure of the actual cortical thickness. The values obtained for cortical thickness were comparable to frozen section samples for the adolescent age range (personal correspondence between Dr. Nancy C. Andreasen and Dr. Karl Zilles). Figure 1 illustrates the cortical surface visualized through this method, as well as its division into sulci and gyri.

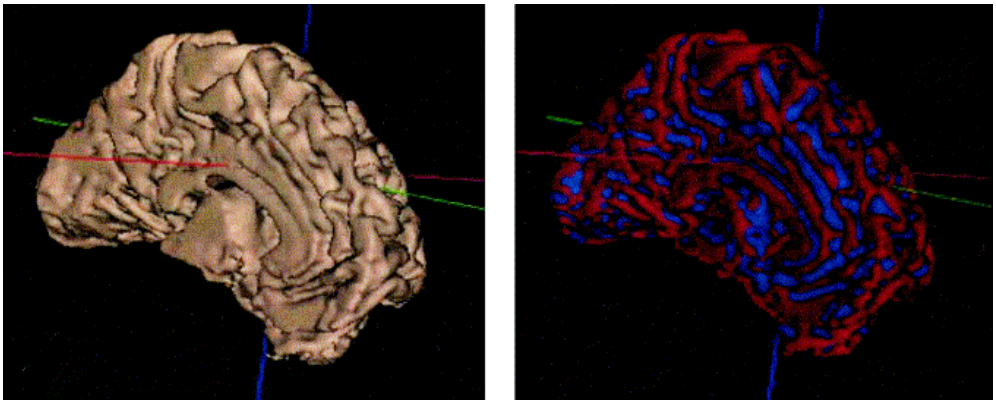


Figure 1. *The figure on the left demonstrates the brain surface at the midpoint of the cortex. The figure on the right is the identical image after calculating and color-coding the curvature of the sulci (blue) and gyri (red). (Figure 3, Back Cover)*

Statistical analysis

Demographic information was analyzed by either a t test for continuous data or ?2 for categorical data. Variables that were grossly nonnormal in their distribution were evaluated utilizing a Wilcoxon rank sum test. Multivariate analysis of covariance (MANCOVA) was used initially to assess group differences. The initial MANCOVA was subsequently followed up with analyses of covariance (ANCOVAs). Since there is no perfect choice of a covariate in brain imaging analyses, two combinations were used. Findings that were consistently significant using both sets of covariates were considered as significant. The two sets of covariates used for these analyses were 1) age and gender, and 2) total brain compartment (TBC) volume (or total intracranial volume), age, and gender.

Results

Surface measures

A MANCOVA of the surface morphology variables was significantly different between patients and controls ($p = .05$). Analyses of covariance with age, gender, and TBC as a covariate demonstrated differences in the measures of both the gyral ($p = .01$) and sulcal ($p = .02$) curvature indices and in cortical thickness ($p = .05$). Data are shown in Table 3. The differences in the cortical thickness and curvature indices remained significant when controlling for age and gender alone. The cortical thickness of the gyral regions (convex regions of the cortex) and the sulcal regions (concave regions of the cortex) were evaluated separately. This analysis demonstrated that the cortical thickness of the sulci was significantly different between patients and controls ($p = .009$), whereas the cortical thickness of the gyri was not different ($p = .10$). Surface area was not different between patients and controls. The pattern of abnormalities observed is shown schematically in Figure 2.

Table 3. Measures of Surface Morphology

Cerebral Surface Measures	Patient	Control	F^a	p
Surface Area (mm^2)	181,165	181,602	.02	*ns*
Cortical Thickness (mm)	4.52	4.90	3.84	.05
Average Gyral Curvature	315.3	311.7	6.59	.01
Cortical thickness of gyri (mm)	5.02	5.42	2.55	*ns*
Average Sulcal Curvature	−284.4	−288.4	5.43	.02
Cortical thickness of sulci (mm)	3.94	4.28	6.33	.009

Regional surface morphology

The surface curvature indices and cortical thickness were evaluated for each of the four cerebral lobes (Table 4). The thickness of the cortex in the sulci was significantly smaller in patients in the frontal (p = .03), parietal (p = .007), and temporal lobes (p = .004), but not in the occipital lobes. The thickness of the cortex in the gyri was reduced only in the temporal lobe of patients (p = .03). The curvature of the sulci was significantly less concave for patients in the frontal (p = .05) and parietal (p = .002) lobes, whereas the gyral curvature was significantly smaller for patients only in the occipital lobe (p = .003). There was no difference in any of the surface morphology measures between neuroleptic naive and nonnaive patients.

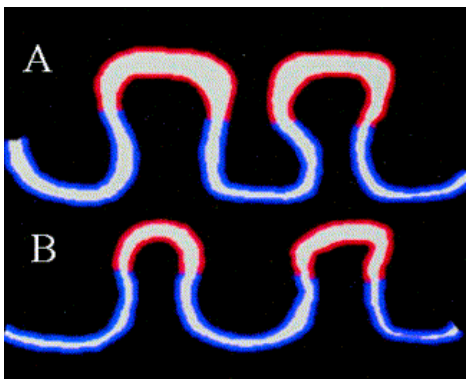


Figure 2. *Differences in the patterns of brain surface morphology between the adolescent patients and controls. The healthy controls (A) demonstrate greater curvature, or an increased concavity, of the sulci (blue) and a more rounded pattern, or smaller convexity, of the gyri (red). The patients (B) have less concavity (more flattening) of the sulci coupled with greater convexity (a more peaked appearance) of the gyri. (Figure 4, Back Cover).*

Discussion

This study utilizes novel measures to quantify the thickness of the cortex and the extent of gyrification in childhood- and adolescent-onset schizophrenia compared to controls. Additionally, the technique is able to separate the gyral and sulcal regions, which have a different histology [Welker 1990]. These young patients demonstrated a variety of general and regional abnormalities when compared to healthy volunteers.

Table 4. *Measures of Lobar Surface Morphology*

	Patient	Control	F^a	p
Frontal Lobe				
Average gyral curvature	296.4	294.2	1.54	*ns*
Cortical thickness of gyri (mm)	5.56	6.00	2.72	*ns*
Average sulcal curvature	−308.9	−312.3	3.96	.05
Cortical thickness of sulci (mm)	3.94	4.24	5.05	.03
Temporal Lobe				
Average gyral curvature	284.5	282.0	2.98	*ns*
Cortical thickness of gyri (mm)	5.14	5.74	5.05	.03
Average sulcal curvature	−298.0	−300.5	1.61	*ns*
Cortical thickness of sulci (mm)	3.80	4.20	8.72	.004
Parietal Lobe				
Average gyral curvature	284.2	279.1	3.20	*ns*
Cortical thickness of gyri (mm)	4.82	5.20	2.39	*ns*
Average sulcal curvature	−318.0	−325.0	10.9	.002
Cortical thickness of sulci (mm)	3.72	4.12	7.94	.007
Occipital Lobe				
Average gyral curvature	268.1	253.9	9.79	.003
Cortical thickness of gyri (mm)	3.16	3.22	.06	*ns*
Average sulcal curvature	−327.5	−329.0	.31	*ns*
Cortical thickness of sulci (mm)	3.76	3.98	2.45	*ns*

First, the patients with schizophrenia had a reduction in the average cortical thickness. Previous neuropathological studies using postmortem tissue have demonstrated a decrease in the cortical thickness without a concomitant loss in neuronal number, interpreted as a result of an increased density of neural fibers secondary to a loss of neuropil because of excessive pruning [Selemon et al 1995]. The present neuroimaging study replicates the postmortem findings of decreased cortical thickness in schizophrenia, using a larger sample, in vivo methods, and a different tool for studying brain anatomy. An interesting additional finding, made possible through the use of in vivo MR, is that the patients showed a greater reduction of cortical thickness in sulci as compared to gyri (Table 3 and Table 4). This finding also replicates work done in a completely separate group of first episode adult patients with schizophrenia, utilizing the same surface-rendering algorithm (Andreasen et al, unpublished data), suggesting that it may define a

critical characteristic of the schizophrenic brain during the early phases of the illness.

The cytoarchitecture of gyri and sulci are considerably different in the normal human brain. The gyri are thicker, as noted in both the patients and controls in the present study, and have a greater density of neuropil, myelinated fiber tracts, and more prominent cell columns [Welker 1990]. The greater spacing of cells within the gyri may buffer the patient/control cytoarchitectural differences, whereas those in the sulci may be more noticeable. Furthermore, there are cytoarchitectural differences in the six layers of the cortex within the sulci and gyri. The superficial cortical layers are thinner and the deeper layers are thicker in the gyri in comparison to the sulci [Welker 1990]. The deeper cortical layers are involved in synaptic connections with the thalamocortical afferents. The thalamocortical afferent fibers arrive at the deeper layers of the cortex during approximately the same developmental window when gyrogenesis begins [Goldman-Rakic and Rakic 1979]. A disruption in these afferents could alter the gyral and sulcal morphology, which may serve as an explanation for the patient/control differences found in the present study. A number of studies have supported aberrant thalamocortical connectivity in schizophrenia [Andreasen et al 1995b and Kim et al 2000].

The mechanisms that lead to the complex gyrification of the mature human brain have been hypothesized in the literature [Armstrong et al 1995, Caviness 1975, Prothero and Sundsten 1984, Van Essen 1997, Welker 1990 and Zilles et al 1989]. Mechanical models of deformation were the earliest theories to account for the gyrification of the brain. These theories are based on the mechanical properties of beams to buckle in regions of unequal thickness ([Welker 1990]); however, the cytoarchitecture is different between the gyri and sulci, and mechanical models do not take into account that gyri require an adequate breadth to allow for the passage of both afferent and efferent neuronal fibers [Prothero and Sundsten 1984 and Welker 1990]. Alternate theories describe differential patterns of growth within the sulci and gyri that account for the fissuration of the cortex.

A tension-based theory is currently perhaps the most widely accepted mechanism of gyrification of the cortex. Within the context of a tension-based theory of morphogenesis of the cerebral cortex, regions that have greater connectivity provide tension that draws them closer together [Van Essen 1997]. This serves to improve efficiency by reducing the time that signals traverse between regions. From the perspective of a tension-based theory of cortical gyrification, the tension within the neural connections serves as the force to induce the warping of the cerebral cortex from the lissencephalic surface that characterizes the fetal brain to the highly gyrified adult brain [Van Essen 1997]. The tensile forces occur in the same direction as the fiber pathways. The histology of the sulci is such that the neural pathways run more tangential to the sulcal surface and form connections with more distant gray matter regions (e.g., frontoparietal connections). This is in contrast to the gyri, which demonstrate more vertical neuronal pathways [Ferrer et al 1986] and form connections with closer regions (e.g., adjacent gyri). Thus, tension within the sulci would tend to be tangential to the cortical surface, whereas the tension would be more vertical in the gyri. Our findings that decreased cortical

thickness occur primarily in the sulci suggest a need to focus future studies on the developmental mechanisms that affect sulcal neuronal connectivity.

During early cortical development, the ventricular system starts relatively large and decreases in proportion to brain development. The process of gyrogenesis can influence the morphology of the lateral ventricles, especially the medial surface of the posterior horn [Welker 1990]. Since enlargement of the ventricular system is a consistently reported abnormality in neurobiological studies of schizophrenia, the ventricular abnormalities may be a neurodevelopmental event that is related to aberrations in the gyrification of the cortex, which in turn is related to aberrant connectivity between regions. The timing of the insult may be hidden within the neurodevelopment of gyrification, occurring in the window of time after the second trimester through the first several years of life.

The patients have abnormalities in measures of both gyral and sulcal curvature (Table 4). The patients have more steeply peaked gyri and more flattened sulci than the controls. These may also reflect developmental differences in the process of gyrification in the two groups. What mechanisms might explain this difference? As in the case of the decreased cortical thickness, an abnormality in pruning is a plausible explanation. Pruning of the connections in the sulci would release tension exerted laterally and result in a broadening or flattening of the sulcal curvature. In addition, the release of tangential connections would affect the tensile forces exerted vertically in U fibers and other connections, leading to the development of tighter curvature. Thus, an aberration in pruning that primarily affects sulci provides a parsimonious explanation for the decrease in cortical thickness and differences in the curvature indices. These findings are consistent with the disconnection theories of schizophrenia that have evolved from functional imaging studies [Andreasen et al 1996 and Friston and Frith 1995].

The gyrification abnormalities observed in this study were not generalized throughout the entire brain but rather affected some regions more significantly. Cortical thickness was reduced in sulci in the frontal, temporal, and parietal lobes. This reduction in sulcal thickness was accompanied by sulcal widening in the parietal and frontal lobes. Furthermore, both gyral and sulcal thickness were decreased in the temporal lobes. These results are consistent with an extensive literature documenting abnormalities in the frontal and temporal lobes in schizophrenia [Andreasen et al 1986, Andreasen et al 1996, Andreasen et al 1997, Barta et al 1997, Benes et al 1991, Breier et al 1992, Buchanan et al 1998, Goldman-Rakic and Selemon 1997, Jacobsen et al 1998, Rajkowska et al 1998 and Weinberger et al 1986]. Our current analysis tools are not specific enough to permit us to examine point-to-point connections that are known to exist between specific cortical regions (e.g., frontoparietal connections), but it seems likely that these more general findings reflect disruptions in regional connectivity.

These results are interesting in light of the recent report of progressive gray matter loss in a small sample of childhood-onset patients who were followed longitudinally [Thompson et al 2001]. In these patients, the gray matter loss appeared initially in the parietal lobes during the index assessment when the subjects were approximately 14 years old. As they were subsequently evaluated

with repeated MR scans over a 5-year period, the gray matter loss moved forward to temporal and frontal regions, finally leading to a pattern affecting all three lobes, as is commonly observed in the late-adolescent or young adult schizophrenic brain. The patients in the present study are comparable in age to those assessed during the late phase of the [Thompson et al 2001] study, and our results are consistent with its findings, in that all three lobes show some type of abnormality.

One weakness of the study is the greater number of female controls, with the potential for a gender bias when comparing patients and controls. Gender differences in surface morphology utilizing these surface rendering methodologies have not been found in both healthy young adults [Magnotta et al 1999b] and adolescents (White et al, unpublished data). In addition, a separate study in adults with first-onset schizophrenia and a gender-matched control group had very similar findings, including a decrease in cortical thickness in the sulci without a difference in the gyri. The curvature measures also demonstrated flattening of the sulci and tighter curvature of the gyri in the adult patients with schizophrenia.

In summary, a large group of adolescent patients with schizophrenia were compared to an age equivalent sample of healthy controls, using newly developed methods to measure cortical surface features. The patients displayed a variety of abnormalities that are consistent with the hypothesis that the illness is a neurodevelopmental disorder that affects regional interconnectivity. This study does not permit us to exactly pinpoint the timing of the developmental injury, but it does indicate that abnormalities are already present during the earliest stages of the illness. Further studies are needed to tease out the specific mechanisms through evaluation of early-onset and high-risk samples using a longitudinal design.

References

Albee et al 1964. Albee G, Lane E, Reuter J (1964): Childhood intelligence of future schizophrenics and neighborhood peers. J Psychology 58:141-144

Andreasen 1983. Andreasen NC (1983): The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, Iowa: The University of Iowa

Andreasen 1984. Andreasen, NC (1984): The Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, Iowa: The University of Iowa

Andreasen 1997. N.C. Andreasen, Linking mind and brain in the study of mental illness: A project for a scientific psychopathology. Science 275 (1997), pp. 1586-1593.

Andreasen et al 1995a. N.C. Andreasen, S. Arndt, D. Miller, M. Flaum and P. Nopolous, Correlational studies of the Scale for the Assessment of Negative Symptoms and the Scale for the Assessment of Positive Symptoms: An overview and update. Psychopathology 28 (1995), pp. 7-17.

Andreasen et al 1993. N.C. Andreasen, T. Cizadlo, G. Harris, V. Swayze, D.S. O'Leary et al., Voxel processing techniques for the antemortem study of neuroanatomy and neuropathology using magnetic resonance imaging. J Neuropsychiatry Clin Neurosci 5 (1993), pp. 121-130.

Andreasen et al 1992a. N.C. Andreasen, G. Cohen, G. Harris, T. Cizadlo, J. Parkkinen, K. Rezai et al., Image processing for the study of brain structure and function: Problems and programs. J Neuropsychiatry Clin Neurosci 4 (1992), pp. 125-133.

Andreasen et al 1992b. N.C. Andreasen, M. Flaum and S. Arndt, The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnoses and psychopathology. Arch Gen Psychiatry 49 (1992), pp. 615-623.

Andreasen et al 1986. N.C. Andreasen, H.A. Nasrallah, V.D. Dunn, S.C. Olson, W.M. Grove, J.C. Ehrhardt et al., Structural abnormalities in the frontal system in schizophrenia. A magnetic resonance imaging study. Arch Gen Psychiatry 43 (1986), pp. 136-144.

Andreasen et al 1996. N.C. Andreasen, D.S. O'Leary, T. Cizadlo, S. Arndt, K. Rezai, L.L.B. Ponto et al., Schizophrenia and cognitive dysmetria: A positron-emission tomography study of dysfunctional prefrontal-thalamic-cerebellar circuitry. Proc Natl Acad Sci U S A 93 (1996), pp. 9985-9990.

Andreasen et al 1997. N.C. Andreasen, D.S. O'Leary, M. Flaum, P. Nopoulos, G.L. Watkins, L.L.B. Ponto et al., Hypofrontality in schizophrenia: Distributed dysfunctional circuits in neuroleptic-naive patients. Lancet 349 (1997), pp. 1730-1734.

Andreasen et al 1982a. N.C. Andreasen, S.A. Olsen, J.W. Dennert and M.R. Smith, Ventricular enlargement in schizophrenia: Relationship to positive and negative symptoms. Am J Psychiatry 139 (1982), pp. 297-302.

Andreasen et al 1982b. N.C. Andreasen, M.R. Smith, C.G. Jacoby, J.W. Dennert and S.A. Olsen, Ventricular enlargement in schizophrenia: Definition and prevalence. Am J Psychiatry 139 (1982), pp. 292-296.

Andreasen et al 1990. N.C. Andreasen, V. Swayze, II, M. Flaum, W.R. Yates, S. Arndt and C. McChesney, Ventricular enlargement in schizophrenia evaluated with computed tomographic scanning. Effects of gender, age, and stage of illness. Arch Gen Psychiatry 47 (1990), pp. 1008-1015.

Andreasen et al 1995b. N.C. Andreasen, V.I.I. Swayze, D.S. O'Leary, P. Nopoulos, T. Cizadlo, G. Harris et al., Abnormalities in midline attentional circuitry in schizophrenia: Evidence from magnetic resonance and positron emission tomography. Eur Neuropsychopharmacol 5 suppl (1995), pp. 37-41.

Armstrong et al 1995. E. Armstrong, A. Schleicher, H. Omran, M. Curtis and K. Zilles, The ontogeny of human gyrification. Cereb Cortex 5 (1995), pp. 56-63.

Barta et al 1997. P.E. Barta, G.D. Pearlson, L.B. Brill, II, R. Royall, I.K. McGilchrist, A.E. Pulver et al., Planum temporale asymmetry reversal in schizophrenia: Replication and relationship to gray matter abnormalities. Am J Psychiatry 154 (1997), pp. 661-667.

Benes et al 1991. F.M. Benes, J. McSparren, E.D. Bird, J.P. SanGiovanni and S.L. Vincent, Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. Arch Gen Psychiatry 48 (1991), pp. 996-1001.

Bogerts et al 1990. B. Bogerts, M. Ashtari, G. Degreef, J.M. Alvir, R.M. Bilder and J.A. Lieberman, Reduced temporal limbic structures volumes on magnetic resonance images in first episode schizophrenia. Psychiatry Res 35 (1990), pp. 1-13.

Breier et al 1992. A. Breier, R.W. Buchanan, A. Elkashef, R.C. Munson, B. Kirkpatrick and F. Gellad, Brain morphology and schizophrenia: A magnetic resonance imaging study of limbic, prefrontal cortex, and caudate structures. Arch Gen Psychiatry 49 (1992), pp. 921-926.

Buchanan et al 1998. R.W. Buchanan, K. Vladar, P.E. Barta and G.D. Pearlson, Structural evaluation of the prefrontal cortex in schizophrenia. Am J Psychiatry 155 (1998), pp. 1049-1055.

Carman et al 1995. G.J. Carman, H.A. Drury and D. Van Essen, Computational methods for reconstructing and unfolding the cerebral cortex. Cereb Cortex 5 (1995), pp. 506-517.

Caviness 1975. V.S. Caviness, Jr, Mechanical model of brain convolutional development. Science 189 (1975), pp. 18-21.

Cohen et al 1992. G. Cohen, N.C. Andreasen, R. Alliger, S. Arndt, J. Kuan, W.T.C. Yuh et al., Segmentation techniques for the classification of brain tissue using magnetic resonance imaging. Psychiatry Res 45 (1992), pp. 33-51.

Dasari et al 1999. M. Dasari, L. Friedman, J. Jesberger, T.A. Stuve, R.L. Findling, T.P. Swales et al., A magnetic resonance imaging study of thalamic area in adolescent patients with either schizophrenia or bipolar disorder as compared to healthy controls. Psychiatry Res 91 (1999), pp. 155-162.

DeLisi et al 1994. L. DeLisi, A. Hoff, C. Neale and M. Kushner, Asymmetries in the superior temporal lobe in male and female first-episode schizophrenia patients: Measures of the planum temporale and superior temporal gyrus by MRI. Schizophr Res 12 (1994), pp. 19-28.

DeLisi et al 1990. DeLisi LE, Gupta SM, Hoff A, Shields A, Schwartz J, Halthore S, et al (1990): Brain morphology in first episode cases of schizophrenia. Schizophr Res 3:20

DeLisi et al 1991. L.E. DeLisi, A.L. Hoff, J.E. Schwartz, G.W. Shields, S.N. Halthore, S.M. Gupta et al., Brain morphology in first-episode schizophrenic-like psychotic patients: A quantitative magnetic resonance imaging study. Biol Psychiatry 29 (1991), pp. 159-175.

DeLisi et al 1983. DeLisi LE, Schwartz CC, Targum SD, Byrnes SM, Spoor EC, Weinberger DR, et al (1983): Ventricular brain enlargement and outcome of acute schizophreniform disorder. Psychiatry Res 9:169-171

Done et al 1994. D.J. Done, T.J. Crow, E.C. Johnstone and A. Sacker, Childhood antecedents of schizophrenia and affective illness: Social adjustment at ages 7 and 11. Br Med J 309 (1994), pp. 699-703.

Ferrer et al 1986. I. Ferrer, I. Fabregues, J. Rairiz and E. Galofre, Decreased numbers of dendritic spines on cortical pyramidal neurons in human chronic alcoholism. Neurosci Lett 69 (1986), pp. 115-119.

Flaum et al 1990. M. Flaum, S. Arndt and N. Andreasen, The role of gender in studies of ventricle enlargement in schizophrenia: A predominantly male effect. Am J Psychiatry 147 (1990), pp. 1327-1332.

Frazier et al 1996. J.A. Frazier, J.N. Giedd, S.D. Hamburger, K.E. Albus, D. Kaysen, A.C. Vaituzis et al., Brain anatomic magnetic resonance imaging in childhood-onset schizophrenia. Arch Gen Psychiatry 53 (1996), pp. 617-624.

Friedman et al 1999. L. Friedman, R.L. Findling, J.T. Kenny, T.P. Swales, T.A. Stuve, J.A. Jesberger et al., An MRI study of adolescent patients with either schizophrenia or bipolar disorder as compared to healthy control subjects. Biol Psychiatry 46 (1999), pp. 78-88.

Friston and Frith 1995. K.J. Friston and C.D. Frith, Schizophrenia: A disconnection syndrome?. Clin Neurosci 3 (1995), pp. 89-97.

Fuller et al 2002. R. Fuller, P. Nopoulos, S. Arndt, D.S. O'Leary, B.C. Ho and N.C. Andreasen, Longitudinal assessment of premorbid cognitive functioning in patients with schizophrenia through examination of standardized scholastic test performance. Am J Psychiatry 159 (2002), pp. 1183-1189.

Goldfarb 1967. Goldfarb W (1967): In: Romano J, editor. The Origins of Schizophrenia. Amsterdam: Excerpta Medica

Goldman-Rakic and Rakic 1979. Goldman-Rakic PS, Rakic P (1979): Experimental modification of gyral patterns. In: Meisami E, Brazier MAB, editors. Neural Growth and Differentiation. New York: Raven Press, 179-192

Goldman-Rakic and Selemon 1997. P.S. Goldman-Rakic and L.D. Selemon, Functional and anatomical aspects of prefrontal pathology in schizophrenia. Schizophr Bull 23 (1997), pp. 437-458.

Harris et al 1999. G. Harris, N.C. Andreasen, T. Cizadlo, J. Bailey, H.J. Bockholt, V. Magnotta et al., Improving tissue classification in MRI: A three-dimensional multispectral discriminant analysis method with automated training class selection. J Comput Assist Tomogr 23 (1999), pp. 144-154.

Huttenlocher 1979. P.R. Huttenlocher, Synaptic density in human frontal cortex-Developmental changes and effects of aging. Brain Res 163 (1979), pp. 195-205.

Huttenlocher and de Courten 1987. P.R. Huttenlocher and C. de Courten, The development of synapses in striate cortex of man. Hum Neurobiol 6 (1987), pp. 1-9.

Huttenlocher et al 1982. P.R. Huttenlocher, C. De Courten, L.J. Garey and H. van der Loos, Synaptic development in human cerebral cortex. Int J Neurol 17 (1982), pp. 144-154.

Jacobsen et al 1998. L.K. Jacobsen, J.N. Giedd, F.X. Castellanos, A.C. Vaituzis, S.D. Hamburger, S. Kumra et al., Progressive reduction of temporal lobe structures in childhood-onset schizophrenia. Am J Psychiatry 155 (1998), pp. 678-685.

James et al 1999. A.C. James, T.J. Crow, S. Renowden, A.M. Wardell, D.M. Smith and P. Anslow, Is the course of brain development in schizophrenia delayed? Evidence from onsets in adolescence. Schizophr Res 40 (1999), pp. 1-10.

James et al 2002. A.C. James, A. Javaloyes, S. James and D.M. Smith, Evidence for non-progressive changes in adolescent-onset schizophrenia: Follow-up magnetic resonance imaging study. Br J Psychiatry 180 (2002), pp. 339-344.

Johnstone et al 1976. E.C. Johnstone, T.J. Crow, C.D. Frith, J. Husband and L. Kreel, Cerebral ventricular size and cognitive impairment in chronic schizophrenia. Lancet 2 (1976), pp. 924-926.

Jones et al 1994. P. Jones, B. Rodgers, R. Murray and M. Marmot, Child development risk factors for adult schizophrenia in British 1946 birth cohort. Lancet 344 (1994), pp. 1398-1402.

Kim et al 2000. J.J. Kim, S. Mohamed, N.C. Andreasen, D.S. O'Leary, G.L. Watkins, L.L.B. Ponto et al., Regional neural dysfunctions in chronic schizophrenia studied with positron emission tomography. Am J Psychiatry 157 (2000), pp. 542-548.

Lewis and Levitt 2002. D.A. Lewis and P. Levitt, Schizophrenia as a disorder of neurodevelopment. Annu Rev Neurosci 25 (2002), pp. 409-432.

Lieberman et al 1992. J.A. Lieberman, J.M. Alvir, M. Woerner, G. Degreef, R.M. Bilder, M. Ashtari et al., Prospective study of psychobiology in first-episode schizophrenia at Hillside Hospital. Schizophr Bull 18 (1992), pp. 351-371.

Lieberman et al 1993. J.A. Lieberman, D. Jody, J.M. Alvir, M. Ashtari, D.L. Levy, B. Bogerts et al., Brain morphology dopamine and eye-tracking abnormalities in first-episode schizophrenia. Prevalence and clinical correlates. Arch Gen Psychiatry 50 (1993), pp. 357-368.

Lombroso 1998. P.J. Lombroso, Development of the cerebral cortex: VII. Growth factors: II. J Am Acad Child Adolesc Psychiatry 37 (1998), pp. 674-675.
Magnotta et al 1999a. V. Magnotta, D. Heckel, N.C. Andreasen, T. Cizadlo, P.W. Corson, J. Ehrhardt et al., Measurement of brain structures with artificial neural networks: Two- and three-dimensional applications. Radiology 211 (1999), pp. 781-790.
Magnotta et al 1999b. V.A. Magnotta, N.C. Andreasen, S.K. Schultz, G. Harris, T. Cizadlo, D. Heckel et al., Quantitative in vivo measurement of gyrification in the human brain: Changes associated with aging. Cereb Cortex 9 (1999), pp. 151-160.
Magnotta et al 2000. V.A. Magnotta, S. Gold, N.C. Andreasen, J.C. Ehrhardt and W.T.C. Yuh, Visualization of subthalamic nuclei with cortex attenuated inversion recovery MR imaging. Neuroimage 11 (2000), pp. 341-346.
Murray et al 1992. R. Murray, E. O'Callaghan, D. Castle and S. Lewis, A neurodevelopmental approach to the classification of schizophrenia. Schizophr Bull 18 (1992), pp. 319-332.
Naegele and Lombroso 1998. J. Naegele and P.J. Lombroso, Development of the cerebral cortex: VII. Apoptosis: Neuronal Hari-Kari. J Am Acad Child Adolesc Psychiatry 37 (1998), pp. 890-892.
Nasrallah et al 1986. H.A. Nasrallah, S.C. Olson, M. McCalley-Whitters, S.M. Chapman and C.G. Jacoby, Cerebral ventricular enlargement in schizophrenia. A preliminary follow-up study. Arch Gen Psychiatry 43 (1986), pp. 157-159.
Nopoulos et al 1996. P. Nopoulos, V. Swayze and N.C. Andreasen, Pattern of brain morphology in patients with schizophrenia and large cavum septi pellucidi. J Neuropsychiatry Clin Neurosci 8 (1996), pp. 147-152.
Nopoulos et al 1995. P. Nopoulos, I. Torres, M. Flaum, N.C. Andreasen, J.C. Ehrhardt and W.T. Yuh, Brain morphology in first-episode schizophrenia. Am J Psychiatry 152 (1995), pp. 1721-1723.
Oldfield 1971. R.C. Oldfield, The assessment and analysis of handedness: The Edinburgh Inventory. Neuropsychologia 9 (1971), pp. 97-113.
Pfefferbaum et al 1988. A. Pfefferbaum, R.B. Zipursky, K.O. Lim, L.M. Zatz, S.M. Stahl and T.L. Jernigan, Computed tomographic evidence for generalized sulcal and ventricular enlargement in schizophrenia. Arch Gen Psychiatry 45 (1988), pp. 633-640.
Prothero and Sundsten 1984. J.W. Prothero and J.W. Sundsten, Folding of the cerebral cortex in mammals. A scaling model. Brain Behav Evol 24 (1984), pp. 152-167.
Rajkowska et al 1998. G. Rajkowska, L.D. Selemon and P.S. Goldman-Rakic, Neuronal and glial somal size in the prefrontal cortex: A postmortem morphometric study of schizophrenia and Huntington disease. Arch Gen Psychiatry 55 (1998), pp. 215-224.
Rapoport et al 1997. J.L. Rapoport, J. Giedd, S. Kumra, L. Jacobsen, A. Smith, P. Lee et al., Childhood-onset schizophrenia. Progressive ventricular change during adolescence. Arch Gen Psychiatry 54 (1997), pp. 897-903.
Rapoport et al 1999. J.L. Rapoport, J.N. Giedd, J. Blumenthal, S. Hamburger, N. Jeffries, T. Fernandez et al., Progressive cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. Arch Gen Psychiatry 56 (1999), pp. 649-654.
Reiss et al 1983. D. Reiss, C. Feinstein, D.R. Weinberger, R. King, R.J. Wyatt and D. Brailier, Ventricular enlargement in child psychiatric patients: A controlled study with planimetric measurements. Am J Psychiatry 140 (1983), pp. 453-456.
Reveley 1985. M.A. Reveley, Ventricular enlargement in schizophrenia. The validity of computerised tomographic findings. Br J Psychiatry 147 (1985), pp. 233-240.
Schulz et al 1982. S.C. Schulz, M. Koller, P.R. Kishore, R.M. Hamer and R.O. Friedel, Abnormal scans in young schizophrenics. Psychopharmacol Bull 18 (1982), pp. 163-164.
Schulz et al 1983a. S.C. Schulz, M.M. Koller, P.R. Kishore, R.M. Hamer, J.J. Gehl and R.O. Friedel, Ventricular enlargement in teenage patients with schizophrenia spectrum disorder. Am J Psychiatry 140 (1983), pp. 1592-1595.
Schulz et al 1983b. S.C. Schulz, P. Sinicrope, P. Kishore and R.O. Friedel, Treatment response and ventricular brain enlargement in young schizophrenic patients. Psychopharmacol Bull 19 (1983), pp. 510-512.
Selemon et al 1995. L.D. Selemon, G. Rajkowska and P.S. Goldman-Rakic, Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. Arch Gen Psychiatry 52 (1995), pp. 805-820.
Sidman and Rakic 1973. R.L. Sidman and P. Rakic, Neuronal migration with special reference to developing human brain: A review. Brain Res 62 (1973), pp. 1-35.
Sisodiya et al 1996. S. Sisodiya, S. Free, D. Fish and S. Shorvon, MRI-based surface area estimates in the normal adult human brain: Evidence for structural organisation. J Anat 188 (1996), pp. 425-438.

Sisodiya and Free 1997. S.M. Sisodiya and S.L. Free, Disproportion of cerebral surface areas and volumes in cerebral dysgenesis. MRI-based evidence for connectional abnormalities. Brain 120 (1997), pp. 271-281.

Swayze et al 1990. V. Swayze, II, N. Andreasen, J. Ehrhardt, W. Yuh, R. Alliger and G. Cohen, Developmental abnormalities of the corpus callosum in schizophrenia. Arch Neurol 47 (1990), pp. 805-808.

Swayze et al 1997. V.W. Swayze, II, V.P. Johnson, J.W. Hanson, J. Piven, Y. Sato and N.C. Andreasen, Magnetic resonance imaging of brain anomalies in fetal alcohol syndrome. Pediatrics 99 (1997), pp. 232-240.

Talairach and Tournoux 1988. J. Talairach and P. Tournoux. Co-Planar Stereotaxic Atlas of the Human Brain, Thieme Medical Publishers, New York (1988).

Thompson et al 2001. P.M. Thompson, C. Vidal, J.N. Giedd, P. Gochman, J. Blumenthal, R. Nicolson et al., Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proc Natl Acad Sci U S A 98 (2001), pp. 11650-11655.

Van Essen 1997. D.C. Van Essen, A tension-based theory of morphogenesis and compact wiring in the central nervous system. Nature 385 (1997), pp. 313-318.

Van Essen and Drury 1997. D.C. Van Essen and H.A. Drury, Structural and functional analyses of human cerebral cortex using a surface-based atlas. J Neurosci 17 (1997), pp. 7079-7102.

Walker and Lewine 1990. E. Walker and R.J. Lewine, Prediction of adult-onset schizophrenia from childhood home movies of the patients. Am J Psychiatry 147 (1990), pp. 1052-1056.

Walker et al 1994. E.F. Walker, T. Savoie and D. Davis, Neuromotor precursors of schizophrenia. Schizophr Bull 20 (1994), pp. 441-451.

Weinberger et al 1986. D.R. Weinberger, K.F. Berman and R.F. Zec, Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. Arch Gen Psychiatry 43 (1986), pp. 114-124.

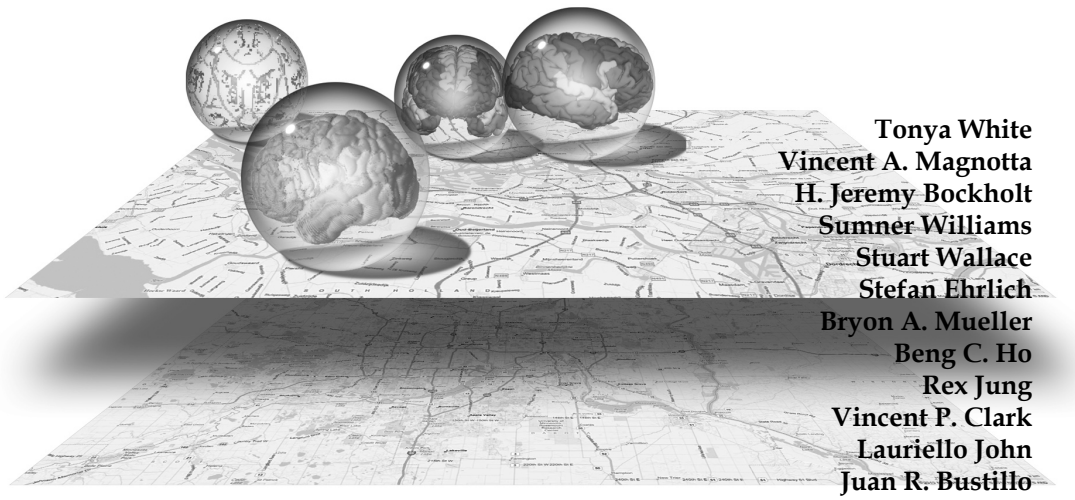
Welker 1990. Welker W (1990): Why does the cerebral cortex fissure and fold? A review of determinants of gyri and sulci. In: Jones EG, Peters A, editors. Cerebral Cortex, vol. 8B. New York: Plenum Press, 3-136

Woods 1998. B.T. Woods, Is schizophrenia a progressive neurodevelopmental disorder? Toward a unitary pathogenetic mechanism. Am J Psychiatry 155 (1998), pp. 1661-1670.

Yakovlev and Lecours 1967. Yakovlev PI, Lecours AR (1967): The myelogenetic cycles of regional maturation of the brain. In: Minowski A, editor. Regional Development of the Brain in Early Life. Oxford, UK: Blackwell, 3-70

Zilles et al 1989. K. Zilles, E. Armstrong, K.H. Moser, A. Schleicher and H. Stephan, Gyrification in the cerebral cortex of primates. Brain Behav Evol 34 (1989), pp. 143-150.

4.1 Global White Matter Abnormalities in Schizophrenia A multisite diffusion tensor imaging study



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Schiz Bull (2009) 35: 204-212

Abstract

***Background*:** Emerging evidence implicates white matter abnormalities in the pathophysiology of schizophrenia. However, there is considerable heterogeneity in the presentation of white matter abnormalities in the existing studies. The object of this study was to evaluate white matter integrity in a large sample of patients with first episode and chronic schizophrenia in comparison to matched control groups. Our goal was to assess whether white matter findings occurred early in the illness or whether these abnormalities developed with the illness over time.

***Methods*:** Participants included 114 patients with schizophrenia (31 first-episode and 83 chronic patients) and 138 matched controls. High-resolution structural and diffusion tensor images were obtained on all participants. Measures of fractional anisotropy were calculated for the four cortical lobes, and the cerebellum and brainstem.

***Results*:** Fractional anisotropy was significant lower in patients versus controls in the whole brain and individually in the frontal, parietal, occipital, and temporal lobes. Fractional anisotropy was not significantly different in the brainstem or cerebellum. Fractional anisotropy differences were significant only in patients with chronic schizophrenia and not in the first-episode group.

***Conclusions*:** We found global differences in the white matter microstructure in patients with chronic, but not first-episode schizophrenia. These findings suggest progressive alterations in white matter microstructure.

Introduction

Evidence from postmortem (1, 2), magnetic resonance spectroscopy (3), and diffusion tensor imaging (DTI) studies (4, 5) implicate brain white matter (WM) abnormalities in the neurobiology of schizophrenia (6). WM forms the basis for high speed communication between brain regions and disruption of WM pathways could potentially partially explain the vast array of clinical and cognitive symptoms associated with the illness.

There have now been more than 60 studies to date that have utilized DTI to evaluate the WM microstructure in patients with schizophrenia (5). One of the challenges in developing a unified understanding of the reported WM abnormalities is the variability in the reported locations of disrupted WM in schizophrenia. A review of DTI studies found an overrepresentation of FA reductions in the corpus callosum (CC), frontal WM, and the cingulum bundle (5). However, diverse abnormalities have also been identified in WM tracts in cortical, subcortical, and cerebellar regions. While the majority of these studies evaluate patients with chronic schizophrenia, studies of first episode (FE) and early-onset schizophrenia (EOS) also have found lower FA in frontal and limbic regions. Finally, there is evidence for a worsening of WM abnormalities as individuals progress from FE to chronic schizophrenia (5, 7), raising the question as to whether duration of illness, medication exposure, cohort effects, or illness-related environmental factors contribute to the greater differences in FA between patients and controls.

The majority of DTI studies utilize voxel-based (VB) techniques to study group differences between patients with schizophrenia and controls. The advantages of VB techniques include the ability to approach the data without a priori assumptions of the implicated regions of interest and greater efficiency of the analysis as compared to region of interest (ROI) analyses. The disadvantages of these techniques include the poor characterization of individual differences in the spatial location of specific brain regions, challenges associated with registration of the brains, and the multiple tests that require statistical correction. VB techniques are valuable and are continually being improved to address these challenges.

Since the majority of the DTI studies of schizophrenia utilize relatively small numbers of patients (n = 20 to 30), the purpose of this study was to utilize a multi-site design to recruit a large number of subjects. The MIND Clinical Imaging Consortium was established as a collaborative multi-site consortium that includes the Massachusetts General Hospital (MGH), the University of Iowa (Iowa), the University of Minnesota (Minn) and the University of New Mexico (NMex) and the Mind Research Network. Our goal was to include FE and chronic patients with schizophrenia, each with an associated matched control group in order to explore aberrant connectivity as a hypothesis in the pathogenesis of schizophrenia. In addition, since schizophrenia is associated with abnormalities that are not localized to any one brain region, our goal was to assess relatively large areas of the brain, including whole brain FA and regional differences within each of the lobes, cerebellum, and the brainstem.

Methods

Participants

The participants consisted of 114 patients with schizophrenia and 138 controls matched by age, sex, and parental education level. Subjects were recruited from four sites: MGH, Iowa, Minn and NMex. All subjects underwent an extensive clinical diagnostic assessment that included either the Structured Clinical Interview for the DSM-IV (SCID) (8) or the Comprehensive Assessment of Symptoms and History (CASH) (9). Both the SCID and CASH utilized DSM-IV-TR criteria for the confirmation of diagnoses. Positive and negative symptoms were rated using the Scale for the Assessment of Positive Symptoms (SAPS) (10) and the Scale for the Assessment of Negative Symptoms (SANS) (11). The consortium study included a total cohort of 385 subjects, however, we report on the subset of those for who had DTI data that could be analyzed. In the image processing methods we indicate the criteria used for exclusion of imaging data. Importantly, the cohort included (65% of the total) did not differ in any systematic way from the total cohort.

Table 1 –*Demographics and Clinical Data of Patients with Schizophrenia and Controls*

	Patients (n = 114)		Controls (n = 138)		
	Chronic (n = 83)	**First-Episode (n = 31)**	**Chronic Controls (n = 95)**	**First-Episode Controls (n = 43)**	**p**
Age (years, SD)	36.4 (11.0)	25.2 (6.7)	34.0 (11.3)	25.2 (6.6)	(ς 1)
Sex (M / F)	62 / 21	22 / 9	57 / 38	24 / 19	(ψ 2)
Hand (R / L / Both)	73 / 3 / 6	26 / 3 / 1	86 / 5 / 3	41 / 2 / 0	
WRAT3RT	46.8 (6.2)	47.1 (6.6)	50.8 (4.5)	50.2 (3.9)	(ς 2)
Father's Education	14.3 (3.3)	13.9 (4.7)	15.0 (3.7)	14.6 (2.3)	
Mother's Education	13.3 (3.6)	14.1 (3.2)	13.8 (2.9)	14.1 (2.3)	

ψ $p < 0.05$
τ $p < 0.01$
ς $p < 0.001$
1 Between chronic and first episode patients
2 Between patients and controls

The clinical group consisted of patients characterized as either chronic or FE schizophrenia. The FE group consisted of patients in their first psychotic episode who had received fewer than 6 months of antipsychotic medications. The sample consisted of 31 FE and 83 chronic patients diagnosed with a schizophrenia spectrum disorder. The demographic information for the patient

and control groups and the clinical characteristics of the patients at each site are provided in Tables 1 and 2, respectively.

Healthy volunteers matched for age, sex, and handedness were recruited

Table 2 – Clinical Characteristics of Patients with First-Episode and Chronic Schizophrenia

Clinical Measures	Chronic Patients		First Episode Patients	
Years of Illness	13.6 (10.8) (n = 83)		2.1 (3.0) (n = 31)	
Dose years Antipsychotics	294.5 (2044.6) CPE (n = 78)		0.64 (1.1) CPE (n = 31)	
Diagnostic Subtypes	**Iowa**	**MGH**	**UMinn**	**NMex**
Paranoid	10	15	19	17
Disorganized	1	3	1	0
Undifferentiated	7	2	3	9
Residual	0	0	0	6
Schizoaffective Disorder	1	1	1	2
Schizophreniform	0	1	3	2
SANS / SAPS Scores				
Positive	4.4 (2.8)		5.6 (2.2)	
Negative	7.2 (3.7)		8.4 (4.1)	
Disorganized	1.5 (1.7)		1.8 (1.9)	

CPE = Chlorpromazine equivalents (A CPE of 100 = 100 mg/day for one year)

from the community through medical clinics and advertisements in local newspapers. The control subjects were excluded if they had a physical or neurological disorder affecting brain function (i.e., head injury, seizure disorder) or a lifetime history of any Axis I psychiatric disorder, including substance abuse or dependence. A diagnosis of schizophrenia or bipolar disorder in a first-degree relative was also exclusionary. Recruitment into the study was performed only after written informed consent was obtained. The study was approved by the institutional review boards at each of the four sites.

Of the 83 patients diagnosed with chronic schizophrenia at the time of scanning, 57 were on atypical antipsychotics only, 8 were on typical antipsychotic only, and 3 were on both an atypical and typical antipsychotic, and 6 were not on medications at the time of scanning. Within the atypical antipsychotic group, 12 were on clozapine only and 5 were on clozapine in addition to another atypical. Twenty-three of those in the first episode group were on atypical antipsychotics and none were on either a typical antipsychotic or clozapine. Antipsychotic dose years (1 dose year = 100 chlorpromazine equivalents per day for one year (12)) for the patient groups are provided in Table 2.

Image Acquisition and Processing

The image acquisition parameters for the high-resolution structural and diffusion tensor images are presented in Table 3. The anatomical images were

Table 3 – High Resolution Structural and Diffusion Tensor Imaging Parameters at each of the Four Sites.

	Iowa	MGH	UMinn	NMex
High Resolution Structural Images				
Scanner	Siemens 1.5T Sonata	Siemens 1.5T Avanto	Siemens 3T TRIO	Siemens 1.5T Avanto
TR (ms)	20	12	2530	12
TE (ms)	6	4.76	3.79	4.76
TI (ms)	n/a	n/a	1100	n/a
Flip Angle (degrees)	20	20	7	20
In Plane Voxel Dimensions (mm)	0.625 x 0.625	0.625 x 0.625	0.625 x 0.625	0.625 x 0.625
Slice Thickness (mm)	1.5	1.5	1.5	1.5
NEX	3	1, acquired 3 identical scans	1, acquired 3 identical scans	1, acquired 3 identical scans
Bandwidth (Hz/pixel)	122	110	181	110
Diffusion Tensor Images				
Scanner	Siemens 3T TRIO	Siemens 1.5 T Sonata	Siemens 3T TRIO	Siemens 1.5 T Sonata
TR (ms)	9,500	8,900	10,500	9800
TE (ms)	90	80	98	86
Voxel Dimensions (mm)	2 x 2 x 2	2 x 2 x 2	2 x 2 x 2	2 x 2 x 2
Diffusion Directions	6	60	12	12
B-Values (s/mm²)	0 / 1,000	0 / 700	0 / 1,000	0 / 1,000
NEX	4	1	2	4
Bandwidth (Hz/pixel)	1,954	1,860	1,342	1,502

analyzed using BRAINS2 (13). This produced a skull stripped T1 weighted image and a white matter mask. The skull stripped T1 weighted image was co-registered with an AC-PC aligned atlas image using a rigid registration to a scaled version of the atlas image to account for linear stretching along each axis. The Talairach parameters were defined for the subject based on an affine registration of the atlas image into the raw subject space allowing the Talairach atlas to be warped onto each subject. The T2 weighted image was then co-registered with the AC-PC aligned T1 weighted image. Tissue classification was performed using a multi-modal tissue classification (14).

The diffusion weighted images were analyzed using the GTRACT program (15). The diffusion weighted images were first co-registered to the B0 image using a mutual-information image registration to correct for motion and distortions caused by eddy currents. The images were median filtered and the diffusion tensor was estimated. Scalar measures for fractional anisotropy (FA) were calculated on the DTI images for all subjects (16).

The B0 image was then co-registered with the skull stripped T1 weighted anatomical image from BRAINS2 using a rigid body transformation and a mutual information metric similarity metric. Next, a B-Spline transform was applied to remove distortion in the echo-planar images resulting from susceptibility changes

at air tissue interfaces (15) . The resulting transforms were applied to the scalar maps placing them into the space of the anatomical image.

Measurements of FA were obtained for the whole brain, cerebellum, and brainstem white matter using the following equation:

$$FA=\sqrt{\frac{3}{2}}\ \frac{\sqrt{(\lambda_1-\bar{D})^2+(\lambda_2-\bar{D})^2+(\lambda_3-\bar{D})^2}}{\sqrt{\lambda_1^2+\lambda_2^2+\lambda_3^2}}$$

Where D is the diffusion tensor and the λ's are the three eigenvalues. In addition, FA measures were obtained for the frontal, temporal, parietal, and occipital lobes as well as lateralized measures for all regions utilizing Talairach parameters (17). The WM mask was defined for each individual as the intersection between the segmented WM obtained from the structural image and FA image with a threshold of 0.1. This combination of structural and DTI images eliminated regions of signal loss resulting from magnetic susceptibility differences. Regional brain measures were obtained using BRAINS2.

Statistical Analyses

The evaluation of differences between patients and controls on demographic measures were initially performed using t-test and χ^2 analyses. Site by demographic differences were evaluated with a one-way ANOVA. Within site demographic variables of patients versus controls were evaluated using χ^2 for discrete data or a one-way ANOVA for continuous data. To assess whether certain structures had greater variability, a coefficient of variability was determined for each brain region. Since there were significant site-related differences in FA, site and age were both used as a covariates in the ANCOVA. FA measures were also converted to z-scores within site and combined to provide measures of effect sizes for the graphical representation of the findings. The evaluation of FA within the brain regions using an ANCOVA with site and age as a covariates was no different from using the z-transformed FA in an ANCOVA with age as a covariate.

Since differences were present in the number of patients and controls within each of the four sites, a secondary analysis was performed using a subset of the data that was closely matched within each site. Matching for this subset was performed using a minimum bipartite matching algorithm which minimizes the sum of edge weights between two sides of a bipartite graph [(18)]. In our case, the two sides were represented by patients and controls and the weights were the age differences between a given patient and control. An independent bipartite graph was also created for each sex at each site, resulting in two matching's per site. Combining these matches results in age and sex matched subjects within each site. This matching algorithm resulted in 103 patients and an equal number of controls in which the above analyses were repeated.

Age-related differences were evaluated utilizing Pearson correlation coefficients. Due to non-linearity in duration of illness and dose years of medication, Spearman rank correlations were used to assess the relationship

between duration of illness, dose years of medication, and FA measures within brain regions. Finally, a localized regression analysis with an optimized smoothing parameter (LOESS procedure in SAS) was performed to obtain qualitative measures of the age related trajectory of FA in both patients and controls. The LOESS procedure has the advantage of being more flexible and allowing for nonlinear registration. All statistical analyses were performed utilizing the SAS statistical package (Cary, NC, USA).

Results

Demographic and Clinical Variables

The mean ages of the patients and controls were 33.3 (SD 11.2) and 31.2 (SD 10.9) years, respectively. There were no differences between the patients and controls in age, handedness, or in the educational level attained by the father or the mother (Table 1). The patient group consisted of 84 males and 30 females (74% male), whereas the control group consisted of 81 males and 57 females (59% male). Thus, the patient group had a higher proportion of males compared to the control group (Chi-Square 6.2, df 1, $p = 0.01$). These sex differences were evaluated by including sex as a covariate in the analyses and by repeating the analyses in a smaller age and sex matched sample.

There was a significant difference between patients and controls on the Wide Range Achievement Test - 3rd Edition, reading test (WRAT3RT) ($t = 5.46$, df = 246, $p < 0.001$). The mean WRAT3RT for patients was 46.9 (SD 6.3) compared to 50.6 (SD 5.0) for the controls. Highest level of education obtained was also significantly different between the patient and control groups ($t = 7.1$, df = 244, $p < 0.0001$). The patients completed on average 13.6 (SD 2.6) years of education compared to 15.7 (SD 2.2) years completed by the controls.

Scores on the SANS and SAPS in the patient group had a mean positive symptom score of 4.7 (SD 2.7), negative symptom of 7.5 (SD 3.8) and disorganized symptoms score of 1.6 (SD 1.8). There were no site differences for the patients on either positive, negative, or disorganized symptoms. The mean number of years since diagnosis and entry into the study was 10.7 (SD 10.7). There were no site-related differences in the log transformed total number of years since diagnosis between sites. The mean number of years since diagnosis for each site is provided in Table 2. To test whether there were site related differences in diagnostic subtypes, we collapsed subtypes to compare with the paranoid subtype. We found no significant site-related differences between the paranoid subtype and the other subtypes of schizophrenia. The diagnostic subtypes for schizophrenia are presented in Table 2. Finally, a one-way ANOVA revealed no site-related differences in total dose years of antipsychotic medications, atypical dose years, or typical dose years.

Site Differences in Measures of Fractional Anisotropy

The mean FA values at the four sites for whole brain, the frontal, temporal, parietal, and occipital lobes, the cerebellum and the brainstem are shown in Figure 1. A one-way ANOVA revealed significant site differences in FA values in all brain regions (F's between 12 and 180). The site differences remained highly significant when controlling for age and sex and thus site was used as a covariate in all analyses. To graphically represent the data, the FA measures were z-transformed within site to preserve the within-site patient control differences, while allowing for a standardization of the scanner platform-dependent differences. This provided for the visual representation of the effect sizes of the pooled data.

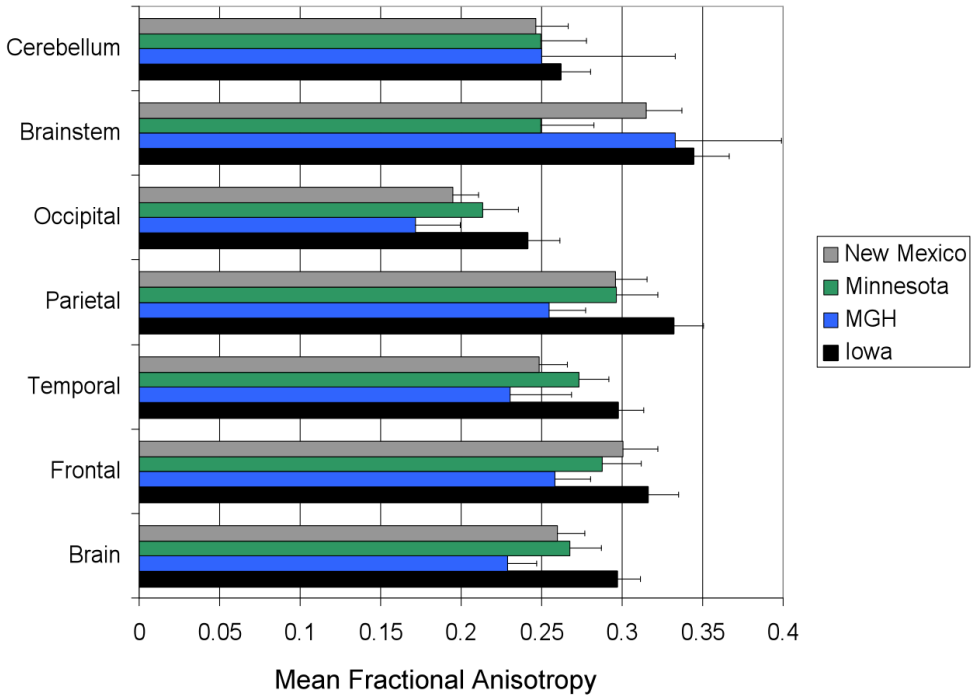


Figure 1 – *Site Related Differences in Mean Fractional Anisotropy Measures within Specific White Matter Brain Regions in Entire Cohort.*

Coefficients of variability (CoV) in FA for each of the brain structures were similar between each of the four sites. The CoV was the lowest for the whole brain measure at Iowa (5.0), MGH (6.7), and NMex (6.5). The lowest CoV at UMinn was the temporal lobe (6.8), with the whole brain close at 7.2. The highest CoV was the occipital lobe for Iowa (7.1) and NMex (8.2); the brain stem at MGH (10.0) and UMinn (13.1).

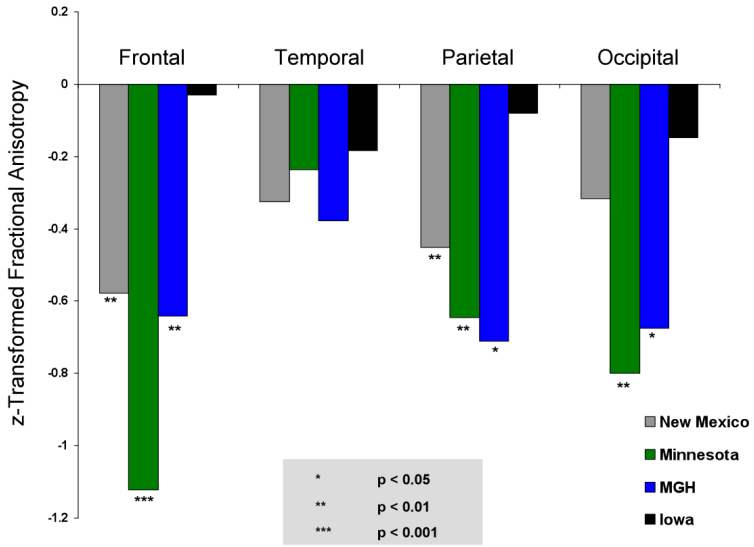


Figure 2 – *Within-Site Differences in Fractional Anisotropy Measures on Cortical White Matter between Patients with Schizophrenia and Controls. The z-transformed data is equivalent to effect sizes for the patient/control differences.*

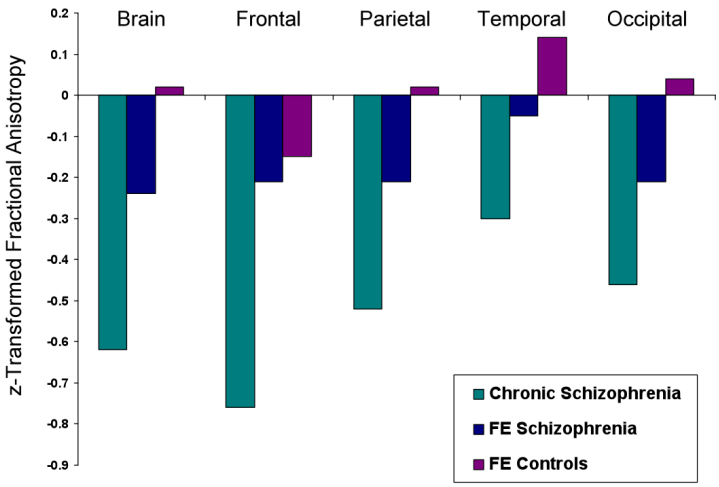


Figure 3 – *Differences in z-Transformed Fractional Anisotropy Measures on Cortical White Matter between FE and Chronic Patients with Schizophrenia and Controls. The z-Score for the chronic control group was set to zero.*

FA Differences between Patients versus Controls

A one-way ANCOVA with site, age, and sex as covariates demonstrated that patients had significantly lower FA in the whole brain ($F_{1,244} = 20.5$, $p < 0.0001$) as well as in the frontal ($F_{1,244} = 23.2$, $p < 0.0001$), parietal ($F_{1,244} = 15.1$, $p < 0.0001$), occipital ($F_{1,244} = 13.7$, $p < 0.0003$), and temporal lobes ($F_{1,244} = 6.9$, $p < 0.009$). FA was not significantly different in either the brainstem ($F_{1,244} = 0.9$, $p = 0.35$) or the cerebellum ($F_{1,244} = 0$, $p = 0.98$). There was a small but significant negative correlation between age and the whole brain FA ($r = -0.28$, $p < 0.0001$), as well as in the frontal ($r = -0.28$, $p < 0.0001$), parietal ($r = -0.29$, $p < 0.0001$), temporal ($r = -0.24$, $p = 0.0001$), and occipital ($r = -0.24$, $p = 0.0002$) lobes. This supports the use of age as a covariate and age effects of FA have been described in the literature (19). Age and FA were not significantly correlated for either the cerebellum ($r = -0.10$) and the brainstem ($r = -0.07$).

To assess whether the regional brain FA differences between patients and controls were present within each site, we performed separate analyses with data within each of the four sites. The effect sizes of the differences between patients and controls for each site are shown in Figure 2. An ANCOVA using age and sex as covariates found significant within site differences at MGH for the whole brain ($F_{1,44} = 5.4$, $p = 0.02$) frontal ($F_{1,44} = 4.5$, $p = 0.04$), parietal ($F_{1,44} = 6.3$, $p = 0.02$), and occipital ($F_{1,44} = 5.2$, $p = 0.03$). Similarly, UMinn had significant differences in the whole brain ($F_{1,45} = 10.3$, $p = 0.002$) frontal ($F_{1,45} = 19.7$, $p < 0.0001$), parietal ($F_{1,45} = 5.3$, $p = 0.03$), and occipital ($F_{1,45} = 8.5$, $p = 0.005$).NMex had significant FA differences in the whole brain ($F_{1,80} = 5.3$, $p = 0.02$) and in the frontal ($F_{1,80} = 8.3$, $p = 0.005$) and parietal ($F_{1,80} = 4.8$, $p = 0.03$) lobes, but not in the occipital lobes. The Iowa site had no significant FA differences between patients and controls in any of the brain regions tested, although the data from Iowa trended in the same direction as from the other sites (Figure 2).

FA Differences in First Episode Patients

A series of ANCOVAs were performed to evaluate differences between the chronic patients, first-episode patients, and their respective controls groups (Tables 4 & 5). FA differences were found in the comparison between the chronic patients and their matched control group (Figure 3). The results paralleled the results seen with the combined FE and chronic comparison. Interestingly, none of the regions that were significant in the chronic patients were found to be significant in a comparison between the FE patient versus FE control groups (Table 4). An ANCOVA comparing the chronic versus FE patients groups found no significant differences between the two groups, although there was a trend for the chronics to have lower FA than the first episode patients ($F_{1,107} = 3.6$, $p = 0.06$).

Paired t-tests were used to evaluate laterality in the measures of FA for each of the brain regions. The right cerebellum had significantly higher mean FA in all four groups; chronic patients ($t = 2.5$, $p = 0.01$), control group matched to the

Table 4 –Measures of Fractional Anisotropy in Chronic and First-Episode Patients and Controls. ANCOVA results used both site and age as covariates.

	Lease Square Mean FA Values				ANCOVA Results			
	Chronic Patients	**Chronic Controls**	**FE Patients**	**FE Controls**	**Combined Patients versus Controls**	**Chronic Patients versus Chronic Controls**	**FE Patients versus FE Controls**	**FE Patients versus Chronic Patients**
Brain	0.257	0.268	0.266	0.267	$F_{1,245} = 20.3$ $p < 0.0001$	$F_{1,171} = 18.7$ $p < 0.0001$	$F_{1,68} = 0.8$ n.s	$F_{1,108} = 0.1$ n.s
Cerebellum	0.249	0.249	0.252	0.246	$F_{1,245} = 0.2$ n.s.	$F_{1,171} = 0.2$ n.s.	$F_{1,68} = 1.5$ n.s	$F_{1,108} = 1.0$ n.s
Brainstem	0.304	0.310	0.316	0.308	$F_{1,245} = 0.4$ n.s	$F_{1,171} = 0.4$ n.s	$F_{1,68} = 1.6$ n.s	$F_{1,108} = 2.4$ n.s
Brain Lobes								
Frontal	0.281	0.298	0.291	0.294	$F_{1,245} = 24.3$ $p < 0.0001$	$F_{1,171} = 27.7$ $p < 0.0001$	$F_{1,68} = 0.3$ n.s	$F_{1,108} = 3.6$ $p = 0.06$
Parietal	0.289	0.300	0.292	0.299	$F_{1,245} = 14.9$ $p < 0.0001$	$F_{1,171} = 13.6$ $p = 0.0003$	$F_{1,68} = 0.7$ n.s	$F_{1,108} = 0.3$ n.s
Temporal	0.258	0.264	0.259	0.263	$F_{1,245} = 5.9$ $p < 0.02$	$F_{1,171} = 5.0$ $p < 0.03$	$F_{1,68} = 0.1$ n.s	$F_{1,108} = 0.1$ n.s
Occipital	0.199	0.208	0.202	0.209	$F_{1,245} = 13.7$ $p = 0.0003$	$F_{1,171} = 11.9$ $p = 0.0007$	$F_{1,68} = 1.2$ n.s	$F_{1,108} = 0.3$ n.s

Table 5 –Measures of Fractional Anisotropy in Chronic and First-Episode Patients and closely Matched Controls. ANCOVA results used both site and age as covariates.

	Lease Square Mean FA Values				ANCOVA Results			
	Chronic Patients	**Chronic Controls**	**FE Patients**	**FE Controls**	**Combined Patients versus Controls**	**Chronic Patients versus Chronic Controls**	**FE Patients versus FE Controls**	**FE Patients versus Chronic Patients**
Brain	0.257	0.270	0.263	0.265	$F_{1,200} = 18.4$ $p < 0.0001$	$F_{1,146} = 20.4$ $p < 0.0001$	$F_{1,48} = 0.5$ n.s	$F_{1,97} = 2.1$ n.s
Cerebellum	0.248	0.251	0.254	0.247	$F_{1,200} = 0$ n.s.	$F_{1,146} = 2.4$ $p < 0.0001$	$F_{1,48} = 1.4$ n.s	$F_{1,97} = 2.1$ n.s
Brainstem	0.303	0.310	0.320	0.311	$F_{1,200} = 0.5$ n.s	$F_{1,146} = 20.4$ $p < 0.0001$	$F_{1,48} = 1.2$ n.s	$F_{1,97} = 2.1$ n.s
Brain Lobes								
Frontal	0.281	0.300	0.293	0.296	$F_{1,200} = 24.9$ $p < 0.0001$	$F_{1,146} = 28.2$ $p < 0.0001$	$F_{1,48} = 0.4$ n.s.	$F_{1,97} = 4.9$ $p < 0.03$
Parietal	0.290	0.301	0.294	0.296	$F_{1,200} = 10.5$ $p = 0.001$	$F_{1,146} = 11.2$ $p = 0.001$	$F_{1,48} = 0.2$ n.s	$F_{1,97} = 0.8$ n.s
Temporal	0.258	0.266	0.262	0.261	$F_{1,200} = 6.4$ $p < 0.01$	$F_{1,146} = 9.0$ $p = 0.003$	$F_{1,48} = 0.1$ n.s	$F_{1,97} = 1.0$ n.s
Occipital	0.200	0.211	0.204	0.206	$F_{1,200} = 12.8$ $p = 0.0004$	$F_{1,146} = 14.7$ $p = 0.0002$	$F_{1,48} = 0.3$ n.s	$F_{1,97} = 0.6$ n.s

Table 6 – Spearman Rank Correlations between FA in Specific Brain Regions and Age, Duration of Illness and Dose Years of Antipsychotic Medications. The sample with n = 93 excludes 21 patients with gaps in their medication histories.

	Age n = 114	Duration of Illness n = 113	Duration of Illness (Adjusting for Dose Years) n = 113	Dose Years of Medication n = 113	Dose Years of Medication (Adjusting for Duration of Illness) n = 113
Brain	r = -0.27 p = 0.003	r = -0.15 n.s.	r = 0.02 n.s.	r = -0.23 p = 0.01	r = -0.19 p = 0.05
Cerebellum	r = 0.0 n.s.	r = 0.09 n.s.	r = 0.17 n.s.	r = -0.06 n.s.	r = -0.16 n.s.
Brainstem	r = -0.01 n.s.	r = 0.02 n.s.	r = -0.06 n.s.	r = 0.11 n.s.	r = 0.12 n.s.
Brain Lobes					
Frontal	r = -0.25 p = 0.007	r = -0.19 p = 0.05	r = -0.05 n.s.	r = -0.22 p = 0.02	r = -0.12 n.s.
Parietal	r = -0.25 p = 0.008	r = -0.13 n.s.	r = 0.02 n.s.	r = -0.20 p = 0.04	r = -0.16 n.s.
Temporal	r = -0.23 p = 0.01	r = -0.11 n.s.	r = 0.05 n.s.	r = -0.21 p = 0.03	r = -0.18 p = 0.06
Occipital	r = -0.21 p = 0.03	r = -0.07 n.s.	r = 0.09 n.s.	r = -0.20 p = 0.03	r = -0.21 p = 0.03

chronic patients ($t = 6.0$, $p < 0.0001$), FE patients ($t = 3.8$, $p < 0.0006$), control group matched to the FE patients ($t = 4.8$, $p < 0.0006$). In addition, the FE patients ($t = 2.6$, $p = 0.01$), the FE controls ($t = 3.3$, $p = 0.002$) and the chronic control ($t = 3.9$, $p = 0.0002$) groups had higher FA in the right occipital lobe. The Chronic patient group had a similar finding in the occipital lobe at the trend level ($t = 1.9$, $p = 0.06$). Repeating the ANOVA on lateralized measures resulted in significant differences between patients and controls in all the same brain regions identified using the combined left and right brain regions.

Analyses between the full cohort of patients and controls, and the additional analyses comparing the chronic patients and their matched controls, FE patients and their matched controls, and chronic versus FE patients were also performed in an age and sex matched subset of the larger sample. This sample included 76 patients with chronic schizophrenia and 27 FE patients with an equally matched number of controls corresponding to each group. Analyses performed on this closely matched sample did not differ significantly from the larger sample (Table 5).

Duration of Illness and Medication Effects on FA

The high intercorrelation between age, duration of illness (DOI), and total dose years of antipsychotics (ADY) provides a challenge for teasing apart the contributions toward reduced FA for each of these variables. The Spearman rank correlation between the duration of illness (DOI) and total dose years of antipsychotics (ADY) for the chronic group was 0.69 ($p < 0.0001$). Subject age was highly correlated with both the DOI ($r = 0.80$, $P < 0.0001$) and ADY ($r = 0.59$, $p < 0.0001$).

Overall, correlations between the brain FA measures with age, duration of illness, and dose years of antipsychotic medications were small (Table 6). These analyses were also performed while adjusting for the dose years of antipsychotics and duration of illness, respectively. There was a negative correlation between DOI and frontal lobe FA ($r = -0.19$, $p = 0.05$). Controlling for ADY, there was no long a significant correlation between the frontal lobe FA and DOI. There was a negative correlation between ADY and FA in multiple brain regions, including the whole brain ($r = -0.23$, $p = 0.01$), and in all four lobes (Table 6).

The FE group included 23 patients who were on medication and 8 patients who were not on medications at the time of scanning. A 4 (site) by 2 (naïve/non-naïve) ANCOVA with age as a covariate found no differences between those who were neuroleptic naïve compared to those who were not.

The large number of subjects coupled with the variability in ADY and DOI allowed for an alternative approach to tease apart duration of illness and medication exposure on global and regional brain FA measures. To assess the effects of medications on brain FA measures, we further analyzed the data from a subgroup of 22 patients who had a DOI between 20 and 40 years. The mean age for this group was 46.3 years (SD 5.3), the range of ADY was between 6.4 and 1,023, and the correlation between the DOI and ADY was 0.07. The Spearman rank

correlations were generally low with the cerebellum (r = -0.23) having the highest absolute correlation, which was non-significant.

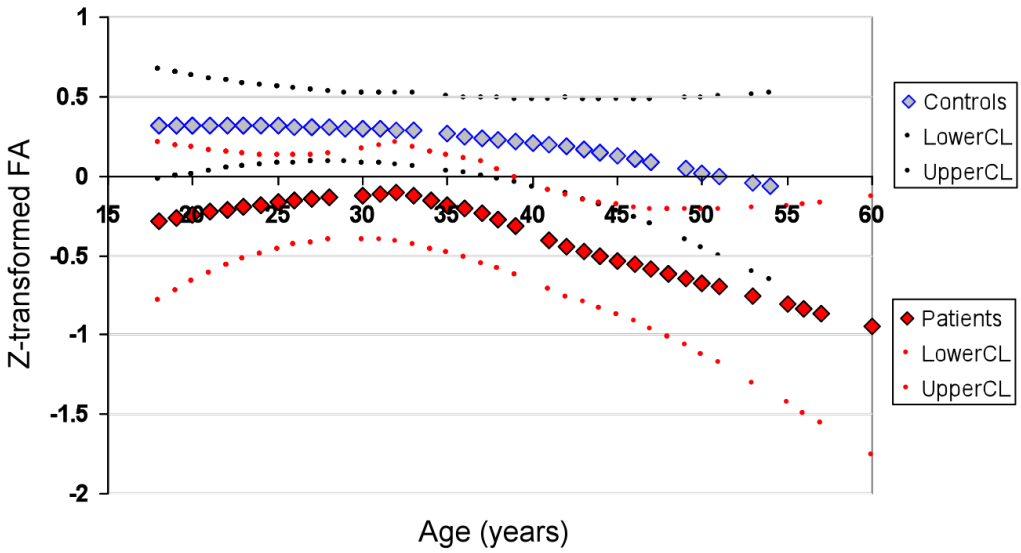


Figure 4 – *Age-Related Trajectories of z-Transformed Fractional Anisotropy Measures in Patients with Chronic Schizophrenia and Controls.*

A subgroup of 45 patients with similar ADY but with considerable variability in DOI was selected for additional analyses. The mean age of this group was 40.1 years (SD 10.0) and the DOI ranged between 0 and 42 years and the ADY was between 17.9 and 145.9. This patient subgroup had a non-significant correlation between the DOI and ADY (r = 0.14, p = 0.36). A Spearman rank correlation resulted in no significant correlations between DOI and FA, although the frontal lobe had the highest negative correlation (r = -0.24, p = 0.10)

Relationship between Clinical Variables and FA

Since there were site-related FA differences in findings between patients and controls, exploratory analyses were performed to evaluate the role of clinical variables. To limit multiple testing, this was performed in a two-stage process. First, either a chi-square for categorical data or an ANOVA for continuous data was performed to assess for site differences in that clinic variable. Those measures that had significant differences in site underwent subsequent analyses to determine if they influenced FA.

A history of alcohol abuse, but not alcohol dependence, had a significant effect of site (χ^2 = 8.2, df = 3, p = 0.04). Iowa and NMex both had lower rates (21% and 17%, respectively) compared to MGH and UMinn, who had rates of 44% and

41%, respectively). A 4 (site) by 2 (alcohol abuse) ANCOVA with age as a covariate found those who did not have a history of alcohol abuse had significantly higher FA in the whole brain ($F_{1,108} = 4.5$, $p = 0.04$), frontal lobe ($F_{1,108} = 5.7$, $p = 0.02$), and parietal lobe ($F_{1,108} = 5.1$, $p = 0.03$).

Both a history of cannabis abuse ($\chi^2 = 10.5$, $df = 3$, $p = 0.02$) and cannabis dependence ($\chi^2 = 15.4$, $df = 3$, $p = 0.001$) had a significant effects of site. For cannabis abuse, once again Iowa and NMex both had lower rates (16% and 23%, respectively) compared to MGH and UMinn, who both had rates of 44%). For cannabis dependence, Iowa had none, whereas NMex had a rate of 10%, MGH had a rate of 41%, and UMinn had a rate of 22%. However, neither cannabis abuse or dependence showed a significant effect on any of the brain measures.

Discussion

We demonstrate global white matter differences in FA in a large sample of patients with schizophrenia. Differences were present in the whole brain, and individually in the frontal, temporal, parietal, and occipital lobes, but were not present in either the brainstem or the cerebellum. The abnormalities were more pronounced in patients with chronic schizophrenia in contrast to the FE patients. The differences in chronic patients also had larger effect sizes compared to the FE patients, lessening the chance that the difference is a result of the larger sample size for the chronic patients. We found that only frontal lobe WM demonstrated a significant difference between the FE and chronic patients.

Disrupted connectivity between brain regions is a potential hypothesis that may explain the global nature and diversity of deficits in schizophrenia (6, 20). Alterations in distributed neural networks, evidenced by abnormalities in WM, could account for the global nature of the clinical, cognitive, and social cognitive symptoms that are a hallmark of the illness. WM disruptions have been supported by the over 60 DTI papers that report aberrant WM microstructure in the pathophysiology of schizophrenia (5). While, there is considerable heterogeneity in the results of these studies, taken together, they provide strong support for WM abnormalities in the pathophysiology of schizophrenia. This is especially notable for pathways involving interhemispheric connections and frontal and limbic WM.

The neuropathological changes responsible for lower FA in patients with schizophrenia are yet unknown. It has been postulated to reflect abnormalities in myelination, oligodendrocytes, neuronal loss, disruption of the integrity of the cell membrane, or alterations in fiber orientation. Perhaps one of the more interesting findings of the current study is the reduced likelihood that altered fiber orientation, such as crossing fibers, would account for the decreases in FA. It is likely that differences as a result of crossing fibers would be equally present in the FE and chronic groups. Although better studied using longitudinal designs, our findings suggest a progressive decrease in FA as patients progress from FE into chronic schizophrenia. Such a progression is also supported in a review of the DTI literature in schizophrenia (5), a recent DTI study comparing FE and chronic patients (7), and is consistent with a number of structural imaging studies (21-25).

Longitudinal MRI studies early in the illness, have documented progression of volume loss in the frontal regions after 2.5 years (26); both cerebral hemispheres after 4 years (22); parietal, frontal, temporal gray matter and hippocampus after 4 years (23, 25); frontal white matter after 3 years (21); and ventricular enlargement after 2.5 years in patients with persistent symptoms (24). It has been proposed that this early brain volume reduction stabilizes in early adulthood (27); however, a recent review suggests that progressive atrophy occurs even later in the illness (28).

What mechanism may account for progressive FA reduction as well as white and gray matter volume losses previously described in adults with schizophrenia? Hypofunction of NMDA receptors in GABAergic interneurons resulting in a paradoxical downstream glutamatergic excitotoxic process has been proposed (29-31) . Consistently, cortical neuropil reductions without neuronal loss or gliosis have been found in postmortem studies (32, 33). Hence, excessive cortical synaptic pruning with neuropil contractions and subsequent axonal microstructural dysfunction may account for the greater FA reductions in chronically-treated compared to the FE patients in our study. However, other possible explanations include a neuroplastic adaptation to psychosocial impoverishment or treatment effects (34).

With the suggestion of a progression of WM changes, we explored underlying factors contributing to these observed group differences. While it was somewhat difficult to tease apart whether lower FA was associated with DOI from the effects of medication, there is evidence that it is more associated with medication effects, although it is difficult to parse out the effects of multicollinear variables. Mori et al. (35) reported an age dependent decline in FA in schizophrenia that was correlated with DOI rather than medication, however the variable of 'current dose of antipsychotic medication' was used and this does not provide information about the cumulative dose of antipsychotic exposure.

Studies tend to lump the different antipsychotics into one or two categories to increase power. However, different atypical antipsychotics will have different effects on the neurochemistry. These neurochemical differences may decrease associations seen between FA and ADY. Overall, we report relatively low correlations between FA measures and duration of illness and total dose years of medications, although the variability in FA in cross-sectional studies likely considerably impacts the correlation. Thus, the question of a progressive decrease in FA and the underlying cause is best addressed in a longitudinal study of FA in schizophrenia.

Interestingly, by including both a FE and chronic sample we were able identify FA differences in the frontal lobe between these two groups. The differences in duration of illness in the current literature may account for some of the heterogeneity of findings. Studies have reported lower FA in the frontal, parietal, temporal, and occipital lobes in patients with schizophrenia (5). The frontal lobe tends to have a larger number of studies identifying abnormalities, although this may be a result of it being a target for a number of region of interest studies. Alternatively, since frontal WM development tends to progress into early

adulthood, greater frontal WM deficits in chronic patients may correspond with a cessation or failure to maintain typical developmental trajectories, rather than WM pathophysiologic changes. Future cross-sectional and longitudinal studies using DTI in schizophrenia will need to consider the stage of the illness and development of the participants.

There are several limitations to the study. We performed a number of different statistical analyses adjusting for multiple comparisons in the initial analysis, but not in the post-hoc tests. Adjusting for multiple comparisons would allow the findings of lower FA in the frontal, parietal, and occipital lobe, but the finding in the temporal lobe in chronic patients would not survive Bonferroni correction. In addition, the finding of lower frontal FA in the chronic compared to the FE patients would also not survive Bonferroni correction. We would expect that random error would affect the patients and controls equally, and we found no brain regions showing higher FA in the controls compared to the patients. Cross-sectional studies of individuals with large age ranges may be susceptible to cohort effects. The patients had a matched control group, which may help reduce some of the cohort effects. The LOESS curve for cortical FA between patients and controls is shown in Figure 4. As can be seen, the trajectory for FA in patients is lower, but parallels the trajectory for FA in controls.

Data collected from multiple sites, using scanners with different field strengths, head coils, and sequence parameters may induce artifacts and increase the variance of the FA measures. While these site related differences cannot be completely removed, we corrected for these differences by using site as a covariate and through the use of relatively large ROIs. In addition, post-processing was performed at one site using the same image processing algorithms. Differences in scanner noise could increase the variance in the measures, resulting in a lowering of the effects sizes and significant findings. The CoV was higher in the occipital lobe and brain stem, thus the higher noise in these smaller structures may have limited smaller effects. Finally, we evaluated differences between patients and controls at each site, and found overlapping patterns in three of the four sites. The differences at the Iowa site may be related to a cohort effect, smaller sample size of patients, a more rural setting, or greater variability due to scanner differences such as obtaining only 6 directions.

Despite these limitations, we report global differences in WM microstructure in patients compared to controls in a large, multi-center study of FE and chronic schizophrenia. Regional FA differences are only observed in the cerebrum, but involve all four lobes. The frontal lobe is the only region that is significantly different between the FE and chronic patient groups, with the lower FA being more pronounced in the chronic cohort. Finally, lower FA appears to be more related to antipsychotic medications, however, due to multicolinearities it is difficult to separate dose years of antipsychotics from duration of illness. Longitudinal studies of WM microstructure will be important to better understand whether these FA differences are a result of the duration of illness, medication, environmental effects, or adaptive processes within the brain as an attempt to

compensate for the illness. Future studies should also assess the functional consequences and prognostic indicators associated with lower cerebral FA.

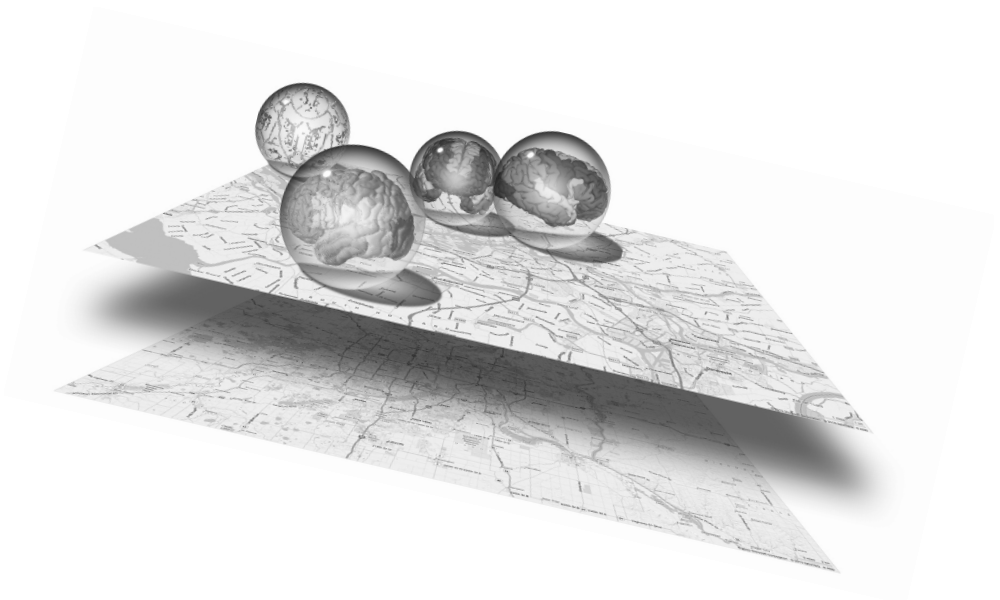
References

1. Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, et al. (2001): Genome-wide expression analysis reveals dysregulation of myelination- related genes in chronic schizophrenia. *Proc Natl Acad Sci U S A*. 98:4746-4751.
2. Heckers S, Heinsen H, Geiger B, Beckmann H (1991): Hippocampal neuron number in schizophrenia. A stereological study. *Arch Gen Psychiatry*. 48:1002-1008.
3. Lim KO, Adalsteinsson E, Spielman D, Sullivan EV, Rosenbloom MJ, Pfefferbaum A (1998): Proton magnetic resonance spectroscopic imaging of cortical gray and white matter in schizophrenia. *Arch Gen Psychiatry*. 55:346-352.
4. Kubicki M, McCarley R, Westin CF, Park HJ, Maier S, Kikinis R, et al. (2007): A review of diffusion tensor imaging studies in schizophrenia. *J Psychiatr Res*. 41:15-30.
5. White T, Nelson M, Lim KO ((In Press)): Diffusion Tensor Imaging in Psychiatric Disorders. *Trends MRI*.
6. Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al. (2003): White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry*. 60:443-456.
7. Friedman JI, Tang C, Carpenter D, Buchsbaum M, Schmeidler J, Flanagan L, et al. (2008): Diffusion tensor imaging findings in first-episode and chronic schizophrenia patients. *Am J Psychiatry*. 165:1024-1032.
8. First M, Spitzer RL, Gibbon M, Williams JB (1997): *Structured Clinical Interview for DSM-IV-TR Axis I Disorders*. Washington, D.C.: American Psychiatric Press, Inc.
9. Andreasen NC, Flaum M, Arndt S (1992): The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. *Arch Gen Psychiatry*. 49:615-623.
10. Andreasen NC (1984): *The Scale for the Assessment of Positive Symptoms (SAPS)*. Iowa City, IA: The University of Iowa.
11. Andreasen NC (1983): *The Scale for the Assessment of Negative Symptoms (SANS)*. Iowa City, IA: The University of Iowa.
12. Miller DD, Flaum M, Nopoulos P, Arndt S, Andreasen NC (1995): The concept of dose years: A reliable method for calculating lifetime psychotropic drug exposure. *Schizophr Res*. 15:159.
13. Magnotta VA, Harris G, Andreasen NC, O'Leary DS, Yuh WT, Heckel D (2002): Structural MR image processing using the BRAINS2 toolbox. *Comput Med Imaging Graph*. 26:251-264.
14. Harris G, Andreasen NC, Cizadlo T, Bailey JM, Bockholt HJ, Magnotta VA, et al. (1999): Improving tissue classification in MRI: a three-dimensional multispectral discriminant analysis method with automated training class selection. *J Comput Assist Tomogr*. 23:144-154.
15. Cheng P, Magnotta VA, Wu D, Nopoulos P, Moser DJ, Paulsen J, et al. (2006): Evaluation of the GTRACT diffusion tensor tractography algorithm: a validation and reliability study. *Neuroimage*. 31:1075-1085.
16. Basser PJ, Mattiello J, LeBihan D (1994): Estimation of the effective self-diffusion tensor from the NMR spin echo. *J Magn Reson B*. 103:247-254.
17. Collins DL, Neelin P, Peters TM, Evans AC (1994): Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. *J Comput Assist Tomogr*. 18:192-205.
18. Buehren M (2004): Matlab Central File Exchange - Functions for the rectangular assignment problem. http://www.mathworks.com/matlabcentral/fileexchange/loadFile.do?objectId=6543&objectType=file.
19. Jones DK, Catani M, Pierpaoli C, Reeves SJ, Shergill SS, O'Sullivan M, et al. (2006): Age effects on diffusion tensor magnetic resonance imaging tractography measures of frontal cortex connections in schizophrenia. *Hum Brain Mapp*. 27:230-238.
20. Friston KJ, Frith CD (1995): Schizophrenia: a disconnection syndrome? *Clin Neurosci*. 3:89-97.
21. Ho BC, Andreasen NC, Nopoulos P, Arndt S, Magnotta V, Flaum M (2003): Progressive structural brain abnormalities and their relationship to clinical outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. *Arch Gen Psychiatry*. 60:585-594.
22. DeLisi LE, Sakuma M, Tew W, Kushner M, Hoff AL, Grimson R (1997): Schizophrenia as a chronic active brain process: a study of progressive brain structural change subsequent to the onset of schizophrenia. *Psychiatry Res*. 74:129-140.

23. Giedd JN, Jeffries NO, Blumenthal J, Castellanos FX, Vaituzis AC, Fernandez T, et al. (1999): Childhood-onset schizophrenia: progressive brain changes during adolescence. *Biol Psychiatry*. 46:892-898.
24. Lieberman J, Chakos M, Wu H, Alvir J, Hoffman E, Robinson D, et al. (2001): Longitudinal study of brain morphology in first episode schizophrenia. *Biol Psychiatry*. 49:487-499.
25. Rapoport JL, Giedd JN, Blumenthal J, Hamburger S, Jeffries N, Fernandez T, et al. (1999): Progressive cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. *Arch Gen Psychiatry*. 56:649-654.
26. Gur RE, Cowell P, Turetsky BI, Gallacher F, Cannon T, Bilker W, et al. (1998): A follow-up magnetic resonance imaging study of schizophrenia. Relationship of neuroanatomical changes to clinical and neurobehavioral measures. *Arch Gen Psychiatry*. 55:145-152.
27. Woods BT (1998): Is schizophrenia a progressive neurodevelopmental disorder? Toward a unitary pathogenetic mechanism. *Am J Psychiatry*. 155:1661-1670.
28. Hulshoff Pol HE, Kahn RS (2008): What happens after the first episode? A review of progressive brain changes in chronically ill patients with schizophrenia. *Schizophr Bull*. 34:354-366.
29. Javitt DC, Zukin SR (1991): Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 148:1301-1308.
30. Olney JW, Farber NB (1995): Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry*. 52:998-1007.
31. Coyle JT (1996): The glutamatergic dysfunction hypothesis for schizophrenia. *Harv Rev Psychiatry*. 3:241-253.
32. Selemon LD, Rajkowska G, Goldman-Rakic PS (1995): Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. *Arch Gen Psychiatry*. 52:805-818; discussion 819-820.
33. Glantz LA, Lewis DA (2000): Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 57:65-73.
34. Weinberger DR, McClure RK (2002): Neurotoxicity, neuroplasticity, and magnetic resonance imaging morphometry: what is happening in the schizophrenic brain? *Arch Gen Psychiatry*. 59:553-558.
35. Mori T, Ohnishi T, Hashimoto R, Nemoto K, Moriguchi Y, Noguchi H, et al. (2007): Progressive changes of white matter integrity in schizophrenia revealed by diffusion tensor imaging. *Psychiatry Res*. 154:133-145.

4.2 Disruption of Hippocampal Connectivity in Children and Adolescents with Schizophrenia

A voxel-based diffusion tensor imaging study



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Schizophr Res (2007) 90: 302-30

Abstract

Introduction: One hypothesis that unifies the diversity of symptoms associated with schizophrenia involves the disruption of connectivity between brain regions. As white matter provides rapid and efficient communication between brain regions, this study was initiated to assess the early disruption of white matter pathways in children and adolescent with schizophrenia.

Materials and Methods: Diffusion Tensor Images were acquired on 14 children and adolescents with schizophrenia, one subject with schizoaffective disorder, and 15 age and gender matched controls. The DTI images were acquired in twelve directions on a 3 Tesla Siemens Trio scanner. The images were transformed into fractional anisotropy and mean diffusivity maps and a group analysis was performed using SPM2.

Results: Children and adolescent patients with schizophrenia demonstrated a significant decrease in FA and associated increase in AD in the left posterior hippocampus ($p<0.001$, Bonferroni corrected on the cluster-level). These diffusion differences were not statistically significant when IQ was used as a covariate in the analysis.

Discussion: These findings suggest hippocampal white matter abnormalities that present early in the development of schizophrenia. The lack of significant differences when IQ is used as a covariate suggests that this hippocampal region is associated with cognitive changes associated with schizophrenia.

Introduction

Schizophrenia is a disabling developmental illness associated with an array of cognitive deficits and clinical symptoms. This diversity of symptoms has led researchers to explore disruptions in cortical and subcortical neuronal connectivity as a pathophysiologic mechanism underlying schizophrenia (1, 2). One hypothesis that may explain the considerable diversity of symptoms in schizophrenia involves aberrations of white matter (WM) pathways. This hypothesis is supported by studies demonstrating abnormal function of myelin-related genes (3), post-mortem studies (4), and structural, diffusion tensor, and magnetization transfer imaging (5).

Diffusion tensor imaging (DTI) offers a non-invasive technique to measure the microstructural integrity of major neuronal fiber pathways in the brain (6). The DTI findings in adults with schizophrenia have been mixed, with some studies demonstrating global WM differences (7-9), whereas others demonstrate either specific WM abnormalities (10, 11), or no differences (12-14). These differences may be partially related to differences in methodologies used (i.e., Price et al. (2005) only a first-episode sample and studied the corpus callosum, Steel et al. (2001) compared 10 patients with schizophrenia and 10 controls in a frontal region of interest). Overall, however, there is converging evidence in DTI studies of individuals with schizophrenia that implicate WM abnormalities in the fronto-thalamic connections (11), cerebellum (11, 15, 16), cingulate bundle (10, 17, 18), hippocampus (19), fornix (20), uncinate fasciculus (21), and the corpus callosum (22).

Whereas childhood and adolescent-onset schizophrenia is not qualitatively different from adult-onset schizophrenia (23), the early-onset form of schizophrenia is associated with greater genetic loading and a worse prognosis (24). The neurobiological findings in children and adolescents with schizophrenia tend to be more pronounced than in those with adult-onset schizophrenia (23). The two studies utilizing DTI in children and adolescents have paralleled the findings in adults, demonstrating WM microstructural abnormalities in the anterior cingulate (25) and frontal pathways (26).

It was the goal of this study to evaluate frontal, striatal, and limbic connectivity in a well defined group of children and adolescents who present early in the course of their illness. Our hypothesis, based on the early onset form of the disorder being a more pronounced variant of the adult form, was that the young patients with schizophrenia would demonstrate widespread disruptions in the integrity of WM connecting these regions, including those regions identified by Kumra et al (2004). These WM disruptions would implicate aberrant connectivity involving multiple neuronal pathways.

Materials and Methods

Table 1 – Demographic and Clinical Characteristics of the Patient and Control Groups

	Patients	Controls	p
Demographic Measures			
Age (years / SD)	15.2 / 2.6	14.5 / 2.7	ns
Sex (M/F)	8 / 7	8 / 7	ns
IQ	91.9 / 17.0	111.9 / 11.8	0.001
SES	41.7 / 11.4	53.1 / 11.5	0.007
Clinical Measures			
* Psychotic Symptoms (mean / SD)	2.2 / 1.4	n/a	n/a
* Negative Symptoms (mean / SD)	2.8 / 1.2	n/a	n/a
* Disorganized Symptoms (mean / SD)	1.6 / 1.1	n/a	n/a
Age of Onset (yrs.) (mean / SD)	12.9 / 1.9	n/a	n/a
⚄ Dose year equivalents for antipsychotic medication (dose years / SD)	4.4 / 4.1	n/a	n/a

* Measures obtained from the SANS and SAPS (28, 29).
⚄⚄ Calculated from (48).

Subjects

The subjects consisted of 14 patients with schizophrenia and 1 with schizoaffective disorder (8 male, 7 female; mean age=15.2 years [SD=2.6]; IQ=92 [SD=17]), and 15 age and gender matched controls (8 male, 7 female; mean age=14.5 years [SD=2.7]; IQ=112 [SD=12]). Table 1 presents demographic and clinical information on the patients and controls. All subjects underwent a semi-structured diagnostic interview using the Schedule for Affective Disorders and Schizophrenia for School-Aged Children – Present and Lifetime Version (K-SADS-PL) (27). In addition, measures of positive and negative symptoms were obtained using the Scale for the Assessment of Positive Symptoms (SAPS) (28) and the Scale for the Assessment of Negative Symptoms (SANS) (29). The exclusion criteria for both the patient and control groups included an IQ less than 70, an active substance use disorder, a lifetime history of substance dependence, a history of a neurological disorder, or a history of a pronounced head injury. Subjects were excluded from the

control group if they had an Axis I, DSM-IV disorder or a first-degree family member with a schizophrenia spectrum disorder. The study was approved by the Institutional Review Board at the University of Minnesota and both informed consent and assent were obtained prior to participation.

MRI Sequence

All MR images were acquired with a 3T Siemens MR System (Erlangen, Germany) at the University of Minnesota Center for Magnetic Resonance Research. After a localizer sequence for orientation, a high resolution, three-dimensional FLASH 3-D volumetric acquisition (TR/TE= 18/4.73, flip angle=25, FoV[read/phase]=240/180 mm, in-plane resolution of 0.625x0.625x1.5 mm, NEX=1) was collected in a coronal orientation on seven subjects using a birdcage coil. As some subjects had significant flow artifacts with the birdcage coil, the high-resolution images acquired using a MP-RAGE sequence (TR/TE= 2530/3.81, flip angle=7, FoV 160 mm, in-plane resolution of 0.625x0.625x1.5 mm, NEX=1) using an 8-channel coil for the remaining participants. Since the use of head coil for the MP-RAGE sequences varied between subjects, we did not use the T1 data in a separate voxel-based morphometry analysis.

Diffusion Tensor Imaging was acquired in all subjects using a full tensor diffusion MRI sequence based on a single shot spin echo planar technique (TR/TE= 11.000/104, FOV=256 mm, in-plane resolution of 2 x 2 mm and a slice thickness of 2.0 mm, average=3). The images were acquired in twelve noncollinear directions (b= 1000 s/mm2) and one acquisition without diffusion encoding (b=0s/mm2).

Diffusion Tensor Analyses

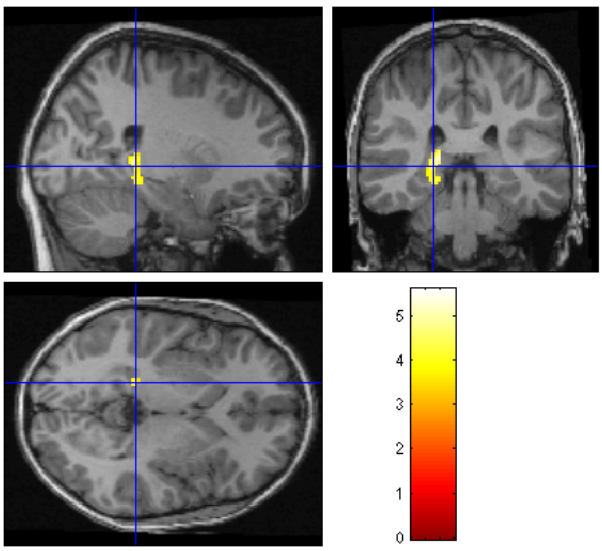
Original MR images registered in DICOM format were converted to ANALYZE format by using MRIcro software (University of Nottingham, UK). Images were corrected for geometric distortion secondary to eddy currents using a registration technique based upon the geometric model of distortions (30). FA and AD maps were obtained by using in-house software running on Matlab 6.5 (Mathworks Inc., MA, USA).

Voxel based fractional anisotropy (FA) and average diffusivity (AD) analyses were performed using MATLAB 6.5.1 (MathWorks) and SPM2 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, London, UK). First a non-linear spatial normalization algorithm was applied to each subject's FA and AD maps to align the images in stereotactic space. The images were then smoothed using a 5 mm FWHM Gaussian filter. The choice of using a 5 mm FWHM filter was based on our prior work that measured the range of structural variability between subjects in regions that are in relatively close proximity to the anterior and posterior commissure (31). An average WM mask was generated in order to include only the voxels belonging to the WM regions. The initial voxel based analyses was performed using a student t-test with significance

defined conservatively by a Bonferroni cluster-level correction of $p < 0.001$. A secondary ANCOVA was performed using IQ as the covariate.

Results

Adolescent patients with schizophrenia demonstrated a decrease in FA in the left posterior hippocampus and posterior limbic regions involved in connections with the posterior cingulate ($p<0.001$ after correcting for multiple comparisons). The patient group also had an increase in AD in the same location as the decrease in FA (Figure 1). There were no brain regions in the typically developing adolescents that demonstrated a decrease in FA or an increase in AD compared to the patients with schizophrenia. In addition, a voxel-wide ANCOVA controlling for IQ differences between the patient and control groups resulted in the disappearance of this finding in both the FA and AD maps. To assess whether this finding was a result of volumetric changes, a voxel based morphometric analysis was performed on the b=0, T2 weighted images. Using the same WM mask in the same stereotactic space, there were no differences between patients and controls in WM volume. These results did not change when the one subject with schizoaffective disorder was removed from the analyses.



A B

Figure 1 – *Group Analyses of Brain Regions with Lower Fractional Anisotropy (A) and Greater Mean Diffusivity (B) in Children and Adolescents with Schizophrenia versus a Matched Control Group. The area identified in yellow identifies the region in the posterior hippocampus that shows the differences between the children and adolescents with schizophrenia and the control group. (Figure 5, Back Cover)*

Discussion

We found that children and adolescents with schizophrenia demonstrated microstructural abnormalities in the left posterior hippocampus, extending posteriorly along caudal neuronal pathways. The WM caudal to the hippocampal formation includes regions with connections between the hippocampus and the fornix and connections between the subiculum and entorhinal cortex and the

posterior cingulate. Our hypothesis that children and adolescents with schizophrenia would demonstrate widespread WM anomalies was not supported .Instead, the patients demonstrated more localized abnormalities in a region that has been implicated in multiple adult studies of schizophrenia (see (32) for a review).

The hippocampus is a limbic structure involved in the encoding and retrieval of multimodal sensory information (33). Although the relationship between disruptions in synaptic connectivity and disruptions in the microstructural integrity of WM is unclear, there is both postmortem (4) and developmental (34) evidence that implicate hippocampal WM abnormalities in schizophrenia. In addition, the posterior regions of the left and right hippocampus have been shown to have approximately a 15% decrease in FA in a DTI study in adults with schizophrenia (19). The early disruption of neuronal fibers communicating with the hippocampus could result in an array of both cognitive and clinical symptoms (32), including altered connectivity with prefrontal regions (35).

Since cognitive deficits have long been associated with the development of schizophrenia, WM abnormalities may be directly related to cognitive deficits associated with the illness. WM abnormalities which result in slowing or disruptions in the flow of neuronal communication could result in a mismatch in the timing of incoming neuronal transmission or alternatively, result in a slowing of cognitive processing. Alternatively, since IQ has been shown to correlate developmentally with alterations in FA and AD (36), developmental factors may be responsible for the patient control differences. It is intriguing that when IQ is used as a covariate, the hippocampal findings no longer reach statistical significance. Interestingly, neuronal fibers traversing the limbic pathways from the posterior hippocampus are connected to prefrontal regions and pathways involved in higher cognition (37). Thus, disruption of these hippocampal output fibers may be associated with cognitive dysfunction (20).

There are several limitations to the present study. Since the subjects were not matched on IQ, it is possible that the hippocampal differences are a generalized finding associated with individuals with a lower IQ. A control sample matched on age, gender and IQ is required to address this question. Since schizophrenia is associated with cognitive decline, the optimum approach would be to have two separate control groups, with one matched for age, sex, and IQ; and the second matched for age, sex and a predicted measure of what their IQ might have been had they never developed schizophrenia. The latter is difficult to predict, although the use of either parental SES, educational level, parental IQs, or the IQ of siblings may provide an indirect estimate of the state variables associated with schizophrenia. .

It is possible that more widespread patient/control differences were not obtained due to the limited power of our small sample size. The power was further reduced due to corrections for multiple testing. Thus, our small sample size may have resulted in type II errors, with greater patient/control differences revealed with larger sample sizes. The presence of our strong findings in the hippocampus with a small sample size, however, may reflect early and more pronounced changes in this region (35). Although the only other study using a voxel-based approach in

children and adolescents with schizophrenia had a larger sample size (25), methodological differences may account for the non-overlapping results. Our approach utilized a WM mask to minimize the effects of partial voluming, higher resolution (2 mm versus 5 mm), and a 5 mm spatial filter (38).

One of the patients had schizoaffective disorder, and although the exclusion of this patient did not alter the findings, the pathophysiology of schizoaffective disorder may be different than schizophrenia, especially in the limbic regions. A study comparing a large sample of patients with schizophrenia and schizoaffective disorder is necessary to address this question.

Although a WM mask was used in the analyses, it is possible that partial-voluming effects secondary to regional volume differences may be contributing to the findings. Whereas MR studies in adults with schizophrenia have consistently demonstrated a reduction in the volume of the hippocampus (20, 32, 39-41), such changes appear to have an age-dependent trajectory in childhood onset illness (42-45). The location of the diffusion abnormalities in the adult studies tend to lie in the anterior regions of the hippocampus, whereas the diffusion differences reported here include regions where neurons project to the posterior cingulate and fornix. In addition, the structural T2 weighted VBM analysis was applied with the identical WM mask without showing any patient control differences, which would be expected with partial voluming or volume differences. Due to significant temporal lobe flow artifacts using the T1 sequence and the birdcage head coil (see Methods Section), we switched to using the 8-channel parallel imaging coil for only the T1 sequence once it became available. As a result, we were unfortunately unable to obtain reliable data using a VBM analysis on the T1 images. The T2 and DTI images were all obtained with the same birdcage head coil.

Since the size of the spatial filter used in voxel-based analyses of scalar diffusion measures can result in dramatically different results (38), we made an a priori decision on filter size based on our prior investigations of structural variability in fMRI data (31). It has been shown in postmortem brains that the intersubject variability of the central sulcus can be upwards of 20 mm for brains which are morphed into a stereotactic space (46). However, subcortical regions may differ by only 1 to 2 mm (31). Thus, when applying the matched filter theorem (47) to group data, both the predicted volume of difference between groups and the extent of structural variability between subjects are important factors when choosing the size of the spatial filter. Since it is challenging in voxel-based diffusion analyses to develop an a priori understanding of the expected differences (38), we utilized a 5 mm spatial filter based on expected structural variability within the white matter mask.

In summary, children and adolescents with schizophrenia demonstrate microstructural abnormalities in the region of the posterior hippocampal formation and limbic pathways. The region includes pathways that connect the hippocampus with the fornix and posterior cingulate. This deficit is associated with cognitive deficits and points to hippocampal disconnectivity early in the course of schizophrenia.

References

1. Friston KJ, Frith CD (1995): Schizophrenia: a disconnection syndrome? *Clin Neurosci*. 3:89-97.
2. Andreasen NC (1997): Linking mind and brain in the study of mental illnesses: a project for a scientific psychopathology. *Science*. 275:1586-1593.
3. Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al. (2003): White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry*. 60:443-456.
4. Heckers S, Heinsen H, Geiger B, Beckmann H (1991): Hippocampal neuron number in schizophrenia. A stereological study. *Arch Gen Psychiatry*. 48:1002-1008.
5. Kubicki M, Park H, Westin CF, Nestor PG, Mulkern RV, Maier SE, et al. (2005): DTI and MTR abnormalities in schizophrenia: Analysis of white matter integrity. *Neuroimage*. 26:1109-1118.
6. Basser PJ, Mattiello J, LeBihan D (1994): MR diffusion tensor spectroscopy and imaging. *Biophys J*. 66:259-267.
7. Agartz I, Andersson JL, Skare S (2001): Abnormal brain white matter in schizophrenia: a diffusion tensor imaging study. *Neuroreport*. 12:2251-2254.
8. Ardekani BA, Bappal A, D'Angelo D, Ashtari M, Lencz T, Szeszko PR, et al. (2005): Brain morphometry using diffusion-weighted magnetic resonance imaging: application to schizophrenia. *Neuroreport*. 16:1455-1459.
9. Lim KO, Hedehus M, Moseley M, de Crespigny A, Sullivan EV, Pfefferbaum A (1999): Compromised white matter tract integrity in schizophrenia inferred from diffusion tensor imaging. *Arch Gen Psychiatry*. 56:367-374.
10. Sun Z, Wang F, Cui L, Breeze J, Du X, Wang X, et al. (2003): Abnormal anterior cingulum in patients with schizophrenia: a diffusion tensor imaging study. *Neuroreport*. 14:1833-1836.
11. Rose SE, Chalk JB, Janke AL, Strudwick MW, Windus LC, Hannah DE, et al. (2006): Evidence of altered prefrontal-thalamic circuitry in schizophrenia: An optimized diffusion MRI study. *Neuroimage*.
12. Foong J, Symms MR, Barker GJ, Maier M, Miller DH, Ron MA (2002): Investigating regional white matter in schizophrenia using diffusion tensor imaging. *Neuroreport*. 13:333-336.
13. Price G, Bagary MS, Cercignani M, Altmann DR, Ron MA (2005): The corpus callosum in first episode schizophrenia: a diffusion tensor imaging study. *J Neurol Neurosurg Psychiatry*. 76:585-587.
14. Steel RM, Bastin ME, McConnell S, Marshall I, Cunningham-Owens DG, Lawrie SM, et al. (2001): Diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (1H MRS) in schizophrenic subjects and normal controls. *Psychiatry Res*. 106:161-170.
15. Okugawa G, Nobuhara K, Minami T, Takase K, Sugimoto T, Saito Y, et al. (2006): Neural disorganization in the superior cerebellar peduncle and cognitive abnormality in patients with schizophrenia: A diffusion tensor imaging study. *Prog Neuropsychopharmacol Biol Psychiatry*.
16. Wang F, Sun Z, Du X, Wang X, Cong Z, Zhang H, et al. (2003): A diffusion tensor imaging study of middle and superior cerebellar peduncle in male patients with schizophrenia. *Neurosci Lett*. 348:135-138.
17. Kubicki M, Westin CF, Nestor PG, Wible CG, Frumin M, Maier SE, et al. (2003): Cingulate fasciculus integrity disruption in schizophrenia: a magnetic resonance diffusion tensor imaging study. *Biol Psychiatry*. 54:1171-1180.
18. Wang F, Sun Z, Cui L, Du X, Wang X, Zhang H, et al. (2004): Anterior cingulum abnormalities in male patients with schizophrenia determined through diffusion tensor imaging. *Am J Psychiatry*. 161:573-575.
19. Kalus P, Buri C, Slotboom J, Gralla J, Remonda L, Dierks T, et al. (2004): Volumetry and diffusion tensor imaging of hippocampal subregions in schizophrenia. *Neuroreport*. 15:867-871.
20. Kuroki N, Kubicki M, Nestor PG, Salisbury DF, Park HJ, Levitt JJ, et al. (2006): Fornix integrity and hippocampal volume in male schizophrenic patients. *Biol Psychiatry*. 60:22-31.
21. Kubicki M, Westin CF, Maier SE, Frumin M, Nestor PG, Salisbury DF, et al. (2002): Uncinate fasciculus findings in schizophrenia: a magnetic resonance diffusion tensor imaging study. *Am J Psychiatry*. 159:813-820.
22. Foong J, Maier M, Clark CA, Barker GJ, Miller DH, Ron MA (2000): Neuropathological abnormalities of the corpus callosum in schizophrenia: a diffusion tensor imaging study. *J Neurol Neurosurg Psychiatry*. 68:242-244.
23. Rapoport JL, Giedd JN, Blumenthal J, Hamburger S, Jeffries N, Fernandez T, et al. (1999): Progressive cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. *Arch Gen Psychiatry*. 56:649-654.

24. Asarnow RF, Nuechterlein KH, Fogelson D, Subotnik KL, Payne DA, Russell AT, et al. (2001): Schizophrenia and schizophrenia-spectrum personality disorders in the first-degree relatives of children with schizophrenia: the UCLA family study. *Arch Gen Psychiatry*. 58:581-588.
25. Kumra S, Ashtari M, Cervellione KL, Henderson I, Kester H, Roofeh D, et al. (2005): White matter abnormalities in early-onset schizophrenia: a voxel-based diffusion tensor imaging study. *J Am Acad Child Adolesc Psychiatry*. 44:934-941.
26. Kumra S, Ashtari M, McMeniman M, Vogel J, Augustin R, Becker DE, et al. (2004): Reduced frontal white matter integrity in early-onset schizophrenia: a preliminary study. *Biol Psychiatry*. 55:1138-1145.
27. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. (1997): Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry*. 36:980-988.
28. Andreasen NC (1984): *The Scale for the Assessment of Positive Symptoms (SAPS)*. Iowa City, IA: The University of Iowa.
29. Andreasen NC (1983): *The Scale for the Assessment of Negative Symptoms (SANS)*. Iowa City, IA: The University of Iowa.
30. Mangin JF, Poupon C, Clark C, Le Bihan D, Bloch I (2002): Distortion correction and robust tensor estimation for MR diffusion imaging. *Med Image Anal*. 6:191-198.
31. White (2001): Anatomic and functional variability: The effects of filter size in group fMRI data analysis. *NeuroImage*. 13:577-588.
32. Harrison PJ (2004): The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. *Psychopharmacology (Berl)*. 174:151-162.
33. Heckers S, Konradi C (2002): Hippocampal neurons in schizophrenia. *J Neural Transm*. 109:891-905.
34. Benes FM (1989): Myelination of cortical-hippocampal relays during late adolescence. *Schizophr Bull*. 15:585-593.
35. Lipska BK, Weinberger DR (2002): A neurodevelopmental model of schizophrenia: neonatal disconnection of the hippocampus. *Neurotox Res*. 4:469-475.
36. Schmithorst VJ, Wilke M, Dardzinski BJ, Holland SK (2005): Cognitive functions correlate with white matter architecture in a normal pediatric population: a diffusion tensor MRI study. *Hum Brain Mapp*. 26:139-147.
37. Goldman-Rakic PS, Selemon LD, Schwartz ML (1984): Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. *Neuroscience*. 12:719-743.
38. Jones DK, Symms MR, Cercignani M, Howard RJ (2005): The effect of filter size on VBM analyses of DT-MRI data. *Neuroimage*. 26:546-554.
39. McCarley RW, Wible CG, Frumin M, Hirayasu Y, Levitt JJ, Fischer IA, et al. (1999): MRI anatomy of schizophrenia. *Biol Psychiatry*. 45:1099-1119.
40. Nelson MD, Saykin AJ, Flashman LA, Riordan HJ (1998): Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. *Arch Gen Psychiatry*. 55:433-440.
41. Shenton ME, Dickey CC, Frumin M, McCarley RW (2001): A review of MRI findings in schizophrenia. *Schizophr Res*. 49:1-52.
42. Levitt JG, Blanton RE, Caplan R, Asarnow R, Guthrie D, Toga AW, et al. (2001): Medial temporal lobe in childhood-onset schizophrenia. *Psychiatry Res*. 108:17-27.
43. Giedd JN, Jeffries NO, Blumenthal J, Castellanos FX, Vaituzis AC, Fernandez T, et al. (1999): Childhood-onset schizophrenia: progressive brain changes during adolescence. *Biol Psychiatry*. 46:892-898.
44. Jacobsen LK, Giedd JN, Castellanos FX, Vaituzis AC, Hamburger SD, Kumra S, et al. (1998): Progressive reduction of temporal lobe structures in childhood-onset schizophrenia. *Am J Psychiatry*. 155:678-685.
45. Jacobsen LK, Giedd JN, Vaituzis AC, Hamburger SD, Rajapakse JC, Frazier JA, et al. (1996): Temporal lobe morphology in childhood-onset schizophrenia. *Am J Psychiatry*. 153:355-361.
46. Talairach J, Tournoux P (1988): *Co-planar Stereotactic Atlas of the Human Brain: 3-Dimensional Proportional System: An Approach to Cerebral Imaging*. Stuttgart/New York: Thieme Verlag.
47. Rosenfeld A, Kak A (1982): *Digital Picture Processing 2*. Orlando, Florida: Academic Press.
48. Woods SW (2003): Chlorpromazine equivalent doses for the newer atypical antipsychotics. *J Clin Psychiatry*. 64:663-667.

4.3 White Matter 'Potholes' in Early-Onset Schizophrenia

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Psychiatric Research: Neuroimaging (2009) 174: 110-115

Abstract

There is considerable evidence implicating white matter abnormalities in the pathophysiology of schizophrenia. Many of the recent studies examining white matter have utilized diffusion tensor imaging (DTI) using either region of interest (ROI) or voxel based approaches. Both voxel-based and ROI approaches are based on the assumption that the abnormalities in white matter overlap spatially. However, this is an assumption that has not been tested and it is possible that aberrations in white matter occur in non-overlapping regions. In order to test for the presence of non-overlapping regions of aberrant white matter, we developed a novel image processing technique that evaluates for white matter 'potholes,' referring to within-subject clusters of white matter voxels that show a significant reduction in fractional anisotropy. We applied this algorithm to a group of children and adolescents with schizophrenia compared to controls and found an increased number of 'potholes' in the patient group. These results suggest that voxel-based and ROI approaches may be missing some white matter differences that do not overlap spatially. This algorithm may be also be well suited to detect white matter abnormalities in disorders such as substance abuse, head trauma, or specifc neurological conditions affecting white matter.

Introduction

White matter (WM) tracts within the brain consist of myelinated neuronal fibers that serve as 'superhighways' for the rapid transfer of information between brain regions. Medical disorders that disrupt these pathways, such as multiple sclerosis or amyotrophic lateral sclerosis can profoundly affect various aspects of cognitive and motor function (1). Schizophrenia is a severe mental illness that involves a constellation of clinical symptoms and global cognitive deficits. While the etiology of schizophrenia is yet unknown, one current hypothesis is a disruption in brain connectivity (2). Thus, cerebral WM has become a source of considerable investigation in schizophrenia, with recent evidence supporting WM abnormalities based on postmortem samples (3-6), genetic analyses (7), and diffusion tensor imaging (DTI) (8-11).

There are now close to 60 studies that have utilized DTI to assess WM microstructure in schizophrenia (10). What is most striking about the combined findings of these studies is the considerable heterogeneity in the locations of the WM differences between patients and controls (8-10). While there does appear to be an over-representation of abnormalities in the corpus callosum, cingulate bundle, and frontal WM, nearly every WM structure has been implicated. Since the majority of DTI studies utilize voxel-based techniques to evaluate regional WM differences, typically only positive findings are reported. However, the whole brain testing inherent in voxel-based approaches is also associated with widespread areas that do not demonstrate significant patient/control differences. These negative results complicate the interpretation of DTI findings in patients with schizophrenia.

While a few early studies have reported a diffuse pattern of WM abnormalities in patients with schizophrenia (12, 13), most DTI studies tend to have focal abnormalities (10). Perhaps one explanation for the variability in the existing studies involves the methodologies applied to determine the underlying deficits. The current methodologies applied to DTI studies involve either region of interest (ROI) or voxel-based techniques. Both ROI and voxel-based approaches make an assumption that WM abnormalities are spatially localized to specific regions in patients. For example, for ROI or voxel-based approaches to detect an abnormality of the cingulate bundle, the WM deficit would need to be spatially localized to the same region of the cingulate bundle in most of the patients. However, a deficit could occur at different locations along the cingulate tract and have a similar contribution to the clinical deficit. While spatial smoothing can be applied to account for this variability (14), it is limited to regions that are proximally located (15). There have been recent studies using tractography approaches that support this weakness of ROI approaches (16).

It is possible that disruptions in WM integrity may occur at different 'points of weakness' in different individuals. Disorders such as tuberous sclerosis (17, 18) and multiple sclerosis (1) may have heterogeneity in affected WM pathways, and there may be similar heterogeneous patterns in the location of WM abnormalities in schizophrenia. While primary WM disorders may not be directly compared to schizophrenia, we only note the assumption of spatially overlapping regions has

not been confirmed. In fact, the heterogeneity of the results in the current studies may be a result of the analytic strategies and voxel based or ROI approaches, that are powerful for evaluating gray matter differences, may not be as well suited to identify specific WM abnormalities.

The goal of this study was to evaluate a novel approach to examine WM abnormalities that does not require spatially overlapping deficits. Much in the way that we would not expect potholes to overlap when highways were placed one on top of one another, we developed an algorithm to detect WM 'potholes.' A 'pothole' is a region of WM where a cluster of voxels fall significantly below its voxel-based mean. This algorithm was applied to a dataset of individuals with early onset schizophrenia, defined as those who develop the illness during childhood or adolescence. EOS has been shown to be on a continuum with the adult form of the illness (19, 20), although those with EOS tend to have greater genetic loading (21) and more pronounced negative symptoms (22, 23) than those with adult-onset schizophrenia.

Methods

Subjects

The participants included 29 patients (18 males and 11 females) with a diagnosis of schizophrenia (n=22), schizoaffective disorder (n=4), or schizophreniform disorder (n=3). The control group consisted of 41 healthy volunteers (25 males and 16 females). Each participant, including the healthy volunteers, underwent a diagnostic evaluation using the Kiddie-SADS-PL (24). Additional clinical measures included the Scale for the Assessment of Negative Symptoms (SANS) (25). Scale for the Assessment of Positive Symptoms (SAPS) (26), and the Anchored Brief Psychiatric Rating Scale for Children (BPRS-C) (27). The mean age of the patient group was 14.8 (S.D. 2.8) years, and the mean age of the healthy volunteers was 14.2 (S.D. 3.4) years (age range 8 to 19).

The control group had no evidence of a past or present psychiatric disorder and no history of schizophrenia or psychosis in a first degree relative. Patients and controls were excluded if they had a history of substance dependence, ongoing substance abuse (within the past month), IQ less than 70, or a neurological disorder, head injury, or medical illness involving the brain. The study was approved by the Institutional Review Board at the University of Minnesota and informed consent and assent were obtained prior to participation.

MRI Sequence

All MR images were acquired with a 3T Siemens MR System (Erlangen, Germany) at the Center for Magnetic resonance Research in University of Minnesota. After a localizer sequence for orientation, a high resolution, three-dimensional FLASH 3-D volumetric acquisition (TR = 18 msec TR = 4.73 sec, flip angle=25, FOV = 240 mm isotropic, in-plane resolution of 0.625 x 0.625 mm and a

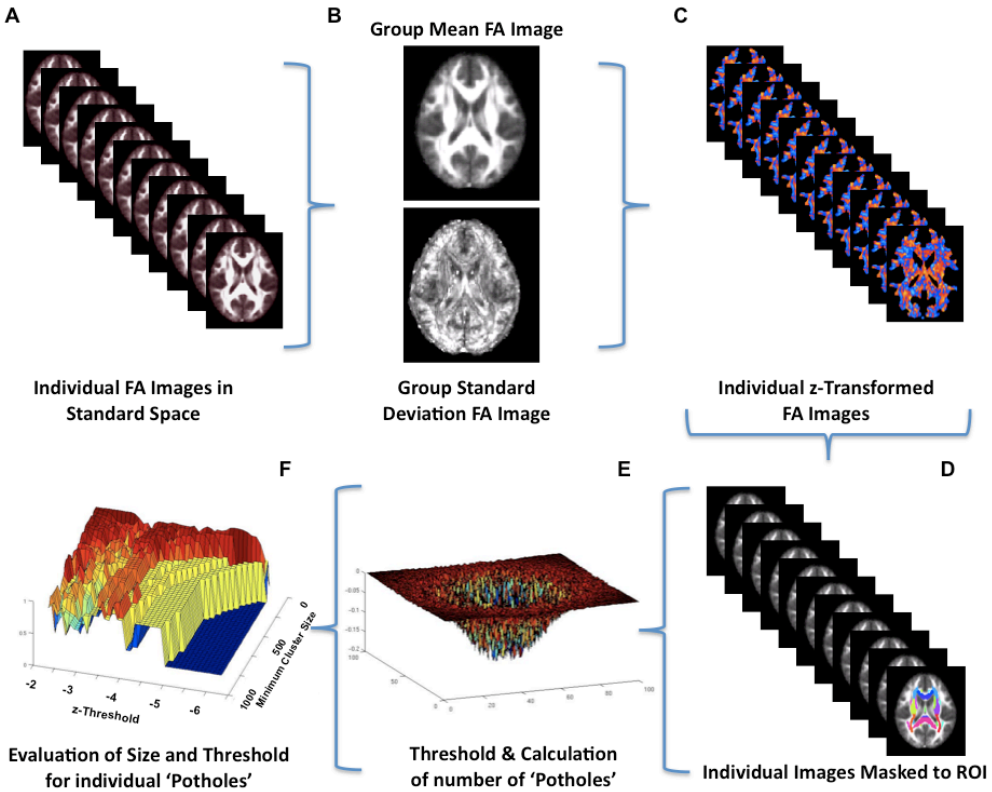
slice thickness of 1.5 mm, average=1) was collected for each subject. From these images, anterior commissure (AC) and posterior commissure (PC) planes were identified.

Diffusion Tensor Imaging was performed with a full tensor diffusion MRI sequence based on a single shot spin echo planar technique (TR=11 sec TE=104 msec, FOV=256 mm isotropic, in-plane resolution of 2 x 2 mm and a slice thickness of 2 mm, averages=3). The images were acquired in twelve non-collinear directions (b=1000 s/mm2) and one image with no diffusion encoding (b=0s/mm2). Diffusion weighted images were acquired along the AC-PC plane.

Image Processing

Pre-processing of the images were performed using Freesurfer (28), FSL (29), and FSL's Tract Based Spatial Statistics (TBSS) (30). Raw diffusion weighted images were converted from DICOM to nifti using Freesurfer (28). Individual images were eddy current corrected, motion corrected, and fractional anisotropy (FA) images were created by fitting a tensor model to the diffusion data using FMRIB's Diffusion Toolbox (FDT) (29). The brain was extracted using BET (31). All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT (32, 33), which uses a b-spline representation of the registration warp field (34). Next, the mean FA image was created and thinned to create a mean FA skeleton which represents the midpoint of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics.

In addition to the group comparison using TBSS, we utilized an in-house program in Matlab in order to quantify the number of WM 'potholes' along the major WM tracts (Figure 1). This process started with FA images that have undergone non-linear registration into MNI space using TBSS. These images did not undergo spatial filtering. The first step generated a voxel-by-voxel mean and standard deviation images of the control subjects. These group and SD images were then used to individually create a voxel-wide z-image for every subject, with each voxel based on the mean and standard deviation of the control group. To ensure the search involved only WM regions, each image was masked with the cortical areas defined by the Johns Hopkins University WM atlas (35, 36). In addition, only regions in which all subjects had an FA > 0.2 were utilized. This resulted in 71 WM masked z-transformed FA images, one for each subject. The individual z-FA images were used to search for clusters of WM that fell below a set z-threshold and were greater than a specified cluster size. Clusters were determined by thresholding each image and labeling the 3-dimensional connectivities (26 neighboring voxels). To evaluate the spatial location of the individual WM potholes, the volume of each pothole was evaluated for each label of the WM atlas.



Statistical Analyses

Figure 1 – *Processing Steps to Determine White Matter Potholes. (A) All subjects FA maps registered to MNI space using Tract-Based Spatial Statistics; (B) Creation of mean and standard deviation images using all subjects; (C) Individual subjects' FA maps were used to create FA z-maps for each subject; (D) z-FA maps masked to the Johns Hopkins white matter atlas; (E) Identification of Potholes by the Identification of Contiguous Voxels that Fall Below a theshholded z-value; (F) Comparison of Patients versus Controls at Different z-Thresholds and Minimum Cluster Sizes.*

Demographic and IQ information was evaluated using chi square for bivariate data and t-tests for continuous data. To provide a comprehensive assessment of this method, we systematically varied the minimum z-threshold and cluster volume. We started at voxels at least one standard deviation below the mean and decremented the threshold by 0.1 to five standard deviations below the mean. For each iteration of the new threshold, we incremented the cluster size from values that exceeded 1 voxel to those that exceeded 1,000 voxels. Statistical analyses on the

imaging data were performed using Matlab (The Mathworks, Natick, MA). To evaluate anatomical regions of interest (ROI) and reduce the number of tests, we performed a 2 (diagnosis) by 48 (region) mixed-model repeated measure ANOVA. Post-hoc tests were performed on individual regions using Wilcoxon rank-order tests. Spearman Correlation Coefficients were utilized to evaluate the relationship between the demographic and clinical measures and the FA potholes. Statistical analyses were performed utilizing the SAS statistical package (Cary, NC, USA).

Results

There were no significant differences between age, sex, and handedness between EOS patients and controls (See Table 1). However, there was a significant difference in SES between patients and controls ($t = 4.4$, $df = 45.2$, $p < 0.01$). There were no statically significant differences in the number of potholes between males and females, however, there was an age effect with potholes smaller than 0.3 cc in volume. Younger subjects had an inverse correlation between age and the number of potholes (Spearman $r = -0.32$, $p < 0.01$) . There were no significant correlations between the number of potholes and age once the minimum number of voxels in the cluster exceeded a volume of 0.3 cc.

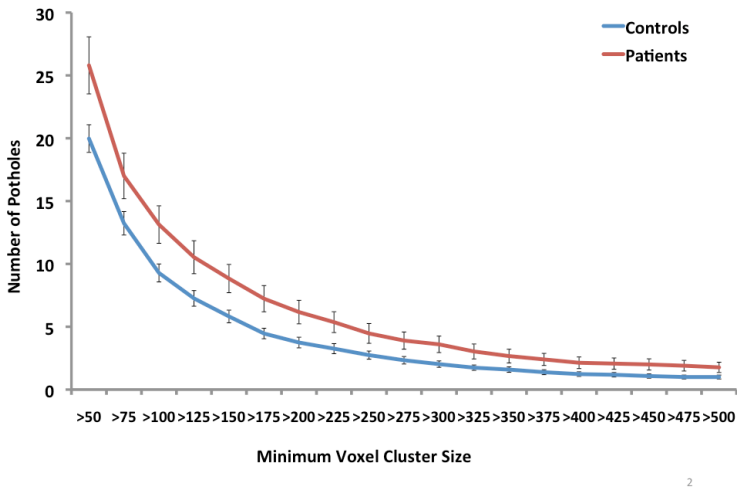


Figure 2 – *Mean Number of Potholes at Different Minimum Pothole Sizes Between Patients and Controls at a Threshold of z = -2.*

Patients with EOS had a greater number of voxel clusters that dipped significantly below the voxel-based group mean. At thresholds of less than 2

standard deviations below the mean, the median number of clusters with at least 50 contiguous voxels was 27.5 in patients and 20 in controls, with a maximum of 59 in patients and 35 in controls. Increasing the number of contiguous voxels to at least 200 resulted in a median of 6 potholes in patients, and 3 in controls, with a maximum number of 23 and 13 in patients and controls, respectively. At none of the thresholds or minimum voxel clusters did controls have a significantly greater number of potholes than patients (Figure 2). Patients had a significantly greater number of potholes compared to controls at 57 different thresholds (Wilcoxon Rank-Order test $p < 0.05$). The p values for different thresholds and minimum cluster sizes can be seen in Figure 1-f.

Anatomically Defined Region of Interest Analyses

The specific ROIs were defined using the Johns Hopkins University Human Brain White Matter Atlas (35, 37). A 2 (diagnosis) by 48 (region) repeated measures mixed model ANOVA found highly significant effects of diagnosis for the volume of potholes within each region ($F_{1,69} = 10.9$, $p < 0.003$) and the mean FA within potholes ($F_{1,69} = 19.3$, $p < 0.0001$). The individual differences between patients and controls that were less than $p < 0.01$ are shown in Table 2. Figure 3-a demonstrates locations of potholes in patients compared to controls. A Spearman rank order correlation was used to evaluate the relationship between the number of potholes and the duration of illness in those regions that were different between patients and controls. There was a significantly positive correlation between the duration of illness and the number of potholes in the right inferior fronto-occipital fasciculus ($r = 0.42$, $p = 0.03$).

Comparison with TBSS Analyses

The voxelwise group analysis using TBSS and the most conservative permutation-based nonparametric algorithm (30) corrected for multiple comparisons at the $p < 0.05$ level found no differences between patients and controls. When relaxing the criteria to a corrected threshold of $p < 0.10$, patients had lower FA in several regions that were also identified using the pothole approach. These regions included the corpus callosum and frontal WM regions (Figure 3-b). Using this same relaxed threshold, there were no regions in which controls had lower FA than patients.

Discussion

Voxel-based and ROI techniques are powerful methods to detect group differences in brain structure and function. However, the underlying assumption of both of these techniques is that the group differences are localized to the same brain region in at least the majority of patients. It is yet unknown if WM pathology occurs in the same location in each patient with schizophrenia. In fact, the currently DTI literature has considerable variability in the location of WM findings (9, 10). If there

are individual differences in the location of the WM abnormalities, both ROI and voxel based approaches may lack the power to identify these differences. In addition, it is possible that variability in the location of WM abnormalities may contribute to the clinical heterogeneity of schizophrenia.

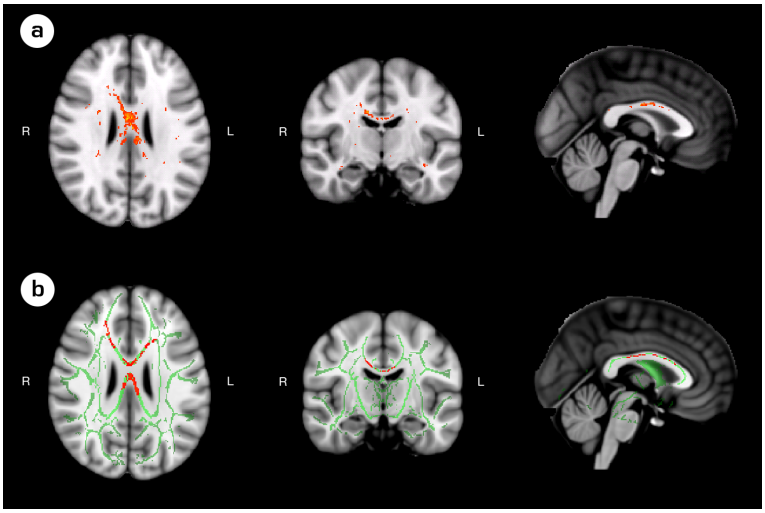


Figure 3 – *The representation of Potholes within the brain in Patients Compared to Controls. The red regions identify potholes in patients and blue regions those in controls. (Figure 6, Back Cover).*

To test for spatially heterogeneous abnormalities in FA, we developed an algorithm to detect contiguous voxels that fall below their voxel-based mean. This method is able to detect clusters between individuals that differ in brain location, but are localized within the WM. These we labeled WM potholes. We found that patients with EOS demonstrated a significantly greater number of WM potholes compared to matched controls. Since this is a novel approach, we tested the method across a number of different z-scores and volume thresholds (see Figure 1-f). The patient group had significantly greater number of 'potholes' at multiple thresholds and cluster sizes. These clusters are most prominent at approximately 2 standard deviations below the mean and have sizes that exceed 1,000 voxels (1 cc), which were found in the corpus callosum. The control group did not have a significantly greater number of 'potholes' than patients at any threshold or volume.

Interestingly, the spatial location of these potholes did emerge in regions that have been identified commonly in studies of schizophrenia. For example, numerous studies have identified aberrant WM regions in the corpus callosum, cingulate bundle, and frontal WM tracts (10, 11). Utilizing the standard TBSS approach, we did not find any regions which were different between patients and controls. However, with a less conservative threshold for multiple testing, we found significant differences in the corpus callosum and frontal WM regions, regions which overlap with the pothole approach. Thus, it is possible that certain regions that have a convergence of WM pathways, such as the corpus callosum, are more likely have an overlap of WM abnormalities, which are then detected using voxel-based techniques.

Considering the variability of WM pathology in disorders such as multiple sclerosis (1), it is possible that similar heterogeneous patterns may also exist in the WM pathology found in schizophrenia. While there may be involvement in multiple regions of the brain, certain areas, such as the cingulate bundle, corpus callosum, or frontal WM tracts, may be more susceptible to alterations. This would account for the overrepresentation of positive findings in these regions using voxel based and ROI studies. However, other individuals or subgroups may have different areas of susceptibility that are based on genetic or environmental influences. One possible etiology would involve disruptions in the distribution or number of oligodendrocytes. Postmortem studies of schizophrenia often focus on specific regions and thus do not provide a gross distribution of abnormalities. However, postmortem studies have identified abnormal numbers of oligodendrocytes in the prefrontal cortex (6, 38) and cingulum (39).

While it was not a goal of this paper to assess developmental differences in the number of potholes, we did find an inverse correlation between potholes and age; i.e., younger children tended to have a greater number of smaller potholes. Since studies using both postmortem and DTI methods have demonstrated an increase in FA that is associated with development, this technique may be beneficial to identify regions associated with neurodevelopmental processes such as myelination. Future work using this technique with larger populations of typically developing children will be necessary to confirm this finding. Finally, we found a significant positive correlation between the duration of illness and the right inferior fronto-occipital fasciculus. Since these EOS patients are in the early stages of their illness (mean duration of illness was 2.3 years), a greater range of illness duration or a longitudinal design would be best suited to evaluate the relationship between illness duration and the number of potholes. This is important since there is evidence that patients with chronic schizophrenia have regions of lower FA has compared to first-episode patients (40).

This is a preliminary investigation of a novel method to assess WM microstructure in clinical populations and we note that there are several limitations to the study. We have a relatively small sample size, although the detection of differences in a small sample actually reflects large effect sizes. It would be beneficial to apply the method to a larger dataset with both a first-episode and chronic patient groups, since a progression of WM abnormalities has been demonstrated between first-episode and chronic groups (40). Finally, we used the Johns Hopkins WM atlas to mask the region in which we searched for contiguous voxels falling below a set threshold. While we attempted to control for multiple tests, some of the potholes may be false positives. However, it would be expected that there would be an equal number of false positives present in both the EOS and control groups. Yet, we demonstrated a greater number of potholes in the patient group across different thresholds and cluster sizes. At any threshold or minimum cluster size, the control group never had a greater number of potholes compared to the patient group.

In summary, we describe a novel approach to detect WM abnormalities that may not be detected using ROI or voxel-based approaches. This algorithm was

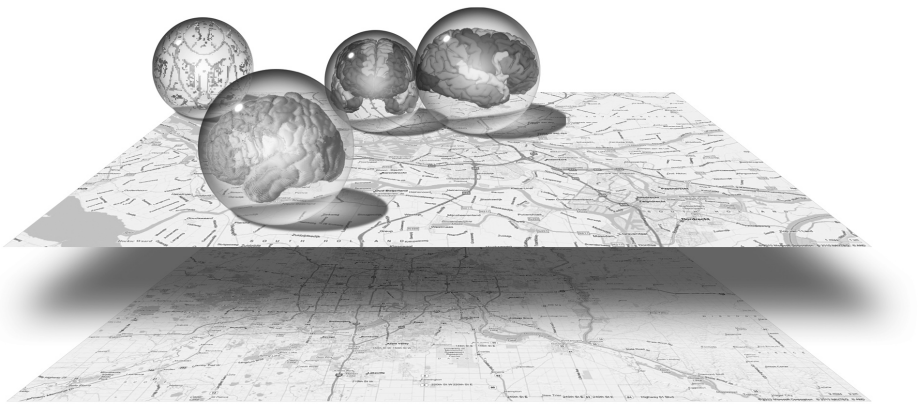
applied to a group of patients with EOS and the patient group had a significantly greater number of WM potholes compared to the control group. Variations of this algorithm could be used to assess for local minima within z-transformed FA maps rather than our approach of thresholding the WM maps. In addition, reductions of the variance may be obtained by regressing variables such as age into the model that have been shown to have an effect on FA.

References

1. Gilmore CP, Donaldson I, Bo L, Owens T, Lowe JS, Evangelou N (2008): Regional variations in the extent and pattern of grey matter demyelination in Multiple Sclerosis: a comparison between the cerebral cortex, cerebellar cortex, deep grey matter nuclei and the spinal cord. J Neurol Neurosurg Psychiatry.
2. Friston KJ, Frith CD (1995): Schizophrenia: a disconnection syndrome? Clin Neurosci. 3:89-97.
3. Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al. (2003): White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry. 60:443-456.
4. Heckers S, Heinsen H, Geiger B, Beckmann H (1991): Hippocampal neuron number in schizophrenia. A stereological study. Arch Gen Psychiatry. 48:1002-1008.
5. Karoutzou G, Emrich HM, Dietrich DE (2008): The myelin-pathogenesis puzzle in schizophrenia: a literature review. Mol Psychiatry. 13:245-260.
6. Uranova NA, Vostrikov VM, Vikhreva OV, Zimina IS, Kolomeets NS, Orlovskaya DD (2007): The role of oligodendrocyte pathology in schizophrenia. Int J Neuropsychopharmacol. 10:537-545.
7. Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, et al. (2001): Genome-wide expression analysis reveals dysregulation of myelination- related genes in chronic schizophrenia. Proc Natl Acad Sci U S A. 98:4746-4751.
8. Kubicki M, McCarley R, Westin CF, Park HJ, Maier S, Kikinis R, et al. (2007): A review of diffusion tensor imaging studies in schizophrenia. J Psychiatr Res. 41:15-30.
9. Kanaan RA, Kim JS, Kaufmann WE, Pearlson GD, Barker GJ, McGuire PK (2005): Diffusion tensor imaging in schizophrenia. Biol Psychiatry. 58:921-929.
10. White T, Nelson M, Lim KO (2008): Diffusion tensor imaging in psychiatric disorders. Top Magn Reson Imaging. 19:97-109.
11. Kyriakopoulos M, Bargiotas T, Barker GJ, Frangou S (2008): Diffusion tensor imaging in schizophrenia. Eur Psychiatry. 23:255-273.
12. Agartz I, Andersson JL, Skare S (2001): Abnormal brain white matter in schizophrenia: a diffusion tensor imaging study. Neuroreport. 12:2251-2254.
13. Flynn SW, Lang DJ, Mackay AL, Goghari V, Vavasour IM, Whittall KP, et al. (2003): Abnormalities of myelination in schizophrenia detected in vivo with MRI, and post-mortem with analysis of oligodendrocyte proteins. Mol Psychiatry. 8:811-820.
14. Jones DK, Symms MR, Cercignani M, Howard RJ (2005): The effect of filter size on VBM analyses of DT-MRI data. Neuroimage. 26:546-554.
15. White T, O'Leary D, Magnotta V, Arndt S, Flaum M, Andreasen NC (2001): Anatomic and functional variability: the effects of filter size in group fMRI data analysis. Neuroimage. 13:577-588.
16. Kanaan RA, Shergill SS, Barker GJ, Catani M, Ng VW, Howard R, et al. (2006): Tract-specific anisotropy measurements in diffusion tensor imaging. Psychiatry Res. 146:73-82.
17. Inoue Y, Nakajima S, Fukuda T, Nemoto Y, Shakudo M, Murata R, et al. (1988): Magnetic resonance images of tuberous sclerosis. Further observations and clinical correlations. Neuroradiology. 30:379-384.
18. Makki MI, Chugani DC, Janisse J, Chugani HT (2007): Characteristics of abnormal diffusivity in normal-appearing white matter investigated with diffusion tensor MR imaging in tuberous sclerosis complex. AJNR Am J Neuroradiol. 28:1662-1667.
19. Rapoport JL, Giedd JN, Blumenthal J, Hamburger S, Jeffries N, Fernandez T, et al. (1999): Progressive cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. Arch Gen Psychiatry. 56:649-654.
20. Rapoport JL, Inoff-Germain G (2000): Update on childhood-onset schizophrenia. Curr Psychiatry Rep. 2:410-415.

21. Asarnow RF, Nuechterlein KH, Fogelson D, Subotnik KL, Payne DA, Russell AT, et al. (2001): Schizophrenia and schizophrenia-spectrum personality disorders in the first-degree relatives of children with schizophrenia: the UCLA family study. Arch Gen Psychiatry. 58:581-588.
22. Rabe-Jablonska JK, Gmitrowticz A (2000): Clinical Picture and duration of prodromal period of schizophrenia in adolescents. Arch Psychiatr Psychother. 2:31-38.
23. Frazier JA, McClellan J, Findling RL, Vitiello B, Anderson R, Zablotsky B, et al. (2007): Treatment of early-onset schizophrenia spectrum disorders (TEOSS): demographic and clinical characteristics. J Am Acad Child Adolesc Psychiatry. 46:979-988.
24. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. (1997): Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 36:980-988.
25. Andreasen NC (1983): The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, IA: The University of Iowa.
26. Andreasen NC (1984): The Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, IA: The University of Iowa.
27. Lachar D, Randle SL, Harper RA, Scott-Gurnell KC, Lewis KR, Santos CW, et al. (2001): The brief psychiatric rating scale for children (BPRS-C): validity and reliability of an anchored version. J Am Acad Child Adolesc Psychiatry. 40:333-340.
28. Dale AM, Fischl B, Sereno MI (1999): Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage. 9:179-194.
29. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. (2004): Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 23 Suppl 1:S208-219.
30. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. (2006): Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage. 31:1487-1505.
31. Smith SM (2002): Fast robust automated brain extraction. Hum Brain Mapp. 17:143-155.
32. Andersson J, Jenkinson M, Smith S (2007): Non-linear optimisation. FMRIB technical report TR07JA1 from www.fmrib.ox.ac.uk/analysis/techrep
33. Andersson J, Jenkinson M, Smith S (2007): Non-linear registration, aka Spatial normalisation. FMRIB technical report TR07JA2 from www.fmrib.ox.ac.uk/analysis/techrep
34. Rueckert D, Sonoda LI, Hayes C, Hill DL, Leach MO, Hawkes DJ (1999): Nonrigid registration using free-form deformations: application to breast MR images. IEEE Trans Med Imaging. 18:712-721.
35. Mori S, Oishi K, Jiang H, Jiang L, Li X, Akhter K, et al. (2008): Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. Neuroimage. 40:570-582.
36. Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S (2004): Fiber tract-based atlas of human white matter anatomy. Radiology. 230:77-87.
37. Oishi K, Zilles K, Amunts K, Faria A, Jiang H, Li X, et al. (2008): Human brain white matter atlas: identification and assignment of common anatomical structures in superficial white matter. Neuroimage. 43:447-457.
38. Uranova NA, Vostrikov VM, Orlovskaya DD, Rachmanova VI (2004): Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. Schizophr Res. 67:269-275.
39. Stark AK, Uylings HB, Sanz-Arigita E, Pakkenberg B (2004): Glial cell loss in the anterior cingulate cortex, a subregion of the prefrontal cortex, in subjects with schizophrenia. Am J Psychiatry. 161:882-888.
40. Friedman JI, Tang C, Carpenter D, Buchsbaum M, Schmeidler J, Flanagan L, et al. (2008): Diffusion tensor imaging findings in first-episode and chronic schizophrenia patients. Am J Psychiatry. 165:1024-1032.

5.1 Evidence for Intact Local but Disrupted Regional Connectivity in Children and Adolescents with Schziophrenia



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Submitted

Abstract

Introduction: It has long been known that specific visual frequencies result in greater blood flow to the striate cortex. These peaks are thought to reflect synchrony of local neuronal firing that is reflective of local cortical networks. Since disrupted neural connectivity is a possible etiology for schizophrenia, our goal was to investigate whether localized connectivity, as measured by aberrant synchrony, is abnormal in children and adolescent with schizophrenia. **Methods**: The subjects included 25 children and adolescents with schizophrenia and 39 age and gender matched controls (age range 8 to 19). Subjects were scanned on a Siemens 3 Tesla Trio scanner while observing flashing checkerboard presented at either 1, 4, 8, or 12 Hz. Pre-processing included time shifting, motion-correction, and normalization. Posprocessing was performed using both a standard GLM model and calculating the number of activated voxels within the occipital lobe region of interest and a Fourier transform analysis. **Results**: Patients had significantly less activation in the occipital lobe compared to controls. There were no differences in the integral or percent signal change of the hemodynamic response function for each of the four frequencies. Finally, both patients and controls demonstrated synchrony, or entrainment in the neural response between 4 and 8 Hz. **Conclusions**: Children and adolescents with schizophrenia demonstrate significantly less activation in the occipital lobe during a multi-frequency flashing checkerboard task. However, there were no differences in the hemodynamic response function and maximum entrainment occurred in both groups at a frequency of 8 Hz. These results may reflect abnormalities in regional connectivity without abnormalities in local connectivity.

Introduction

Schizophrenia is a debilitating illness associated with an array of clinical symptoms and cognitive deficits (1). The neurobiological findings in schizophrenia have been reported in multiple cortical and subcortical brain regions, including all four lobes of the brain, the limbic system, thalamus, striatum, and the cerebellum (1-5). One of the principle theories that integrates the global nature of the illness with the clinical phenotype postulates a disruption in brain connectivity (6-8). Multiple studies using a variety of techniques, including postmortem brain samples (9-11), diffusion tensor imaging (5), and functional magnetic resonance imaging (2) have provided support for the theory of disrupted connectivity in the pathogenesis of schizophrenia.

Connectivity can be parsed into connections residing within local networks (i.e., those that involve communication either within the same or neighboring cortical columns) (12), or long range connections between remote brain regions. The vast majority of neuroimaging studies to date have focused on long range connections between different brain regions using techniques that measure functional and effective connectivity. Measurements of local connectivity has been difficult to measure without invasive techniques.

One potential technique to measure local connectivity is by tapping into the frequency response of the visual cortex during oscillating visual input. There are now multiple studies that demonstrate that flashing stimuli at 8 Hz evokes the greatest neural response in the striate cortex. This 8-Hz peak has been found using PET (13, 14), fMRI (15-20), and ERP studies (15, 21). This 8 Hz peak is thought to be a result of synchrony involving local neuronal firing, which has been defined as neuronal entrainment. Since this synchrony is thought to reflect localized connectivity, it was our goal to study whether entrainment, or local connectivity, is disrupted in children and adolescents with schizophrenia. Disruption of entrainment could reflect either a primary impairment in local connectivity, or alternatively, top-down abnormalities related to higher-order connectivity.

Methods

Subjects

A total of 64 subjects participated in the study, consisting of 25 children and adolescents with schizophrenia (18 males and 7 females) and 39 controls (23 males and 16 females). The demographic information for the two groups is provided in Table 1. All participants underwent a diagnostic evaluation using the Kiddie-SADS-PL (22). Of the children and adolescents with schizophrenia, also known as early-onset schizophrenia (EOS) Additional clinical measures included the Scale for the Assessment of Negative Symptoms (SANS) (23) and the scale for the Assessment of Positive Symptoms (SAPS) (24). The mean age of the patient and control groups were 14.8 (S.D. 3.0) and 14.6 (S.D. 3.3) years, respectively. The age range for both groups was between 8 to 19 years.

The control group had no evidence of a past or present psychiatric disorder and no history of schizophrenia or psychosis in a first degree relative. Patients and controls were excluded if they had a history of substance dependence, ongoing substance abuse (within the past month), IQ less than 70, or a neurological disorder, head injury, or medical illness involving the brain. The study was approved by the Institutional Review Board and informed consent and assent was obtained prior to participation.

Table 1 – *Demographic Characteristics for the Patient and Control Groups and Clinical Characteristics for the Patient Group*

	Patients n = 25	**Controls n = 39**	**p**
Demographic Measures	Mean / SD	Mean / SD	
Age (years / SD)	14.8 / 3.0	14.6 / 3.3	ns
Sex (M/F)	18 / 7	23 / 16	ns
Parental SES	39.2 (13.7)	52.9 (9.3)	<0.001
Clinical Measures			
Age of Onset (years / SD)	12.0 (3.3)		
Psychotic Symptoms (mean / SD)	2.5 / 1.0		
Negative Symptoms (mean / SD)	2.8 / 0.7		
Disorganized Symptoms (mean / SD)	2.0 / 1.0		
Diagnoses			
Schizophrenia	n = 19		
Schizoaffective Disorder	n = 3		
Schizophreniform Disorder	n = 3		

Functional Imaging Paradigm

The fMRI paradigm consisted of a flashing checkerboard that was back projected onto a screen located within the bore. The flashing checkerboard was presented using a block design with four different frequencies (1, 4, 8, and 12 Hz). The timing for each fMRI run are shown in Figure 1. After a 20 second rest period, the stimuli were presented sequentially with a frequency of 20 seconds (10 seconds on followed by a 10 seconds off). Each 20 second block was present twice within each run and there were two runs, each lasting 180 seconds.

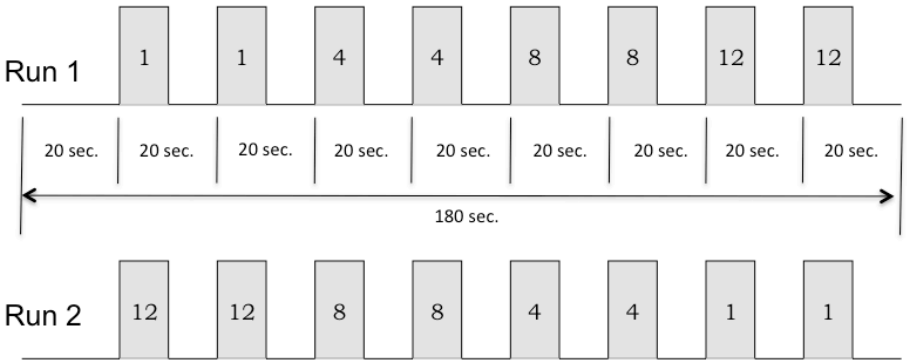

Figure 1 - *Timing for the two runs of the fMRI Flashing Checkerboard Paradigm. The numbers in each block reflect the flashing frequency .presented during the scanning paradigm.*

MRI Sequence

All MR images were acquired with a 3T Siemens MR System (Erlangen, Germany) at the Center for Magnetic resonance Research in University of Minnesota. Head immobilization was performed using a vacuum bag. Following an initial localizer, a series of high-resolution slices were obtained to find the coronal midline. Next a series of high resolution sagittal images were acquired along the midline of the brain. These were used to orient the slice with the midpoint of the posterior slice at the calcarine fissure and oriented along the anterior/posterior commissure (ACPC) plane.

Functional images were acquired using a gradient echo sequence in 16 contiguous axial slices with an in plane resolution of 3.5 x 3.5 mm and a 2 mm slice thickness (Figure 2). Additional parameters were: TE = 30 ms, TR = 1,000 ms, flip angle = 60^{o}, FoV = 224 mm, FoV phase 100%. A total of 180 measurements were obtained in each of the two runs.

Image Processing

The functional images were processed using a combination of Analysis of Functional NeuroImages (AFNI, http://afni.nimh.nih.gov/) (25) and FSL's FMRIB's Software Library (http://www.fmrib.ox.ac.uk/fsl/) (26). Following the conversion from DICOM to Nifti format, slice timing correction and motion correction were performed using AFNI (25). Subjects who had greater than 2.5 mm maximum head motion in either the x, y, or z directions were excluded from the analyses. Following preprocessing, two different algorithms were then utilized to

assess the hemodynamic response function and extent of the activations between the patients and controls.

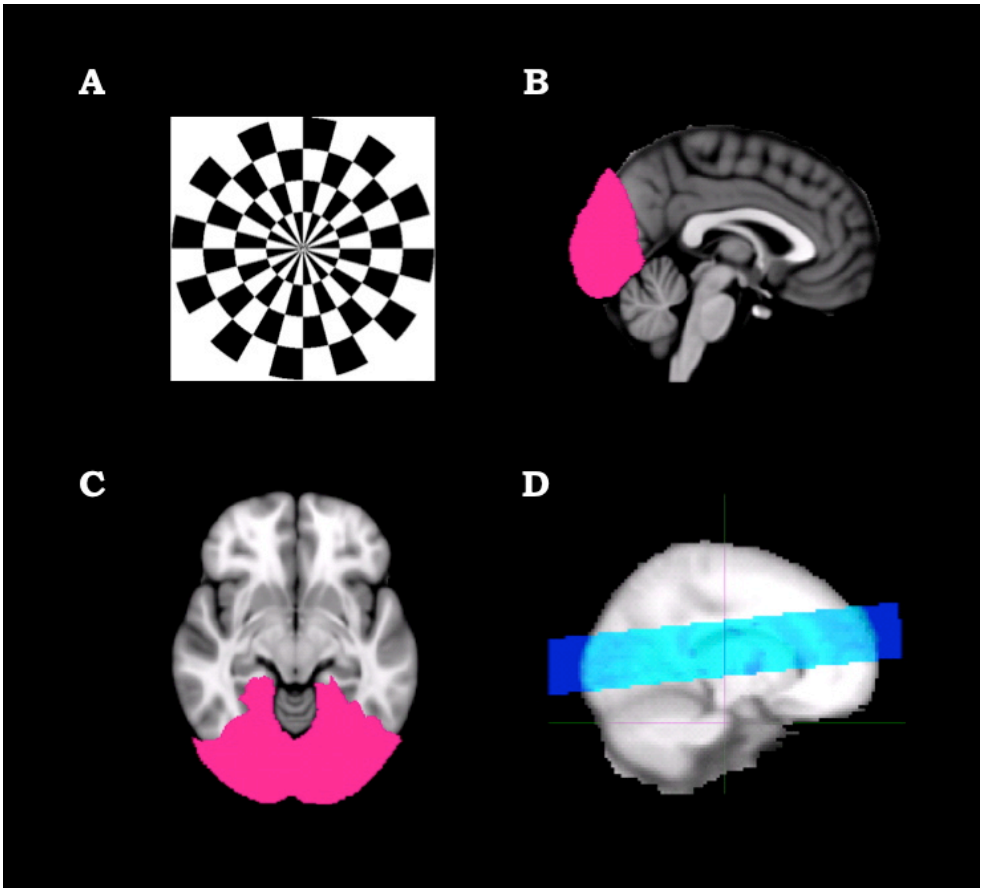

Figure 2 – *(A) Flashing Checkerboard Image that was alternated with it's negative image and presented at four different frequencies; (B) Sagittal and (C) axial sections that show the occipital lobe mask; (D) Slice Prescription for the fMRI acquisition.*

Region of Interest Analysis

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The preprocessed images were oriented to standard MNI space utilizing FSL in a three-stage process. First, a mean EPI image was generated from the fMRI time series for each individual. This mean EPI image was registered to Montreal Neurological Institute space through a 12-parameter transformation (27, 28).

Finally, the 12-parameter transform was applied to the entire fMRI time series for each individual and each run. Single-subject analyses were performed using FMRIB's fMRI Expert Analysis Tool FEAT (http://www.fmrib.ox.ac.uk/fsl/feat5/index.html). The flashing checkerboard was modeled as a square wave with a 0.05 Hz frequency. This time series was convolved with the hemodynamic response function (HRF) that was modeled from a linear combination of gamma functions. Next, a general linear model (GLM) was implemented using FMRIB's Improved Linear Model (FILM). A singular value decomposition (SVD) was utilized to assess the fit of each voxel to the design matrix using local autocorrelation (29). The two within-subject runs were combined using a fixed effects model.

A mask of the occipital lobe in MNI space was obtained from the Harvard-Oxford Atlas within FSL. The volume of activation was obtained using an in-house MATLAB program which calculated the number of voxels within the occipital mask that that exceeded a set threshold, determined as $p < 0.05$ corrected at the cluster level.

Fourier Analysis

To assess the hemodynamic waveform without a priori assumptions of its shape, a voxel-wide Fourier transform was performed on time series data within each run. The first 10 TRs were excluded to allow for a symmetric time series. Voxels which had a peak frequency corresponding to 0.05 Hz (corresponding to the block design with 20 second periods) were identified in the dataset. Next, the largest contiguous set of voxels responding to this frequency was extracted, yielding a set of voxels which had a frequency response identical to the stimulus presentation. The anatomic location of these voxels were evaluated to assure that they were in the occipital lobe. The time series for each voxel within the Fourier identified region of interest in the occipital lobe were averaged and parsed to correspond with either the 1, 4, 8 or 12 Hz stimuli. Finally, the frequency-dependent HRF for each flashing checkerboard frequency was integrated to determine the area under the curve.

Statistical Analyses

The demographic data was assessed using chi-square for categorical and t-tests for continuous data. Assessment of the volume of activation in the occipital lobe was performed using t-tests and ANCOVA with age and sex as covariates. Developmental trends were evaluated using a 2 (group) by 3 (age group) ANOVA. The maximum signal change and integral of the HRF for each of the frequencies were evaluated using a (2) diagnosis by (4) frequency repeated measures mixed model ANOVA, with diagnosis and frequency as the fixed effects, and subject as the random variable. Paired t-tests were used post hoc to evaluate differences between the individual frequencies.

Results

There were no differences in either the mean age or sex distributions between the patient and control groups. There was a significant difference in the parental SES between groups (t = x, df = y, p < 0.001). Of the EOS patients, 19 patients had schizophrenia, 3 had schizoaffective disorder, and 3 had schizophreniform disorder. The age of onset of psychotic symptoms was 12.0 years (SD 3.3). The positive and negative symptoms had a mean within the mild to moderate level and the disorganized symptoms tended to be more mild (Table 1).

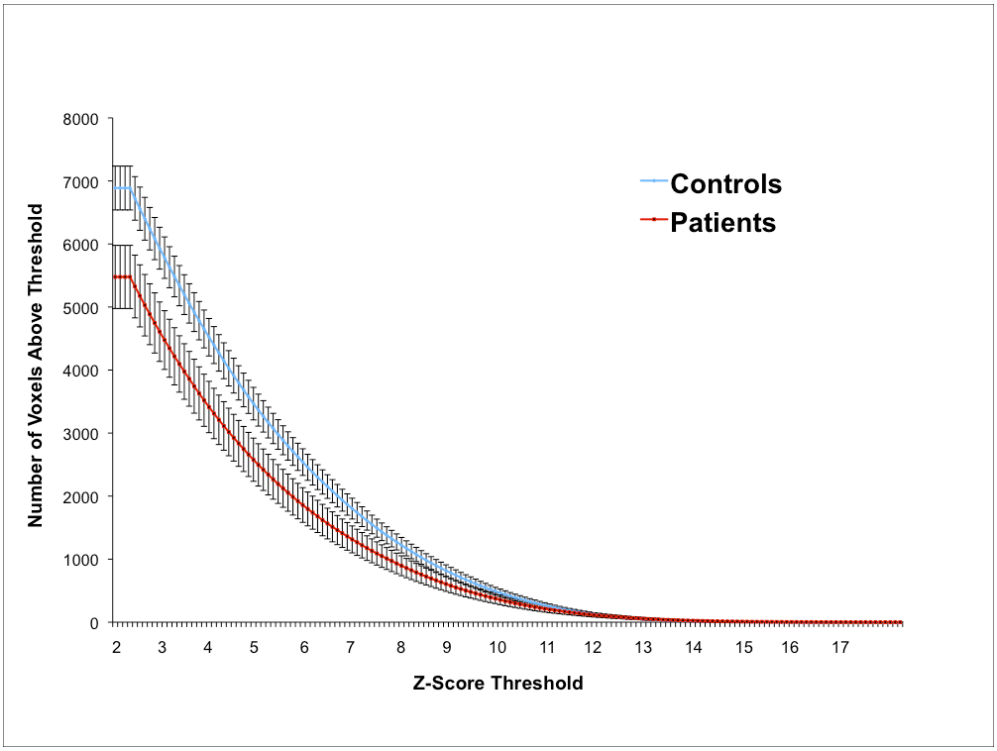


Figure 3 – *Volume of Activation between Patients and Controls at Different Thresholds of Significance.*

Region of Interest Analysis

The volume of activation within the occipital lobe was significantly greater in controls compared to patients (t = 2.0, df 62, p = 0.05). The mean volume of activation within the occipital lobe was 35.8 cc in patients compared to 45.0 cc in controls. Importantly, the difference in was not a result of an arbitrary threshold, since the patient/control difference was present across multiple thresholds (Figure

3). In addition, the difference remained significant when controlling for age ($F_{1,61}$ = 4.4, p = 0.04), age and gender ($F_{1,60}$ = 5.2, p = 0.02), but not when controlling for SES. There were no differences between patients and controls in either the mean z-score of activated voxels.

Developmental effects of occipital lobe activation were evaluated by dividing the subjects into three different age groups (8 to 12, 13 to 16, and 17 to 19 year olds). This resulted in 13 controls in each of the three age groups, and 7, 9, and 9 patients in each of the three age groups, respectively. A 2 (diagnosis) by 3 (age group) ANOVA was performed with an effect of diagnosis ($F_{1,60}$ = 4.4, p = 0.04), but without an effect of age group or an age group by diagnosis interaction. Thus, occipital lobe activation appears to be relatively stable from childhood through older adolescence.

Fourier Analysis

A (2) group by (4) frequency repeated measures mixed model ANOVA was performed to evaluate for differences between patients and controls in the integral of the HRF at the different frequencies. There was a significant effect of frequency ($F_{3,111}$ = 7.1, p = 0.0002), without a significant effect of group or a group by frequency interaction. Since there was not a significant group effect, the patients and controls were pooled for the post hoc analyses of frequency. Paired t-tests found that the 1 Hz flashing checkerboard evoked a significantly less HRF integral than either 4 Hz (t = 3.7, df 38, p = 0.0006) and 8 Hz (t = 4.5, df 38, p < 0.0001). In addition, 8 Hz had a significantly greater HRF integral compared to 12 Hz (t = 2.2, df 38, p = 0.03). These analyses were repeated for the peak percent signal change of the HRF for each frequency and the results were unchanged. The HRF and values for the integral of the HRF for each frequency and group are shown in Figure 4a & 4b, respectively.

Discussion

Several important findings emerge from this work. The first finding is that local connectivity, as measured through entrainment, appears to remain intact in patients with EOS. Entrainment involves a local cooperation of neural networks that when stimulated, becomes time locked with the driving frequency of the visual stimuli (20, 30). Studies performed with PET (13, 14), fMRI (16-20) and EEG (21) have shown that the driving frequency that produces the maximum synchrony is between 8 to 10 Hz and its associated harmonics. We found no differences in the EOS patients and controls in the peak percent signal change or the integral of the HRF at any of the four frequencies tested. In addition, both groups had peak activations at around 8 Hz, paralleling studies performed in healthy adult populations.

Both the patient and control groups had significant differences between the flashing checkerboard between 1 and 4 Hz, and between 1 and 8 Hz. There was also a significant difference between 8 Hz and 12 Hz, but no significant difference

between 4 and 8 Hz. These findings are very similar to the work of Ozus et al (16), who found a significant increase in the maximum percent signal change from 1 Hz to 4 Hz, with a plateau between 4 Hz and 8 Hz and a slight decrease, although not significant, at 12 Hz.

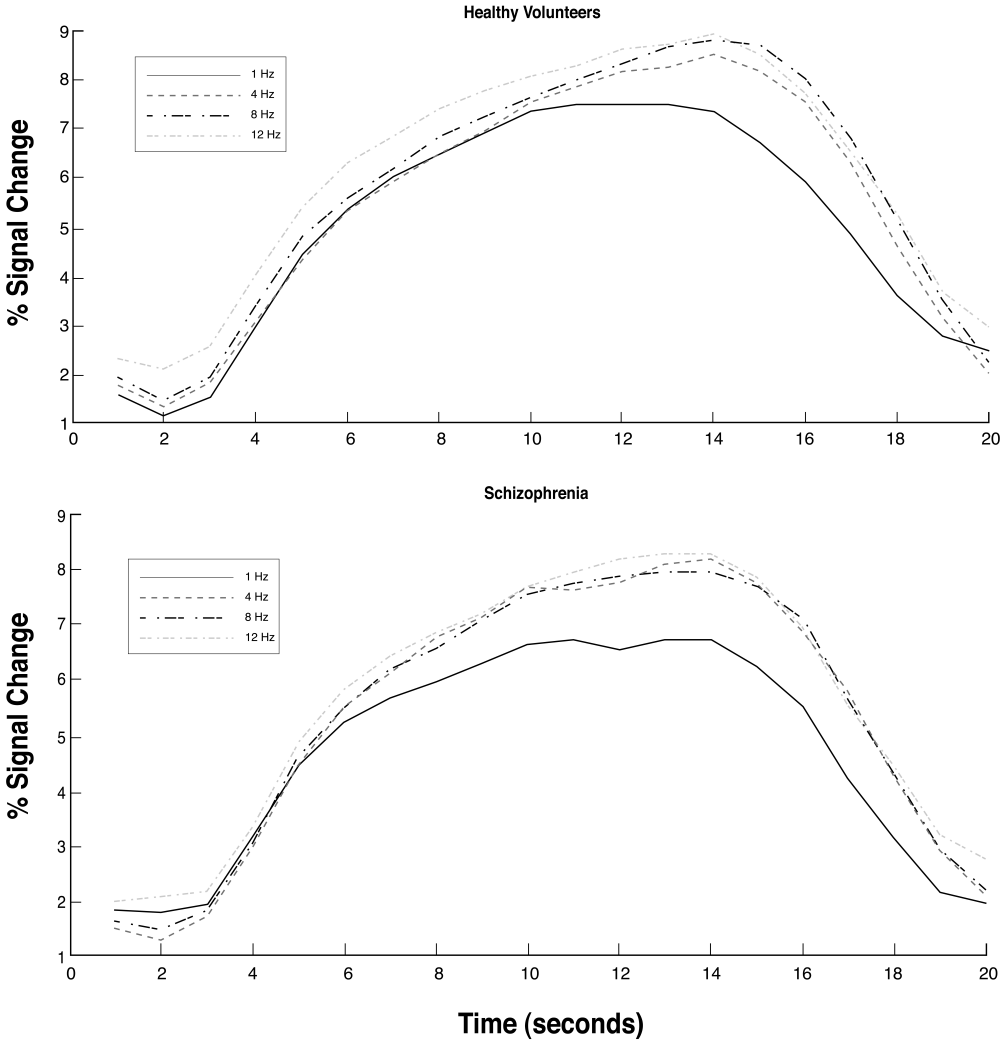


Figure 4 – *Hemodynamic Response Curves at Different Frequencies for the Healthy Volunteers (Top) and the Patients with Schizophrenia (Bottom)*

The second important finding is that although entrainment occurred in both groups, children and adolescents with schizophrenia had a significantly

smaller volume of activation extended throughout the occipital lobe compared to controls. This may reflect intact local connectivity, but alterations in how information is conveyed between remote brain regions.

There has been a growing literature supporting abnormalities in early-stage visual processes in schizophrenia (31). For example, early visual deficits, identified as decreased occipital lobe activation has been shown in EOS patients performing a visuospatial working memory (WkM) task (32). While there is a considerably body of work focusing on higher-level cognitive abnormalities in schizophrenia, there is also evidence that more fundamental processes involving simple sensory networks are disrupted (32-34). This disruption could be due to either direct abnormalities in the primary sensory networks, or alternatively, could represent disruptions as a result of top-down modulation of these neural circuits. It is thought that even lower level perceptual information is processed and modulated through distributed cortical networks (35, 36). However, the nature of how these networks are disrupted in schizophrenia is unclear.

The primary visual cortex (V1) involves a complex local network where 90% of the cells are subject to suppression by neighboring cells (37). Interestingly, postmortem studies in schizophrenia have identified abnormalities in pyramidal cells in layer four of the cortical shell. These cells are involved in longer-range communication between brain regions. Thus, one possibility is that the connectivity within the superficial layers of the cortex are intact in schizophrenia, whereas the abnormalities arise in the deeper layers of the cortex, which are more involved in the long distance transmission of signals.

There are several limitations to the current study. First, we did not parse the stimuli in such a manner in which we could investigate the HMF related to the parvocellular and magnocellular pathways. Given recent findings of abnormalities related to the magnocellular pathways, designing stimuli that would parse out these different pathways might provide greater detail of the aberrant neural pathways. Second, as is true with most fMRI studies, we are assuming a direct relationship between the BOLD effect, which is related to blood flow and oxygen extraction, and neuronal activity. Third, the use of a complementary event-related paradigm may have allowed us to better model the hemodynamic wave form for each of the different frequencies, as well as evaluating additive effects. However, the block paradigm also allowed for the additional Fourier analysis. Finally, a time-course of 10 seconds off and ten seconds on does not allow for a full return of the HRF to baseline, which requires 15 to 20 seconds on average (38), however, having two sequential blocks at the same frequency does allow for accurate modeling of the HRF. The second block of visual activation is only influenced by the frequency of the former block, which is at the same frequency. This design allowed for a 30 second gap between different frequencies when evaluating the HRF.

In summary, we found that children and adolescents with schizophrenia show a similar patterns of entrainment as a matched control group during visual stimulation of the occipital cortex. This may reflect intact local neural connectivity. Children and adolescents with schizophrenia did, however, have significantly less overall volume of activation in the occipital lobe compared to controls. This may

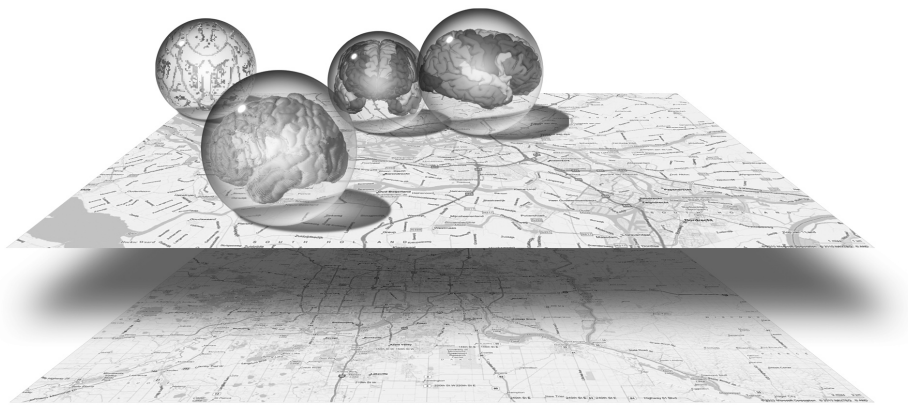
reflect aberrant connectivity from V1 to other regions in the occipital lobe or aberrant top-down control of neuronal signals moving from V1 to other brain regions. Future work in evaluating entrainment coupling EEG with finer, high-resolution imaging (i.e., spin-echo techniques at higher field strengths) may help to better parse the occipital lobe and identify aberrant functional connectivity.

References

1. Andreasen NC, Paradiso S, O'Leary DS (1998): "Cognitive dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical-subcortical-cerebellar circuitry? Schizophr Bull. 24:203-218.
2. MacDonald AW, 3rd, Thermenos HW, Barch DM, Seidman LJ (2009): Imaging genetic liability to schizophrenia: systematic review of FMRI studies of patients' nonpsychotic relatives. Schizophr Bull. 35:1142-1162.
3. Pearlson GD, Marsh L (1999): Structural brain imaging in schizophrenia: a selective review. Biol Psychiatry. 46:627-649.
4. White T, Cullen K, Rohrer LM, Karatekin C, Luciana M, Schmidt M, et al. (2008): Limbic structures and networks in children and adolescents with schizophrenia. Schizophr Bull. 34:18-29.
5. White T, Nelson M, Lim KO (2008): Diffusion tensor imaging in psychiatric disorders. Top Magn Reson Imaging. 19:97-109.
6. Friston KJ (1998): The disconnection hypothesis. Schizophr Res. 30:115-125.
7. Andreasen NC (1997): Linking mind and brain in the study of mental illnesses: a project for a scientific psychopathology. Science. 275:1586-1593.
8. Weinberger DR, Berman KF, Suddath R, Torrey F (1992): Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: A magnetic resonance and regional cerebral blood flow study of discordant monozygotic twins. American Journal Psychiatry. 149:890-897.
9. Dean B, Boer S, Gibbons A, Money T, Scarr E (2009): Recent advances in postmortem pathology and neurochemistry in schizophrenia. Curr Opin Psychiatry. 22:154-160.
10. Lewis DA, Akil M (1997): Cortical dopamine in schizophrenia: strategies for postmortem studies. J Psychiatr Res. 31:175-195.
11. Perlman WR, Weickert CS, Akil M, Kleinman JE (2004): Postmortem investigations of the pathophysiology of schizophrenia: the role of susceptibility genes. J Psychiatry Neurosci. 29:287-293.
12. Douglas RJ, Martin KA (2007): Mapping the matrix: the ways of neocortex. Neuron. 56:226-238.
13. Fox PT, Raichle ME (1984): Stimulus rate dependence of regional cerebral blood flow in human striate cortex, demonstrated by positron emission tomography. J Neurophysiol. 51:1109-1120.
14. Fox PT, Raichle ME (1985): Stimulus rate determines regional brain blood flow in striate cortex. Ann Neurol. 17:303-305.
15. Singh M, Kim S, Kim TS (2003): Correlation between BOLD-fMRI and EEG signal changes in response to visual stimulus frequency in humans. Magn Reson Med. 49:108-114.
16. Ozus B, Liu HL, Chen L, Iyer MB, Fox PT, Gao JH (2001): Rate dependence of human visual cortical response due to brief stimulation: an event-related fMRI study. Magn Reson Imaging. 19:21-25.
17. Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, et al. (1992): Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc Natl Acad Sci U S A. 89:5675-5679.
18. Thomas CG, Menon RS (1998): Amplitude response and stimulus presentation frequency response of human primary visual cortex using BOLD EPI at 4 T. Magn Reson Med. 40:203-209.
19. Zhu XH, Kim SG, Andersen P, Ogawa S, Ugurbil K, Chen W (1998): Simultaneous oxygenation and perfusion imaging study of functional activity in primary visual cortex at different visual stimulation frequency: quantitative correlation between BOLD and CBF changes. Magn Reson Med. 40:703-711.
20. Parkes LM, Fries P, Kerskens CM, Norris DG (2004): Reduced BOLD response to periodic visual stimulation. Neuroimage. 21:236-243.

21. Herrmann CS (2001): Human EEG responses to 1-100 Hz flicker: resonance phenomena in visual cortex and their potential correlation to cognitive phenomena. Exp Brain Res. 137:346-353.
22. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. (1997): Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 36:980-988.
23. Andreasen NC (1983): The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, IA: The University of Iowa.
24. Andreasen NC (1984): The Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, IA: The University of Iowa.
25. Cox RW (1996): AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res. 29:162-173.
26. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. (2004): Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 23 Suppl 1:S208-219.
27. Jenkinson M, Smith S (2001): A global optimisation method for robust affine registration of brain images. Med Image Anal. 5:143-156.
28. Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage. 17:825-841.
29. Woolrich MW, Ripley BD, Brady M, Smith SM (2001): Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage. 14:1370-1386.
30. Rager G, Singer W (1998): The response of cat visual cortex to flicker stimuli of variable frequency. Eur J Neurosci. 10:1856-1877.
31. Butler PD, Javitt DC (2005): Early-stage visual processing deficits in schizophrenia. Curr Opin Psychiatry. 18:151-157.
32. Haenschel C, Bittner RA, Haertling F, Rotarska-Jagiela A, Maurer K, Singer W, et al. (2007): Contribution of impaired early-stage visual processing to working memory dysfunction in adolescents with schizophrenia: a study with event-related potentials and functional magnetic resonance imaging. Arch Gen Psychiatry. 64:1229-1240.
33. Butler PD, Schechter I, Zemon V, Schwartz SG, Greenstein VC, Gordon J, et al. (2001): Dysfunction of early-stage visual processing in schizophrenia. Am J Psychiatry. 158:1126-1133.
34. Butler PD, Zemon V, Schechter I, Saperstein AM, Hoptman MJ, Lim KO, et al. (2005): Early-stage visual processing and cortical amplification deficits in schizophrenia. Arch Gen Psychiatry. 62:495-504.
35. McIntosh AR (1999): Mapping cognition to the brain through neural interactions. Memory. 7:523-548.
36. Mesulam MM (1990): Large-scale neurocognitive networks and distributed processing for attention, language, and memory. Ann Neurol. 28:597-613.
37. Jones HE, Grieve KL, Wang W, Sillito AM (2001): Surround suppression in primate V1. J Neurophysiol. 86:2011-2028.
38. Leniger-Follert E, Hossmann KA (1979): Simultaneous measurements of microflow and evoked potentials in the somatomotor cortex of the cat brain during specific sensory activation. Pflugers Arch. 380:85-89.

5.2 Disrupted Functional Brain Connectivity during Verbal Working Memory in Children and Adolescents with Schizophrenia



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Cerebral Cortex (In Press)

Abstract

Children and adolescents who develop schizophrenia tend to have greater symptom severity than adults who develop the illness. Since the brain continues to mature into early adulthood, developmental differences in brain structure and function may provide clues to the underlying neurobiology of schizophrenia. With an emerging body of evidence supporting disrupted connectivity contributing to the underlying pathophysiology of schizophrenia, it was our goal to assess differences in functional connectivity in children and adolescents who develop schizophrenia. Participants included a total of 28 children and adolescents (14 patients with schizophrenia and 14 age and gender matched controls). All subjects underwent a functional magnetic resonance imaging (fMRI) scan involving a modified Sternberg Item Recognition Paradigm with three working memory loads. Patients had poorer performance at all three working memory loads, without a load by diagnosis interaction. Functional imaging results demonstrated three specific brain networks disrupted in children and adolescents with schizophrenia. These networks include (1) the anterior cingulate and the temporal lobes, bilaterally; (2) the cerebellum with subcortical regions; and (3) the occipital lobe and the cerebellum. Patients with early onset schizophrenia demonstrate abnormal functional connectivity in networks involving limbic, temporal lobe, cerebellum, and early visual processing streams.

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Introduction

Much work over the past several decades supports the theory that schizophrenia involves an impairment in orchestrating the multiple neural networks that participate in higher order cognitive functions (1-4). The development of interconnected brain regions arising during normal development may be altered, leading to the recruitment of either inappropriate regions for task execution, or alternatively, adding additional processing requirements in expected regions. Early studies in adults with schizophrenia utilizing ^{133}Xenon found lower blood flow in frontal regions compared to posterior regions, describing an overall 'hypofrontality' (5). Later studies using both glucose metabolism (FDG-PET) and H_2O^{15} PET also demonstrated hypofrontality in the prefrontal cortex (PFC) during tasks of executive function (6-11).

While these early imaging studies also demonstrated hypofrontality in patients with schizophrenia performing tasks which tap working memory (WkM) (10, 12, 13), these studies were designed neither to specifically tap WkM circuits, nor were patients and controls matched on performance. There is evidence that once subjects are matched on WkM task performance, patients with schizophrenia demonstrate hyperfrontality (14, 15). One thought is that patients recruit greater cognitive resources in order to match their performance with controls, however, once the task difficulty exceeds their capacity, they demonstrate hypofrontality, fitting an inverted 'U' shaped pattern of activity (16).

An alternate thought is that different regions within the PFC show either hypo- or hyperactivity, depending on their location (17). Regions in the anterior, ventral, or medial PFC may demonstrate hyperactivity, while the dorsolateral PFC (DLPFC) shows hypoactivity (17, 18). This would imply that patients are tapping different neural networks to compensate for aberrant DLPFC activity. It would also highlight that differences in image processing strategies, such as spatial filtering, region of interest (ROI) versus voxel based approaches, and block versus event related paradigms, could influence the results of the current studies (19).

In addition to differences in differences between patients and controls in the PFC, a recent meta-analysis of fMRI imaging studies that utilize the n-back paradigm found that patients show increased activation in the caudal anterior cingulate cortex (ACC) (18). Since the majority of studies using alternate cognitive tasks find hypoactivity in the ACC in patients with schizophrenia, Ragland et al. (17) postulates that the increased ACC activity may be related to less conflict in controls, who are making less errors during the task. Patients, however, who are making more errors, are in turn activating the ACC to a greater extent.

It is an open question whether patients with schizophrenia are tapping different neural networks compared to controls. Recent image processing algorithms have been designed to measure functional connectivity between brain regions and under different conditions (20, 21). Functional connectivity is defined as the temporal correlation of the hemodynamic waveforms (i.e., BOLD signal for a specific voxel or regional clusters of voxels over time), between different brain regions (22). One technique to study functional connectivity involves an

independent component analysis (ICA) and is able to identify spatially distinct and temporally coherent components of brain activity (23, 24). When ICA is combined with a specific task, it provides measures of both functional connectivity and task-related functional connectivity. This allows for the identification of neural networks involving a specific cognitive task as well as to test which of these networks are affected by schizophrenia (25). Using a verbal WkM paradigm, Kim et al. (26) found aberrant connectivity in regions of the dorsolateral PFC (DLPFC) and ventrolateral PFC (VLPFC) in adults with schizophrenia. There have been no studies to date evaluating functional connectivity in children and adolescents with schizophrenia, also known as early-onset schizophrenia (EOS).

Children acquire specific cognitive abilities during specific points in development (27). Gross and fine motor function, language, and the various aspects of social cognition all develop and mature within specific windows of neurodevelopment. Higher order cognitive processes, complex problem solving skills and the ability to engage in abstract reasoning show the most robust development after the first decade of life (28, 29). It is known that the PFC has continued development through adolescence shown by studies of synaptic pruning (30), GM changes (31, 32), and myelination (33-36).

There have been two fMRI studies to date that utilized fMRI to evaluate WkM in EOS (37, 38). Haenschel et al. (38) reported abnormal activations during a visual WkM paradigm in temporal and occipital regions during both encoding and retrieval, without any patient/control differences in prefrontal regions. They concluded that patients with EOS have abnormalities in early visual processing streams which impair WkM performance. Pauly et al. (37) using a verbal n-back paradigm combined with neutral and adverse odors found that adolescent patients had hypoactivation in the DLPFC, parietal cortex, and the ACC.

Since EOS has been shown to be on a continuum with adult onset illness (39), and as WkM continues to mature during late adolescence and early adulthood (28, 29), a better understanding of the role of prefrontal regions in EOS is crucial for our understanding the neurobiology of schizophrenia. This is especially true in light of the considerable research documenting PFC abnormalities in adults with schizophrenia (18). If EOS patients do not have abnormalities in prefrontal regions, it is possible that aberrant activity related to these structures are downstream, or developmental effects of the illness (40). Our goal for this study was to investigate the functional connectivity of verbal WkM in EOS, with a primary interest in evaluating for the presence of aberrant PFC function.

Methods

Subjects

The participants included a total of 28 children and adolescents, including 14 patients with a schizophrenia spectrum disorder and 14 age and sex matched controls. The age of inclusion was between 8 and 19 years of age with the mean age of the patient and control groups was 15.1 (S.D. 2.6) and 15.0 (S.D. 2.8) years,

respectively and both groups consisted of 12 boys and 2 girls (Table 1). Both the patients and controls underwent a thorough diagnostic assessment using the Kiddie-SADS-PL (41). Females who had started their menses underwent pregnancy testing and none were found pregnant. Of the patients, 11 had a diagnosis of schizophrenia, 2 had schizoaffective disorder, and 1 had schizophreniform disorder. Additional clinical measures included the Scale for the Assessment of Negative Symptoms (SANS) (42) and the Scale for the Assessment of Positive Symptoms (SAPS) (43).

Table 1 – ***Demographic and Clinical Characteristics for the Patient and Control Groups***

	Patients n = 14	**Controls n = 14**	**p**
Demographic Measures	Mean / SD	Mean / SD	
Age (years / SD)	13.4 / 2.6	13.5 / 2.8	ns
Sex (M/F)	12 / 2	12 / 2	ns
Parental SES	40.4 (10.2)	53.7 (7.4)	0.0007
Clinical Measures			
Psychotic Symptoms (mean / SD)	2.5 / 1.2		
Negative Symptoms (mean / SD)	2.6 / 0.8		
Disorganized Symptoms (mean / SD)	2.0 / 1.2		
Antipsychotic Medications	**Number**	**Mean Dose**	
Aripiprazole	3	23.3	
Ziprasidone	2	40	
Risperidone	3	3.2	
Quetiapine	2	250	
None	2		

The control group had no evidence of a past or present psychiatric disorder and no history of schizophrenia or psychosis in a first degree relative. Patients and controls were excluded if they had a history of substance dependence, ongoing substance abuse (within the past month), IQ less than 70, or a neurological disorder, head injury, or medical illness involving the brain. In addition subjects were screened for any contraindications for participating in MRI or if they wore braces. Twelve of the 14 patients were on medication at the time of the scanning (Table 1).

The study was approved by the Institutional Review Board at the University of Minnesota and informed consent and assent was obtained prior to participation.

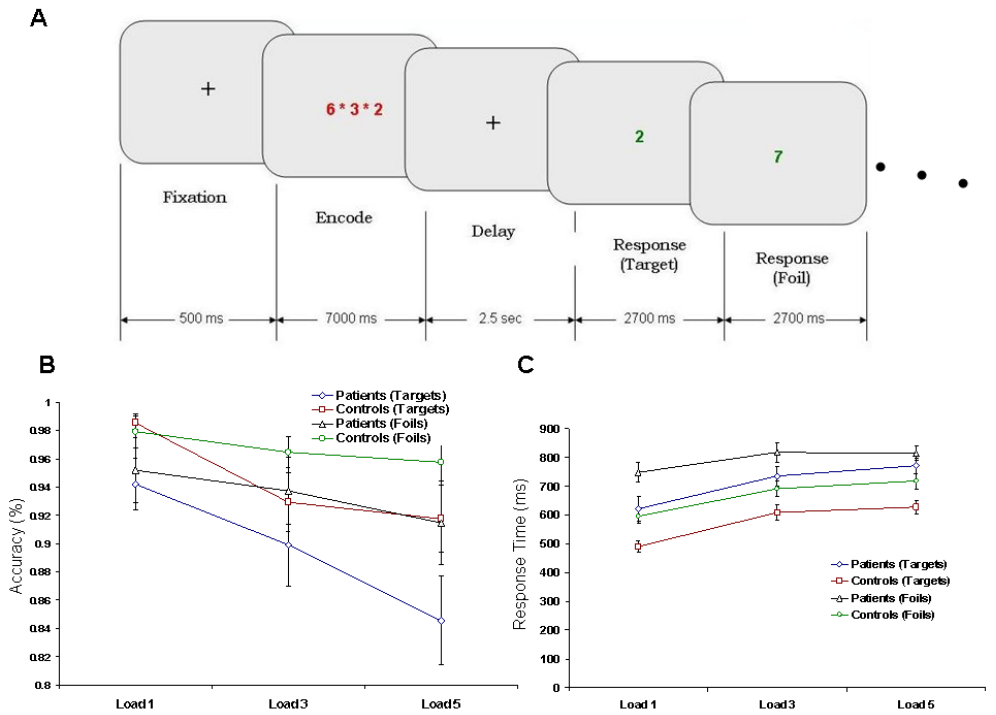


Figure 1 – *(A) Timing for one Trial of the Sternberg Item Recognition Paradigm. Each block consisted of loads of either 1, 3, or 3 digits (3 are shown in this example), followed by a delay, and a string of 16 targets or foils. (B) Behavioral Results for Accuracy in Patients and Controls. Accuracy is shown for both the targets and foils. (C) Response Time for targets and foils for the patient and control groups.*

Working Memory Paradigm

Subjects rested comfortably in the scanner with head mobilization performed using a vacuum bag. The stimulus presentation paradigms were programmed using E-Prime (Psychology Software Tools, Inc.) and were triggered following the fourth repetition time (TR) of the scanner. Prior to scanning, subjects had two practice sessions, one while seated in a chair in front of a monitor, and the second session in a mock scanner with back-presented stimuli, identical to that used during the scanning session. The subjects practiced until it was observed that they understood and were comfortable performing the task. Subjects were told to respond as quickly as possible, but to try to make correct responses.

The stimuli consisted of a modified version of the Sternberg Item Recognition Paradigm (SIRP) (44) (Figure 1-A) and was identical to that used by the Mind Clinical Imaging Consortium study of adults with schizophrenia (26, 45). Subjects were presented the word 'Learn,' which was followed by either 1, 3, or 5 digits presented simultaneously on the screen for 7 seconds. This was followed by a series of sixteen single digits presented sequentially at a rate of 2.7 seconds for each digit. The participants wore a set of gloves with buttons and were instructed to push the right thumb button if the number on the screen matched a number they had seen during the 'learn' set (target), or the left thumb button if the number did not match a number in the 'learn' set (foil). There were three runs, each lasting five minutes and 58 seconds. Each run consisted of two blocks of the 1, 3, and 5 digit memory loads.

MRI Sequence

All MR images were acquired with a 3T Siemens MR System (Erlangen, Germany) at the Center for Magnetic Resonance Research at the University of Minnesota. Following an initial localizer, a coronal scout image (12 slices; field of view (FoV) 224 mm, TR 2000 ms; TE 72 ms; resolution 2.3 x 1.8 x 2 mm) was obtained to locate the coronal midline. Following alignment, sagittal images were acquired along the coronal midline (12 slices; FoV 224 mm; TR 2040 ms, TE 62 ms; resolution 1.2 x 0.9 x 2 mm). These sagittal slices were used to orient the volume along the anterior/posterior commissure (ACPC) plane.

Functional images were acquired using a gradient echo sequence with 27 axial slices and an in plane resolution of 3.4 x 3.4 mm with a 4 mm slice thickness and a 1mm gap. Additional sequence parameters include: TE = 30 ms, TR = 2000 ms, flip angle = 90^{o}, FoV = 220 mm. A total of 177 volumes were obtained within each of the three runs, for a total of 531 volumes.

Image Processing

The functional images were processed using a combination of Analysis of Functional NeuroImages (AFNI, http://afni.nimh.nih.gov/) (46) and FSL's FMRIB's Software Library (http://www.fmrib.ox.ac.uk/fsl/) (47). Following the conversion from DICOM to Nifti format, slice timing correction and motion correction were performed using AFNI (46). Subjects who were unable to complete three runs of the SIRP or subjects who had greater than 2.5 mm of motion in either the x, y, or z directions were excluded from the analyses.

Images were oriented to standard MNI space utilizing FSL in a three-stage process. First, a mean EPI image was generated from the fMRI time series for each individual. This mean EPI image was registered to Montreal Neurological Institute space through a 12-parameter transformation (48, 49). Finally, the 12-parameter transform was applied to the entire fMRI time series for each individual and each run. The data were spatially smoothed with an 8mm full width at half-maximum Gaussian kernel (50). The resulting coordinates were converted to the Talairach and

Tournoux standard space for anatomical mapping (51) using MNI2Tal (Mathew Brett, http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach).

Independent Component Analysis

Following preprocessing, a group ICA was performed on the preprocessed data (23, 24). The methods prescribed by this process were performed via the group ICA of fMRI (GIFT: Matlab toolbox version 1.3c http://icatb.sourceforge.net). ICA is a statistical and computational data-driven technique that attempts to extract temporally related signals that are hidden within sets of random or unrelated variables. It assumes that the fMRI data are linear mixtures of independent source signals and attempts to extract maximally independent signals and their mixing coefficients. The principle behind ICA is that these independent source signals represent coherent groupings of blood oxygen level dependent (BOLD) signal change, often referred to as component maps, that are thought to be functionally relevant. Since ICA is a data-driven approach, the functional networks are generated without any assumptions about the shape of the hemodynamic time courses.

The spatial maps generated by ICA were averaged together across the three scans sessions and resulted in 27 independent component spatial maps for every subject. These 27 independent component spatial maps represent the regions of the brain related to a specific timecourse. Every voxel within a component spatial map contains a z-score, with high z-scores reflecting a greater contribution to the associated timecourse.

Component Selection

One of the strengths of ICA is its ability to find noise related components that represent head motion, ventricle activity, eyeball movement, and other signal artifacts. Thus, we first evaluated each of the spatial maps and eliminate those with motion or other artifacts. These were readily identified by symmetric activations on the opposite sides of the skull, activations within the ventricles, or activation within the eye itself. This resulted in the removal of 8 components related to artifacts.

The second phase consisted of identifying and limiting the components to only those that were task-related. A regression was performed on the ICA component timecourses using a SPM5 GLM design matrix coded for encoding and retrieval at each of the three WkM loads. This resulted in a set of beta weights for each experimental regressor associated with a particular subject and component. The resulting beta weights represent the degree to which the component was modulated by the WkM task load relative to the fixation baseline (i.e., a high beta weight represents a large task-related modulation of a component for a given regressor). Finally, only components that showed an effect of load for either encoding or retrieval were included. This resulted in the elimination of all but 13

components. These 13 components were used to assess for patient and control differences using a mixed-model repeated-measures ANOVA.

Statistical Analyses

The demographic data was assessed using chi-square for categorical data and t-tests for continuous data. A 2 (diagnosis) x 2 (encode/retrieval) x 3 (load) mixed-model repeated-measures ANOVA was performed using diagnosis, task, and load as the fixed effects, and subject as the random variable. The task related beta-weights for each of the individual components were entered into a 2 (diagnosis) by 3 (load) mixed-model repeated-measures ANOVA. To control for performance differences between patients and controls, a 2 (group) by 3 (load) by 3 (run) mixed model ANOVA was performed using response time (RT) as a covariate.

Results

There were no differences in age or sex between the patients with schizophrenia and the control group (Table 1). There was a significant difference in the socioeconomic status (SES) between the two groups, with the controls coming from families with a significantly higher SES. The patients had on average mild to moderate negative and positive symptoms at the time of scanning and mild disorganized symptoms (Table 1). All but two of the patients were on antipsychotic medications during the scanning session.

Behavioral Results

Both patients and controls responded faster to the probes than the foils (paired $t = 13.3$, $df = 80$, $p < 0.0001$). There were significant effects of both load ($F_{2,50} = 52.1$, $p < 0.0001$) and diagnosis ($F_{1,25} = 11.5$, $p = 0.002$) for the probe RT using a 2 (group) by 3 (load) mixed model repeated measures ANOVA (Figure 2). Similarly there was also an effect of load ($F_{2,50} = 35.7$, $p < 0.0001$) and diagnosis ($F_{1,25} = 10.4$, $p = 0.004$) for the foil RT. Neither the probe nor the foil RT conditions had an interaction between load and diagnosis. Evaluating accuracy, a 2 (group) by 3 (load) mixed model repeated measures ANOVA found a significant effect of load for both the probes ($F_{2,50} = 11.2$, $p < 0.0001$) and the foils ($F_{2,50} = 3.4$, $p < 0.05$), without significant effects of either diagnosis or a load by diagnosis interactions.

Imaging Results

The thirteen components that passed the selection criteria were grouped depending on whether they were significantly related to the encoding phase, retrieval phase, or both. There were three independent components related solely to encoding, six components related solely to retrieval, and four components related to both encoding and retrieval. To determine which brain regions were representative of a component, a random-effects analysis in SPM5 was performed

across all subjects on the raw spatial maps for each individual component using a false discovery rate (FDR) correction (FDR, $p < 1 \times 10^{-12}$)(52). The statistical analyses of the spatial maps do not determine whether a given component is task-related, but merely represents a statistical visualization of the relevant regions for that component under a statistically relevant threshold.

Table 2 – Brain Networks Associated with Working Memory Load and their differences between Patients and Controls

Brain Network	**Effect of Load**		**Effect of Diagnosis**	**Load by Diagnosis Interaction**
Encode Only	**$F_{2,52}$ / p**		**$F_{1,26}$ / p**	**$F_{2,52}$ / p**
Occipital Lobe – Cerebellum	5.7 / 0.005			4.8 / 0.01
Bilateral Temporal – Anterior Cingulate	3.5 / 0.04		3.8 / 0.05	
Cerebellum	5.14 / 0.009			
Retrieval Only				
ACC – Medial Frontal	8.1 / 0.0008			
Occipital Lobe	6.1 / 0.004			
Left Sup. Parietal – Left Motor – Right Cerebellum	11.0 / 0.0001			
Medial Parietal – Orbitofrontal Cortex	3.2 / 0.05			
Anterior PFC – Cerebellum – Motor	9.4 / 0.0003			
Occipital Lobe	6.9 / 0.0003			
Encoding and Retrieval	**Encode**	**Retrieval**	**Retrieval**	
DLPFC – Premotor – ACC – Bilateral Cerebellum	15.6 / < 0.0001	35.9 / < . 0001		
PFC – ACC – Bilateral Parietal – PCC	4.1 / 0.02	26.3 / < . 0001		
Bilat. DLPFC – Bilateral Parietal	3.9 / 0.03	7.5 / 0.001		
Striatum - Cerebellum	6.1 / 0.003	6.9 / 0.002	4.2 / 0.05	

Networks Associated with Encoding

The three independent components (ICs) that are solely associated with encoding include a network involving (1) the occipital lobe and cerebellum; (2) the

anterior cingulate cortex (ACC) and the temporal lobes bilaterally; and (3) a cerebellar network (Table 2). Of these three independent components, the network involving the ACC and temporal lobes demonstrated an effect of diagnosis and the network involving the occipital lobe and cerebellum had a diagnosis by load interaction. Thus two out of the three encode-related IC networks showed differences between patients and controls. See Figure 2-A for the spatial distribution of the individual components and Figure 2-D for the IC networks that were different between patients and controls.

Networks Associated with Retrieval

The six ICs associated solely with retrieval are shown in Table 2 and Figure 2-B. These included a network involving the ACC and medial frontal lobe, two networks involving the occipital lobe; two networks involving motor function, including a network involving the left superior parietal, left motor, and right cerebellum; and a network involving the medial PFC, motor, and cerebellum. Finally, there was a network involving the medial parietal lobe and the orbitofrontal cortex (OFC). While all of these components were significantly related to load, none of them demonstrated statistically significant differences between patients and controls.

Networks Associated with both Encoding and Retrieval

The remaining four components (Table 2), showed significant effects of load during both the encoding and retrieval phases of the WkM task. These five ICs include networks associated with (1) dorsolateral prefrontal cortex (DLPFC), premotor, ACC, and bilateral cerebellum; (2) the PFC, bilateral parietal, and the posterior cingulate cortex (PCC); (3) bilateral DLPFC and bilateral parietal; and (4) the cerebellum and striatum (Figure 2-C). While many of these networks include brain regions typically associated with WkM performance, only the networks involving the cerebellum and striatum showed statistically significant differences between patients and controls (Figure 2-D & Figure 3-C).

Brain Connectivity and Behavioral Data

To assess whether the differences between patients and controls were secondary to differences in performance, a 2 (group) by 3 (load) by 3 (run) mixed model ANOVA was performed using RT as a covariate. In this analysis, runs were not averaged, allowing for a direct matching between the beta weights of the ICA analysis within each run with the behavioral performance for the corresponding run. When using the mean RT for accurate probes as a covariate, the significant findings remained unchanged. The network between the ACC and bilateral temporal lobes showed a significant effect of diagnosis ($F_{1,30.1} = 7.5$, $p = 0.007$) and there was a load by diagnosis interaction for the network involving the occipital lobe and the cerebellum ($F_{2,207} = 5.8$, $p = 0.004$). During retrieval, the network

involving the striatum and cerebellum was no longer significant, however, a network involving the cerebellum, motor strip, and frontal pole was significant ($F_{1,26.2} = 5.5$, $p = 0.03$).

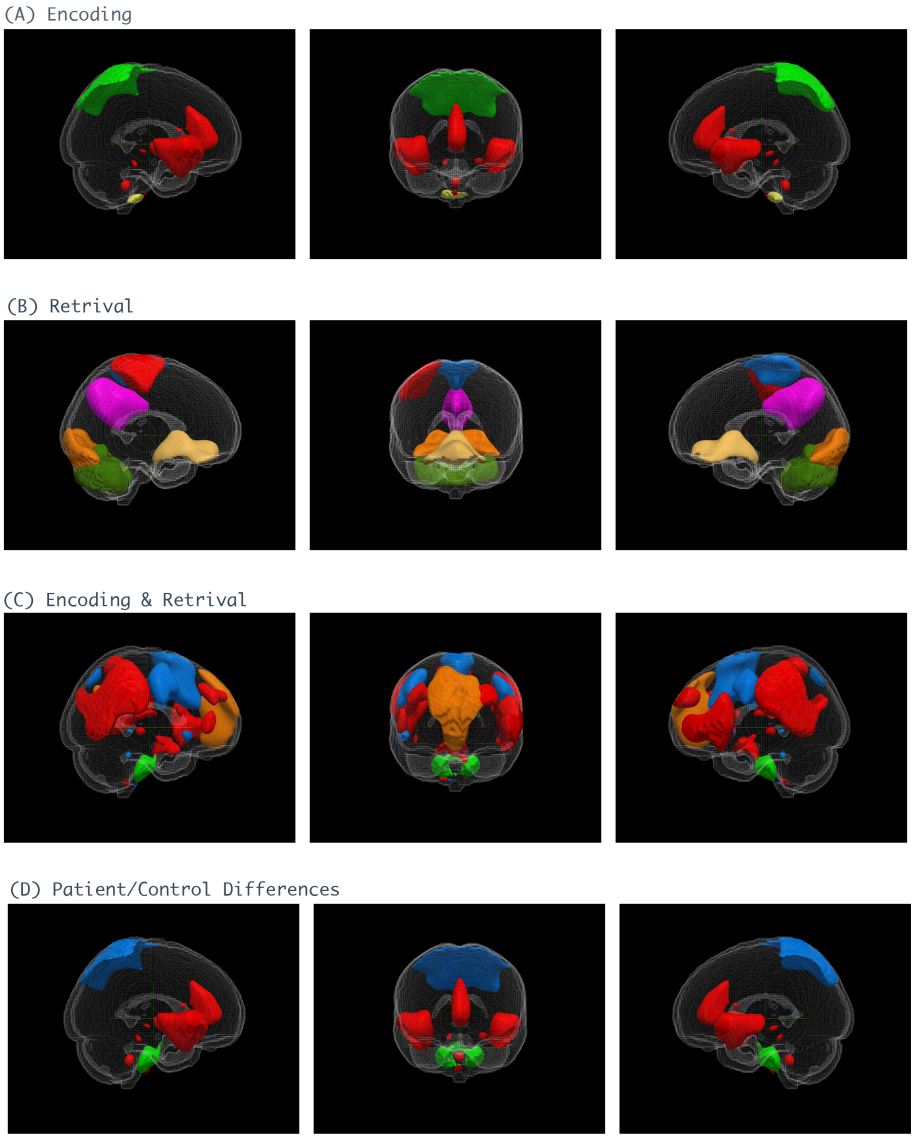


Figure 2 – *Brain Regions which demonstrated significant effects of load during (A) Encoding. (B) Retrieval, and. (C) during both Encoding and Retrieval. (D) Brain Regions*

which demonstrated significant differences in Functional Connectivity between Patients and Controls. (Figure 9, Back Cover)

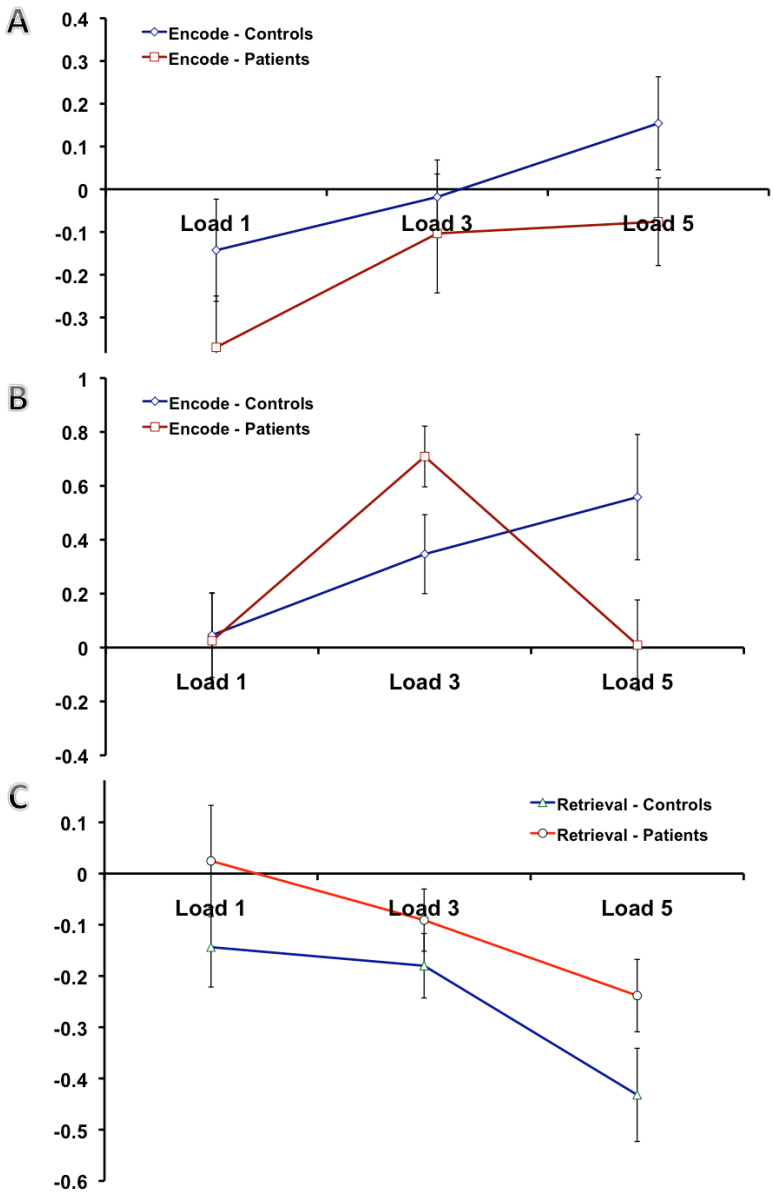


Figure 3 –*Patient/Control Differences in the Task-Related Beta Weights for (A) connectivity between the anterior cingulate cortex and the temporal lobes bilaterally; (B) connectivity between the occipital lobe and the cerebellum; and (C) connectivity between the cerebellum and striatum.*

When using the mean RT for accurate foils as a covariate, the results also remained quite similar. The load by diagnosis interaction for the network involving the occipital lobe and the cerebellum remained significant ($F_{2,207}$ = 6.1, p = 0.003), whereas the network between the ACC and bilateral temporal lobes showed a trend effect of diagnosis ($F_{1,30.1}$ = 3.4, p = 0.07). During retrieval, the network involving cerebellum, motor strip, and frontal pole trended to significance ($F_{1,26.2}$ = 4.0, p = 0.056).

Discussion

Functional connectivity is defined as the temporal correlation of the hemodynamic waveforms (i.e., BOLD signal for a specific voxel or regional clusters of voxels over time), between different brain regions (22). The theory is that distributed neural networks within the brain mediate the performance on a specific task. Different tasks tap both separate and common distributed networks. With EOS schizophrenia being on a continuum with adult onset schizophrenia (39), and with the considerable evidence identifying DLPFC abnormalities in adults with schizophrenia (17, 18), we predicted disrupted functional connectivity between the DLPFC and regions known to involve working memory networks, as has been shown in studies of adults with schizophrenia (26).

Importantly, we found that patients and controls were both tapping neural networks known to be involved in WkM performance (Table 2) (53-55). These networks were highly significant for the effect of load and included widespread networks that included the DLPFC, posterior parietal lobe, anterior and posterior cingulate cortex, temporal lobe, and the striatum (Figure 2). In addition, brain regions known to involve motor performance were shown to have regional connectivity during the retrieval phase of the WkM task. This was expected, since the motor response was present only during this phase of the task. Thus, there is good evidence that both EOS patients and controls were tapping neural networks known to be active during WkM tasks.

There were three networks that showed differences in functional connectivity between patients and controls (Figure 2-D & Figure 3). The first difference occurred during encoding and involved network connections between the ACC and the temporal lobe (Figure 3-A). The second abnormal circuit, also present during encoding, involved connectivity between the occipital lobe and the cerebellum. The differences in this second network demonstrated a load by diagnosis interaction, as shown in Figure 3-B. During retrieval, a network connecting the cerebellum and striatum demonstrated statistically significant differences between patients and controls (Figure 3-C).

Interestingly, none of these networks that showed differences between patients and controls included regions in the DLPFC. Haenschel et al. (38) also did not find differences in the DLPFC in adolescent patients performing a visuospatial WkM task, however, Pauly et al. (37) used a verbal n-back paradigm combined with neutral and adverse odors and reported hypoactivation in the DLPFC, parietal cortex, and the ACC in adolescents with schizophrenia. It is possible that the added

component of adverse and neutral odors altered the task resulting in differences in prefrontal activation patterns (56). No studies to date have studied functional connectivity in adolescents with schizophrenia.

Structural imaging studies of childhood-onset schizophrenia (COS), referring to those who have the onset of schizophrenia before 13 years of age, have identified important differences in the developmental neurobiology of schizophrenia. More pronounced differences occur in parietal lobe regions, which progress to prefrontal regions as the children go from early to later adolescence (57, 58). Also during this period of development, functional imaging studies have demonstrated a shift from more diffuse patterns of activity to localized patterns, as brain maturation occurs (59). Thus, the combination of less GM differences in younger patients, coupled with more diffuse activation patterns associated with development, may result in smaller effect sizes for patients and controls, and thus less likely to be identified over the underlying noise. Finally, there is evidence that prefrontal dopamine homeostasis is different in adolescents than in adults (60),

There are several limitations to the current study, the first being the relatively small sample size. However, all subject performed three separate runs with performance rates greater than chance. In addition, we found significant activations within WkM networks as well as patient/control differences in several brain networks. All but two of the patients were on antipsychotic medications at the time of the scan, which may alter functional connectivity. However, there is evidence in adults that antipsychotic medications tend to normalize functional connectivity (61).

In summary, we found evidence of disrupted connectivity in networks involving the anterior cingulate, cerebellum, striatum, and occipital lobe in patients with EOS. We did not find evidence for aberrant connectivity in regions of the PFC in this study. Since WkM performance continues to develop through adolescence and into early adulthood (28, 29), one possible explanation is that aberrant PFC function tends to be a downstream effect that occurs with continued development. Alternatively, the PFC may be inherently noisier in earlier development and thus is less likely to show differences. If the former is true, however, it would suggest that aberrant PFC is a developmental downstream effect of an earlier insult, perhaps involving the limbic system (40). Future studies should evaluate larger populations with EOS across different developmental periods. Differences in the patterns of functional connectivity between children and adolescents and adults with schizophrenia may provide a window into the neurodevelopmental course of the illness.

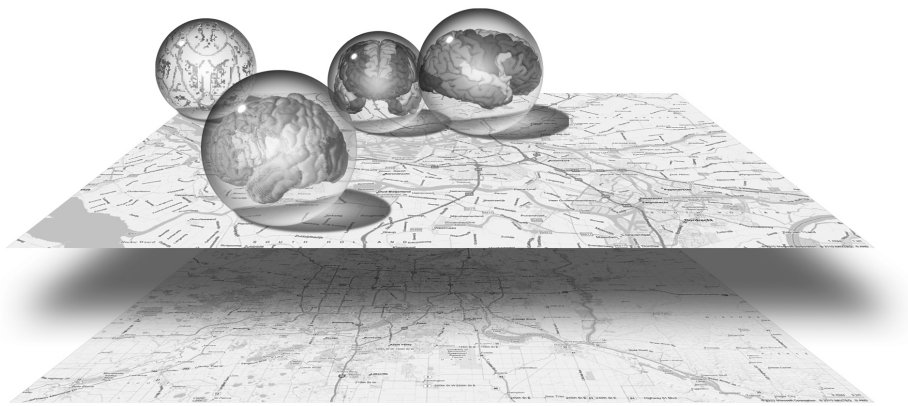
References

1. Weinberger DR, Berman KF, Suddath R, Torrey EF (1992): Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: a magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. Am J Psychiatry. 149:890-897.
2. Andreasen NC (1997): Linking mind and brain in the study of mental illnesses: a project for a scientific psychopathology. Science. 275:1586-1593.
3. Friston KJ, Frith CD (1995): Schizophrenia: a disconnection syndrome? Clin Neurosci. 3:89-97.
4. Fuster JM (1999): Synopsis of function and dysfunction of the frontal lobe. Acta Psychiatr Scand Suppl. 395:51-57.
5. Franzen G, Ingvar DH (1975): Absence of activation in frontal structures during psychological testing of chronic schizophrenics. J Neurol Neurosurg Psychiatry. 38:1027-1032.
6. Andreasen NC, Cohen G, Harris G, Cizadlo T, Parkkinen J, Rezai K, et al. (1992): Image processing for the study of brain structure and function: problems and programs. J Neuropsychiatry Clin Neurosci. 4:125-133.
7. Weinberger D, Berman KF (1988): Speculation on the meaning of cerebral metabolic hypofrontality in schizophrenia. Schizophrenia Bulletin. 14:157-168.
8. Weinberger DR, Berman KF, Suddath R, Torrey F (1992): Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: A magnetic resonance and regional cerebral blood flow study of discordant monozygotic twins. American Journal Psychiatry. 149:890-897.
9. Andreasen NC, O'Leary DS, Flaum M, Nopoulos P, Watkins GL, Boles Ponto LL, et al. (1997): Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. Lancet. 349:1730-1734.
10. Buchsbaum MS, Wu JC (1987): Hypofrontality in schizophrenia as assessed by PET. Am J Psychiatry. 144:122-123.
11. Carter CS, Perlstein W, Ganguli R, Brar J, Mintun M, Cohen JD (1998): Functional hypofrontality and working memory dysfunction in schizophrenia. Am J Psychiatry. 155:1285-1287.
12. Andreasen NC, Rezai K, Alliger R, Swayze VW, 2nd, Flaum M, Kirchner P, et al. (1992): Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia. Assessment with xenon 133 single-photon emission computed tomography and the Tower of London. Arch Gen Psychiatry. 49:943-958.
13. Weinberger DR, Berman KF (1988): Speculation on the meaning of cerebral metabolic hypofrontality in schizophrenia. Schizophr Bull. 14:157-168.
14. Manoach DS, Press DZ, Thangaraj V, Searl MM, Goff DC, Halpern E, et al. (1999): Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. Biol Psychiatry. 45:1128-1137.
15. Callicott JH, Bertolino A, Mattay VS, Langheim FJ, Duyn J, Coppola R, et al. (2000): Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb Cortex. 10:1078-1092.
16. Manoach DS (2003): Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr Res. 60:285-298.
17. Ragland JD, Yoon J, Minzenberg MJ, Carter CS (2007): Neuroimaging of cognitive disability in schizophrenia: search for a pathophysiological mechanism. Int Rev Psychiatry. 19:417-427.
18. Glahn DC, Ragland JD, Abramoff A, Barrett J, Laird AR, Bearden CE, et al. (2005): Beyond hypofrontality: a quantitative meta-analysis of functional neuroimaging studies of working memory in schizophrenia. Hum Brain Mapp. 25:60-69.
19. Carter CS, Heckers S, Nichols T, Pine DS, Strother S (2008): Optimizing the design and analysis of clinical functional magnetic resonance imaging research studies. Biol Psychiatry. 64:842-849.
20. Calhoun VD, Adali T, Kiehl KA, Astur R, Pekar JJ, Pearlson GD (2006): A method for multitask fMRI data fusion applied to schizophrenia. Hum Brain Mapp. 27:598-610.
21. Meyer-Lindenberg A, Poline JB, Kohn PD, Holt JL, Egan MF, Weinberger DR, et al. (2001): Evidence for abnormal cortical functional connectivity during working memory in schizophrenia. Am J Psychiatry. 158:1809-1817.
22. Friston KJ, Frith CD, Liddle PF, Frackowiak RS (1993): Functional connectivity: the principal-component analysis of large (PET) data sets. J Cereb Blood Flow Metab. 13:5-14.
23. Calhoun VD, Adali T, Pearlson GD, Pekar JJ (2001): Spatial and temporal independent component analysis of functional MRI data containing a pair of task-related waveforms. Hum Brain Mapp. 13:43-53.

24. Calhoun VD, Adali T, Pearlson GD, Pekar JJ (2001): A method for making group inferences from functional MRI data using independent component analysis. Hum Brain Mapp. 14:140-151.
25. Calhoun V, Adali T, McGinty V, Pekar J, Watson T, Pearlson G (2001): fMRI activation in a visual-perception task: network of areas detected using the general linear model and independent components analysis. Neuroimage. 14:1080-1088.
26. Kim D, Andreasen NC, Belger A, Bockholt HJ, Brown G, Clark VP, et al. ((In Press)): An analysis of the fMRI Multisite Function BIRN and MIND studies show a unique pattern in the modulation of functional networks related to working memory in schizophrenia. Hum Brain Mapp.
27. Thompson RA, Nelson CA (2001): Developmental science and the media. Early brain development. Am Psychol. 56:5-15.
28. De Luca CR, Wood SJ, Anderson V, Buchanan JA, Proffitt TM, Mahony K, et al. (2003): Normative data from the CANTAB. I: development of executive function over the lifespan. J Clin Exp Neuropsychol. 25:242-254.
29. Luciana M, Nelson CA (2000): Neurodevelopmental assessment of cognitive function using CANTAB: Validation and future goals. In: Ernst, Rumsey, editors. Functional Neuroimaging in Child Psychiatry. Cambridge: Cambridge University Press, pp 379-397.
30. Huttenlocher PR (1979): Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res. 163:195-205.
31. Sowell ER, Thompson PM, Holmes CJ, Batth R, Jernigan TL, Toga AW (1999): Localizing age-related changes in brain structure between childhood and adolescence using statistical parametric mapping. Neuroimage. 9:587-597.
32. Sowell ER, Thompson PM, Holmes CJ, Jernigan TL, Toga AW (1999): In vivo evidence for post-adolescent brain maturation in frontal and striatal regions. Nat Neurosci. 2:859-861.
33. De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, et al. (2001): Sex differences in brain maturation during childhood and adolescence. Cereb Cortex. 11:552-557.
34. Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, et al. (1999): Structural maturation of neural pathways in children and adolescents: in vivo study. Science. 283:1908-1911.
35. Bartzokis G, Beckson M, Lu PH, Nuechterlein KH, Edwards N, Mintz J (2001): Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study. Arch Gen Psychiatry. 58:461-465.
36. Yakovlev PI, Lecours AR (1967): The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, editor. Regional Development of the Brain in Early Life. Oxford: Blackwell, pp 3-70.
37. Pauly K, Seiferth NY, Kellermann T, Backes V, Vloet TD, Shah NJ, et al. (2008): Cerebral dysfunctions of emotion-cognition interactions in adolescent-onset schizophrenia. J Am Acad Child Adolesc Psychiatry. 47:1299-1310.
38. Haenschel C, Bittner RA, Haertling F, Rotarska-Jagiela A, Maurer K, Singer W, et al. (2007): Contribution of impaired early-stage visual processing to working memory dysfunction in adolescents with schizophrenia: a study with event-related potentials and functional magnetic resonance imaging. Arch Gen Psychiatry. 64:1229-1240.
39. Rapoport JL, Inoff-Germain G (2000): Update on childhood-onset schizophrenia. Curr Psychiatry Rep. 2:410-415.
40. White T, Cullen K, Rohrer LM, Karatekin C, Luciana M, Schmidt M, et al. (2008): Limbic structures and networks in children and adolescents with schizophrenia. Schizophr Bull. 34:18-29.
41. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. (1997): Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 36:980-988.
42. Andreasen NC (1983): The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, IA: The University of Iowa.
43. Andreasen NC (1984): The Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, IA: The University of Iowa.
44. Sternberg S (1966): High-speed scanning in human memory. Science. 153:652-654.
45. Roffman JL, Gollub RL, Calhoun VD, Wassink TH, Weiss AP, Ho BC, et al. (2008): MTHFR 677C --> T genotype disrupts prefrontal function in schizophrenia through an interaction with COMT 158Val --> Met. Proc Natl Acad Sci U S A. 105:17573-17578.
46. Cox RW (1996): AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res. 29:162-173.
47. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. (2004): Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 23 Suppl 1:S208-219.

48. Jenkinson M, Smith S (2001): A global optimisation method for robust affine registration of brain images. Med Image Anal. 5:143-156.
49. Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage. 17:825-841.
50. White T, O'Leary D, Magnotta V, Arndt S, Flaum M, Andreasen NC (2001): Anatomic and functional variability: the effects of filter size in group fMRI data analysis. Neuroimage. 13:577-588.
51. Talairach J, Tournoux P (1988): Co-planar Stereotactic Atlas of the Human Brain: 3-Dimensional Proportional System: An Approach to Cerebral Imaging. Stuttgart/New York: Thieme Verlag.
52. Genovese CR, Lazar NA, Nichols T (2002): Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage. 15:870-878.
53. Smith EE, Jonides J (1999): Storage and executive processes in the frontal lobes. Science. 283:1657-1661.
54. Muller NG, Knight RT (2006): The functional neuroanatomy of working memory: contributions of human brain lesion studies. Neuroscience. 139:51-58.
55. Goldman-Rakic PS (1999): The physiological approach: functional architecture of working memory and disordered cognition in schizophrenia. Biol Psychiatry. 46:650-661.
56. Compton MT, McKenzie Mack L, Esterberg ML, Bercu Z, Kryda AD, Quintero L, et al. (2006): Associations between olfactory identification and verbal memory in patients with schizophrenia, first-degree relatives, and non-psychiatric controls. Schizophr Res. 86:154-166.
57. Thompson PM, Vidal C, Giedd JN, Gochman P, Blumenthal J, Nicolson R, et al. (2001): Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proc Natl Acad Sci U S A. 98:11650-11655.
58. Gogtay N (2008): Cortical brain development in schizophrenia: insights from neuroimaging studies in childhood-onset schizophrenia. Schizophr Bull. 34:30-36.
59. Durston S, Davidson MC, Tottenham N, Galvan A, Spicer J, Fossella JA, et al. (2006): A shift from diffuse to focal cortical activity with development. Dev Sci. 9:1-8.
60. Wahlstrom D, White T, Hooper CJ, Vrshek-Schallhorn S, Oetting WS, Brott MJ, et al. (2007): Variations in the catechol O-methyltransferase polymorphism and prefrontally guided behaviors in adolescents. Biol Psychiatry. 61:626-632.
61. Stephan KE, Magnotta VA, White T, Arndt S, Flaum M, O'Leary DS, et al. (2001): Effects of olanzapine on cerebellar functional connectivity in schizophrenia measured by fMRI during a simple motor task. Psychol Med. 31:1065-1078.

5.3 Increased Limbic and Temporal Lobe Activity during Working Memory in Children and Adolescents with Schizophrenia



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In Revision

Abstract

Objective: Similar to adults, children and adolescents with schizophrenia present with significant working memory (WkM) deficits. However, unlike adults, findings of abnormal activity in the prefrontal cortex in early-onset schizophrenia (EOS) are not consistently reported. Since WkM continues to develop through adolescence and into early adulthood, patterns of activation in adolescents may be different than those found in adults. The goal of this study was to evaluate the functional neurobiology of WkM in patients with EOS. **Method**: Participants included 22 patients with EOS (mean age 15 ±2.8 years) and 24 controls (mean age 15.0 ±3.0 years). Diagnoses were confirmed using the KIDDIE-SADS-PL. All subjects underwent a functional MRI paradigm involving a visuospatial working memory task with three separate loads. **Results**: The behavioral results demonstrated deficits in EOS patients at all three WkM loads. On functional imaging, EOS patients demonstrated increased activation in the anterior cingulate cortex (ACC), medial temporal limbic structures, the insula, and bilateral lateral temporal lobes. **Conclusions**: Patients with EOS demonstrate increased activity in limbic structures and regions involved in processing primary and secondary sensory information. Unlike studies in adults, we did not find that EOS patients had activation differences in frontal cortical regions. One possibility is that abnormalities in PFC function are related to secondary downstream or developmental processes which are 'unmasked' during development. Finally, our findings support growing evidence that EOS patients have aberrations in limbic and temporal lobe regions.

Introduction

Working memory (WkM) deficits are considered a core feature of schizophrenia (1-3) and have been reported not only in adults and first-episode patients (2), but also in children and adolescents with schizophrenia (4-10). In addition, WkM deficits are present during the schizophrenia prodrome (11-15) and even reports of pre-illness WkM deficits (16). While there have been a series of different definitions of WkM since Baddeley's pioneering work (17), all definitions agree that WkM involves components of encoding, maintenance, and retrieval of information (18).

Electrophysiological and neuroimaging studies have been very effective in mapping the functional regions associated with WkM performance (19-21). Efficient brain function involves the efficient transmission of information through distributed networks, so it is not surprising that multiple brain regions have been associated with WkM performance. Brain regions most commonly identified during verbal and visuospatial WkM paradigms include the dorsolateral prefrontal cortex (DLPFC, Brodmann's area 9, 46) (19, 20), inferior frontal gyrus (BA 6/44) (22, 23), the superior (BA 7) and inferior (BA 40) parietal lobes (20, 24), and the medial temporal lobe (25). In addition, the anterior cingulate cortex (ACC) (26) and the basal ganglia (27) have also been shown to be involved in WkM performance. It is not surprising that these regions have been shown to have abnormal activity during functional imaging studies in adults with schizophrenia (28-30).

One of the major questions that has arisen from functional imaging studies in schizophrenia is the question of hyper- versus hypofrontality (28, 31). While early H_2O^{15}-PET studies also demonstrated hypofrontality in patients with schizophrenia performing tasks which tap WkM (32-34), these studies were designed neither to specifically tap WkM circuits, nor were patients and controls matched on performance. There is evidence that once subjects are matched on WkM task performance, patients with schizophrenia demonstrate hyperfrontality. The thought of hyperfrontality is that patients are recruiting greater cognitive resources in order to match their performance with controls (35, 36), however, once the task difficulty exceeds their capacity, they demonstrate hypofrontality (37).

There have been two studies to date that evaluate WkM in children and adolescents with schizophrenia, or early-onset schizophrenia (EOS) (4, 38). Haenschel et al. (4) reported abnormal activations during a visual WkM paradigm in temporal and occipital regions during both encoding and retrieval, without any patient/control differences in prefrontal regions. Pauly et al. (38) using a verbal n-back paradigm which included neutral and adverse odors, found that adolescent patients had hypoactivation in the DLPFC, parietal cortex, and the ACC. Since EOS has been shown to be on a continuum with adult onset illness (39), and as WkM continues to mature during late adolescence and early adulthood (40), a better understanding of the role of prefrontal regions in EOS is crucial for our understanding of the neurobiology of schizophrenia. This is especially true in light of the considerable research documenting PFC abnormalities in adults with schizophrenia. If EOS patients do not have abnormalities in prefrontal regions, it is

possible that aberrant activity related to these structures are downstream, or developmental effects of the illness (41). Our goal was to investigate the neurobiology of spatial WkM in EOS, with a primary interest in studying aberrant PFC function.

Methods

Subjects

The participants included a total of 46 children and adolescents, including 22 patients with a schizophrenia spectrum disorder and 24 controls. The mean age of the patient group was 15.0 (S.D. 2.8) years and consisted of 15 males and 7 females. The mean age of the control group was 15.0 (S.D. 3.0) years and consisted of 16 boys and 8 girls (Table 1). Both the patients and controls underwent a diagnostic assessment using the Kiddie-SADS-PL (42). Of the patients, 19 had a diagnosis of schizophrenia, 1 had schizoaffective disorder, and 2 had schizophreniform disorder. Additional clinical measures included the Scale for the Assessment of Negative Symptoms (SANS) (43) and the Scale for the Assessment of Positive Symptoms (SAPS) (44).

The control group had no evidence of a past or present psychiatric disorder and no history of schizophrenia or psychosis in a first degree relative. Patients and controls were excluded if they had a history of substance dependence, ongoing substance abuse (within the past month), IQ less than 70, a neurological disorder, head injury, or a medical illness involving the brain. Twenty-one of the 22 patients were on medication at the time of the scanning (Table 1). The study was approved by the Institutional Review Board at the University of Minnesota, and informed consent and assent was obtained prior to participation.

Visuospatial Working Memory Paradigm

The WkM task was a visuospatial adaptation of the Sternberg Item Recognition Paradigm (SIRP) (45). The visuospatial SIRP consisted of a 'learn' phase, followed by either 1, 2, or 3 small black circles presented sequentially in one of 10 different locations on the screen (Figure 1). Each small circle was presented for 2.5 seconds, after which there was a 5 second delay. The recall phase consisted of a series of black dots that were presented sequentially, every 2.5 seconds, in one of the ten different locations. The participants wore MR-compatible push-button gloves and pushed the right thumb button if the location of the dot matched the location of one of the learned dots (target), and the left thumb button if it was in a different location (foil).

Each subject participated in two runs, each lasting five minutes and 30 seconds. Each run consisted of two blocks of the 1, 2, and 3 spatial memory loads, and the order of these blocks were randomly counterbalanced for each subject. Prior to scanning, subjects had two practice sessions, one while seated in a chair in front of a monitor, and the second session in a mock scanner with the stimuli back-

presented as was also performed during the scanning session. The subjects were able to practice until they were comfortable with the task. Subjects were told to respond as quickly as possible but try not to make any mistakes. The tasks were programmed using E-Prime (Psychological Software Tools, Inc., Pittsburgh, PA) and the behavioral outcome measures included response time (RT) and accuracy for each response. The stimulus presentation was triggered by the scanner following the fourth repetition time (TR). Subjects rested comfortably in the scanner with head mobilization performed using a vacuum bag.

Table 1 – *Demographic and Clinical Characteristics for the Patient and Control Groups*

	Patients n = 22	**Controls n = 24**	**p**
Demographic Measures	Mean / SD	Mean / SD	
Age (years / SD)	15.0 (2.8)	15.0 (3.0)	ns
Sex (M/F)	15 / 7	16 / 8	ns
SES	41.5 (14.8)	53.9 (7.5)	0.002
Clinical Measures			
Psychotic Symptoms (mean / SD)	2.6 / 1.1		
Negative Symptoms (mean / SD)	2.8 / 0.8		
Disorganized Symptoms (mean / SD)	1.9 / 1.0		
Antipsychotic Medications	**Number**	**Mean Dose**	
Aripiprazole	4	25 (12.9)	
Clozapine	4	250 (70.7)	
Olanzapine	1	20	
Ziprasidone	1	40	
Risperidone	8	3.5 (1.4)	
Quetiapine	5	500 (324)	
None	1		

MRI Sequence

All MR images were acquired with a 3T Siemens MR System (Erlangen, Germany) at the Center for Magnetic resonance Research in University of Minnesota. A series of localizer sequences were first acquired to orient the functional sequence. Following an initial localizer, a coronal scout image (12 slices;

FoV read 224 mm; FoV phase 100%; TR 2000; TE 72 ms; resolution 2.3 x 1.8 x 2 mm) was obtained to locate the coronal midline. Following alignment, sagittal images were acquired along the coronal midline (12 slices; FoV read 224 mm; FoV phase 100%; TR 2040; TE 62 ms; resolution 1.2 x 0.9 x 2 mm). These sagittal slices were used to orient the volume along the anterior/posterior commissure (ACPC) plane.

Functional images were acquired using a gradient echo sequence in 27 axial slices with an in plane resolution of 3.4 x 3.4 mm and a 4 mm slice thickness and a 25% distance factor. Additional sequence parameters include: TE = 30 ms, TR = 2,000 ms, flip angle = 90°, FoV read = 220 mm and the FoV phase was 100%. A total of 177 volumes were obtained for each of the two runs.

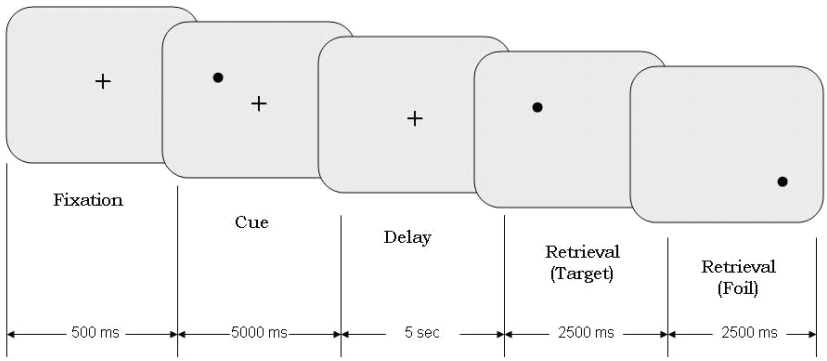


Figure 1 – Visuospatial Adaptation of the Sternberg Item Recognition Paradigm. The delay period is followed by the retrieval phase, of which there are ten randomly presented probes that consist of either a 'target' or a 'foil.' An example of a 'target' and a 'foil' within the retrieval phase are presented below.

Image Processing

The functional images were processed using a combination of Analysis of Functional NeuroImages (AFNI, http://afni.nimh.nih.gov/) (46) and FSL's FMRIB's Software Library (http://www.fmrib.ox.ac.uk/fsl/) (47). Following the conversion from DICOM to Nifti format, slice timing correction and motion correction were performed using AFNI (46). Subjects who had greater than 2.5 mm of motion in either the x, y, or z directions were excluded from the analyses. An 8-mm FWHM Gaussian spatial filter was applied prior to registration to standard space (48).

Images were oriented to standard MNI space utilizing FSL in a three-stage process. First, a mean EPI image was generated from the fMRI time series for each individual. This mean EPI image was registered to a MNI-152 EPI image through a

12-parameter transformation (49, 50). Finally, the 12-parameter transform was applied to the entire fMRI time series for each individual and each run.

Single-subject analyses were performed using FMRIB's fMRI Expert Analysis Tool FEAT (http://www.fmrib.ox.ac.uk/fsl/feat5/index.html). The time series for the behavioral conditions modeled three different encoding and three different probe conditions, these reflecting the three memory loads. This modeled time series was convolved with the hemodynamic response function (HRF) that was modeled from a linear combination of gamma functions. Next, a general linear model (GLM) was implemented using FMRIB's Improved Linear Model (FILM). A singular value decomposition (SVD) was utilized to assess the fit of each voxel to the design matrix using local autocorrelation (51). The two within-subject runs were combined using a fixed effects model. The higher-level group analysis, which compared patients and controls for each of the contrasts, was performed using FMRIB's Local Analysis of Mixed Effects (FLAME). The difference images between patients and controls were corrected for multiple comparisons using random Gaussian fields and significance was set at $p < 0.05$, corrected.

Results

The demographic data was assessed using either chi-square for ordinal and t-tests for continuous data. There were no differences between the age and sex distributions between the two groups (Table 1). There was a significant difference in SES between the patient and control groups ($t = 3.5$, $df = 42$, $p < 0.002$).

Behavioral Results

A 2 (diagnosis) x 3 (load) repeated measures mixed-model ANOVA was performed to assess d' for the visuospatial SIRP. There was a main effect of diagnosis ($F_{1,42} = 14.2$, $p < 0.001$) and load ($F_{2,84} = 37.0$, $p < 0.0001$), without a significant load by diagnosis interaction. Measures of d-prime for the patients and controls are shown in Figure 2.

Imaging Results

The imaging data was analyzed for encoding and retrieval phases both with and without behavioral performance as a covariate. Several brain regions demonstrated differences between patients and controls irrespective of whether performance was used as a covariate. These regions included increased activity in patients primarily in limbic structures and in the temporal lobes (Table 2 & 3 and Figures 3 & 4). There were no differences in functional activation between patients and controls at the lowest load of 1 spatial location, even though performance was significantly different. When controlling for performance, only the highest load (remembering 3 spatial locations) had significant differences between patients and controls. However, when performance was not used as a covariate, significant differences were present at both the medium and highest load levels (Table 2 & 3).

Table 2 – *Regional Differences in Brain Activation Between Patients and Controls during the Encode Condition of the Visuospatial SIRP.*

Brain Region	Cluster Size	Cluster z-Maximum	Local z-Maximum	x	y	z
Encode Load = 2 (P > C)						
Anterior Cingulate Gyrus	5,550	3.86		-12	36	-4
Planum Polare / Heschl's Gyrus / Superior Temporal Gyrus			3.69	-50	-14	-2
Left Temporal Pole			3.66	-32	12	-42
Cerebellum / Brainstem	2,587	3.73		-10	-16	-40
Posterior Cingulate Gyrus			3.48	-6	-54	22
Left Anterior Cerebellum			3.42	-2	-52	-6
Left Hippocampus			3.16	-26	-10	-20
Right Middle Temporal Gyrus	2,309	5.59		58	4	-22
Right Orbital Frontal Cortex			3.25	26	14	-20
Right Hippocampus / Amygdala			3.22	24	-6	-22
Encode Load = 3 (C > P)						
Parietal Lobe / Precuneous	3,733	3.27		6	-58	50
Superior Parietal Lobe			3.04	38	-52	46
Lateral Occipital Cortex			3.04	-22	-68	44
Encode Load = 3 (Covaried for Performance) (P > C)						
Anterior cingulate	3,158	5.18		-2	28	-6
Left Orbital frontal cortex			3.39	-12	26	-24
Left Temporal Lobe	2,118	3.96		-44	4	-22
Planum Polare / Heschl's Gyrus			3.75	-46	-20	-4
Left Temporal Pole			3.73	-32	12	-42
Superior Temporal Gyrus			3.54	-52	-10	-6
Left Mid & Inf. Temporal Gyrus			3.24	-56	-8	-26
Planum Polare / Heschl's Gyrus	2,003	3.74		44	-18	-2
Right Temporal Pole			3.3	36	14	-22
Right Sup. & Mid Temporal Gyrus			3.3	50	-6	-18
Right Insula			3.21	36	14	-18

P > C – Regions in which patients had greater activation compared to controls.
C > P – Regions in which controls had greater activation compared to patients.

Table 3 – *Regional Differences in Brain Activation Between Patients and Controls during the Retrieval Condition of the Visuospatial SIRP. The Children and Adolescents with Schizophrenia had greater activity in all regions and there were no regions in which the controls had greater activity than the patients.*

Brain Region	Cluster Size	Cluster z-Maximum	Local z-Maximum	x	y	z
Probe Load = 2						
Left Temporal Pole	5,198	3.64		-28	10	-38
Middle Temporal Gyrus			3.43	-56	-8	-24
Left Hippocampus / Parahippocampal Gyrus				-20	-8	-28
Paracingulate Gyrus			3.25	-14	42	-6
Probe Load = 3						
Left Middle Temporal Gyrus	3,632	3.61		-60	-2	-18
Left Temporal Pole			3.46	-46	10	-24
Left Parahippocampal Gyrus			3.31	-16	-14	-22
Left Hippocampus / Amygdala			3.21	-16	-10	-16
Probe Load = 3 (Covaried for Performance)						
Anterior Cingulate	34,262	5.3		-2	32	0
Planum Polare/ Heschl's Gyrus			5.04	44	-18	-2
Right Cerebellum			4.46	20	-74	-34
Left Thalamus			4.42	-6	-28	10
Right Hippocampus			4.4	40	-22	-10
Right Amygdala / Hippocampus			4.36	30	-4	-22

Encoding

The anterior cingulate, planum polare, Heschl's gyrus, and regions within the superior, middle and inferior temporal lobe all had significantly greater activity for both the middle load level (not covaried) and for the highest load level (with covarying for performance). However, these structures were not significant for the highest load when performance was not used as a covariate. In fact, for the latter, controls had greater activation in the parietal and occipital lobes (Table 2). The left and right hippocampus and cerebellum had significantly increased activity when not controlling for performance, however, these findings were not present when performance was used as a covariate.

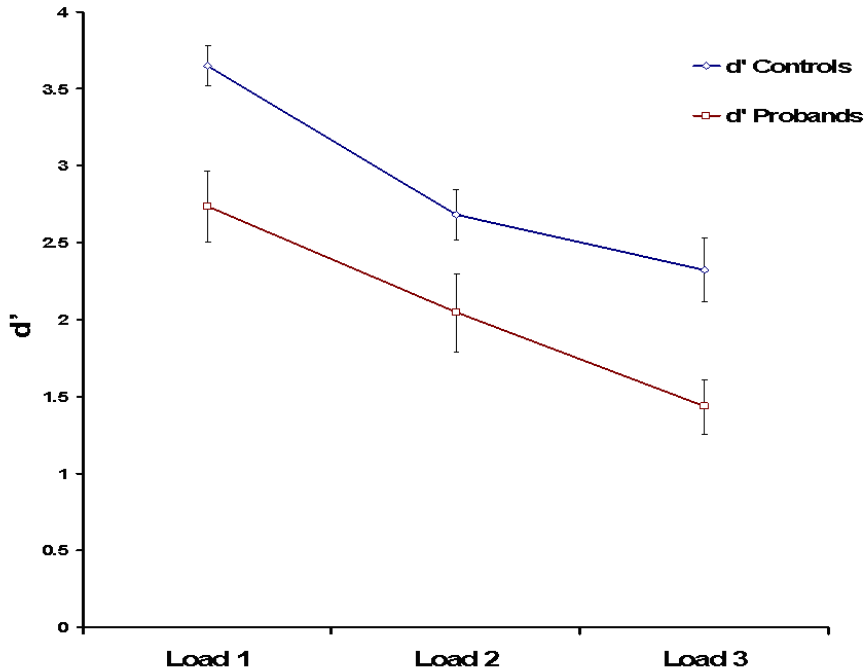


Figure 2 – *Measures of d' between Patients with Early-Onset Schizophrenia and Matched Controls.*

Retrieval

When performance was not used as a covariate, the results between the middle and highest loads are nearly identical. Both loads show significant differences between patients and controls in the left temporal pole, left middle temporal gyrus, and in the left hippocampus and parahippocampal gyrus (Table 3). Interestingly, when controlling for performance, none of these regions remain significant. Controlling for performance resulted in differences between patients and controls in regions that include the anterior cingulate, planum polare, Heschle's gyrus, the right cerebellum, the left thalamus, and the right hippocampus and amygdala (Table 3).

Discussion

We demonstrated that WkM deficits in children and adolescents with schizophrenia were associated with altered brain activity in the anterior cingulate and medial and lateral temporal lobes. The patients showed increased activation in

these brain regions during encoding and retrieval with and without controlling for performance. During encoding, patients had increased activation in the anterior cingulate, the left and right temporal pole, the right insula, and regions encompassing the left superior, middle, and inferior temporal gyri (Table 2). The anterior cingulate also demonstrated increased activation in patients during retrieval, as did the planum polare, Heschl's gyrus, the left thalamus, and the right hippocampus and amygdala (Table 3). Interestingly, we did not find significant differences between patients and controls in prefrontal regions.

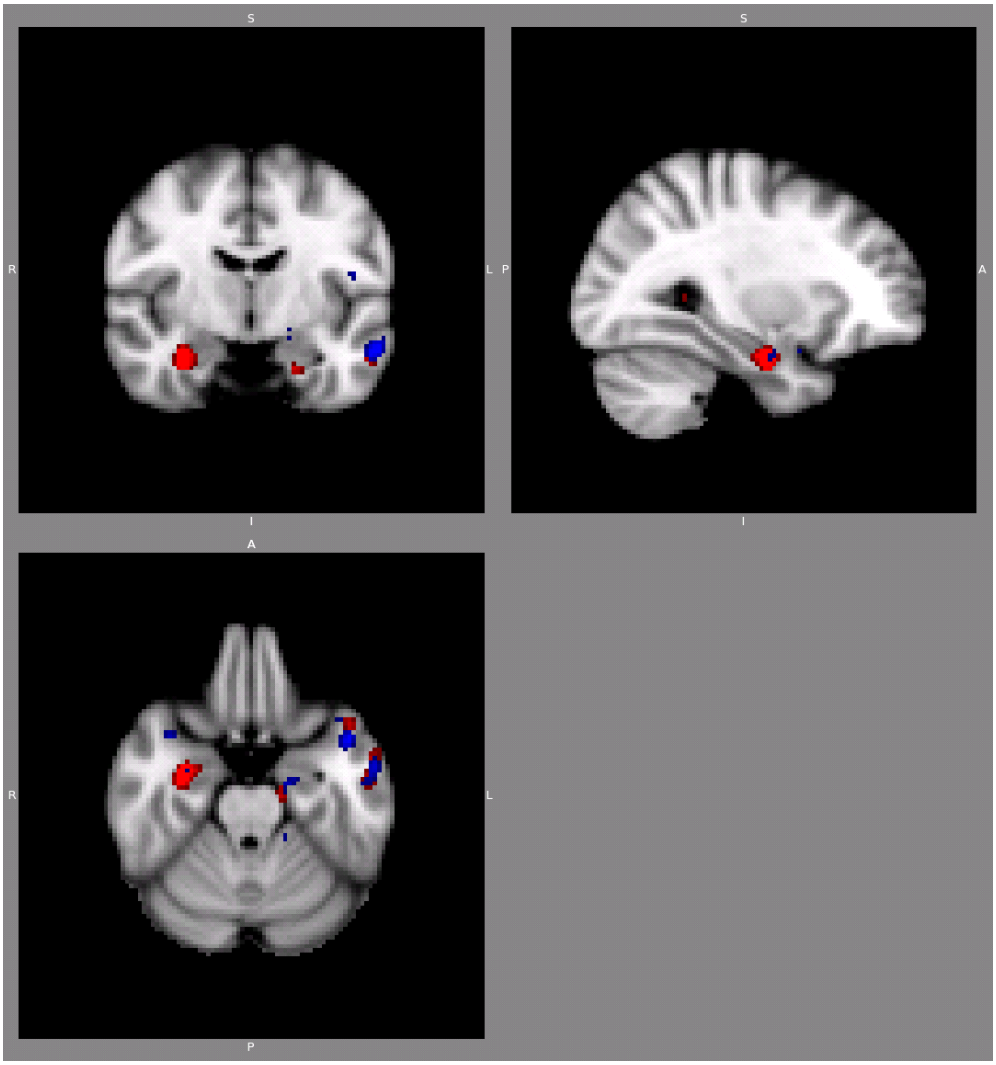


Figure 3 – *Group Analysis of Visuospatial SIRP ANCOVA. Patients had increased activity in: Blue = Encoding load 3; Red = Retrieval load 3 (Figure 7, Back Cover).*

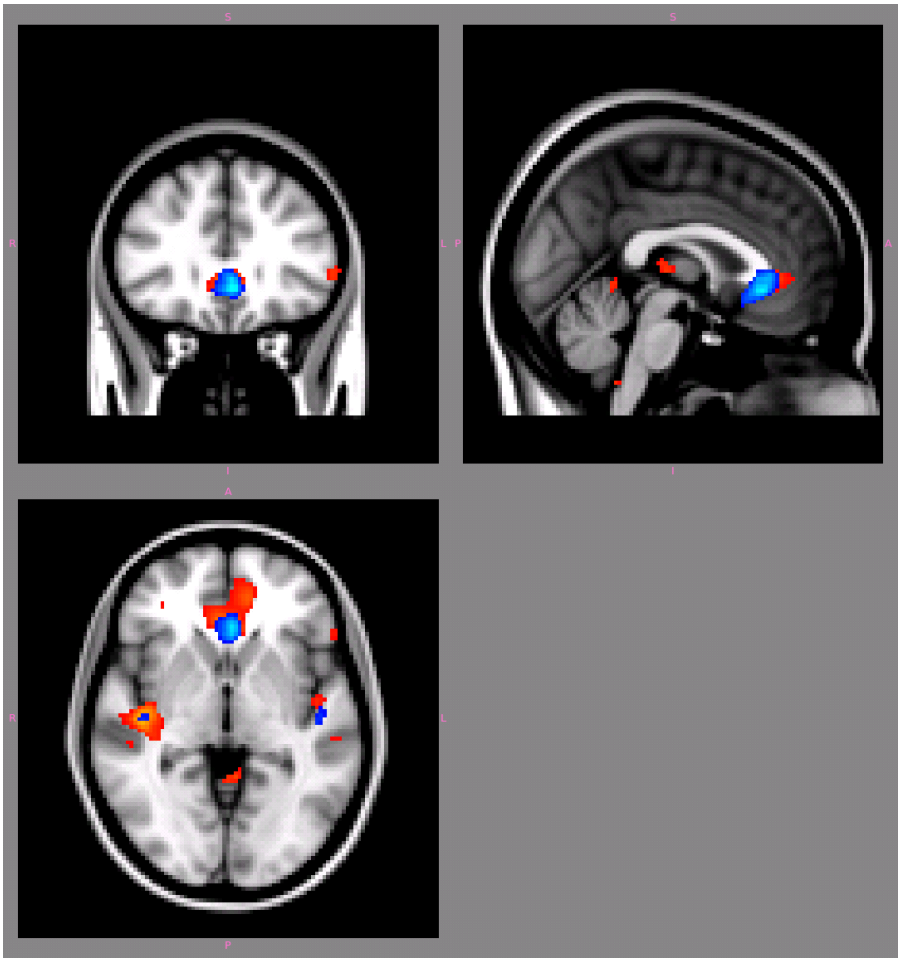


Figure 4 – Group Analysis of Visuospatial SIRP ANCOVA with performance (d') as a covariate. Patients had increased activity in: Blue = Encoding load 3; Red = Retrieval load 3 *(Figure 8, Back Cover).*

For decades, neuroimaging studies in adults with schizophrenia have highlighted the role of the DLPFC, first as 'hypofrontality' (52), then later, when controlling for performance, as 'hyperfrontality' (35, 53). Considering this wealth of data from adult studies, the lack of differences between EOS patients and controls in prefrontal regions is surprising. Irrespective of whether we covaried for performance, we found no differences in the PFC between EOS patients and controls. A lack of differences in PFC activation between EOS patients and controls was also reported by Haenschel et al. (4), who studied WkM load using a delayed discrimination task. They reported differences in regions of the left middle and

superior temporal lobe, the right inferior temporal gyrus and the middle occipital gyrus during encoding. During retrieval, they reported differences in the left middle and right inferior temporal gyrus and in the left and right middle occipital gyrus.

The only other neuroimaging study of WkM in EOS used a verbal n-back task and included negative and neutral olfactory stimuli (38). Evaluating WkM during the neutral olfactory condition, they found hypoactivation in the right and left lateral PFC, anterior cingulate, and inferior parietal lobe. Similar to our findings, they also reported hyperactivation in the superior temporal pole and occipital regions. The activations of PFC regions, which were not reported by either Haenschel et al. (4) or found in our study, could be a result of using a verbal WkM paradigm, the n-back versus WkM load-dependent tasks, or the influence of the negative emotion paradigm on the WkM task.

The lack of differences in the PFC during visuospatial WkM raises the question as to whether the finding of abnormal PFC function in adult PET and fMRI studies of schizophrenia are related to a downstream or developmental process. There is now considerable evidence that structural (54), functional (40), and neurochemical (55) development of the PFC continues throughout adolescence and into early adulthood. In addition, studies of childhood-onset schizophrenia (COS) find that the loss of GM occurs first in the parietal regions and progresses to encompass the PFC by the time the subjects reach older adolescence (56). One possible explanation for the lack of patient/control differences in the PFC is that these abnormalities are related to connectivity that mature during later adolescence and early adulthood (57). As these later developing prefrontal structures mature, disrupted connectivity from earlier developing structures, such as limbic regions, are unmasked (58).

While we found no differences in PFC function in our EOS sample, we did find abnormally increased activity in the anterior cingulate during WkM. This is a finding that has been consistently reported in studies of adults with schizophrenia (28). The increased activation was present during both the encoding and retrieval phases of the WkM task. A review of WkM performance in schizophrenia have highlighted the role of encoding deficits as a primary factor (2). Encoding reflects the components of attention and processing speed and the ACC has been linked to attentional control (59). Thus, the increased ACC may be related to patients' impairment in attentional control. Alternatively, since EOS patients made more errors than controls, especially at higher loads, the increased ACC activation in EOS patients may reflect greater error detection and conflict monitoring (60, 61). Finally, connections exist between the ACC and PFC (62), and with the continued maturation of the PFC into early adulthood, the EOS patients may be utilizing different neural circuits for task completion. This latter explanation may account for the increased activation in limbic structures in the medial temporal lobe, the insula, and in lateral temporal lobe regions.

Limitations

There are several limitations to the current study. All but one of the patients were on an assortment of antipsychotic medications at the time of the study (Table 1). It is possible that some of the differences between patients and controls may be related to medication effects, although medication is more likely to normalize brain activity (63). In addition, medication effects cannot account for differences between the adult studies and our sample, since most fMRI studies of adults use medicated patients. While the stimulus presentation paradigm was designed to parse out encoding from retrieval, we did not parse the analysis of the retrieval phase into probes and foils and model only correct answers in the design matrix. A true event related design could parse the task into additional retrieval-related components and test whether the increase in ACC activation was secondary to the greater number of errors made by the patient group (60). Finally, we did not have a comparison group of adults with schizophrenia who performed this visuospatial SIRP. Such an adult patient group could assess when in development that neurobiological deficits begin to emerge in the PFC.

Conclusions

In summary, children and adolescents with schizophrenia demonstrate increased activity in the ACC and regions in the left and right temporal lobes. Unlike studies in adults, we did not find differences in activation in the PFC between patients and controls. One possibility is that aberration in PFC function is related to secondary downstream or developmental processes which are 'unmasked' during development. Finally, our findings support a growing evidence that EOS patients have aberrations in limbic regions that occur prior to the abnormalities in prefrontal regions (41).

References

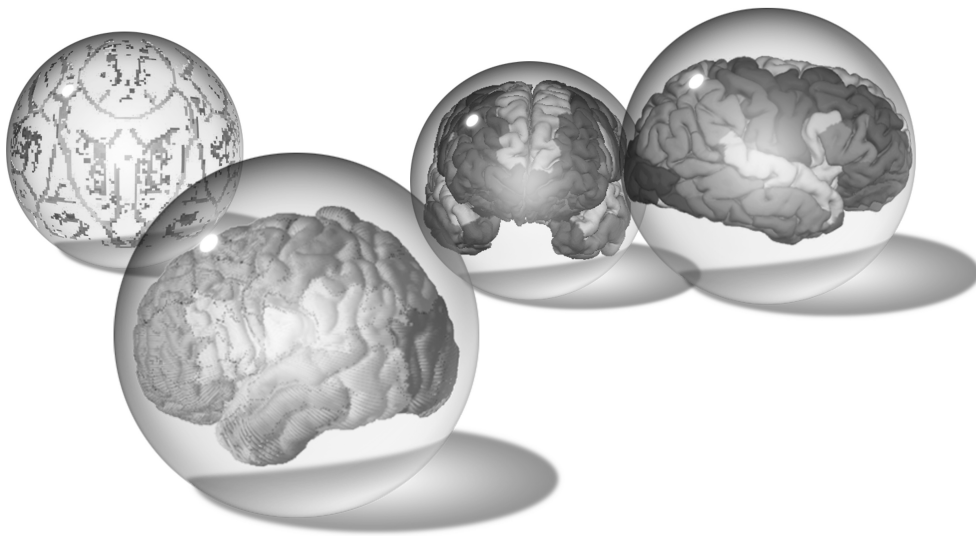
1. Aleman A, Hijman R, de Haan EH, Kahn RS (1999): Memory impairment in schizophrenia: a meta-analysis. Am J Psychiatry. 156:1358-1366.
2. Lee J, Park S (2005): Working memory impairments in schizophrenia: a meta-analysis. J Abnorm Psychol. 114:599-611.
3. Goldman-Rakic PS (1994): Working memory dysfunction in schizophrenia. J Neuropsychiatry Clin Neurosci. 6:348-357.
4. Haenschel C, Bittner RA, Haertling F, Rotarska-Jagiela A, Maurer K, Singer W, et al. (2007): Contribution of impaired early-stage visual processing to working memory dysfunction in adolescents with schizophrenia: a study with event-related potentials and functional magnetic resonance imaging. Arch Gen Psychiatry. 64:1229-1240.
5. White T, Ho BC, Ward J, O'Leary D, Andreasen NC (2006): Neuropsychological performance in first-episode adolescents with schizophrenia: a comparison with first-episode adults and adolescent control subjects. Biol Psychiatry. 60:463-471.
6. Cervellione KL, Burdick KE, Cottone JG, Rhinewine JP, Kumra S (2007): Neurocognitive deficits in adolescents with schizophrenia: longitudinal stability and predictive utility for short-term functional outcome. J Am Acad Child Adolesc Psychiatry. 46:867-878.

7. Kenny JT, Friedman L, Findling RL, Swales TP, Strauss ME, Jesberger JA, et al. (1997): Cognitive impairment in adolescents with schizophrenia. Am J Psychiatry. 154:1613-1615.
8. Vance A, Hall N, Bellgrove MA, Casey M, Karsz F, Maruff P (2006): Visuospatial working memory deficits in adolescent onset schizophrenia. Schizophr Res. 87:223-227.
9. Karatekin C, Asarnow RF (1998): Working memory in childhood-onset schizophrenia and attention- deficit/hyperactivity disorder. Psychiatry Res. 80:165-176.
10. Øie M, Sunde K, Rund BR (1999): Contrasts in memory functions between adolescents with schizophrenia or ADHD. Neuropsychologia. 37:1351-1358.
11. Hawkins KA, Addington J, Keefe RS, Christensen B, Perkins DO, Zipurksy R, et al. (2004): Neuropsychological status of subjects at high risk for a first episode of psychosis. Schizophr Res. 67:115-122.
12. Keefe RS, Perkins DO, Gu H, Zipursky RB, Christensen BK, Lieberman JA (2006): A longitudinal study of neurocognitive function in individuals at-risk for psychosis. Schizophr Res. 88:26-35.
13. Pukrop R, Ruhrmann S, Schultze-Lutter F, Bechdolf A, Brockhaus-Dumke A, Klosterkotter J (2007): Neurocognitive indicators for a conversion to psychosis: comparison of patients in a potentially initial prodromal state who did or did not convert to a psychosis. Schizophr Res. 92:116-125.
14. Smith CW, Park S, Cornblatt B (2006): Spatial working memory deficits in adolescents at clinical high risk for schizophrenia. Schizophr Res. 81:211-215.
15. Wood SJ, Pantelis C, Proffitt T, Phillips LJ, Stuart GW, Buchanan JA, et al. (2003): Spatial working memory ability is a marker of risk-for-psychosis. Psychol Med. 33:1239-1247.
16. Erlenmeyer-Kimling L, Rock D, Roberts SA, Janal M, Kestenbaum C, Cornblatt B, et al. (2000): Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: the New York High-Risk Project. Am J Psychiatry. 157:1416-1422.
17. Baddeley A (1986): Working Memory. New York: Oxford University Press.
18. Miyake A, Shaw P (1999): Models of working memory: Mechanisms of active maintenance and executive control. New York: Cambridge Univesity Press.
19. D'Esposito M, Aguirre GK, Zarahn E, Ballard D, Shin RK, Lease J (1998): Functional MRI studies of spatial and nonspatial working memory. Brain Res Cogn Brain Res. 7:1-13.
20. Wendelken C, Bunge SA, Carter CS (2008): Maintaining structured information: an investigation into functions of parietal and lateral prefrontal cortices. Neuropsychologia. 46:665-678.
21. Goldman-Rakic PS (1996): The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. Philos Trans R Soc Lond B Biol Sci. 351:1445-1453.
22. Buckner RL, Koutstaal W (1998): Functional neuroimaging studies of encoding, priming, and explicit memory retrieval. Proc Natl Acad Sci U S A. 95:891-898.
23. MacLeod AK, Buckner RL, Miezin FM, Petersen SE, Raichle ME (1998): Right anterior prefrontal cortex activation during semantic monitoring and working memory. Neuroimage. 7:41-48.
24. Tuladhar AM, ter Huurne N, Schoffelen JM, Maris E, Oostenveld R, Jensen O (2007): Parieto-occipital sources account for the increase in alpha activity with working memory load. Hum Brain Mapp. 28:785-792.
25. Axmacher N, Mormann F, Fernandez G, Cohen MX, Elger CE, Fell J (2007): Sustained neural activity patterns during working memory in the human medial temporal lobe. J Neurosci. 27:7807-7816.
26. Jansma JM, Ramsey NF, de Zwart JA, van Gelderen P, Duyn JH (2007): fMRI study of effort and information processing in a working memory task. Hum Brain Mapp. 28:431-440.
27. Chang C, Crottaz-Herbette S, Menon V (2007): Temporal dynamics of basal ganglia response and connectivity during verbal working memory. Neuroimage. 34:1253-1269.
28. Glahn DC, Ragland JD, Abramoff A, Barrett J, Laird AR, Bearden CE, et al. (2005): Beyond hypofrontality: a quantitative meta-analysis of functional neuroimaging studies of working memory in schizophrenia. Hum Brain Mapp. 25:60-69.
29. Barch DM, Csernansky JG (2007): Abnormal parietal cortex activation during working memory in schizophrenia: verbal phonological coding disturbances versus domain-general executive dysfunction. Am J Psychiatry. 164:1090-1098.
30. Barch DM, Csernansky JG, Conturo T, Snyder AZ (2002): Working and long-term memory deficits in schizophrenia: is there a common prefrontal mechanism? J Abnorm Psychol. 111:478-494.
31. Ragland JD, Yoon J, Minzenberg MJ, Carter CS (2007): Neuroimaging of cognitive disability in schizophrenia: search for a pathophysiological mechanism. Int Rev Psychiatry. 19:417-427.
32. Andreasen NC, Rezai K, Alliger R, Swayze VW, 2nd, Flaum M, Kirchner P, et al. (1992): Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia.

Assessment with xenon 133 single-photon emission computed tomography and the Tower of London. Arch Gen Psychiatry. 49:943-958.
33. Weinberger DR, Berman KF (1988): Speculation on the meaning of cerebral metabolic hypofrontality in schizophrenia. Schizophr Bull. 14:157-168.
34. Buchsbaum MS, Wu JC (1987): Hypofrontality in schizophrenia as assessed by PET. Am J Psychiatry. 144:122-123.
35. Manoach DS, Press DZ, Thangaraj V, Searl MM, Goff DC, Halpern E, et al. (1999): Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. Biol Psychiatry. 45:1128-1137.
36. Callicott JH, Bertolino A, Mattay VS, Langheim FJ, Duyn J, Coppola R, et al. (2000): Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb Cortex. 10:1078-1092.
37. Manoach DS (2003): Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr Res. 60:285-298.
38. Pauly K, Seiferth NY, Kellermann T, Backes V, Vloet TD, Shah NJ, et al. (2008): Cerebral dysfunctions of emotion-cognition interactions in adolescent-onset schizophrenia. J Am Acad Child Adolesc Psychiatry. 47:1299-1310.
39. Rapoport JL, Inoff-Germain G (2000): Update on childhood-onset schizophrenia. Curr Psychiatry Rep. 2:410-415.
40. Luciana M, Nelson CA (2000): Neurodevelopmental assessment of cognitive function using CANTAB: Validation and future goals. In: Ernst, Rumsey, editors. Functional Neuroimaging in Child Psychiatry. Cambridge: Cambridge University Press, pp 379-397.
41. White T, Cullen K, Rohrer LM, Karatekin C, Luciana M, Schmidt M, et al. (2008): Limbic structures and networks in children and adolescents with schizophrenia. Schizophr Bull. 34:18-29.
42. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. (1997): Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 36:980-988.
43. Andreasen NC (1983): The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, IA: The University of Iowa.
44. Andreasen NC (1984): The Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, IA: The University of Iowa.
45. Sternberg S (1966): High-speed scanning in human memory. Science. 153:652-654.
46. Cox RW (1996): AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res. 29:162-173.
47. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. (2004): Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 23 Suppl 1:S208-219.
48. White T, O'Leary D, Magnotta V, Arndt S, Flaum M, Andreasen NC (2001): Anatomic and functional variability: the effects of filter size in group fMRI data analysis. Neuroimage. 13:577-588.
49. Jenkinson M, Smith S (2001): A global optimisation method for robust affine registration of brain images. Med Image Anal. 5:143-156.
50. Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage. 17:825-841.
51. Woolrich MW, Ripley BD, Brady M, Smith SM (2001): Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage. 14:1370-1386.
52. Weinberger D, Berman KF (1988): Speculation on the meaning of cerebral metabolic hypofrontality in schizophrenia. Schizophrenia Bulletin. 14:157-168.
53. Callicott JH, Mattay VS, Verchinski BA, Marenco S, Egan MF, Weinberger DR (2003): Complexity of prefrontal cortical dysfunction in schizophrenia: more than up or down. Am J Psychiatry. 160:2209-2215.
54. Sowell ER, Thompson PM, Holmes CJ, Jernigan TL, Toga AW (1999): In vivo evidence for post-adolescent brain maturation in frontal and striatal regions. Nat Neurosci. 2:859-861.
55. Wahlstrom D, White T, Hooper CJ, Vrshek-Schallhorn S, Oetting WS, Brott MJ, et al. (2007): Variations in the catechol O-methyltransferase polymorphism and prefrontally guided behaviors in adolescents. Biol Psychiatry. 61:626-632.
56. Thompson PM, Vidal C, Giedd JN, Gochman P, Blumenthal J, Nicolson R, et al. (2001): Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proc Natl Acad Sci U S A. 98:11650-11655.
57. Benes FM (1989): Myelination of cortical-hippocampal relays during late adolescence. Schizophr Bull. 15:585-593.

58. White T, Kendi AT, Lehericy S, Kendi M, Karatekin C, Guimaraes A, et al. (2007): Disruption of hippocampal connectivity in children and adolescents with schizophrenia--a voxel-based diffusion tensor imaging study. Schizophr Res. 90:302-307.
59. Davis KD, Hutchison WD, Lozano AM, Tasker RR, Dostrovsky JO (2000): Human anterior cingulate cortex neurons modulated by attention-demanding tasks. J Neurophysiol. 83:3575-3577.
60. Carter CS, Braver TS, Barch DM, Botvinick MM, Noll D, Cohen JD (1998): Anterior cingulate cortex, error detection, and the online monitoring of performance. Science. 280:747-749.
61. Taylor SF, Stern ER, Gehring WJ (2007): Neural systems for error monitoring: recent findings and theoretical perspectives. Neuroscientist. 13:160-172.
62. Kondo H, Osaka N, Osaka M (2004): Cooperation of the anterior cingulate cortex and dorsolateral prefrontal cortex for attention shifting. Neuroimage. 23:670-679.
63. Stephan KE, Magnotta VA, White T, Arndt S, Flaum M, O'Leary DS, et al. (2001): Effects of olanzapine on cerebellar functional connectivity in schizophrenia measured by fMRI during a simple motor task. Psychol Med. 31:1065-1078.

6.1 Summary



Tonya White

Summary

Schizophrenia is a serious psychiatric illness that effects between 0.5 to 1 percent of the population, worldwide. Symptoms of schizophrenia include hallucinations, delusions, disorganized thought and behavior, and negative symptoms. Negative symptoms include avolition, apathy, alogia, and flattened affect and have been shown to be the most closely associated with functional outcome. The typical age of onset for schizophrenia occurs between eighteen to twenty-two years of age, although non-specific prodromal symptoms often precede the full onset of the illness. Thus, schizophrenia occurs relatively early in life and often, but not always, steals a large portion of an individual's future.

There has been much work over the past several decades to determine the underlying etiology of this devastating illness, however, there are more uncertainties than certainties. It is known that schizophrenia has both genetic and environmental components and that the genetic components likely involve multiple genes that are common in the population. It is also known that global cognitive deficits are typically present in both adolescents and adults with schizophrenia (Chapter 2.1), and these cognitive deficits appear to be associated with abnormalities in encoding (Chapter 2.2), which is a fundamental cognitive process. Thus, the combination of the global nature of the cognitive deficits, coupled with the diversity of symptoms associated with schizophrenia, have pointed toward etiologic hypotheses that involve global brain processes.

One of the principle working hypotheses that incorporates disrupted global brain processes is the theory of disrupted connectivity between brain regions. This theory, when combined with developmental neuroscience approaches, takes into account the age-dependent changes of the brain associated with typical neurodevelopment. Typical neurodevelopment involves a cascade of events, starting with the early formation of the brain at approximately two weeks of fetal life and continues throughout childhood, adolescence, and into early-adulthood (Chapter 3.1). The developmental neuroscience theory of the neurobiology of schizophrenia points toward an interaction between development and neuronal connectivity. This theory is supported by the findings in this thesis.

The major neurodevelopmental events that occur between childhood and young adulthood include continued myelination, especially in the association cortices and the prefrontal cortex. Myelination can be quantified by measuring white matter volumes with structural MRI. Additionally, white matter microstructure, or the coherence of neuronal fiber bundles, can be measured using diffusion tensor imaging (DTI). First-episode patients with schizophrenia tend to have less white matter aberrations than those with chronic schizophrenia (Chapter 4.1). Thus, it is possible that early changes are more focal, but then progress over time. Evidence in adolescents point toward disruptions of white matter pathways in the limbic system, including the hippocampus (Chapter 4.2). However, there is considerable heterogeneity in the location of the microstructural abnormalities in schizophrenia. Most of the DTI studies utilize either voxel-based or region-of-

interest approaches. Both of these techniques require an assumption, being that microstructural abnormalities are spatially homogeneous. Yet, most neurological illnesses that affect white matter tend to do so in a spatially heterogeneous manner, which raises the question as why schizophrenia would be any different. In fact, I recently developed a recent novel technique that does not require spatially homogeneous white matter abnormalities (Chapter 4.3). We applied this technique to a group of children and adolescents wtih schizophrenia and found significant differences in white matter anisotropy in a non-spatially dependent manner (Chapter 4.3).

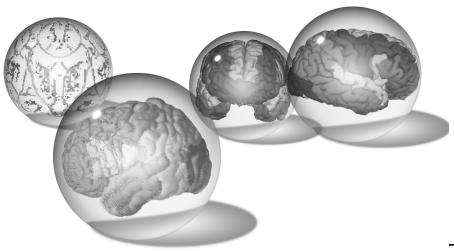
In addition to continued myelination, synaptic pruning also continues throughout adolescence and into early adulthood. This pruning of connections can alter the surface morphology of the brain in a consistent manner and can be measured (Chapter 3.1). The characteristic fissures and folds of the brain, the gyri and sulci, may be formed as a direct result of their underlying connectivity. Thus, as pruning takes place, the morphology of the brain may change in a manner consistent with the underlying histology. Since adolescents with schizophrenia show differences in the gyri and sulci of the brain, these differences may reflect developmental differences related to the underlying connectivity in the cortex, most notably in the frontal and temporal lobes (Chapter 3.2).

Finally, connectivity can be measured using functional neuroimaging techniques. Functional connectivity is defined as the temporal correlation between the fMRI time series between two distinct brain regions. Functional connectivity is guided by the principle that: "what is wired together, fires together." Early functional imaging studies of adults with schizophrenia tended to show hypofrontality, or less activation in the frontal lobes during tasks of executive function. Later studies that matched for performance found hyperactivation in the frontal cortex in adults with schizophrenia, challenging these early findings. Together though, there is considerable evidence to support abnormalities in the prefrontal cortex in adults with schizophrenia. These studies were exciting from the perspective of a cognitive developmental neuroscientist, as the prefrontal cortex is an area that continues to develop during adolescence and early-adulthood. However, our studies in adolescence are not finding the same prefrontal abnormalities as is found in adults (Chapter 5.1 & 5.2). Interestingly, our studies using functional MRI tend to find abnormalities in the limbic system, cerebellum, parietal lobe, and early visual processes (Chapter 5).

In conclusion, schizophrenia is a serious psychiatric disorder that affects a significant proportion of the population worldwide. While the etiology of schizophrenia is unclear, there is evidence for disrupted connectivity between distant brain regions. In adolescents, there is less evidence for prefrontal cortical abnormalities, and greater evidence for limbic, parietal, cerebellum, and occipital lobe abnormalities. However, the brain functions as a distributed neural network, and thus there should be caution in excluding specific regions that don't 'light up' using specific neuroimaging techniques. Yet it is intriguing to speculate that an early insult in, for example, the hippocampus, in a genetically vulnerable individual, could create a cascade of downstream developmental effects. Such an

insult would not result in the full spectrum of symptoms early in life, as there are collateral neural networks that compensate. But with the progression of typical developmental processes (i.e., synaptic pruning of collateral networks or alterations as a result of myelination) the brain connectivity is altered in such a way that the effects of the insult become 'unmasked.'

6.2 Samenvatting



Tonya White

Vertaling met hulp van Dr. Th. M. J. Machielse, MRICS, Prof.dr. F.C. Verhulst, Dr. Hanan El Marroun, en Dr. Mijke Zeegers

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Schizofrenie is een ernstige psychiatrische stoornis die 0,5 tot 1% van de wereldbevolking treft. De symptomen van schizofrenie kunnen ondergebracht worden in twee symptoomclusters: positieve symptomen en negatieve symptomen. Voorbeelden van positieve symptomen zijn: hallucinaties, wanen en ongeorganiseerd denken en gedrag. Voorbeelden van negatieve symptomen zijn: verlies van initiatief, apathie, spraak- en gedachtenarmoede en vermindering van de expressieve vermogens. Van alle symptomen zijn de negatieve symptomen het sterkst geassocieerd met het dagelijks functioneren. De typische leeftijd waarop de symptomen zich voor het eerst manifesteren ligt tussen de achttien en tweeëntwintig jaar, hoewel er vaak niet-specifieke prodromale symptomen zijn die voorafgaan aan de ontwikkeling van het volledige ziektebeeld. Schizofrenie komt dus relatief vroeg in het leven voor en vaak, maar niet altijd, heeft het grote negatieve consequenties voor het gehele verdere leven van een individu.

Er is de afgelopen decennia veel onderzoek verricht om de onderliggende oorzaken van deze ernstige aandoening te ontrafelen echter met als resultaat meer

onzekerheden dan zekerheden. Het is bekend dat de oorzaak van schizofrenie uit zowel genetische als omgevingscomponenten bestaat heeft en dat de genetische component waarschijnlijk uit meerdere genen bestaat die ook in de algemene bevolking voorkomen. Het is ook bekend dat globale cognitieve defecten kenmerkend zijn voor zowel adolescenten als volwassenen met schizofrenie (Hoofdstuk 2.1), en dat deze cognitieve defecten geassocieerd zijn met afwijkende 'encoding' (hoofdstuk 2.2), een fundamenteel cognitief proces. Zo heeft de combinatie van het globale karakter van de cognitieve defecten en de diversiteit van symptomen geassocieerd met schizofrenie, geleid tot het formuleren van etiologische hypothesen waar algemene hersenprocessen bij betrokken zijn.

Een van de belangrijkste werkhypothesen die rekening houdt met verstoorde algemene hersenprocessen is de theorie van verstoorde connectiviteit tussen hersengebieden. In combinatie met een ontwikkelingsneurobiologische (developmental neuroscience) benadering houdt deze theorie, rekening met leeftijdsafhankelijke veranderingen van de hersenen. De hersenontwikkeling omvat een cascade van gebeurtenissen, die begint met de vroege vorming van de hersenen in ongeveer de tweede week van het foetale leven en die zich voortzet gedurende de kinderjaren en adolescentie tot in de jong-volwassenheid (hoofdstuk 3.1).

De developmental neuroscience theorie van de neurobiologie van schizofrenie wijst in de richting van een interactie tussen ontwikkeling en neuronale connectiviteit. Deze theorie wordt gesteund door de bevindingen in dit proefschrift.

Een van de belangrijkste ontwikkelingsneurobiologische gebeurtenissen die plaatsvindt tussen de kinderleeftijd en volwassenheid is de voortgaande myelinisatie, vooral de myelinisatie met name in de associatieve hersenschorsen en de prefrontale cortex. Myelinisatie kan worden gekwantificeerd door het meten van witte stof volumes met structurele MRI. Ook kan de witte stof microstructuur, of de samenhang van neuronale vezelbundels, worden gemeten met behulp van Diffusion Tensor Imaging (DTI).

Duidelijk wordt in dit proefschrift aangetoond dat eerste-episode patiënten met schizofrenie over het algemeen minder witte stof afwijkingen hebben dan chronisch schizofrene patiënten (hoofdsstuk 4.1). Het is mogelijk dat vroege veranderingen meer focaal zijn en zich vervolgens uitbreiden in de loop van de tijd. Bevindingen bij adolescenten wijzen in de richting van onderbrekingen van de witte stof trajecten in het limbische systeem, met inbegrip van de hippocampus (hoofdstuk 4.2). Er is echter een grote heterogeniteit in de localisatie van de microstructurele afwijkingen bij schizofrenie. Het merendeel van de DTI studies gebruikt of een "voxel-based" of een "region-of-interest" benadering. Beide technieken hanteren als aanname dat microstructurele afwijkingen in de hersenen ruimtelijk homogeen zijn. Bij de meeste neurologische ziekten van de witte stof zijn de microstructurele afwijkingen juist ruimtelijk homogeen en men kan zich dan ook afvragen waarom dit bij schizofrenie anders zou zijn. Er is door mij recent een nieuwe techniek ontwikkeld die niet vereist dat er ruimtelijk homogene witte stof afwijkingen zijn (Hoofdstuk 4.3) Deze techniek werd toegepast op een groep kinderen en adolescenten met schizofrenie en er werden significante verschillen

gevonden in de witte stof anisotropie op een niet-ruimtelijk afhankelijke manier.

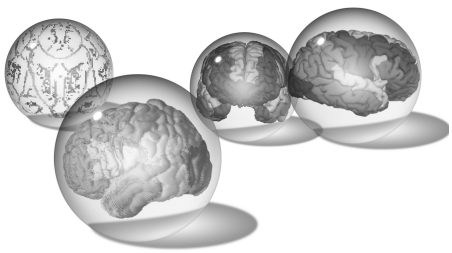
Naast de voortgaande myelinisatie vindt er ook synaptische pruning plaats gedurende de adolescentie tot in de vroege volwassenheid. Dit "wegsnoeien" van eerder gevormde verbindingen kan de morfologie van de hersenschors op een consistente wijze veranderen. Die veranderingen kunnen worden gemeten (hoofdstuk 3.1). De karakteristieke fissuren en plooien van de hersenen, de gyri en sulci, kunnen worden gevormd als een direct gevolg van onderliggende connectiviteit. Als pruning plaatsvindt, kan de morfologie van de hersenen veranderen op een wijze die in overeenstemming is met de onderliggende histologie. Aangezien adolescenten met schizofrenie verschillen vertonen in de gyri en sulci van de hersenen, kunnen deze verschillen de verschillen weerspiegelen in de ontwikkeling van de onderliggende connectiviteit in de cortex, vooral in de frontale en temporale kwabben. Dit proefschrift ondersteunt deze visie, maar kan geen bewijs leveren (hoofdstuk 3.2).

Tenslotte kan de connectiviteit worden gemeten met behulp van functionele neuro-imaging technieken. Functionele connectiviteit wordt gedefinieerd als de temporele correlatie die bestaat tussen de fMRI tijdreeksen tussen twee verschillende gebieden van de hersenen. Functionele connectiviteit wordt geleid door het principe: 'what is wired together, fires together' Vroege functionele imaging studies van schizofrene volwassenen toonden verminderde frontale activiteit aan tijdens het uitvoeren van executieve functietaken. Latere studies vonden juist hyperactivatie van de frontale cortex bij volwassenen met schizofrenie, daarmee de eerdere bevindingen ter discussie stellend. Alles tezamen echter, is er een aanzienlijke hoeveelheid bewijs om het bestaan van afwijkingen in de prefrontale cortex bij volwassenen met schizofrenie te ondersteunen. Deze studies waren interessant vanuit het perspectief van cognitieve neurowetenschappers, omdat de prefrontale cortex een gebied is dat zich blijft ontwikkelen tijdens de adolescentie en de vroege volwassenheid. Echter, de in dit proefschrift beschreven studies bij adolescenten vinden de prefrontale afwijkingen niet die bij volwassenen gevonden worden (hoofdstukken 5.1 & 5.2). In de functionele MRI studies in dit proefschrift worden afwijkingen gevonden in het limbische systeem, cerebellum, pariëtele kwab, en vroege visuele verwerkingsprocessen (hoofdstukken 5.1-5.3). Dit proefschrift ondersteunt de stelling dat er verschillen zijn tussen kinderen en volwassenen in de functionele activiteit van de prefrontale kwab. Daarbij steunen deze verschillen op vroeg optredende afwijkingen in de limbische, temporale kwab, en de kleine hersenen.

Concluderend kunnen we stellen dat schizofrenie een ernstige psychiatrische stoornis is, die een aanzienlijk deel van de wereldbevolking treft. Hoewel de etiologie van schizofrenie onduidelijk is, zijn er aanwijzingen dat er sprake is van verstoorde connectiviteit tussen hersengebieden. Bij adolescenten is er minder bewijs voor prefrontale corticale afwijkingen en meer bewijs voor limbische, parietale, cerebellum, en de ocipitale kwab afwijkingen. De hersenen functioneren echter als een verspreid neuraal netwerk en daarom is voorzichtigheid geboden bij het uitsluiten van specifieke regio's die niet "oplichten" bij het gebruik van specifieke neuroimaging technieken. Het is interessant om te speculeren dat

een vroege beschadiging van bijvoorbeeld de hippocampus bij een genetisch kwetsbaar individu een opeenvolging van elkaar versterkende negatieve effecten op de ontwikkeling van de hersenen zou kunnen veroorzaken. De beschadiging veroorzaakt niet het gehele spectrum van symptomen op jonge leeftijd omdat er collaterale netwerken zijn die voor de beschadiging compenseren. Echter, door synaptische pruning en myelinisatie als typische ontwikkelingsprocessen, wordt de hersenconnectiviteit zodanig beïnvloed dat de gevolgen van de beschadiging tot uiting komen en als het ware ontmaskerd worden.

6.3 Acknowledgements Dankwoord



There are many people who I would like to thank for their contribution to this work, whether directly or indirectly. First I would like to thank the children and their families who participated in these studies. Schizophrenia can be a devastating illness and our work forms a symbiotic relationship, with researchers working to find ways to help the patients and their families, and patients and families hoping that the work can contribute to helping those afflicted, even if it be for future generations. Without their participation, none of this work would be possible and I am hopeful that these studies will add to the understanding of the developmental neurobiology of schizophrenia so that one day we will be able to prevent or better treat the illness.

I would like to heartedly thank my two promotors, Prof. dr. Frank C. Verhulst and Prof. dr. Nancy C. Andreasen. Nancy, I will be forever grateful for your mentorship, not only during my postdoctoral research fellowship, but extending far beyond. Even after my postdoctoral fellowship, I have been very fortunate to be involved in the collaborative work of the MIND Research Network. Your help and guidance

has been wonderful, and I thank you. Frank, thank you for your guidance and encouragement in putting together this thesis and for your help with the translation. Also, thanks for opening the door for me to come to Rotterdam. I am grateful for all that you have done, not only with the thesis, but also in helping with my adjustment to life in the Netherlands. You're a fabulous department head and I'll look forward to working for and with you over the years to come.

I am also very thankful to the members of my thesis committee. First, I would like to thank Prof. dr. Irving I. Gottesman, who due to recent health problems is unable to attend my defense. Irv, thanks so much for your mentorship during the time that I was in Minnesota. Your door was always open, your advice always sound, and your science impeccable. I still appreciate all the articles that you send me. I'm very sorry that you are unable to attend my defense and I wish you a speedy and full recovery.

Ingrid, I admire your work and I thank you for your willingness to serve on my committee. It's always wonderful to see you at HBM and ICOSR and to catch up. Aad, it is wonderful working with you and I thank you for your willingness to serve as 'secretaris' for my 'leescommissie.' Wiro, thanks so much for your willingness to serve on my committee. I am impressed with your group and will look forward to working together in merging methodology with developmental neuroscience and population based imaging. I'll look forward to working with you and Aad in the years to come. Henning, thanks also for your willingness to serve on my committee and for all your help since my move to the Netherlands. I appreciate your sound advice. It's great to share an office with you and it will be great working with you over the years to come. Thank you also Prof. dr. Franken for your willingness to serve on my committee. I hope that we have the opportunity to work together in the future.

I heartedly thank my two paranymphs. Cindy, you're really a great friend and I really appreciate your flying over from 'Minnesnowta' to participate in my defense. Your exploration into the historical role of the paranymphs, who carried swords to defend the defender, was interesting to hear. It's good that times have changed. I'll look forward to hearing the reaction from your family and friends when you tell them that you traveled to Holland to be a paranymph. Mijke, I don't know how to thank you for all that you've done to help with my transition to life in the Netherlands. Your help with everything from enlightening me on the protocol related to the 'verdedigen,' helping with the tranlation, and your advice about the borrel and other events has been very helpful. I also really, really, really appreciate your opening your home to me during the time when my apartment was being renovated. The two weeks that I spent there was like a breath of fresh air. Thanks!

I would like to especially thank dr. Th. M. J. Machielse for his help in translating the 'Summary' into the 'Samenvatting.' As a true scholar, you not only assisted with the translation, but also made suggestions regarding content and helped improve the manuscript. Thanks also for your kindness in volunteering your time, not only to assist with the translation, but I also very much appreciate the time that you have given to help me learn the Dutch language and culture. As you have given to me, so shall I give to others. Thanks also to Hanan Marroun and Mijke Zeegers for your help with scientific aspects of the translation. "Wetenschappelijke taal kan soms een beetje raar zijn."

I want to thank my mentors and colleagues at the University of Minnesota. Since much of the work was as a part of a career development award funded by the National Institutes of Mental Health and by two separate NARSAD Awards, I had a number of mentors and consultants who I could turn to for assistance. I especially would like to thank Kelvin O. Lim, M.D. for his mentorship during my career development award and my first NARSAD. I appreciate all your help Kelvin and I learned a great deal from your mentorship. Thanks again Irv for your mentorship on my NARSAD award. It's appreciated. I also would like to thank other mentors who I learned a tremendous amount from during my years at the University of Minnesota, including S. Charles Schulz, M.D., Charles A. Nelson, Ph.D., Kamil Ugurbil, Ph.D., Stephen Strother, Ph.D., and Vince Calhoun, Ph.D. These people gave generously of their time and I thank them.

Two of my Minnesota colleagues who I especially would like to thank are Canan Karatekin, Ph.D. and Monica Luciana, Ph.D. Canan and Monica, I miss working with you both and I thank you for all of our collaborative projects. We really were quite productive and I'll keep my eyes open for future collaborative projects. Thanks Afshan Anjum, M.D., for your help with the KIDDIE-SADS and other aspects of the research program. I would like to thank my mentee turned colleague, Katie Cullen, M.D., who assisted with various aspects of the research project. I thank all my co-authors, and because of the collaborations, they are many. There names are all listed in the book. Thanks to all the folks at the CMRR, including Melissa, Sholom, Silvia, Diane, Mike, Tommy, Noam, Brian, Bryon, Uta, Karl, Deb, Gulin, Xiaoping, John, Steen, Cheryl, and Pierre Francois, Geoff, Greg, Essa, Pierre-Gilles, Ghosha, Curt, Eddie, and Gregor. You made the CMRR an exciting place to work. I especially would like to thank those who attended the weekly MIND Research Network weekly calls, including Randy Gollub, M.D., Ph.D., Vincent Clark, Ph.D., Stefan Ehrlich, M.D., Ph.D., Beng C. Ho, M.D., H. Jeremy Bockholt, and Vincent Calhoun, Ph.D. It's great to have such a wonderful network of colleagues. Thanks also Margaret King for all your coordinating and scientific efforts.

I would like to thank my Minnesota team, who were invaluable in their dedication and self-motivation. I especially thank Angela Guimaraes and Marcus Schmidt. Angie, it was wonderful working with you performing all of the KIDDIE-SADS that we did, but your work in the everyday details of keeping the lab running,

interacting with the families, organizing, scheduling, planning, was invaluable. I know that you are enjoying your new role as a mom. Marcus, your help in running the scanner, scripting the image processing pipeline, generating beautiful graphics, and your all around computer knowledge has been invaluable. I'm very happy that you agreed to join the new team here in the Netherlands. I would like to thank all those who assisted with various aspects of data collection and imaging, including Jill Pluhar, Al Fox, Donaya Hongwanishkul, and Lisa Michelle Rohrer.

I would like to thank the KNICR team here, who are a terrific group. It is this team also that worked together to coordinate the symposium scheduled for September 2. I would like to thank dr. Hanan El Marroun, Alette Walstra, Gerbrich van de Bosch, Sabine Mous, and again, Marcus Schmidt.Thanks to the students, Anouk Geraets, Anne Kampstra, Elles van den Broek, Kary Mok, Iris Haitsma, Josefien Mellegers, Kimberley Slingerland, Madhvi Moelchand, and Nikita Schoemaker. It's great to have such a hardworking and motivated team. Hanan, I turned to your proefshrift frequently as a example, so thanks for doing such great work on yours.

I also thank my colleagues at the Westzeedijk, who were always willing to help when I had questions regarding various aspects of the proefschrift. Thanks Kristin Greaves-Lord, Floor van Oort, Jan van de Ende, and Henning Tiemeier, your help is appreciated.

Thanks Laureen Pauling for your help with some of the administrative aspects related to my PhD program and defense, not to mention my move to Rotterdam.

And since the First shall be last and the last shall be first, I would like to again thank my family for their help and support. Thanks so much Mom and Dad and I'm glad that you've come over to be present for my defense. Thanks for the genes, the environment, and the gene by environment interaction. I hope that you will enjoy the defense and surrounding festivities. Thanks to my three wonderful boys, Kristof, Andreas, and Jon Erik, being such easy boys to parent has been wonderful. Thanks for doing everything that I ask all the time… Ha! You all have worked very hard in the renovation of my apartment here, and it is appreciated. I also want to thank other family members: Sally, Cynthia, Grandma Kerstin, and Gordon & Joyce,. the farm is always such a wonderful place to relax and feel at home.

PhD Portfolio

I was matriculated and had completed all the course work for a Ph.D. in Biomedical Engineering at the University of Minnesota, Twin Cities Campus. I had completed a total of 65.332 points and had cumulative grade point average of 3.843/4.000.

Courses	Year	Workload
Functional Biomedical Imaging	2004	3.0
Biomedical Engineering Seminar	2004	1.0
Advanced MRI Physics	2005	3.0
Biomedical Engineering Seminar	2004	1.0
Graduate Seminar in Psychology	2006	3.0
Ethics of Human Subjects Research	2006	3.0
Theory of Statistics	2006	4.0
Data Processing with PC-SAS	2007	1.0
Cell Engineering	2007	3.0
Software Programming Courses		
Analysis of Functional Neuroimaging (AFNI Course)	2005	1.4
IDL Programming for Medical Imaging	2006	1.4
FSL Course	2007	1.4
UCLA Institute for Pure and Applied Mathematics	2008	2.8

National and International Conferences, Seminars, Meetings, & Workshops

2004	
Winter Workshop for Schizophrenia Research, Davos, Switzerland	1.4
Society for Biological Psychiatry, New York, USA	0.6
2005	
International Congress on Schizophrenia Research, Savannah, Georgia, USA	1.4
American Academy of Child and Adolescent Psychiatry, Chicago, IL, USA	1.1
American College of Neuropsychopharmacology, Big Island, Hawaii, USA	1.4
Biomedical Engineering in Medicine, Minneapolis, Minnesota, USA	0.3
2006	
Winter Workshop for Schizophrenia Research, Davos, Switzerland	1.4
International Society for Magnetic Resonance in Medicine, Seattle, WA, USA	2.0
Organization for Human Brain Mapping, Florence, Italy	1.1
2007	
International Congress on Schizophrenia Research, Colorado Springs, CO, USA	1.1
International Society for Magnetic Resonance in Medicine, Berlin, Germany	2.0
Organization for Human Brain Mapping, Chicago, Illinois, USA	1.1
2008	
Winter Workshop for Schizophrenia Research, Montreux, Switzerland	1.4
International Society for Magnetic Resonance in Medicine, Toronto, Canada	1.4
American Academy of Child and Adolescent Psychiatry, Chicago, IL, USA	1.4
International Conference on Early Psychosis, Melbourne, Australia	0.6

2009

International Congress on Schizophrenia Research, San Diego, CA, USA	1.1
International Society for Magnetic Resonance in Medicine, Honolulu, HI, USA	1.4
Organization for Human Brain Mapping, San Francisco, California, USA	1.1
Vogt-Brodmann Symposium, Juelich, Germany	0.7

2010

Organization for Human Brain Mapping, Barcelona, Spain	1.1

Publications

Peer Reviewed Papers

1. Kim DI, Sui J, Rachakonda S, White T, Manoach DS, Ho BC, Schulz SC, Calhoun VD (In Press) Identification of imaging biomarkers in schizophrenia: A coefficient-constrained independent component analysis of the MIND multi-site schizophrenia study. Neuroinformatics.
2. White T, Schmidt M, Kim DI, Calhoun VD (In Press) Disrupted functional brain connectivity during verbal working memory in children and adolescents with schizophrenia. Cereb Cortex.
3. White T, Su S, Schmidt M, Kao CY, Sapiro G (In Press) The development of gyrification in children and adolescents. Brain & Cognition.
4. Sui J, Adali T, Pearlson GD, Yang H, Sponheim SR, White T, Calhoun VD (In Press) A CCA+ICA based model for multi-task brain imaging data fusion and its application to schizophrenia. NeuroImage.
5. Cullen K, Guimaraes A, Wozniak J, Anjum A, Schulz SC, White T (In Press) Trajectories of social withdrawal and cognitive decline in the schizophrenia prodrome. Clin Schiz Rel Psychos.
6. White T, Hilgetag C (In Press) Gyrification and neural connectivity in schizophrenia. Development and Psychopathology.
7. Ehrlich S, Morrow EM, Roffman JL, Wallace SR, Naylor M, Bockholt J, Lundquist A, Yendiki A, Ho BC, White T, Manoach DS, Clark VP, Calhoun VD, Gollub RL, Holt DJ (In Press) The COMT Val108/158Met polymorphism and medial temporal lobe volumetry in patients with schizophrenia and healthy adults. NeuroImage.
8. Wahlstrom D, Collins P, White T, Luciana M (In Press) Developmental changes in dopamine neurotransmission in adolescence: Behavioral implications and issues in assessment. Brain & Cognition.
9. Davenport N, Karatekin C, White T, Lim K (In Press) Differential fractional anisotropy abnormalities in adolescents with ADHD or schizophrenia. Psychiatry Research: Neuroimaging.
10. Michael A, Baum S, White T, Demirci O, Andreasen NC, Segall J, Jung R, Pearlson, G, Clark V, Gollub R, Schulz SC, Roffman J, Lim KO, Ho BC, Bockholt HJ, Calhoun VC (In Press) Does Function Follow Form?: Methods to Fuse Structural and Functional Brain Images Show Decreased Linkage in Schizophrenia. NeuroImage.
11. Kim D, Manoach DS, Turner J, Brown G, Belger A, Bockholt HJ, Clark VP, Ford JM, Gollub R, Lauriello J, Mathalon D, White T, Wible C, Leary D, Mueller B, Lim KO, Andreasen NC, Potkin S, Calhoun VD (In Press) An analysis of the fMRI BIRN and MIND studies shows a dysmodulation of functional networks in schizophrenia during working memory. Hum Brain Mapp.
12. White T, Magnotta VA, Bockholt HJ, Williams S, Gollub RL, Mueller BA, Ho BC, Jung R, Clark VP, Lauriello J, Bustillo JR, Schulz SC, Andreasen NC, Calhoun VD, Lim KO (In Press) Progressive White Matter Abnormalities in Schizophrenia: A multisite diffusion tensor imaging study. Schiz Bull 35: 204-212.
13. Karatekin C, White T, Bingham C (In Press) A Comparison of Parent and Teacher Ratings of Behavior Between Youth-Onset Psychosis and ADHD. J of Attention Disorders.
14. Karatekin C, Bingham C, White T (In Press) Oculomotor and pupillometric indices of pro- and antisaccade performance in youth-onset psychosis and attention-deficit/hyperactivity disorder. Schiz Bull.

15. Wahlstrom D, White T, Luciana M (2010) Neurobehavioral evidence for changes in dopamine system activity during adolescence. Neurosci Biobehav Rev 34: 631-648.
16. White T, Schmidt M, Karatekin C (2009) White matter 'potholes' in early-onset schizophrenia: a new approach to evaluate white matter microstructure using diffusion tensor imaging. Psychiatry Res 174: 110-115.
17. Demirci O, Stevens MC, Andreasen NC, Michael A, Liu J, White T, Pearlson GD, Clark VP, Calhoun VD (2009) Investigation of Relationships between fMRI Brain Networks in the Spectral Domain using ICA and Granger Causality Reveals Distinct Differences between Schizophrenia Patients and Healthy Controls. NeuroImage 46: 419-431.
18. Karatekin C, Bingham C, White T (2009) Regulation of cognitive resources during an n-back task in youth-onset psychosis and attention deficit hyperactivity disorder (ADHD). Int J Psychophysiol 73: 294-307.
19. Karatekin C, White T, Bingham C (2009) Incidental and Intentional Sequence Learning in Youth-Onset Psychosis and Attention-Deficit/Hyperactivity Disorder (ADHD). Neuropsychology 23: 445-59.
20. White T (2009) The Ethics are in the Numbers: A Bayesian Approach to the Management of Incidental Findings in Pediatric Magnetic Resonance Research. Am J Case Rep 10: 22-26.
21. White T, Miller J, Smith GL, McMahon WM (2009) Adherence and Psychopathology in Children and Adolescents with Cystic Fibrosis. Eur J Child Adolesc Psychiatr 18: 96-104.
22. Segall JM, Turner JA, van Erp TGM, White T, Bockholt HJ, Gollub RL, Ho BC, Magnotta V, Jung RE, McCarley RW, Schulz SC, Lauriello J, Clark VP, Voyvodic JT, Diaz MT, Calhoun VD (2009) Voxel-based morphometric multi-site collaborative study on schizophrenia. Schiz Bull 35(1) 82-95.
23. Karatekin C, White T, Bingham C (2008) Divided Attention in Youth-Onset Psychosis and Attention-Deficit/Hyperactivity Disorder (ADHD). J Abnormal Psychol 117: 881-95.
24. Roffman JL, Gollub RL, Calhoun VD, Wassink TH, Weiss AP, BC Ho BC, White T, J Fries, Andreasen NC, Goff DC, Manoach DS (2008) MTHFR 677CàT genotype disrupts prefrontal and dopaminergic function in schizophrenia. Proc Natl Acad Sci 105(45) 17573-8.
25. Wozniak JR, Block EE, White T, Jensen J, Schulz SC (2008) Clinical and Neurocognitive Course in Early-Onset Psychosis: A longitudinal study of adolescents with schizophrenia spectrum disorders. Early Intervention in Psychiatry 2: 169-177.
26. Jenson J, Oberstar J, Anjum A, White T, Wozniak J, Kumra S, Schulz SC (2008) A Comparative Study of Second-Generation Antipsychotics in Children and Adolescents with Schizophrenia Spectrum Disorders. J Child Adolesc Psychopharm 18(4) 317-326.
27. Kendi M, Kendi ATK, Lehericy S, Ducros M, Lim KO, Ugurbil K, Schulz SC, White T: (2008) Structural and diffusion tensor imaging of the fornix in childhood and adolescent-onset schizophrenia. J Am Acad Child Adolesc Psychiatry 47: 826-832.
28. White T, Nelson M, Lim KO (2008) Diffusion Tensor Imaging in Psychiatric Disorders. Trends MRI 19: 97-109.
29. White T, Cullen R, Rohrer LM, Karatekin C, Luciana M, Schmidt M, Hongwanishkul D, Kumra S, Schulz SC, Lim KO: (2008) Limbic structures and networks in children and adolescents with schizophrenia. Schiz Bull 34: 18-29.
30. White T, Kendi ATK, Lehéricy S, Kendi M, Karatekin C, Guimaraes A, Davenport N, Schulz SC, Lim KO (2007) Disruption of Hippocampal Connectivity in Children and Adolescents with Schizophrenia – A voxel based diffusion tensor imaging study, Schizophrenia Research 90: 302-307.
31. Karatekin C, Marcus DJ, White T (2007) Oculomotor and manual indices of spatial sequence learning in middle childhood and adolescence. Journal of Experimental Child Psychology 96: 107-130.
32. Wahlstrom D, White T, Hooper CJ, Vrshek-Schallhorn S, Luciana, M (2007) Variations in the catechol O-methyltransferase polymorphism and prefrontally-guided behaviors in adolescents, Biological Psychiatry 61: 626-632.
33. Vrshek-Schallhorn S, Wahlstrom D, Benolkin K, White T, Luciana M (2006) Affective bias and response modulation following tyrosine depletion in healthy adults, Neuropsychopharmacology 31: 2523-2536.
34. White T, Anjum A, Schulz SC (2006) The Schizophrenia Prodrome, Am J Psychiatry 163(3): 376-380.
35. White T, Ho BC, Ward J, Arndt S, O'Leary D, Andreasen NC (2006) Neuropsychological performance in first-episode adolescents with schizophrenia: A comparison with first-episode adults and adolescent control subjects, Biological Psychiatry 60: 463-471.

36. White T, Magnotta, V (2005) The application of magnetic resonance imaging to develop anatomically correct brain models, International Journal of Bioelectromagnetism 7(1): 150-154.
37. White T, Andreasen NC, Nopoulos P, Magnotta, V (2003) Gyrification abnormalities in childhood and adolescent onset schizophrenia. Biological Psychiatry 52: 418-426.
38. White T, Andreasen NC, Nopolous P, Arndt S (2002) Brain volumes and surface morphology in monozygotic twins. Cerebral Cortex 12: 486-493.
39. Gray D, Parker-Cohen NY, White T, Clark ST, Seiner SH, Achilles J, McMahon WM (2001) A comparison of individual and family psychology of adolescents with chronic fatigue syndrome, rheumatoid arthritis, and mood disorders, J Dev Beh Pediatrics 22: 234-242.
40. White T, O'Leary DS, Magnotta V, Flaum M, Arndt S, Andreasen NC (2001) Anatomic and functional variability: The effects of filter size in fMRI data analysis. NeuroImage, 13: 577-588.
41. Stephan KE, Magnotta VA, White T, Flaum M, O'Leary DS, Andreasen NC (2001) Effects of olanzapine on cerebellar functional connectivity in schizophrenic patients during a simple motor task. Psychological Medicine, 31: 1065-1078.
42. White T, Schulz, SK (2000) Naltrexone in a 3½-year-old boy with self injurious behavior: Am J Psychiatry, 157: 1574-1582.

Book Chapters

1. White T, Lim KO (In Press) Diffusion Tensor Imaging in Psychiatric Disorders. Ed: Derik Jones. Oxford University Press (anticipated publication, 2010).
2. Anjum A, Gait P, Cullen K, White T (In Press) Schizophrenia in Adolescents and Young Adults. Eds: Jon E. Grant & Marc N. Potenza. Oxford Univesity Press (In Press).
3. Karatekin, C, Couperus, JW, Marcus DJ, & White T (in press). Effects of delay and retrieval mode on memory-guided saccades in 10-year-olds and adults. To be published in N. B. Johansen (Ed.), New Research on Short-Term Memory. Happauge, NY: Nova Science Publishers.
4. White T, Hilgetag C (2009) Gyrification and Development of the Cerebral Cortex. Eds: Charles Nelson & Monica Luciana. Handbook of Developmental Cognitive Neuroscience. MIT Press, Cambridge, Massachusettes.
5. White T (2008) Schizophrenia. In: Diseases and Disorders. Vol. 3. Brown Reference Group, London, England.
6. White T (2008) Psychotic Disorders. In: Diseases and Disorders. Vol. 3. Brown Reference Group, London, England.
7. White T (2004) Brain imaging in childhood and adolescent psychiatric disorders. Minnesota Association for Children's Mental Health, Networker 13, 2-3.
8. White T, Nelson CA (2005) Neurobiological Development During Childhood and Adolescence. Eds: S. Charles Schulz & Robert Findling. Johns Hopkins University Press. Baltimore, MD.
9. Wozniak J, White T, Schulz SC (2005) Neuropsychological Factors in Early-Onset Schizophrenia. In: Juvenile Schizophrenia. Eds: S. Charles Schulz & Robert Findling. Johns Hopkins University Press. Baltimore, MD.
10. Drell MJ, White TJH: (2005) Children's reaction to illness and hospitalization. In: Kaplan & Sadock's Comprehensive Textbook of Psychiatry – 8th Edition, Eds: Harold I. Kaplan and Virginia Sadock.
11. Drell MJ, White TJH: Children's reaction to illness and hospitalization. Comprehensive Textbook of Psychiatry – 7th Edition, Eds: Harold I. Kaplan and Benjamin J. Sadock (2000).

Master's Thesis

White T, Design and Noise Analysis of an Operational Amplifier Photodiode Pair for Use in Fiber Optic Sensors, Masters Thesis in Electrical Engineering, University of Illinois at ChampaignUrbana, October 1987.

Grants (Principle Investigator)

Biomedical Genetics Center at the University of Minnesota: $5,375 for one year. Feb 1, 2007 – Jan 31, 2008. Project title: "Developmental Genomics of the Adolescent Brain."

National Alliance for Research in Schizophrenia and Affective Disorders (NARSAD): $30,000 per year for two years. Jul 2006 – Jul 2006. Project title: "A Study of Prefrontal Structure and Function in Children and Adolescents with Velocardiofacial syndrome."

National Institutes of Mental Health Career Development Award (K-08 MH068540) – Jun 1, 2004 to May 31, 2009. $908,652 over five years Project Title: "Functional Connectivity in Schizophrenia."

Mental Illness and Neuroscience Discover (MIND) Institute - $50,000 per year from Jan 1, 2003 to Dec 31, 2005. Project Title: "Disregulation of Optimized Cognition in Schizophrenia."

National Alliance for Research in Schizophrenia and Affective Disorders (NARSAD): $30,000 per year for two years. Jul 2002 – Jul 2004. Project title: "Brain Surface Morphology in Childhood and Adolescent Schizophrenia." Role: Principle Investigator.

Center for Neurobehavioral Development – (PI: Tonya White & Canan Karatekin, Ph.D.) $9,230 from Mar 2002 to Mar 2003. Project Title: "A Multi-Method Study of Prefrontal-Meditated Tasks in Children and Adolescents with Schizophrenia & Their Siblings."

Mental Illness and Neuroscience Discover (MIND) Institute - $41,281 from Jan 1, 2002 to Dec 31, 2002. Project Title: "An fMRI study of spatial working memory in children and adolescents with schizophrenia."

Eli Lilly Pilot Research Award - $10,000 Granted through the Office of Research, American Academy of Child and Adolescent Psychiatry, Mar 1995 to Dec 1995. Project: "The relationship between family functioning, perceived compliance, and psychopathology in children and adolescents with cystic fibrosis." Mentor: William McMahon, M.D.