MICROSTRUCTURAL CHANGES IN FRONTAL WHITE MATTER ASSOCIATED WITH CANNABIS USE IN EARLY PHASE PSYCHOSIS: A DIFFUSION TENSOR IMAGING (DTI) STUDY

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
August 2014

In dedication to the patients and family members who are affected by schizophrenia spectrum and other psychotic disorders

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Abstract

Introduction: This project aimed to examine the impact of cannabis use on the brain white matter (WM) tissue abnormalities associated with psychosis, in individuals at the early phase of psychosis (< 5 years from diagnosis). Using diffusion tensor imaging (DTI), it was predicted that there would be evidence of WM disruption in patients with psychosis compared to healthy controls, and more so in patients with earlier and heavier lifetime cannabis use. Methods: The primary outcome measure was fractional anisotropy (FA) which reflects WM integrity. There were two groups of subjects: 1) Patients with early phase psychosis and 2) Healthy controls with no cannabis use. Scans were acquired on a 1.5T MRI and imaging analysis was done both within a volume of interest (VOI - left superior longitudinal fasciculus) and at the full-brain level. Results: A trend level decrease in mean VOI FA was found in patients relative to healthy controls, but no difference was found at the full-brain level. Within the patient group, there was a significant positive correlation found between cumulative lifetime cannabis use and mean VOI FA (but not mean full-brain FA), however there was no relationship between age of onset of cannabis use and mean FA either at the full-brain level or within the VOI. Discussion: There was trend level evidence of WM abnormalities in the VOI (but not at the full-brain level) in patients at the early phase of psychosis. Furthermore, contrary to what was hypothesized, there was evidence of *increased* WM integrity in patients with heavier lifetime cannabis use within the VOI. By accounting for concomitant alcohol and substance use, this study provides novel insight about WM microstructure, as it relates to early phase psychosis and adolescent cannabis use.

List Of Abbreviations Used

η^2	partial eta squared
ω	Larmor frequency
ADC	apparent diffusion coefficient
AxD	axial diffusivity
B_0	static magnetic field
BET	FMRIB's Brain Extraction Tool
BPRS	Brief Psychiatric Rating Scale
CB1/CB2	cannabinoid 1 / 2 receptors
CDSS	Calgary Depression Scale for Schizophrenia
DNA	deoxyribonucleic acid
DTI	diffusion tensor imaging
DUP	duration of untreated psychosis
DSM	Diagnostic and Statistical Manual of Mental Disorders
e.g.	*exempli gratia*
EIS	early intervention services
et al.	*et alia*
FA	fractional anisotropy
FDT	FSL's Diffusion Toolbox
FMRIB	Functional MRI of the Brain
FLIRT	FMRIB's Linear Image Registration Tool
GE	General Electric
HAM-A	Hamilton Anxiety Scale

i.e.	*id est*
MD	mean diffusivity
MINI	Mini International Neuropsychiatric Interview Screen
MRI	magnetic resonance imaging
NMDA	N-methyl-D-aspartate
NMR	Nuclear Magnetic Resonance
PANSS	Positive and Negative Syndrome Scale
PANSS-N	PANSS – negative scale
PANSS-P	PANSS – positive scale
PANSS-G	PANSS – General psychopathology scale
PSP	Personal and Social Performance scale
r	Pearson's r correlation
r_s	Spearman's rho correlation
$r_{partial}$	Partial correlation
RaD	radial diffusivity
SCID-1	Structured Clinical Interview for the DSM-IV Axis I Disorders
SCIP	Screen for Cognitive Impairment in Psychiatry
SCIP-global	SCIP global index
SCIP-PST	SCIP Psychomotor Speed Test
SCIP-VFT	SCIP Verbal Fluency Test
SCIP-VLT_D	SCIP Verbal Learning Test – Delayed
SCIP-VLT_I	SCIP Verbal Learning Test – Immediate
SCIP-WMT	SCIP Working Memory Test

T1	longitudinal relaxation time
TE	echo time
THC	tetrahydrocannabinol
TLFB	Timeline Followback
TR	repetition time
WM	white matter

Acknowledgements

I would like to acknowledge my all of my supervisors, especially Dr. Philip Tibbo and Dr. Denise Bernier, for the time and energy they put into helping me complete this research from the very beginning as well as my extended supervisory team, Dr. Aaron Newman and Dr. Sherry Stewart, for their expertise and input. I am grateful for the support and guidance of the CIP program director, Dr. Jason Berman, and program manager, Jesslyn Kinney. A big thanks also to Carl Helmick, who was crucial in guiding me through the many hours of DTI analysis.

I would also like to acknowledge my colleagues and friends (too many to name); although they are informal supports, they provided me with much encouragement and strength during this project. I also have to give thanks to my parents, Ben and Judy Cookey, my brother Tamuno Cookey, and especially my wife, Lauren Cookey, as they were the backbone upon which I could continue to work, especially during the more stressful times.

If it wasn't for all these supports, and the grace of God, I could not have completed this thesis.

CHAPTER 1 – INTRODUCTION

1.1 Schizophrenia and Other Psychotic Disorders

1.1.1 Epidemiology, Clinical Manifestations, Diagnosis

Schizophrenia and other psychotic disorders are a group of mental disorders characterized by psychotic symptoms (e.g. hallucinations and delusions), cognitive dysfunction, as well as significant functional and social impairment. The prevalence of psychotic disorders is approximately 3%. Schizophrenia (one of the more severe psychotic disorders), with a prevalence of 1%, is ranked by the World Health Organization as one of the top ten causes of the global disease burden (Lopez and Murray, 1998; McGrath et al., 2008; Perälä et al., 2007). Typically the onset of illness is in adolescence or early adulthood and in many cases it can follow a lifelong course.

In 1887, the German physician, Emil Kraepelin, first described schizophrenia and the other psychotic disorders (then known as Dementia Praecox) as discrete mental disorders (Kraepelin, 1919), and they have since been recognized to be among the most debilitating and perplexing illnesses known to exist. Several years later, the Swiss psychiatrist, Eugen Bleuler (1857-1939) expanded our understanding of these disorders, and was the first to describe them as "the group of schizophrenias" (Bleuler, 1950). Even at that time, there were descriptions of different categories of symptoms, and over time phenomenological descriptions began to separate symptoms into a 'positive' cluster (e.g. hallucinations, delusions, disorganization) and a 'negative' cluster (e.g. alogia, anhedonia, flat affect). The negative symptoms were initially considered as 'fundamental' symptoms when first described by Kraeplin and

Bleuler, but over time the positive symptoms have come to the forefront of clinical attention, perhaps driven by the fact that pharmacotherapy development has a significantly better impact on positive symptoms compared to negative symptoms.

Although the symptomatology of psychosis, and other mental disorders is manifold and diverse, the Diagnostic and Statistical Manual of Mental Disorders (DSM) provides a detailed description and categorization. The DSM began as a way of collecting census and psychiatric hospital statistics, but through several iterations now describes psychiatric symptoms and classifies mental disorders into various categories and diagnoses. The current version of DSM is the DSM-5 (fifth edition), and describes the psychotic disorders in a chapter entitled 'schizophrenia spectrum and other psychotic disorders'(APA, 2013). It is important to distinguish the difference between psychosis and schizophrenia. Psychosis refers to a disordered mental state, characterized by a disturbance in the perception of reality whereas schizophrenia is one of several diagnosable psychotic disorders according to the DSM, which is characterized by the presence of psychotic, as well as other symptoms and accompanied by significant social and functional deterioration.

The diagnosis of schizophrenia spectrum and other psychotic disorders is clinical, following diagnostic criteria as per the DSM. To diagnose most psychotic disorders, the presence of one or more of the following symptoms is required: hallucinations, delusions, disorganized speech, disorganized or abnormal motor behaviour (e.g. catatonia), and negative symptoms. In addition, there must be evidence of significant social/occupational dysfunction. Finally, other mental disorders associated with psychosis (such as mood disorders) must be ruled out, along

with other potential causes of psychosis, including general medical conditions and substance misuse. This study focuses on the chronic primary psychotic disorders including, schizophrenia and schizoaffective disorder (APA, 2000). The word 'psychosis' or 'psychotic disorder' will refer to the clinical picture consistent with these diagnoses. Further, this study focuses on the early phase of psychosis.

1.1.2 Early Phase Psychosis

The clinical course of psychosis has been described as a series of stages beginning with the period prior to the onset of illness (premorbid stage), followed by a period of non-specific behavioural change (prodromal stage), then leading to the development of psychotic symptoms (onset/active stage), and finally patients will fall into various patterns of relapse/recovery, for the remainder of their lives (residual/chronic stage).

Although onset of psychotic disorders can be abrupt, typically there is a gradual progression of sub-syndromal symptoms (the prodrome) leading up to the onset of active psychosis. The prodrome can consist of a variety of non-specific symptoms including attenuated positive symptoms (e.g. illusions), mood symptoms (e.g. dysphoria), and cognitive symptoms (e.g. concentration difficulties), among others (Lieberman et al., 2001). Once patients enter the active stage of psychosis, it is referred to as the 'first episode' or 'first break'. Often there can be a delay of several months or even years before patients enter treatment following their first episode, which can have a negative impact. Studies have reported that the longer the patient experiences untreated psychosis (termed 'duration of untreated psychosis' (DUP)),

the worse the outcomes as measured by severity of global psychopathology, positive symptom severity, and negative symptom severity (Perkins et al., 2005). With this knowledge, early intervention services (EIS) for psychosis have developed worldwide. The goals for EIS include improving clinical outcomes for those diagnosed with psychosis which run counter to the pessimism and therapeutic nihilism which existed previously. Specifically, EIS programs aim to intervene in the early phase of psychosis as this is thought to represent a 'critical period' of the illness in terms of determining long-term outcome of clinical and social functioning (Birchwood et al., 1998; McGlashan and Johannessen, 1996). In addition this period represents an ideal time for intervention with important patient and family factors being more responsive to change (Birchwood, 2000). From the point of view of investigating biopsychosocial factors that are core to psychosis, a focus of research on patients in the early phase (typically the first 5 years) of their illness allows minimization of the confounding effects of years with medication (e.g. antipsychotics) use as well as the neurotoxic effects of psychosis itself (Olney and Farber, 1995; Wyatt, 1991).

1.1.3 Etiology and Pathophysiology

Despite the wealth of knowledge describing the phenomenology of psychosis, as well as the rapidly growing body of genetic and epidemiological evidence, the pathophysiology of the disorder is still unclear. However, there is good evidence from twin studies that there is both a strong genetic component as well as an environmental component. Generally, the pair-wise concordance between monozygotic twins (who

share 100% of their DNA) is about 40%, suggesting a strong genetic link, but given the 60% remaining who are not concordant, there is likely a significant role of the environment as well (Kringlen, 2000). Theories focusing on predominantly psychosocial and environmental factors were more prevalent early in the 20th century. Sigmund Freud (1856-1939) postulated a theory of psychosis resulting from defects in ego development; Margaret Mahler (1897-1985) understood psychotic symptoms to arise from distortions in the mother-child relationship; and Harry Stack Sullivan (1892-1949) viewed psychosis as the result of cumulative lifetime trauma culminating in a disturbance in interpersonal relatedness (Sadock et al., 2007).

A gradual shift to a more neurobiologically oriented understanding of psychosis has developed since then. For example, the pathophysiology of psychosis can also be understood at the level of neurotransmitters. In particular dopaminergic and glutamatergic neurotransmitter systems have been most frequently implicated. The dopamine hypothesis postulates that overactivity of dopamine in the mesolimbic pathway of the brain causes the positive symptoms of psychosis, while the relative underactivity of dopamine in meso-cortical pathways contributes to negative symptoms (Davis et al., 1991; Sadock et al., 2009). The main driver of the dopamine hypothesis was evidence that dopamine blocking agents had a significant antipsychotic effect (Creese et al., 1996; Weinberger, 1987). There was also the additional observation that drugs that increased dopaminergic activity (e.g. cocaine and amphetamine) elicited psychotic symptoms (Sadock et al., 2009). More recently abnormalities in the glutamatergic system have been hypothesized as a cause of psychosis. Evidence for the glutamate hypothesis is derived from observations of

psychosis induced by glutamate antagonists (e.g. ketamine) in both patients as well as healthy volunteers (Javitt and Zukin, 1991; Lahti et al., 2001). Accordingly, drugs enhancing the effect of glutamate at the N-methyl-D-aspartate (NMDA) receptor (via the glycine coagonist site), were found to ameliorate psychotic symptoms (Meador-Woodruff and Healy, 2000). Furthermore, genetic studies examining post-mortem brain tissue of patients with psychosis have found abnormalities in critical structural and functional subunits of NMDA-glutamate receptors (Geddes et al., 2011).

Currently, given the wealth of accumulated evidence that complex interrelated biopsychosocial elements are likely involved in the etiology of psychosis, a broader explanatory model of the disorder has emerged, known as the neurodevelopmental model. This model postulates that a fixed lesion early in life (e.g. perinatally) interacts with the complex anatomical and functional maturation of the brain, resulting in the onset of the disorder later in life (Rapoport et al., 2005; Weinberger, 1987). In other words, psychosis is the clinical end state of an abnormal neurodevelopmental process that begins early in life, but does not fully manifest until several years later in adolescence or early adulthood (Rapoport et al., 2012).

There is now significant evidence supporting the broad neurodevelopmental model from diverse lines of research. Epidemiological studies examining pre/peri-natal risk factors have found evidence of an increased risk of psychosis related to obstetrical complications and pre/peri-natal infection amongst other risk factors (Brown and Derkits, 2010; M. Cannon, 2002). Part of the neurodevelopmental model also includes abnormal environmental exposures which can negatively affect the developing brain; a complex process that continues into young adulthood. As such,

studies examining risk factors more proximal to illness onset (i.e. during childhood and adolescence) have found that living in an urban environment, being exposed to childhood abuse/trauma, as well as substance abuse, particularly cannabis abuse, are associated with increased risk of developing psychosis (Morgan and Fisher, 2007; Sadock et al., 2009; van Os et al., 2010). Neuroimaging studies provide further evidence for the neurodevelopmental hypothesis with studies of those at high-risk of psychosis (siblings/twins of patients with psychosis) as well as those with diagnosed psychotic disorders, having demonstrable reductions in cortical grey matter (T. D. Cannon et al., 2002; Ettinger et al., 2011; Greenstein et al., 2006).

Another developmental model of psychosis that takes into account the complex interplay between genes and environment is known as the "two-hit" hypothesis. This model postulates that a genetic or environmental disruption during early development (the "first hit") would make the brain vulnerable to a "second hit" that would precipitate the onset of psychosis (Maynard et al., 2001). It goes on to explain that neither insult is sufficient in and of itself to produce the disorder, but rather the first hit (likely occurring perinatally) "primes" the system for the second, at which point psychosis emerges (Maynard et al., 2001). This is consistent with another widely accepted developmental model, which emphasizes that the presence of neuropathological vulnerability is an essential substrate of the illness, which later can manifest as psychosis with exposure to biopsychosocial stressors (Cornblatt et al., 2003). In regards to these developmental models of psychosis, cannabis exposure may represent a critical environmental trigger (second hit), leading to the onset of psychosis (Malone et al., 2010).

1.2 Cannabis Use and Early Phase Psychosis

1.2.1 Epidemiology of Cannabis Use

Cannabis comes from a species of flowering plants called *Cannabis Sativa* and is the most widely used illicit drug in the world (Leggett, 2006). The prevalence of cannabis use has significantly increased over the past two decades and recent Canadian statistics report 12 million people (approximately 34% of Canadian population) have used cannabis in their lifetime. It is notable that Canada's youth in particular (between ages 11-15 years old) have the highest 12-month prevalence rate of cannabis use (28%) out of all developed countries (Adamson, 2013). Furthermore, 6.8% of all Canadians have symptoms of cannabis abuse or dependence, a rate that is greater than all other illicit drug abuse/dependence combined (4.0%) (Pearson et al., 2013). Cannabis use in psychosis is much higher than in the general population, with rates up to 43%, and with the majority of users being younger patients (Bersani et al., 2002).

1.2.2 Biological Effects of Cannabis Use

There are over 400 compounds found in cannabis many of which have various antagonistic or synergistic effects (Ashton, 2001). There are over 60 cannabinoids, and the various biological effects of these compounds are largely unknown. Eventually it was discovered that the primary psychoactive ingredient in cannabis is delta-9-tetrahydrocannabinol (THC) (Mechoulam and Gaoni, 1965). The potency of the THC content of cannabis has gradually increased over the past two decades and

now can reach up to 15% (DSM-5) (APA, 2013). Cannabis acts in the brain primarily on Cannabinoid 1 (CB1) and CB2 receptors which are part of an endogenous cannabinoid receptor system in the brain. These endocannabinoid receptors (particularly CB1 receptors) are widespread in the brain in both grey matter and WM (Glass et al., 1997; Romero et al., 1997) and are involved in a variety of functions including memory, mood and reward processing (Bossong et al., 2014). In fact, CB1 receptors are the most abundant G-protein-coupled receptors in the mammalian brain and can induce a variety of behavioural and psychological changes including anxiety, euphoria, impaired motor coordination, impairment in short-term/working memory as well as psychotic symptoms (Bossong et al., 2014). Furthermore, cannabinoids are highly lipid-soluble and therefore can accumulate over time in the lipid membranes of neurons, a phenomenon that may help explain some of its more long-term neurobiological effects (Hollister, 1986). Finally, it is of particular interest that the endocananbinoid system is involved in the regulation of both the glutamatergic and dopaminergic neurotransmitter systems, with recent evidence implicating a role in the pathophysiology of psychotic disorders (Bossong et al., 2014). The following section explores the link between cannabis and psychotic disorders, with particular focus on the early phase of the illness.

1.2.3 Role of Cannabis Use in Early Phase Psychosis

Starting with the Swedish longitudinal study (Andréasson et al., 1987), epidemiological evidence has accumulated supporting the notion that cannabis use plays a causal role in the development of some psychotic illnesses (Arseneault, 2002;

Casadio et al., 2011). These studies demonstrated an increased risk of developing psychosis with a higher frequency of cannabis use, with up to a 6-fold increased risk for psychosis in heavy users (over 50 lifetime occasions) compared to non-users (Andréasson et al., 1987). In terms of age of cannabis use, early age of onset is associated with a particularly increased risk of developing psychosis, and could be much more damaging to the brain relative to later cannabis use (early users being defined as younger than 17 years old) (Casadio et al., 2011; Malone et al., 2010). Furthermore, cannabis use is associated with a significantly younger age of onset of psychosis, which in turn predicts a poorer prognosis of the disorder (Veen et al., 2004).

There is a hypothesis that was prominent especially during the 1990's postulating that substances, such as cannabis, are used in order to self-medicate to relieve the distress associated with psychotic symptoms (Blanchard et al., 2000; Khantzian, 1997). This hypothesis however has now fallen out of favour as the bulk of the data has shown that substance use patterns do not fit with symptom patterns in individuals with psychotic disorders (Blanchard et al., 2000).

Complimentary epidemiological evidence examining cohorts of patients early in the course of psychotic disorders have found that the prevalence of cannabis use is well above that of the general population with the majority starting use at least three years prior to the onset of illness (Bersani et al., 2002). Also, individuals early in their course of psychotic illness were found to be more likely to abuse cannabis which in turn was related to exacerbated psychiatric symptoms as measured by the Brief Psychiatric Rating Scale (BPRS) (Kovasznay et al., 1997).

Additional lines of evidence, particularly from neuroimaging and neuropsychological studies also demonstrate a greater detrimental impact of early adolescent initiation of cannabis use, reported for visual reaction times (Ehrenreich et al., 1999), cognitive performance (Pope et al., 2003), and volumetric brain tissue abnormalities (Wilson et al., 2000), supporting the idea that the adolescent brain may be especially vulnerable to cannabis exposure. A recent review and synthesis of currently available neuroimaging and neuropsychological studies also reinforces the important impact of cannabis use in psychosis with a focus on the endocannabinoid system (Bossong et al., 2014). By examining challenge studies looking at the acute effects of THC on brain functions implicated in psychosis, Bossong et al. (2014) found that there was significant overlap between THC-induced abnormalities, and those found in psychosis over a number of cognitive domains including memory encoding, working memory, executive function and emotional processing (Bossong et al., 2014).

Recent evidence has implicated that cannabis use may exert this potential triggering effect leading to psychosis through its effects on white matter (WM) development. Specifically, significant levels of endocannabinoid receptors have been found in WM tracts of the brain, as well as on the glial cells responsible for the production and maintenance of WM (e.g. astrocytes and oligodendrocytes), especially early on in neurodevelopment (Molina-Holgado et al., 2002; Romero et al., 1997). It has therefore been postulated that early cannabis exposure interacts with these processes and adversely affects the trajectory of WM development, ultimately triggering psychosis in individuals who are vulnerable to the illness (Bava et al.,

2009). The investigation of WM therefore is important in examining the role of cannabis use in the development of psychotic disorders.

1.3 White Matter Disturbances in Early Phase Psychosis

1.3.1 What is White Matter?

White matter provides the physical foundation for functional connectivity in the brain. It consists of axons as well as the oligodendrocyte-produced lipid sheaths surrounding them, known as myelin. Myelin acts to insulate axonal membranes and helps improve the conduction of action potentials. Despite the fact that WM constitutes almost half of the human brain, traditionally the focus of neuroscience research has been mostly on gray matter (Fields, 2008a). In recent decades however, there has been a shift towards a focus on WM (Fields, 2008b).

1.3.1.1 White Matter Tracts/Families?

Functionally related myelinated axons bundle together into groups known as WM tracts. These tracts are able to carry large amounts of information from one brain region to another. WM tracts can be categorized into five major WM tract families based on their functionality: 1) Brainstem fibers, 2) Projection fibers, 3) Association fibers, 4) Limbic fibers and 5) Commissural fibers (Wakana et al., 2004).

Brainstem fibers include the main tracts of WM in the brain stem. This includes the corticospinal tract (relaying ascending and descending somatosensory information), cerebellar peduncles (coordination of motor and control of equilibrium and muscle tone) and the medial lemniscus (carries ascending somatosensory

information to the thalamus). Projection fibers include the main connections between the cortex to the lower structures including the spinal cord, brainstem, and thalamus. These fibers carry ascending and descending information across a wide variety of functional systems including the motor, somatosensory, and reticular activating systems. Association fibers are those connecting one part of the cortex with another (cortex-cortex), and are responsible for high level cognitive processes including executive functions, working/spatial memory and complex sensory integration, amongst many other cognitive functions. Association fibers include the superior/inferior longitudinal fasciculi (SLF/ILF), superior/inferior fronto-occipital fasciculi, and the uncinate fasciculus. Limbic fibers are centered around the temporal limbic structures, and they are involved with memory encoding and emotional processing. Limbic fibers include fibers related to the cingulum, fornix and stria terminalis. Finally, the commissural fibers (also known as callosal fibers) connect the left and right cerebral hemispheres and are involved with interhemispheric communication and cooperation. These fibers include the corpus callosum, forceps major/minor as well as the tapetum.

1.3.2 Rationale for Studying White Matter in Early Phase Psychosis

There is a growing body of evidence suggesting that disturbances in connectivity between different brain regions, rather than abnormalities within the separate regions themselves, are responsible for the clinical symptoms and cognitive dysfunctions observed in schizophrenia and other psychotic disorders (Davis et al., 2003). This disconnectivity hypothesis of psychosis has now gained support from a

variety of different lines of evidence, which strongly implicates WM as playing a key role in psychosis (Davis et al., 2003; Friston and Frith, 1995; Luck et al., 2011). Therefore in recent years WM, the connective infrastructure of the brain, has come into focus.

Various lines of evidence point to irregularities of WM in psychosis, in particular with regards to myelin maintenance and repair involving oligodendroglial dysfunction (Davis et al., 2003; Karlsgodt et al., 2012). Additionally, there is post-mortem evidence of decreased number and density of oligodendrocytes as well as associated damage to myelin sheaths (Hof et al., 2003; Uranova et al., 2001; 2007). Neuroimaging is a way to investigate potential brain abnormalities in a variety of illnesses in living patients. Neuroimaging methods sensitive to subtle abnormalities in WM microstructure play a key role in characterizing brain changes in psychosis. Diffusion tensor imaging (DTI) is currently one of the most effective methods available to investigate WM *in vivo.*

1.4 Diffusion Tensor Imaging (DTI) in Early Phase Psychosis

1.4.1 Basic Principles of MRI

Magnetic Resonance Imaging (MRI), is an imaging technique with a range of applications, primarily used in biomedical settings as both a clinical and research tool. MRI employs the principles of nuclei magnetic resonance (NMR) which uses the magnetic properties of atomic nuclei, to produce images of the human body (e.g. brain).

The human body is approximately 63% hydrogen, mostly coming from water and fat, and so the images acquired with MRI are primarily a result of the signal from hydrogen atoms (Hornak, 2010). Atomic nuclei have a fundamental property called spin, which can be thought to be analogous to a magnetic field. When these nuclei are placed in an external magnetic field (i.e. the MRI scanner magnet), they align with it resulting in a bulk magnetization moment. In addition to their spins, nuclei display another resonance phenomenon known as precession, where the nuclei rotate about the magnetic field axis at a particular frequency (know as the larmor frequency ω). The larmor frequency is directly proportional to the applied magnetic field strength (B_0) as well as the inherent properties of the nuclei themselves. In order to obtain an MRI image, the object of interest must be placed in the external magnetic field, at which point a radio frequency electromagnetic pulse is applied transverse to the B_0 axis, which rotates the nuclei 90-degrees such that they rotate around B_0 at ω. Once the radio frequency pulse is stopped, the nuclei return to their equilibrium and line up parallel to B_0 once again. During this process, the oscillating magnetic field associated with the nuclei is detected by a receiver coil in the MRI machine as an electrical signal. It is this voltage that is the basis for the MRI signal (Hornak, 2010).

1.4.2 Basic Principles of DTI

The MRI signal can be used to quantify the diffusional properties of nuclei in the brain. Diffusion tensor imaging (DTI) is an in vivo MRI tool capable of providing an index of the micro-structural integrity of WM tissue (Beaulieu, 2002), as it measures the diffusion of molecules (generally water) moving through the brain

(Basser et al., 1994; Mori and J. Zhang, 2006). The random movement of water in the brain (Brownian motion), if left unhindered, would form a spherical shape, as the water molecules have the freedom to move in all directions (isotropic diffusion). Brain tissue structures such as axonal bundles prevent free diffusion of water molecules, which will naturally move more easily along these tracts rather than perpendicular to them (figure 1) (anisotropic diffusion) (Mori and J. Zhang, 2006). So by measuring the amount of anisotropy it is possible to estimate the axonal organization of the brain (Mori and J. Zhang, 2006).

To do this the application of two opposing (bipolar), unidirectional, magnetic field gradients is required, such that calculating the difference between the gradients allows for the detection of movement. This bipolar gradient has no measurable end effect on molecules that are stationary because the first gradient which dephases the nuclei spins is cancelled out by the second gradient which rephases the spins. However, actively diffusing water molecules are affected, and therefore can be detected and quantified (Johansen-Berg and Behrens, 2009). Since WM in the brain can take any orientation in 3D space, it is necessary to apply this bipolar gradient along multiple orientations. However as it would be impractical to obtain images for all of the thousands of possible directions, the diffusion tensor model was proposed (Basser et al., 1994). This model takes measurements of diffusion along any number of directions in space and fits it into a 3D ellipsoid model which is calculated for each voxel (smallest 3D volume element of and MRI scan) (Mori and J. Zhang, 2006). The matrix that allows the transformation of the diffusion directions into the 3 X 3

ellipsoid model is called the tensor; thus ‘diffusion tensor imaging’ (see Figure 2 for illustration of the diffusion tensor) (Mori and J. Zhang, 2006).

1.4.3 DTI Measures

Fractional anisotropy (FA) is the most widely used DTI measure reported in the literature, and it is computed by comparing the water diffusion along the longest axis of water movement (the course of WM tracts) relative to the other axes. Ultrastructural studies directly comparing DTI parameters with corresponding tissue pathology have associated reduced water diffusivity measures with dysmyelination or hypomyelination of white matter tracts; other tissue alterations that might influence water diffusivity are axonal pathology and changes in cell densities (Ruest et al., 2011). Another common diffusion measure is mean diffusivity (MD), which is a measure of the average magnitude of diffusion in all directions. In areas without barriers (e.g. cerebrospinal fluid) MD is high, whereas in more restricted areas (e.g. WM), MD is low; thus MD is usually inversely correlated with FA. Two other diffusivity measures from the family of parameters derived from the diffusion tensor which provide useful structural information are radial diffusivity (RaD) and axial diffusivity (AxD). In white matter, RaD represents the average water diffusion perpendicular to axonal fibers, while AxD represents the water diffusion parallel to axonal fibers (Basser, 1995; Song et al., 2002).

1.5 Application of DTI to Early Phase Psychosis and Cannabis Use: Review of the Literature

The majority of DTI studies of psychosis have assessed people in the *chronic* phase of illness. The most consistent finding in this population is a reduction in FA values compared to healthy controls, observed in a number of major WM tracts (Whitford et al., 2011). Specifically, findings point to WM disruption most frequently in the corpus callosum, frontal WM and SLF (Konrad and Winterer, 2008; Kyriakopoulos et al., 2008; White et al., 2008). There are fewer DTI studies assessing people in the *early phase* of psychosis (within the first 5 years of illness onset). This cohort is important, as it allows for the investigation of pathology core to the illness and at time of illness onset, with a minimal impact of confounders such as medication, age, and length of time with illness.

An up-to-date systematic literature review was completed in association with this thesis, examining DTI studies of early phase psychosis and of cannabis use (Table 1) (Cookey et al., 2014). The studies included in the review were categorized into three groups, as follows: 1) early phase psychosis (illness effect), 2) cannabis use in otherwise healthy individuals (drug effect), and 3) early phase psychosis with concurrent cannabis use (combined illness and drug effects) (Table 1). Results are outlined below.

1.5.1 DTI in Early Phase Psychosis: Illness Effect

A total of 72% (13/18) of studies found evidence of WM abnormalities (decreased FA values) in early phase psychosis compared to healthy controls, while 28% (5/18) reported no group differences (Cookey et al., 2014). Interestingly, another

recent review of DTI studies in this population (Peters et al., 2010) also found evidence of widespread WM abnormalities, supported by 71% (15/21) of the compiled studies. It is notable that although this earlier review included less stringent criteria (which is why it included more studies), it had a very similar pattern of findings. Of note, calculation of the magnitude of effect based on findings from studies that found between-group differences revealed an effect size of r = .454, which corresponds to a moderate to large effect.

A comparison of studies with (13/18 studies) and without (5/18 studies) group differences in FA values reveals that studies with group differences mainly used a whole brain VBA approach (10/13 studies), often clearly excluded participants with current substance dependence (7/13 studies), and were more likely to be acquired at a magnetic field of 1.5 T (11/13 studies). Studies with no group differences used a whole brain VBA approach in 3/5 studies, were less likely to clearly exclude substance dependence (1/5 studies), and less likely to be acquired at lower (1.5 T) magnetic field (2/5).

The association fiber tracts were most often implicated, namely a) the fronto-occipital fasciculus (Cheung et al., 2008; James et al., 2011; Kyriakopoulos et al., 2009; Pérez-Iglesias et al., 2010; Szeszko et al., 2008; Wang et al., 2011; White et al., 2009), and b) the SLF (James et al., 2011; Kyriakopoulos et al., 2009; Luck et al., 2011; Pérez-Iglesias et al., 2010; Szeszko et al., 2008; White et al., 2009), along with a callosal fiber tract, the splenium of the corpus callosum (Cheung et al., 2008; Dekker et al., 2010; Gasparotti et al., 2009; James et al., 2011; Kyriakopoulos et al., 2009).

The fronto-occipital fasciculus connects parts of the para-striate and parietal cortex to the frontal lobe. A close neuroanatomical analysis of this WM tract suggests a role in high order motor control and spatial attention which are important for functions such as coordination and selecting some stimuli over others (Schmahmann and Pandya, 2007), both functions known to be disrupted in psychosis (Danckert et al., 2002; Franck, 2001; Gruzelier et al., 1988; Park and Holzman, 1992).

The SLF connects parts of the frontal lobe with the parietal, occipital and temporal lobes (Afifi and Bergman, 2005), and it is involved in working memory (Karlsgodt et al., 2008; Makris et al., 2005). This is consistent with findings of impaired working memory in patients with psychosis (Goldman-Rakic, 1994). Recent empirical evidence and meta-analysis suggest that abnormal maturation of the SLF in adolescence may be key in the development of psychotic disorders (Peters et al., 2012).

The corpus callosum is the primary connection between the two cerebral hemispheres. It is involved with interhemispheric communication and cooperation. Disturbances in its structural integrity are implicated in psychosis in terms of decreased FA values and WM density (Hulshoff Pol et al., 2004; Patel et al., 2011). There is also evidence of abnormalities of interhemispheric cooperation and connection in this patient population (Crow, 1998; Mohr et al., 2000).

Other implicated WM structures (although implicated with less consistency), are the inferior longitudinal fasciculus (association fibers), body of the corpus callosum (callosal fibers), and the internal/external capsules (projection fibers)

(Cheung et al., 2008; Gasparotti et al., 2009; Kyriakopoulos et al., 2009; Pérez-Iglesias et al., 2010; Wang et al., 2011; White et al., 2009).

1.5.2 DTI in Cannabis Use: Drug Effect

Cannabis use is an often overlooked, yet likely important confounding factor in DTI studies of psychosis. As previously discussed, cannabis use is prevalent in the early phase psychosis population and likely plays a causal role in some psychotic disorders.

Two DTI studies were retained in the review reporting on the effects of cannabis use in otherwise healthy participants, as eight studies with full-text review were excluded due to small sample sizes, and two were excluded due to concomitant alcohol and/or illicit drug use (Table 1; Table 2). One study (Gruber et al., 2013) assessed FA values in several WM regions. Decreased FA in cannabis users versus healthy controls was found in the genu of the corpus callosum and left internal capsule. Importantly, younger age at initiation of regular cannabis use was associated with greater severity of WM disruption among the cannabis users. In the other study (Zalesky et al., 2012), complex WM network measures assessed connectivity maps in the brain. Cannabis users displayed decreased connectivity in the splenium of the corpus callosum and right fimbria relative to controls. Once more, younger age at initiation of regular cannabis use (onset prior to age 16) was a key factor in determining the severity of WM disruption. Both of these studies postulated that early cannabis exposure has a detrimental neurobiological impact which is unique to the developing adolescent brain.

1.5.3 DTI in Early Phase Psychosis with Cannabis Use: Combined Illness and Drug Effects

Only one DTI study was retained (see Table 1 and Table 2) assessing early phase psychosis with concurrent cannabis use in the review, designed with clear exclusion criteria for coexisting alcohol misuse and illicit drug use (James et al., 2011). Two other studies with full-text review were excluded due to concomitant alcohol and/or illicit drug use (Dekker et al., 2010; Peters et al., 2009). Of note, these two studies found evidence of higher FA values (reflecting greater WM integrity) in cannabis using patients with early phase psychosis compared to non-cannabis using patients (Dekker et al., 2010) as well as to healthy controls (Peters et al., 2009). This has led to the speculation that cannabis using patients with early phase psychosis may represent a distinct clinical subgroup with 'hyperconnectiviy', and perhaps less vulnerability to the illness as they develop psychosis after exposure to a significant amount of cannabis (a likely potential trigger of psychosis), whereas other patients develop psychosis even without this exposure (Dekker et al., 2010; Peters et al., 2009). James et al. (2011), found significant widespread WM disruption (decreased FA) in patients relative to healthy controls. These WM abnormalities occurred in four of the five major WM tract families (Wakana et al., 2004), including the association, callosal, projection and brainstem fibers. Interestingly, a subgroup analysis showed that cannabis using patients had a widespread reduction of FA compared to non-cannabis using patients. This evidence suggests that, when other drugs are controlled for, cannabis use may lead to deleterious WM abnormalities in people with early

phase psychosis. It is important to note however that this study focused on a very young population of patients with adolescent onset schizophrenia (aged 13 to 18 years). Furthermore, although they controlled for recent substance misuse, there was no reported exclusion for lifetime history of alcohol or drug misuse, and no data presented on lifetime amounts of substance exposure.

1.5.4 Conclusion

By grouping the DTI findings in terms of the five major fiber tract families (Table 3), we see that association fiber tracts were most often affected in early phase psychosis, while limbic and brain stem families were the least frequently affected. In otherwise healthy cannabis users, deficits in WM tracts were found more frequently in callosal fibers, but also in projection and limbic fibers. In cannabis users with early phase psychosis, deficits in WM integrity were observed in all fiber tract families except for limbic fibers.

These findings (Table 2) support the assumption that psychosis is associated with impairment in WM tissue early in the course of the illness, and that within this patient group, cannabis use may cause WM disruption especially when initiated in early adolescence. However, it is notable that this review found only one DTI study of early phase psychosis with concurrent cannabis use, designed with exclusion for active alcohol misuse and illicit drug use; and even this study failed to report or control for lifetime exposure to substances, and had limited generalizability as it focused only on those developing psychosis between the ages of 13 to 18 years old (James et al., 2011). Also noteworthy, is the paucity of available data investigating

the relationship between cannabis use, WM integrity and symptom/function measures in psychosis.

Therefore, the present study is unique in that it excluded lifetime substance misuse, reported on (and assessed for) the impact of concomitant alcohol and cannabis use, and included a broader, and more generalizable, patient population. Furthermore, it extends previous findings by examining relationships between WM integrity and important clinical measures such as symptomatology and function.

1.6 Study Objectives

1.6.1 Purpose

In summary, there is a high prevalence of cannabis use in early phase psychosis; cannabis exposure likely has a detrimental impact on both brain structure and function; and there is a paucity of evidence currently available investigating the interaction between cannabis and early phase psychosis. The purpose of this study was to expand the literature in this field by examining the impact of cannabis use on WM in patients with early phase psychosis. In order to accomplish this, we employed DTI to examine WM tracts in the full-brain as well as within the SLF as a region of particular interest (as discussed in section 1.5.1) in those individuals with early phase psychosis in comparison to healthy control participants. Using correlation analyses, we also wished to explore a potential relationship between a) the amount of lifetime cannabis exposure and b) the age at which cannabis use was initiated, with the mean FA (integrity) of WM (both at the full-brain level and within the SLF), in patients.

1.6.2 Hypotheses

Primary Hypotheses

1) It is hypothesized that people with early phase psychosis will have measurable abnormalities in full-brain and SLF WM, as measured by decreased mean FA values, compared to healthy controls with minimal cannabis exposure (less than 10 lifetime occasions).

2) It is hypothesized that the cumulative lifetime amount of cannabis usage will negatively correlate with mean FA values, both at the full-brain level and within the SLF in the patient group.

3) It is hypothesized that the age at first cannabis exposure, will positively correlate with the mean FA values, both at the full-brain level and within the SLF in the patient group.

Secondary Hypotheses

It is expected that abnormalities in values from DTI indices (FA, MD, AxD, RaD), will be associated with greater severity of psychotic symptoms and poorer functioning. These findings are exploratory in nature and will be used to inform future work.

CHAPTER 2 – MATERIALS AND METHODS

2.1 Power Analysis

A priori power calculations were completed using the software package Gpower (Faul et al., 2007). Calculations used existing DTI data that reported mean FA in early phase psychosis populations (comparing mean FA in patients versus healthy controls). This data examined the same area of interest (left SLF), and indicated an effect size of d = 1 (Szeszko et al., 2008). With a 2-tailed alpha of .05 and power of .8, an n of 17 subjects per comparison group would be required to find significant differences in mean FA.

2.2 Participants

This study initially included 42 participants, however due to data corruption (described in section 3.1) 40 participants were included in the final analysis: a) young adults in their early phase of psychosis (n = 18, mean age = 24.9), and b) cannabis-naïve healthy controls (n = 22, mean age = 24.0) (Table 4). Based on the power calculations above, our sample size was adequately powered to detect significant between-group differences on the primary outcome measure (mean FA). Early phase psychosis was defined as less than five years from diagnosis of psychosis and initiation of appropriate medical treatment. Cannabis use history was determined via interview using a drug questionnaire based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I; see section 2.3 for full description) (First et al., 2002). Non-cannabis users were defined as people who were cannabis-naive or who had very minimal experimentation with cannabis (lifetime use of less than 10

cannabis joints) (Gruber et al., 2011). Cumulative lifetime usage of cannabis was estimated in grams.

Recruitment of patients with early phase psychosis, and between 19 – 35 years of age, was conducted at the Nova Scotia Early Psychosis Program (NSEPP). The NSEPP is an EIS for psychosis, treating youth and young adults from age 12 – 35 years of age. Currently the NSEPP has approximately 300 individuals who are active in the clinic, with approximately 60 to 70% of them with a history of cannabis use. Diagnosis of patients was provided by the treating psychiatrist using DSM-IV-TR as per NSEPP protocol, and confirmed by consensus with the PI of the study and the Director of the NSEPP. Medications were obtained by patient history.

Healthy controls were 19 – 35 years of age, with no lifetime diagnosis of psychiatric disorder, no medical comorbidities, taking no prescribed medications, and had no first degree relatives (sibling, mother, or father) with a lifetime diagnosis of psychosis or bipolar disorder. They had minimal cannabis exposure (defined above). Both the mean age, and the male to female gender ratio was similar to the patient group. They were recruited through websites and by poster advertisements.

All participants met MRI inclusion criteria as per the MRI safety checklist at the 1.5 Tesla scanner (see Table 5).

Other criteria applied to all participants included: exclusion of those with history or current substance abuse or dependence (as per DSM-IV-TR criteria) other than cannabis and nicotine abuse/dependence in the patient group; exclusion of those with a history of head injury with loss of consciousness or seizure; exclusion of those with a BMI below normal range (less than 18.5), or above class I obesity (above 35);

and participants had to be naïve or have had minimal experimentation with all other illicit drugs (less than 20 lifetime occasions for each drug).

This study was approved by the Capital District Health Authority research ethics board as well as the Izaak Walton Killam (IWK) Hospital for Children's research ethics board. The IWK ethics board approval was obtained solely due to the location of the MRI scanner being at the IWK. No subjects below the age of 19 years were recruited.

2.3 Clinical Measures

The following clinical measures were obtained from the study participants. The Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Axis-I (SCID-1) using DSM-IV-TR (fourth edition, text revision of DSM) criteria was administered to patients for diagnostic confirmation (First et al., 2002). DSM-IV-TR was used instead of the DSM-5 as this study began prior to the publication of DSM-5 (APA, 2013). The Mini International Neuropsychiatric Interview Screen (MINI screen 6.0.0), designed as a brief structured interview for the major Axis I psychiatric disorders in DSM-IV, was administered to healthy controls in order to rule out Axis I disorders (Sheehan et al., 1998). The Positive and Negative Syndrome Scale (PANSS) was administered to patients to assess current severity of psychotic symptoms (Kay et al., 1987; Van den Oord et al., 2006). The Personal and Social Performance scale (PSP) was used to measure personal and social functioning in patients across various domains (Morosini et al., 2000). Scores on the PSP have been correlated with severity of clinical symptoms and global function measures in

people with psychotic disorders. Current anxiety and depressive symptoms were measured in patients using the Hamilton Anxiety Scale (HAM-A) and the Calgary Depression Scale for Schizophrenia (CDSS) respectively (Addington et al., 1990; Maier et al., 1988). An author-compiled substance use questionnaire based on the SCID-I as well as elements of the Timeline Followback (TLFB) method was administered to all participants in order to collect detailed information about past history and current use of a large variety of illicit substances, alcohol and nicotine (Sobell et al., 1996). In particular, unlike previous studies in the literature, each participant's drug use history was chronicled, beginning with age and pattern of first use, up to and including present use, for each class of drug (e.g. tobacco, cannabis, opiates, alcohol, etc.). In this way estimates were produced for cumulative lifetime amounts of substance use as well as age of onset and pattern of substance use. The Screen for Cognitive Impairment in Psychiatry (SCIP) was administered to all participants to assess five cognitive domains: working memory, immediate verbal learning, delayed verbal learning, verbal fluency, and psychomotor speed. There is support for criterion validity for each subscale of this test, as well as evidence that the global score provides an efficient metric for detection of impairment in patients versus healthy controls (Pino et al., 2006; Purdon, 2005). Finally, the Edinburgh Handedness Inventory was administered, a quantitative scale to assess handedness supported by the known distribution of values in a normal population (Oldfield, 1971). Handedness is an important variable to measure, as there is evidence that it can have an impact on WM microstructure as measured with DTI, in particular with regards to brain lateralization (Fujiwara et al., 2007; Westerhausen et al., 2004)

2.4 DTI Acquisition and Data Analysis

Neuroimaging data was acquired with a GE 1.5 Tesla scanner equipped with a multichannel head coil. Scans were acquired using a gradient-echo, echo planar pulse sequence supplied by the manufacturer. A localizer scan was acquired to determine where the imaging slices should be placed and for general alignment of the scan plane. A calibration scan was acquired to smooth out variations between the scanning coils. A T1-weighted structural scan was acquired from each participant using a SPGR pulse sequence with a 256 x 256 matrix, 162 slices, 1 mm isotropic resolution, TR = 11.3 s; TE = 4.2 ms; flip angle = 20 degrees. Diffusion weighted images were collected using the following parameters: 60 axial slices; TR = 8.5 s; TE = 80-90 ms; flip angle 90 degrees; 54 noncollinear diffusion weighting directions with a b-factor of 1000 s/mm^2; six acquisitions with a b-factor approximately 0 s/mm^2; 256 x 256 acquisition matrix; 260 mm field of view, generating 1.02 x 1.02 x 3 mm^3 voxels (see Table 6).

Analyses were performed using Functional MRI of the Brain (FMRIB) Software Library (FSL) (Smith et al., 2004). T1-weighted structural images were skull-stripped using FMRIB's Brain Extraction Tool (BET), followed by manually removing any remaining voxels belonging to skull/meninges. FMRIB's Automated Segmentation Tool (FAST), was then applied to this image to segment the brain image into different tissue types, as well as to correct for variations in spatial intensities (Y. Zhang et al., 2001).

DTI data was processed and analyzed using FSL's Diffusion Toolbox (FDT). The raw images were visually inspected for motion artifacts and image corruption. After separating all volumes from the 4D DTI dataset, the first 6 volumes (b=0) were averaged in order to improve signal-to-noise ratio. BET was used to eliminate all non-brain tissue. Eddy current correction and simple head motion correction were completed using affine registration to the reference volume. The diffusion tensor model was then fit to each anatomical voxel (the smallest volumetric unit of MR images) using DTIFIT.

The WM tract of particular interest in this study was the left SLF as this has been strongly implicated in playing a role in psychosis (as explained in section 1.5.1), and additionally has been the WM region to more consistently demonstrate FA abnormalities. Therefore, this region was chosen as the volume of interest (VOI). The VOI mask was calculated from the headers of the raw image acquired during image acquisition (see Figure 3 for images of VOI). A transformation matrix was calculated and the T1 image space was registered to the DTI image space using FMRIB's Linear Image Registration Tool (FLIRT). A VOI WM mask was created by selecting only the voxels within the VOI corresponding to the WM tissue type, which was then also registered into DTI space. Finally, the DTI outcome measures were then calculated for the full-brain WM mask as well as the VOI WM mask (Figure 4 illustrates a color-coded FA map).

2.5 DTI Statistical Analysis

Statistical analyses were conducted using SPSS version 20. Statistical analyses were two-tailed with alpha set as $p < .05$ unless otherwise specified. Data were visually inspected for outliers, boxplots were used, and a cutoff of +/- 3 SDs to identify extreme values. Independent samples t-tests were done to compare mean FA values in patients versus non-cannabis using healthy controls, at both the full-brain level as well as within the VOI (left SLF)(Table 7).

Pearson's correlation was used to assess a potential relationship between cumulative lifetime cannabis use and mean FA value (VOI and full brain) in the patient group ($n = 18$). Spearman's was also used in order to add complimentary correlation information to account for the limited sample size (i.e. less than $n = 30$), and in the case that parametric assumptions were not met. Spearman's rho was used to assess a potential relationship between age of onset of cannabis use and mean FA value (VOI and full brain). The data representing the age of onset of cannabis use had to be ranked (and therefore analyzed non-parametrically) as assigning zero to the patients who had never smoked cannabis would skew the data rendering the correlations inaccurate. Follow-up partial correlation analyses were used to control for the effects of alcohol as a potentially confounding variable given that it has previously been associated with changes in diffusion measures (Baker et al., 2013).

For exploratory analyses within the patient group ($n = 18$), bivariate correlations were computed between the four full-brain and VOI DTI measurements on the one hand, and the following clinical variables on the other hand (see Tables 8 – 11): Illness effects (duration of illness); cognitive measures (SCIP); symptom/function measures (PANSS, PSP, HAM-A and Calgary depression scores);

substance use measures (cannabis age of onset and cumulative lifetime usage; alcohol age of onset and cumulative lifetime usage; tobacco cumulative lifetime usage; antipsychotic use). Again, given the relatively small sample size in this study, both parametric (Pearson's r) and non-parametric (Spearman's rho) correlations were used, to avoid leverage effects of a few data points, which could lead to spurious correlations when measured parametrically. Given this situation of multiple comparisons, the threshold for true statistical significance was set at $p < .01$ for each type of correlation (parametric and non-parametric), for a specific comparison to be considered for full discussion. However, given these correlations are exploratory in nature, correlations at the $p < .05$ level are also mentioned for the purposes of informing future work.

Finally, in the case that there was a correlation of true statistical significance between one of the clinical variables and the diffusion measures, that particular clinical variable was then included as a covariate in a follow-up between-group comparison of mean FA between patients and controls as it may have confounding effects on the primary outcome measure. These results were also reported in Table 7.

CHAPTER 3 – Results

3.1 Clinical and Demographic Variables

On visual inspection of the raw image data, two subjects were excluded from the study due to missing/corrupted DICOM data (one from the patient group and one from the healthy control group). Therefore the total number of subjects included in the analysis was 18 patients and 22 healthy controls (see Table 4). For clinical/demographic variables that were at least interval level data, the means were compared between healthy controls and the clinical group using independent-sample *t*-tests. Means and standard deviations for both groups are presented in Table 4, as a function of group. A Levene test for equality of error variances was conducted to assess between-group differences in variance around the mean; variances that differed between groups included lifetime number of occasions of cannabis use (p = .000), cumulative lifetime cannabis use (p = .000), cumulative lifetime drinks (p =.002), lifetime number of cigarettes (.000), SCIP-global (p = .027), SCIP-VLT_I (p = .018), and SCIP-WMT (p = .019). For these variables, tests with appropriately adjusted degrees of freedom were used. Variances did not differ between groups on any other demographic/clinical measure. The *t*-tests that showed significant between-group differences included: Years of education (greater in controls, p = .003), BMI (greater in patients, p = .017), weight (greater in patients, p = .046), lifetime cannabis use – occasions (greater in patients p = .002), lifetime cannabis use – cumulative amount (greater in patients, p = .011), lifetime number of cigarettes (greater in patients, p = .023), other illicit drug use – lifetime occasions (greater in patients, p = .018) SCIP-global (greater in controls, p = .007), SCIP-VLT_I (greater in controls, p = .022),

SCIP-WMT (greater in controls, p = .009), and SCIP-PST (greater in controls, p = .004). The differences in lifetime cannabis use (occasions and cumulative amount) was an effect resulting from the study design, as healthy controls were screened out for cannabis and illicit drug use. There were no other between-group differences found.

3.2 Effect of Early Phase Psychosis (Hypothesis 1)

The first pass method of identifying outlying and missing data was completed by plotting all of the raw data in the form of scatterplots. Also, an analysis using a cutoff of +/- 3 SDs, identified one subject (from the healthy control group) with abnormally high (outlying) mean RaD and mean MD values in the VOI. As would be expected, this corresponded to the mean FA value being the lowest of all mean FA values; therefore this subject was removed from DTI calculations. Thus, final group sample sizes for testing hypothesis 1 were: patients (n = 18), and controls (n = 21). This did not affect testing of the other hypotheses as these were all tests within the patient group.

An independent samples t-test was conducted to evaluate group differences in full-brain mean FA. The t-statistic was not significant, t(37) = -1.139, p = .262, r = .184, showing that the two groups did not differ in mean full-brain FA. Means, standard deviations, t-statistics and p values are presented in Table 7, as a function of group.

A second independent samples t-test was conducted to evaluate group differences in VOI (left SLF) mean FA. The t-statistic was not significant, t(37) = -

.635, p = .529, r = .104, showing that the two groups did not differ in mean VOI FA. Means, standard deviations, t-statistics and p values are presented in Table 7, as a function of group.

Follow-up ANCOVAs were calculated (see Table 7) due to significant associations that were found between age of onset of alcohol use and mean full-brain FA (see section 3.4.4) as well as cumulative lifetime cannabis use and mean VOI FA (see section 3.3.1). After controlling for these variables, there was still no significant difference found between patients and healthy controls in mean full-brain FA, F (1,36) = 1.079, p = .306. There was, however, a trend-level effect found in mean VOI FA, F(1,36) = 3.847, p = .058 with a small to moderate effect size of *partial* η^2 = .097. Both cumulative lifetime cannabis use (p = .006) and age of onset of alcohol (p = .002) were confirmed to be significant covariates in the ANCOVA analysis.

3.3 Associations: Cannabis Use and DTI Measures (Hypotheses 2&3)

3.3.1 Correlation Between Lifetime Cannabis Use and Mean FA

In the patient group (n = 18), there was a significant positive relationship between the cumulative lifetime amount of cannabis use and mean FA in the VOI (left SLF), r = .61, p = .008 (see Table 9). This corresponds to 37% of the variance in mean FA of the VOI being accounted for by the lifetime amount of cannabis use. This relationship remained significant after controlling for alcohol as analyzed using partial correlation with age of onset of alcohol and cumulative lifetime drinks added as control factors, $r_{partial}$ = .74, p = .006. There was no significant relationship between the cumulative lifetime amount of cannabis use and mean FA of the full

brain WM (see Table 11). There was no significant relationship between cumulative lifetime amount of cannabis use and mean FA in the full-brain or within the VOI as measured by Spearman's rho (see Tables 8 and 10)

On inspection of the raw data, the scatter plot of lifetime cannabis use on the X-axis and mean FA on the Y-axis, revealed 2 outlying data points corresponding to 2 patients whose lifetime cannabis use was significantly greater than the rest of the patient group. When the Pearson correlation was re-run excluding these 2 subjects, there was no significant correlation found between cumulative lifetime cannabis use and mean VOI FA, $r = .062$, p = .818.

3.3.2 Correlation Between Age of Onset of Cannabis Use and Mean FA

In the patient group (n = 18) the age of onset of cannabis use was not significantly correlated with mean FA in the VOI or with mean full-brain FA (see Tables 8 and 10).

Also of note, in cannabis using patients with available data (n = 13), the age of onset of cannabis use positively correlated with the age of illness onset, Pearson's $r = .625$, p = .023. However when measured non-parametrically, this correlation was non-significant, $r_s = .235$, p = .439.

3.4 Exploratory Analyses

3.4.1 Illness Effects

There was no significant relationship between the duration of illness and mean VOI diffusion measures (Tables 8 and 9).

It is notable that there was a significant negative correlation between the duration of illness and mean full-brain FA, r_s = -.668, p = .002, accounting for 45% of the variance in the full-brain mean FA (Table 10). There was also a significant positive correlation between duration of illness and mean full-brain RaD, r_s = .588, p = .010, accounting for 35% of the variance in the full-brain mean RaD (Table 10). When measured parametrically, both of these correlations were found to be non-significant at the p < .01 level, but were found to be significant at the p < .05 level, r = -.482, p = .043, and r = .474, p = .047, respectively (Table 11). No other full-brain diffusion measures were significantly correlated with duration of illness.

3.4.2 Cognitive Measures

There was a significant relationship found between SCIP-VFT and mean AxD within the VOI, r_s = .521, p = .027. There was also a positive correlation found between SCIP-PST and mean AxD at the level of the full-brain, r_s = .489, p = .039. All other correlations (both Pearson's and Spearman's) between SCIP measures (global index and subtest scores) and diffusion measures (Full brain and VOI) were non-significant (Tables 8 – 11).

3.4.3 Symptom/Function Measures

The PANSS-G was found to have a significant positive correlation with mean full-brain FA values, r_s = .482, p = .043. Other PANSS scores were not significantly related to any of the DTI measures (Full brain or VOI).

The PSP score had a significant negative correlation with mean full-brain FA values, r = -.508, p = .031, and a positive correlation with mean full-brain RaD, r = .491, p = .039. Both of these correlations were also found to be significant using Spearman's, r_s = -.477, p = .045, and r_s = .503, p = .033 respectively. There were no other significant correlations between PSP and diffusion measures.

In addition, the CDSS scores were found to be significantly positively correlated with mean VOI MD, r_s = .541, p = .02, as well as with mean full-brain FA, r = .505, p = .033. CDSS scores did not significantly correlate with any other diffusion measures.

HAM-A scores were not significantly correlated with DTI measures (Full Brain or VOI) (Tables 8 – 11).

3.4.4 Substance Use Measures

There was an inverse relationship between cumulative lifetime amount of cannabis use and mean VOI RaD, r = -.625, p = .006, accounting for 39% of the variance of the mean VOI RaD (Table 9). There was also a significant negative correlation with lifetime cannabis use and mean MD within the VOI, r = -.494, p = .037. There was no significant correlation between cumulative lifetime cannabis use and the other VOI or full-brain DTI measures (Tables 8 – 11).

Of note, a significant correlation was found between the age of onset of regular alcohol use and mean full-brain FA, r = .70, p = .005, accounting for 49% of the variance in mean full-brain FA (Table 11). This correlation was also significant at the $p < .05$ level when measured non-parametrically, r_s = .630, p = .016 (Table 10).

This relationship remained significant after controlling for cannabis use as analyzed using partial correlation with cumulative lifetime cannabis exposure as a control factor, $r_{partial} = .706$, $p = .007$. At the $p < .05$ level, there were also correlations between age of onset of alcohol use and both mean MD and RaD, $r_s = -.610$, $p = .021$, and $r_s = -.628$, $p = .016$. For RaD, this association was also significant as measured by Pearson's, $r = -.623$, $p = .017$. There was no other significant relationship between age of onset of regular alcohol use and DTI measures. Furthermore, there was no relationship between cumulative lifetime amount of alcohol and DTI measures.

Lifetime number of cigarettes, mean daily antipsychotic dose, and duration of treatment with antipsychotics all did not significantly correlate with the DTI measures (Tables 8 – 11).

3.5 Results Summary

In summary, with regards to hypothesis 1, there was trend-level evidence of decreased mean VOI FA in early phase psychosis (but no difference in mean full-brain FA). With regards to hypothesis 2, findings indicated that greater lifetime cannabis exposure was associated with increased mean FA values; a relationship opposite to what was expected. Finally, there was also no evidence found to support hypothesis 3, with no association found between age of onset of cannabis use and mean FA values (full-brain and within the left SLF).

Regarding the secondary hypothesis (exploratory analyses), there was a significant effect of illness as a longer duration of illness was associated with decreased mean FA at the full-brain level. Exploration of cognitive measures revealed

that SCIP-VFT and SCIP-PST were positively correlated with mean AxD values. In terms of symptom/function measures PANSS-G was positively correlated with mean full-brain FA; PSP was negatively correlated with mean full-brain FA and positively correlated with mean full-brain RaD; and greater CDSS scores were associated with greater mean VOI MD and mean full-brain FA. Finally, with regards to substance use, cumulative lifetime cannabis use was negatively correlated with mean VOI MD and mean VOI RaD; and at the level of the full-brain, younger age of onset of regular alcohol use was associated with decreased mean FA, increased mean MD and increased mean RaD.

CHAPTER 4 – Discussion

The present study is aimed at examining the impact of cannabis use on WM in patients with early phase psychosis. There are a paucity of DTI studies in this area, and failing to control for concomitant alcohol and illicit drug misuse has limited the specificity and utility of existing data. In this DTI study, we were able to control for these factors. Another aim of this study was to replicate existing DTI data supporting the disconnectivity hypothesis of psychosis (Cookey et al., 2014; Luck et al., 2011), namely to confirm the existence of WM microstructural changes in the brain as measured by decreased FA values in patients compared to healthy controls.

4.1 Primary Outcome Measures

4.1.1 Effect of Early Phase Psychosis (Hypothesis 1)

A review of existing DTI studies suggests that WM changes in early phase psychosis seem to occur throughout the brain, as opposed to any one specific region. On analysis of all the WM tract families that have been shown to be affected, the association fibers (which include the SLF) are affected most (Cookey et al, 2014). Therefore, it was hypothesized that people with early phase psychosis would have measurable WM abnormalities at the full-brain level as well as within the SLF as a specific region of interest, compared to healthy controls with minimal cannabis exposure. However, we failed to find group-level evidence supporting these findings as our analysis revealed no significant difference in mean VOI FA and mean full-brain FA between patients and healthy controls. Although these findings do not support our hypothesis, they are not completely unexpected as there are considerably

mixed findings in the literature with approximately 28% of DTI studies of early phase psychosis finding no significant WM changes as measured by FA (Cookey at al, 2014). These findings suggests that at both the specific VOI (left SLF) as well as the whole brain level, there is no DTI evidence of abnormalities in WM microstructure associated with early phase psychosis; a finding that would not support the disconnectivity hypothesis (Davis et al., 2003; Friston and Frith, 1995). Although this between-group effect was not significant, this effect might have proven significant with a larger sample size, given that the effect sizes were small and therefore would require greater power to find group differences (r = .184 and r = .104 for mean full-brain and VOI FA respectively). Additionally, our control group was specifically chosen to exclude cannabis users, so the testing of hypothesis 1 was between patients (a mix of cannabis users and non-users), and non-cannabis using healthy controls. Given the fact that cumulative cannabis use was found to positively correlate with mean FA values within the VOI, this may have increased the mean FA in the patient group thus reducing the between group effect of the illness itself. Indeed the follow-up ANCOVA of the mean VOI FA with the inclusion of cumulative cannabis exposure as a covariate revealed trend-level evidence of decreased FA values in the patient group as well as an increase in the effect size from $\eta^2 = .034$ to $\eta^2 = .097$.

4.1.2 Effect of Cannabis Use (Hypothesis 2 & 3)

Although data is limited, existing evidence indicates that cannabis use may be associated with WM abnormalities both in healthy controls and in patients with early phase psychosis (Cookey et al., 2014). It was hypothesized in this study, that

cumulative lifetime amount of cannabis usage would negatively correlate with mean FA values in the patient group (hypothesis 2). The results showed that there was a significant positive relationship between the cumulative lifetime amount of cannabis use and mean FA in patients in the VOI (left SLF). That is, for patients, the greater the amount of cannabis exposure, the greater the mean FA value. This suggests that, in direct opposition to hypothesis 2, cannabis use may be associated with increased WM integrity in the left SLF, a novel finding. There was however no relationship found between cumulative exposure to cannabis and mean FA value at the level of the full-brain. This would indicate that there may be specific families of WM tracts that are more vulnerable to the effects of cannabis than others.

Two previous studies have also found evidence of increased WM integrity as measured by increased FA in WM, in cannabis using patients with early phase psychosis compared to non-cannabis using patients. However because the effects of hard drug and alcohol use were not controlled for, conclusions could not be drawn about the specific effects of cannabis exposure (Dekker et al., 2010; Peters et al., 2009). The present study excluded those with substance misuse so that there was no major confounding effects due to hard drugs or alcohol. In addition, the relationship between cumulative lifetime amount of cannabis use and mean FA in the VOI remained significant even after controlling for alcohol use.

The present study is also different from these previous findings, in that both a full-brain and VOI specific approach linked with clinical variables were employed. In particular, the VOI (left SLF) was chosen using a clinically driven hypothesis based on previous evidence from both neuroimaging studies as well as clinical studies

(Goldman-Rakic, 1994; Karlsgodt et al., 2008). Peters et al (2009), on the other hand, explored specific VOIs including: corpus callosum, frontal WM, parieto-occipital WM, internal capsule, uncinate fasciculus, arcuate fasciculus and the dorsal cingulum; however this study did not examine areas based on clinical findings. Dekker et al (2010), used DTI to examine full-brain WM, but also did not explore potential links between DTI findings and clinically relevant variables.

One potential interpretation of these findings may be that the increased mean FA represents the direct effect of cannabis use on WM in the brain. In this case, increasing the WM integrity. As mentioned in section (1.5.3), another potential explanation for these findings is that patients with early phase psychosis who use cannabis heavily, may represent a distinct subgroup of patients with a greater propensity to seek out and use cannabis. It may be that this subgroup has increased WM integrity as a neurocellular biomarker relative to non-cannabis using patients. Future studies could employ a longitudinal study design in order to differentiate between these two possibilities: 1) cannabis causing increased WM integrity versus, 2) pre-existing increased WM integrity associated with increased propensity to seek out and use cannabis. Yet another interpretation, in light of the finding of no significant association when the outlying data from two subjects were removed from the analysis, is that there may not be any association between lifetime amount of cannabis use and microstructural WM in the brain. If this was the case, it may help explain the variability of findings in the literature as there have been studies finding increased FA with cannabis use (Dekker et al., 2010; Peters et al., 2009) as well as decreased FA with cannabis use in this patient population (James et al., 2011).

It is important to note that there is currently no clear explanation with regards to the specific underlying microstructural changes implicated with a finding of increased mean FA values. It may reflect increased myelination, a crossing of axonal fibers, increased packing density of axons or even changes in axonal diameters. Also, it is unclear whether this increase is something that improves the function in these WM tracts, or causes dysfunction. For example there have been findings of increased FA values in other clinical populations (e.g. mood disorders) which would suggest that increased FA values (like decreased FA) may also reflect dysfunctional changes in WM microstructure (Sexton et al., 2009).

Also of note is the finding that within our sample, when measured non-parametrically, cumulative lifetime cannabis use was not found to be correlated with mean FA, neither at the full-brain level nor within the VOI. This may suggest that given the limited sample size, some of the more extreme data points within the sample could have disproportionately swayed the results to show a larger correlation; and that these leverage effects were then negated by using ranked data. For these reasons, future studies with larger sample sizes will be required to clarify this relationship.

It was also hypothesized that the age at first cannabis exposure, would positively correlate with the mean FA values in the patient group (hypothesis 3). This idea is born out of previous work indicating that the earlier the exposure to cannabis the greater the detrimental impact may be on the brain (Casadio et al., 2011; Malone et al., 2010). The results showed that age at first exposure was not related to mean FA values either at the VOI or the full-brain level, in patients with early phase psychosis.

This would suggest that the age at which one is exposed to cannabis may not significantly impact the brain's WM in those who have early phase psychosis. Although this runs counter to the prevailing hypotheses in the literature, there is actually limited direct DTI evidence of a detrimental impact specifically due to earlier age of onset of cannabis use on WM microstructure in this patient population. Dekker et al (2010) was the one DTI study that did compare early (< 15 years old) versus late (> 17 years old) onset cannabis use in patients with early phase psychosis. However, they did not find any differences between these two groups. The results of the present study replicate the findings of Dekker et al (2010) and advance this work by improving the specificity as confounding effects of alcohol and illicit substance misuse were controlled for.

On the other hand, the fact that age of onset of cannabis use was positively correlated with age of onset of psychosis, suggests that there may be a significant effect of cannabis whereby the earlier one is exposed to cannabis, the more detrimental the effect as manifested by earlier onset of illness. Caution must be used when interpreting this finding however as this correlation was not significant when measured non-parametrically, and may be due to leverage effects of outlying data. Alternatively, it may be a true effect that non-parametric testing was unable to detect given the limited power compared to parametric testing.

This potential correlation between age of onset of cannabis use and age of illness onset, is consistent with prior literature, and may indicate that despite there be a significant negative impact of earlier cannabis use clinically, this may not necessarily be reflected at the level of WM microstructure as measured via DTI. It is

also notable that on average, patients in this study began using cannabis 7.1 years prior to the time that their illness was diagnosed. This finding would call into question the theory that individuals with psychosis use cannabis in order to treat or self-medicate their symptoms, as one would expect that cannabis use should follow symptom onset rather than preceed it (Blanchard et al., 2000).

4.2 Exploratory analyses

Exploratory analysis was done in order to explore the relationships between the four full-brain and VOI DTI measurements on the one hand (FA, MD, RaD, and AxD), and a number of clinical variables that were collected as part of the assessment. The following findings are not hypothesis driven and should be interpreted as preliminary in nature only.

4.2.1 Illness Effects

There have been several DTI studies with evidence of WM microstructural abnormalities, as measured by decreased FA values, in those with early phase psychosis compared to healthy controls (Cookey et al., 2014; Peters et al., 2010). Therefore we expected that patients with a longer duration of illness would likely have decreased mean FA values. Our findings showed a significant correlation between duration of illness (measured in months) and mean FA (negative correlation) and RaD (positive correlation) values at the full-brain level; however, no correlation was found within the VOI. This result supports the hypothesis that the duration of psychosis itself may have a detrimental impact on WM microstructure, widespread

throughout the brain, which can be detected even at an early stage in the illness. The fact FA and RaD, and not AxD or MD, were significantly affected, would suggest a potential abnormality in myelination, more so than other microstructural changes such as axonal damage or packing density (Baker et al., 2013). Other illnesses with a similar pattern of findings (decreased FA, and increased RaD) include multiple sclerosis and Alzheimer's Disease among others (Alexander et al., 2007).

4.2.2 Cognitive Measures

There is limited data linking DTI measures to cognitive functioning in early phase psychosis literature. However, given that patients have been found to have abnormalities in a number of cognitive domains (on average 1.5 standard deviations below normal) compared to healthy controls (Bilder et al., 2000), it would be reasonable to hypothesize that some of these abnormalities may have observable microstructural correlates as measured by DTI. Our findings however show no significant correlations at the $p < .01$ level between cognitive functioning as measured using the SCIP, and any of the diffusion measures either at the full brain level or within the VOI. This may be due to lack of power with our relatively small sample size, because there are significant correlations with a moderate magnitude effect at the $p < .05$ level. Namely, the SCIP-VFT was positively correlated with mean VOI AxD, and the SCIP-PST was positively correlated with mean full-brain AxD. It is worthwhile noting here that these isolated increases in AxD may reflect increased axonal caliber and/or orientation in WM tracts. However, caution must be taken when interpreting these findings as these correlations were not statistically significant when

measured parametrically. This calls into question the existence of this association or alternatively may suggest that these relationships are non-linear as Pearson's tests only for a linear relationship.

Otherwise, the relatively limited associations between cognitive measures and WM microstructure may also, in part, be due to the fact that changes in diffusion values can be very small, and so in order to detect WM changes related to specific cognitive functions, it may be necessary to employ targeted, smaller VOI's corresponding to areas of the brain known to be involved in each of these cognitive domains separately. For example, if a cognitive task (e.g. task involving interhemispheric cooperation) is known to involve a specific WM tract (e.g. splenium of the corpus callosum), targeting only the splenium would reduce the size of the VOI thereby limiting variability and making any changes in mean FA within that VOI more likely to be detected (Alexander et al., 2007).

4.2.3 Symptom/Function Measures

There is also limited data linking DTI measures to clinical symptomatology and social functioning in early phase psychosis. Given the wide variation in clinical presentation of these patients, as well as the variability in the DTI literature itself, it may be that certain symptom clusters (e.g. positive symptoms versus negative symptoms) may be associated with WM microstructure in different ways thus impacting the results in the existing DTI literature. In fact, there is currently evidence in the chronic psychosis literature that there may be increased WM integrity (as measured by increased FA) in certain WM tracts related to auditory areas (e.g. arcuate

fasciculus) in patients with significant auditory hallucinations, compared to patients without these symptoms (Mulert et al., 2012; Shergill et al., 2007). However, the results of the present study revealed no significant correlations at the $p < .01$ level, between symptom/function measures and any DTI outcome measures either at the full brain level or within the VOI.

Of note, however, there were some associations found at the $p < .05$ level. Within the left SLF, there was evidence that depression scores were positively correlated with mean MD, which would indicate abnormal WM pathology. However, MD alone is fairly non-specific, with a number of different potential pathologies causing changes to either the axons themselves or the surrounding myelin. Also, this correlation was not found to be significant when measured non-parametrically which may reflect leverage effects of extreme values within this sample, or alternatively this may be due to the lower power-efficiency inherent in non-parametric statistical testing. At the full-brain level, it was found that as PANSS-G scores increased, so did mean FA scores. This would imply that increased general symptomatology is associated with increased WM integrity. This finding is out of keeping with existing literature, but should be included in future investigation. Also, given that this correlation was non-significant when measured parametrically, this calls into question the existence of this association or alternatively may suggest that these relationships are non-linear as Pearson's tests only for a linear relationship. With larger sample size and sub-group analysis, it may be that certain symptoms now viewed within the general psychopathology sub-test influence (or are influenced by) WM microstructure. Also, at the full brain level, there was evidence of WM changes in

association with the PSP scores. The directionality of this association (negative correlation with mean FA and positive correlation with mean RaD), would suggest that the higher the level of social functioning, the less WM integrity at the level of the full brain. Again, this finding is counter-intuitive, with no clear rationale available in existing literature, but would warrant future investigation.

In this study, symptomatology did not seem to have strong associations with underlying WM microstructure. It is particularly interesting that PANSS scores (other that PANSS-G) were not found to be related to WM microstructural abnormalities, particularly because duration of illness did have a significant association with WM. However, it is possible that we failed to find any significant relationships at the $p < .01$ level due to a combination of small sample size as well as the fact that clinical measures can be somewhat subjective and non-specific and therefore may not have clear, measurable associations with underlying neurological tissue as measured using current DTI technology. For instance, it may be that the underlying neurological processes associated with specific symptomatology are either too small to be detected with the present sample size, or perhaps may be functional (rather than structural) in nature, thus not readily detectable using DTI measures. Also, the PANSS scores in our sample were relatively low (see Table 4) when compared to average PANSS scores in the literature, corresponding to fairly low clinical severity (Kay et al., 1987; Leucht et al., 2005). So another possibility could be that the effects that clinical symptomatology might otherwise have on WM, was limited within this particular sample.

4.2.4 Substance Use Measures

There is growing evidence that adolescent substance misuse can detrimentally impact WM microstructure in the developing brain. In particular alcohol and cannabis (two of the most commonly misused substances), have been shown to cause WM abnormalities as measured by DTI in a number of studies (Baker et al., 2013; Bava et al., 2013). Generally, these abnormalities have been reflected by findings of decreased FA values in various WM areas. There have also been the opposite findings (increased FA) (Baker et al., 2013), which may also reflect a detrimental change in WM microstructure given there have been findings of increased FA associated with other clinical populations (e.g. affective disorders) (Sexton et al., 2009) , however this remains unclear. Our findings demonstrate a significant negative correlation between cumulative lifetime amount of cannabis use and mean RaD and MD in the VOI, such that the more cannabis exposure a patient had, the lower the mean RaD and MD values. These findings, in combination with the above mentioned finding of increased mean VOI FA with greater lifetime cannabis use (discussed with hypothesis 2), would suggest increased WM integrity, most likely reflecting a process related to axonal myelination within the left SLF. Of note, when tested non-parametrically, this association was not found to be significant. This may reflect leverage effects of extreme values within this sample, or alternatively this may be due to the lower power-efficiency inherent in non-parametric statistical testing.

Our results also showed a significant positive correlation (as measured parametrically and non-parametrically) between the age of onset of regular alcohol use and mean full brain FA. This suggests that the earlier that one begins regularly

drinking alcohol (≥ one occasion per week), the more significant the deficits in WM integrity in the brain. This seems to be a generalized effect as this was found at the level of the full brain (and not within the VOI). This particular association was found to be very strong, with age of onset of regular alcohol use explaining almost half (49%) of the variance found in mean full brain FA. This relationship may reflect an effect of early alcohol use on the developing brain, or alternatively may represent a biomarker of a propensity to use alcohol from an early age. This association also remained significant after controlling for cannabis use, which increases confidence that this finding is a true effect.

4.3 Summary

In summary, regarding our first hypothesis, there was a trend-level decrease found in mean VOI FA in patients compared to healthy controls, with no difference found at the level of the full-brain. Although a significant minority of studies have had similar findings, the results of this study may also represent a lack of power resulting from small group sample sizes given the low magnitude of effect size (as discussed in section 4.1.1). In regards to the second hypothesis, there was evidence of an effect of cumulative lifetime cannabis exposure on WM microstructure in our patient group, however in the opposite direction to what was hypothesized based on much of the previous literature. It remains unclear whether these findings reflect a true effect whereby cannabis exposure increases WM integrity, or whether those who smoke cannabis more heavily represent a clinically distinct, and perhaps more resilient, subgroup of patients who develop psychosis only after being exposed to

larger amounts of cannabis (which represents a potential trigger of the illness). We did not find support for our third hypothesis, and so did not find evidence that the age at which patients begin to use cannabis has any impact on WM microstructure as measured using DTI. Again, the limiting factor was likely our small sample size thus reducing the power to find a relationship (if such a relationship exists).

Finally, exploration of the data revealed some interesting findings. First, of note, within the patient group, the duration of illness was found to be correlated with a decreased mean FA at the level of the full brain, which would suggest a globalized detrimental effect of the illness over time on WM integrity. This would lend support the hypothesis that the duration of psychosis itself may be associated with WM deficits. Secondly, we found evidence that along with a positive correlation with mean FA, a negative correlation with mean RaD and MD was also associated with cumulative lifetime cannabis exposure. This pattern of findings (increased FA and decreased RaD/MD) suggests an increase in WM integrity related to myelination in those with a greater cannabis exposure; a finding that seems to be specific to our VOI of interest (the left SLF). In addition, our exploratory analyses revealed a very strong correlation between mean age of onset of regular alcohol use and mean full brain FA, suggesting that the earlier the exposure to alcohol, the more detrimental the effect on the developing brain.

4.4 Conclusion

In conclusion, we failed to find evidence supporting the disconnectivity hypothesis of psychosis as our results revealed no WM microstructural abnormalities

in patients with early phase psychosis relative to healthy controls. Although this finding is out of keeping with the majority of the existing DTI literature, there are a number of DTI studies with similar results. Results also failed to reveal any impact of age of onset of cannabis use on WM microstructure, however did indicate that cumulative lifetime amount of cannabis was associated with significantly increased WM integrity in this patient group. Depending of the directionality of causation, this finding may represent an effect of cannabis on the developing brain, or alternatively, there may be a subgroup of patients predisposed to cannabis use who have increased WM integrity.

Additional findings indicated that in those with early phase psychosis, the duration of illness likely has a detrimental effect on WM integrity. Also, a younger age of onset of alcohol consumption may similarly have a significant detrimental impact on the WM in the developing brain.

Although our results do not support the stated hypotheses, the findings do underline an important point; the fact that differences in mean FA values (presuming they exist), even between two very distinct clinical groups, can be quite small. This means that current DTI technology is still far from being clinically useful (i.e. for diagnostic/prognostic purposes), at least in this patient population, at the individual patient level.

4.5 Study Limitations

There are a number of limitations to the current study. One potential limitation is that despite having adequate power, there was still a relatively small patient sample

size, and therefore this study may have had limited power to test the hypotheses, especially given the small effect size of the primary outcome measure. Another important limitation is with the DTI technology and analysis itself. There are several different methods of acquisition and post-processing analyses, making it difficult to compare findings between different groups/studies. In addition, despite our best efforts, people with psychosis represent a fairly heterogeneous patient population, which can limit the specificity of findings given the high number of potential confounding factors. Finally, as mentioned, DTI utilizes the measurement of a very weak signal effect (water diffusion), and so it can be difficult to gain enough power to find true between-group differences, making type II errors more likely. This was confirmed in our finding of the small effect size of our primary outcome measure (mean FA values).

4.6 Future Directions

Future studies should strive to employ more consistent (and perhaps more clearly prescribed) DTI analysis methodology. Also, although we attempted to limit substance misuse, studies should include more stringent exclusion of alcohol misuse and illicit substance use in both healthy control and in patient groups. However, it should be acknowledged that this can be difficult given the high prevalence of polysubstance misuse, particularly in this age group and patient population. This would help better distinguish the specific impacts of psychosis versus substances, on WM tissue. In addition, future studies should employ longitudinal designs in order to clarify the relationship between the emergence of WM abnormalities and the onset of

psychotic illness and cannabis use. Finally, future studies may benefit from using several imaging modalities to assess WM microstructure, which would bring a more precise interpretation of WM abnormalities.

4.7 Implications

In practical terms, these results call into question whether or not there are WM abnormalities (at the full-brain level and within the left SLF) in individuals with early phase psychosis compared to healthy controls. At the very least, it should promote caution when interpreting current DTI studies as alterations in diffusion measures may be smaller than anticipated with several potential confounding factors existing in the literature (such as patterns of substance use). This study also supports the idea that in people with early phase psychosis, exposure to substances (in particular cannabis and alcohol), may have a significant impact on WM tracts in the developing brain. In the present study, cannabis use impacted WM diffusivity measures opposite to what was expected; the implication being that the relationship of cannabis exposure to WM microstructure is complex and may have different effects in different clinical populations. The effect of age of onset of regular alcohol use on WM was in the direction expected (causing decreased mean FA values), and given the large effect size, may have a more significant impact on WM than cannabis.

Tables

Table 1. Outline of literature search: count of studies as a function of each population (Cookey et al., 2014)

	DTI studies of early phase schizophrenia (illness effect)	DTI studies of cannabis use (drug effect)	DTI studies of early phase schizophrenia and cannabis use (combined illness and drug effects)
Identified by search	66	35	4
Not relevant based on abstract review*	22	23	1
Full text studies evaluated	44	12	3
Excluded based on inclusion criteria	26	10	2
- Sample size too small	24	8	0
- No reported FA	2	0	0
- Substance abuse	0	2	2
Included in this review	18	2	1

*Studies were deemed 'not relevant' for a number of reasons, most commonly: not early-phase population, no reported FA measures, and no examination of white matter structure.

Table 2: Diffusion Tensor Imaging studies retained in literature review (Cookey et al., 2014)

First author (year)	Group (n)	Age, years: Mean(SD)	Gender (M/F)	Substance use	Offline analysis	Results
A. Early phase schizophrenia						
(Lu et al., 2011)	EPS (21) HC (18)	22(5) 24(5)	17/4 10/8	Exclusion of abuse other than cannabis	VBA	EPS = controls in FA values
(White et al., 2011)	EPS (31) HC (43)	25(7) 25(7)	22/9 24/19	Exclusion not clearly stated	VBA	EPS = controls in FA values
(Chan et al., 2010)	EPS (39) HC (64)	27(7) 32(10)	30/9 38/26	Exclusion of abuse in past three months	VBA & ROI	EPS = controls in FA values
(Pérez-Iglesias et al., 2010)	EPS (62) HC (54)	31(10) 30(8)	19/43 18/36	Exclusion of dependence	VBA	EPS < controls in FA, in association, callosal, projection and brainstem tract families
(Tang et al., 2010)	EPS (38) HC (38)	16(1) 17(1)	20/18 20/18	Exclusion not clearly stated	VBA	EPS < controls in FA, in limbic fiber tract family
(Hao et al., 2006)	EPS (21) HC (21)	24(6) 25(5)	12/9 10/11	Exclusion of dependence	VBA	Pts < controls in FA, in association, callosal, projection, limbic and brainstem tract families
(Szeszko et al., 2008)	EPS (33) HC (30)	25(4) 26(5)	21/12 18/12	Exclusion not clearly stated	VBA	Pts < controls in FA, in association fiber tract family
(Cheung et al., 2008)	EPS (25) HC (26)	29(9) 28(9)	13/13 11/14	Exclusion of abuse	VBA & ROI	Pts < controls in FA, in association, callosal, projection and brainstem fiber tract families
(Gasparotti et al., 2009)	EPS (21) HC (21)	29(9) 27(7)	11/10 13/8	Exclusion of abuse and dependence	ROI	Pts < controls in FA, in callosal fiber tract family
(Price et al., 2005)	EPS (20) HC (29)	25(?) 28(?)	14/6 11/18	Exclusion of current use	ROI	Pts = controls in FA, in callosal fiber tract family
(Friedman et al., 2008)	EPS (40) HC (39)	26(6) 25(6)	30/10 28/11	Excluded based on urine test	ROI	Pts = controls in FA, in callosal and association fiber tracts
(Zou et al., 2008)	EPS (21) HC (18)	29(10) 31(1)0	13/8 11/7	Exclusion of abuse and dependence	ROI	Pts < controls in FA, in projection fiber tract family
(Wang et al., 2011)	EPS (68) HC (100)	24(8) 26(8)	32/36 52/48	Exclusion of abuse	VBA	Pts < controls in FA in association and callosal fiber tract families
(White et al., 2009)	EPS (29) HC (40)	14(3) 15(3)	18/11 25/16	Exclusion of dependence and of abuse in past month.	TBSS	Pts < controls in FA, in association, callosal, projection, limbic and brainstem tract families
(Kyriakopoulos et al., 2009)	EPS (34) HC(34)	20(3) 20(3)	26/8 23/11	Exclusion of substance use disorder	VBA	EPS < controls in FA, in association, callosal, and brainstem fiber tract families

Table 2: Diffusion Tensor Imaging studies retained in literature review (Cookey et al., 2014)

First author (year)	Group (n)	Age, years: Mean(SD)	Gender (M/F)	Substance use	Offline analysis	Results
(Kumra et al., 2005)	EPS (26) HC (34)	15(2) 15(3)	14/12 20/14	Exclusion of abuse and dependence	VBA	Pts < controls in FA, in callosal and limbic fiber tract families
(Guo et al., 2012)	EPS (20) HC (26)	24(5) 24(4)	9/11 14/12	Exclusion of abuse	VBA	Pts < controls in FA, in association, limbic and projection fiber tract families
B. Cannabis use in healthy volunteers						
(Zalesky et al., 2012)	CAN+(59) CAN– (33)	33(11) 32(12)	28/31 14/19	Exclusion of other illicit drugs	VBA & tractography (streamlines)	CAN + < CAN – in FA, in callosal and limbic fiber tract families
(Gruber et al., 2013)	CAN+(25) CAN– (18)	23(6) 23(4)	18/7 7/11	Exclusion of other illicit drugs	TBSS & ROI	CAN + < CAN – in FA, in callosal and projection fiber tract families
C. Early phase schizophrenia with concurrent cannabis use						
(James et al., 2011)	EPS (32) -16 CAN+ -16 CAN– HC (28) -28 CAN–	16(1) 16(1)	22/10 18/10	Exclusion of other illicit drugs	TBSS & VBA	Pts < controls in FA, in association, brainstem, callosal and projection fiber tract families. CAN + Pts < CAN – Pts in FA, in brainstem, projection and association fiber tract families

EPS = early phase schizophrenia; HC = healthy control; SD = standard deviation; VBA = full brain, voxel-based analysis; ROI = region of interest; FA = fractional anisotropy; TBSS = tract-based spatial statistics; CAN = cannbis/marijuana; CAN + = cannabis use; CAN – = no cannabis use

Table 3: Affected group of fiber tracts in early phase schizophrenia (Cookey et al., 2014)

Fiber tract families	↓ FA (first author, year)	= FA (first author, year)
Association fibers: Superior longitudinal Superior fronto-occipital Inferior longitudinal Inferior fronto-occipital Uncinate fasciculus	Pérez-Iglesias, 2010 Hao, 2006 Szesko, 2008 Cheung, 2008 Wang, 2011 Kyriakopoulos, 2009 Guo, 2012 White, 2009 Luck, 2011	Lu, 2011 White, 2011 Chan, 2010 Friedman, 2008 Tang, 2010 Kumra, 2005
	60% (9/15 studies)	**40% (6/15 studies)**
Callosal fibers: Corpus callosum body splenium Forceps minor Forceps major Tapetum	Pérez-Iglesias, 2010 Hao, 2006 Cheung, 2008 Gasparotti, 2009 Wang, 2011 Kyriakopoulos, 2009 White, 2009 Kumra, 2005	Lu, 2011 White, 2011 Chan, 2010 Szesko, 2008 Price, 2005 Guo, 2012 Friedman, 2008 Tang, 2010
	50% (8/16 studies)	**50% (8/16 studies)**
Limbic fibers: Cingulum Fornix Stria terminalis	Hao, 2006 Guo, 2012 Tang, 2010 White, 2009 Kumra, 2005	Lu, 2011 White, 2011 Chan, 2010 Pérez-Iglesias, 2010 Szesko, 2008 Cheung, 2008 Wang, 2011 Kyriakopoulos, 2009 Luck, 2011
	36% (5/14 studies)	**64% (9/14 studies)**
Projection fibers Corticobulbar tract Thalamic projections Internal capsule anterior medial posterior External capsule	Pérez-Iglesias, 2010 Hao, 2006 Cheung, 2008 Zou, 2008 Guo, 2012 White, 2009	Lu, 2011 White, 2011 Chan, 2010 Szesko, 2008 Wang, 2011 Kyriakopoulos, 2009 Tang, 2010 Kumra, 2005
	43% (6/14 studies)	**57% (8/14 studies)**
Brain stem fibers Cerebral peduncles superior middle inferior Corticospinal tract Medial lemniscus	Pérez-Iglesias, 2010 Hao, 2006 Cheung, 2008 Kyriakopoulos, 2009 White, 2009	Lu, 2011 White, 2011 Chan, 2010 Szesko, 2008 Wang, 2011 Guo, 2012 Tang, 2010 Kumra, 2005
	38% (5/13 studies)	**62% (8/13 studies)**

Note. FA, fractional anisotropy

Table 4: Means and standard deviations (or frequencies) for demographic and clinical characteristics

Characteristic	Early Phase Psychosis n = 18	Healthy Controls n = 22	t	p
Age, years (sd)	24.9 (4.3)	24.0 (3.4)	.691	.494
Gender (M/F)	12 / 6	15 / 7	-	-
Education, years	14.2 (3.2)	17.0 (2.1)	-3.225**	.003
Handedness (R/L)	14R / 4L	20R / 2L	-	
Age of illness onset, years	22.9 (4.4)	-	-	-
Duration of illness, months	26 (28)	-	-	
BMI	27.1 (4.2)	23.9 (3.7)	2.51*	.017
Height, inches	69 (4)	68 (4)	.425	.673
Weight, lbs	184 (37)	161 (34)	2.062*	.046
Cannabis use, lifetime occasions§	1302 (1531)	3 (3)	3.601**	.002
Cannabis use, lifetime – grams §	1323 (1959)	2 (2)	2.862*	.011
Cannabis use, age of onset	15.8 (3.3)	-	-	-
Alcohol, lifetime drinks§	1734 (2384)	810 (1044)	1.641	.109
Alcohl, onset of regular use	18.2 (2.9)	17.8 (1.3)	.421	.678
Tobacco, lifetime # cigarettes§	13691 (22642)	359 (1554)	2.493*	.023
Other illicit drug use, lifetime occasions	14.6 (22.8)	0.5 (1.4)	2.627*	.018
Antipsychotic use, CPZ (average mg/day)	345 (213)	-	-	-
PSP	72.2 (7.5)	-	-	-
PANSS-P	10.3 (4.3)	-	-	-
PANSS-N	11.3 (2.9)	-	-	-
PANSS-G	23.9 (7.9)	-	-	-
PANSS Total	45.4 (13.3)	-	-	-
SCIP – Global Index§	15.5 (2.1)	17.1 (1.4)	-2.840**	.007
SCIP-VLT_I§	21.6 (3.4)	23.8 (2.4)	-2.387*	.022
SCIP-WMT§	19.1 (2.9)	21.1 (1.6)	-2.761**	.009
SCIP-VFT	18.8 (4.5)	19.6 (4.6)	-.488	.629
SCIP-VLT_D	6.3 (2.4)	7.1 (1.7)	-1.193	.240
SCIP-PST	11.4 (2.6)	13.7 (2.1)	-3.076**	.004
Calgary Depression	2.7 (3.7)	-	-	-
Hamilton Anxiety	5.2 (7.4)	-	-	-

* Significant values at p < .05.

** Significant values at p < .01.

§ characteristics for which adjusted degrees of freedom were used due to significant Levene's test. Numbers represent means (sd); all p-values are two-tailed. t = independent samples t-test. Regular alcohol use defined as once a week or more. M/F = male/female; R/L = right/left; BMI = body mass index; CPZ = chlorpromazine equivalents; PSP = Personal and Social Performance scale; PANSS = Positive and Negative Syndrome Scale (subscales: P=positive symptoms, N=negative symptoms, G=general psychopathology); SCIP = Screen for Cognitive Impairment in Psychiatry (subscales: VLT_I=verbal learning test_immediate, WMT=working memory test, VFT=verbal fluency test, VLT_D=verbal learning test_delayed, PST=psychomotor speed test).

Table 5: MRI Safety Checklist

Pacemaker/biostimulator	Yes / No	Removable dentures	Yes / No
Cerebral aneurysm clip	Yes / No	Dental apparatus	Yes / No
Joint replacement	Yes / No	Jewelry	Yes / No
Fracture treated with metal	Yes / No	Body piercings	Yes / No
Metal worker	Yes / No	Watches	Yes / No
History of ocular foreign body	Yes / No	Eye/Makeup	Yes / No
		Wig	Yes / No
Shrapnel wound	Yes / No	Artificial limb	Yes / No
Chance of pregnancy	Yes / No	Implants	Yes / No
Inner ear implant	Yes / No	Checked for loose metal	Yes / No
Pump for medication	Yes / No		
Hearing aid	Yes / No	Valuables stored	Yes / No
Dry hair	Yes / No	No styling gel	Yes / No

Table 6. Outline of neuroimaging acquisitions

Type	Parameters	Min
Localizer and calibration		2
3D SPGR T_1-weighted, for online placement of VOI and offline tissue segmentation	256 x 256 matrix; 170 sagittal slices; 1 mm isotropic resolution, no inter-slice gap; TR = 11.3 s; TE = 4.2 ms; flip angle = 20 deg.	7
Diffusion-weighted images	TR = 8.5 s; TE 80-90 ms; flip angle = 90 deg; 54 non-collinear diffusion weighting directions, b-factor of 1000 s/mm^2; 6 acquisitions, b-factor of ~ 0 s/mm^2; 256 x 256 matrix; 260 FOV; 1.02 x 1.02 x 3 mm^3 voxels; NEX = 1; and acquisition of field maps.	25

3D = 3-dimentional; SPGR = Spoiled Gradient Echo; VOI = volume of interest; T1 = longitudinal relaxation time; ms = milliseconds; TR = repetition time; TE = echo time; FOV = field of view; NEX = number of excitations

Table 7: DTI measures

Characteristic	Early Phase Psychosis (patients) N = 18	Healthy Controls N = 21	Statistic	P value	Effect size
FULL BRAIN					
FA	369 (21)	375 (15)	t = -1.139 F = 1.079	.262 .306	r = .184 η^2 = .029
MD	.800 (.020)	.797 (.023)	t =.508	.615	r = .083
RaD	.633 (.026)	.627 (.024)	t = .719	.477	r = .117
AxD	1.133 (.018)	1.135 (.022)	t = -.221	.826	r = .036
VOI					
FA	395 (36)	402 (31)	t = -.635 F = 3.847	.529 .058	r = .104 η^2 = .097
MD§	.741 (.022)	.732 (.015)	t = 1.485	.146	r = .237
RaD	.578 (.033)	.567 (.022)	t = 1.195	.240	r = .193
AxD	1.068 (.034)	1.062 (.039)	t = .462	.647	r = .076

Numbers represent means (sd). All diffusion values = multiplied by 1000. FA = fractional anisotropy; MD = mean diffusivity; RaD = radial diffusivity; AxD = axial diffusivity; VOI = volume of interest (left SLF).
t = independent-samples t-test statistic
F = F-test statistic, as calculated via ANCOVA (with age of onset of alcohol as covariate in full-brain comparison and cumulative lifetime cannabis use as covariate in VOI comparison).
r = pearson's coefficient
η^2 = partial eta squared
§ characteristics for which adjusted degrees of freedom were used due to significant Levene's test.

Table 8: Non-parametric correlation matrix - VOI

Correlations – Spearman's rho	mean FA VOI	mean MD VOI	mean RaD VOI	mean AxD VOI
Duration of Illness (months)	.152	-.177	-.190	.117
SCIP - Global index score (total)	.134	.296	.036	.466
SCIP - Verbal Learning - Immediate	-.188	.344	.268	.192
SCIP - Working memory test	-.005	.284	.096	.169
SCIP - Verbal Fluency test	.383	.052	-.217	.521*
SCIP - Verbal Learning - Delayed	.090	.119	-.025	.355
SCIP - Psychomotor Speed test	.109	.270	.082	.443
PANSS - Total score	-.003	.216	.146	.252
PANSS - Positive score	-.240	.241	.313	-.047
PANSS - Negative score	.134	.099	-.038	.252
PANSS - General Symptoms score	-.025	.273	.201	.245
Personal and Social Performance score	-.083	-.145	-.006	-.176
Hamilton Anxiety Score	.174	.210	.077	.352
Calgary Depression Score	-.214	.541*	.443	.094
Cannabis cumulative lifetime (grams)	.316	-.463	-.414	.032
Cannabis use - Number of occasions	.248	-.356	-.332	.020
Cannabis age onset (years)	.110	.034	-.073	.199
THC_onset_rank	.025	.093	.003	.139
EtOH cumulative lifetime (drinks)	-.027	-.343	-.208	-.080
EtOH age onset regular use (>1x/week)	.116	-.051	-.064	.051
EtOH_onset_rank	.098	.145	.047	.074
Lifetime # of cigarettes	-.026	-.234	-.149	-.036
Mean daily antipsychotic use (mg/day) - CPZ Equivalents	-.091	-.022	.063	-.229
Duration of antipsychotic use (months)	.176	-.417	-.343	-.135

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9: Parametric correlation matrix - VOI

Correlations – Pearson's r	mean FA VOI	mean MD VOI	mean RaD VOI	mean AxD VOI
Duration of Illness (months)	.186	-.326	-.288	-.100
SCIP - Global index score (total)	.114	.257	.043	.442
SCIP - Verbal Learning - Immediate	-.128	.308	.221	.197
SCIP - Working memory test	-.074	.189	.139	.121
SCIP - Verbal Fluency test	.274	.063	-.151	.422
SCIP - Verbal Learning - Delayed	.217	.018	-.150	.337
SCIP - Psychomotor Speed test	.047	.312	.126	.381
PANSS - Total score	-.138	.360	.296	.150
PANSS - Positive score	-.234	.306	.335	-.037
PANSS - Negative score	.087	.151	.034	.240
PANSS - General Symptoms score	-.137	.385	.303	.185
Personal and Social Performance score	.014	-.160	-.085	-.160
Hamilton Anxiety Score	-.047	.395	.255	.304
Calgary Depression Score	-.151	.384	.297	.202
Cannabis cumulative lifetime (grams)	.605**	-.494*	-.625**	.217
Cannabis use - Number of occasions	.335	-.320	-.387	.106
Cannabis age onset (years)	-.012	.233	.102	.279
THC_onset_rank	-.014	.112	.061	.111
EtOH cumulative lifetime (drinks)	-.047	-.338	-.196	-.297
EtOH age onset regular use (>1x/week)	.213	-.133	-.228	.185
EtOH_onset_rank	.104	.075	-.035	.216
Lifetime # of cigarettes	.317	-.285	-.341	.091
Mean daily antipsychotic use (mg/day) - CPZ Equivalents	-.194	-.031	.144	-.342
Duration of antipsychotic use (months)	.177	-.427	-.349	-.180

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 10: Non-parametric correlation matrix – full-brain

Correlations – Spearman's rho

	mean FA brain	mean MD brain	mean RaD brain	mean AxD brain
Duration of Illness (months)	-.668**	.499*	.588**	-.155
SCIP - Global index score (total)	-.127	.041	.055	.177
SCIP - Verbal Learning - Immediate	-.249	.129	.198	.139
SCIP - Working memory test	.059	-.217	-.194	-.078
SCIP - Verbal Fluency test	-.220	.138	.141	.091
SCIP - Verbal Learning - Delayed	-.195	.095	.156	.182
SCIP - Psychomotor Speed test	.229	.063	-.089	.489*
PANSS - Total score	.349	-.020	-.150	.415
PANSS - Positive score	.224	-.029	-.086	.267
PANSS - Negative score	.283	-.039	-.161	.238
PANSS - General Symptoms score	.482*	-.140	-.276	.423
Personal and Social Performance score	-.477*	.411	.503*	.047
Hamilton Anxiety Score	.303	-.198	-.262	.123
Calgary Depression Score	.358	-.270	-.313	.264
Cannabis cumulative lifetime (grams)	.413	-.262	-.364	.014
Cannabis use - Number of occasions	.497*	-.350	-.447	-.017
Cannabis age onset (years)	.140	.158	.023	.458
THC_onset_rank	-.294	.334	.357	.191
EtOH cumulative lifetime (drinks)	.096	-.028	-.068	.033
EtOH age onset regular use (>1x/week)	.630*	-.610*	-.628*	-.029
EtOH_onset_rank	.199	-.174	-.144	-.055
Lifetime # of cigarettes	-.112	-.037	.003	-.220
Mean daily antipsychotic use (mg/day) - CPZ Equivalents	-.081	-.007	.014	-.222
Duration of antipsychotic use (months)	-.435	.168	.318	-.398

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 11: Parametric correlation matrix – full-brain

Correlations – Pearson's r	mean FA brain	mean MD brain	mean RaD brain	mean AxD brain
Duration of Illness (months)	-.482*	.341	.474*	-.215
SCIP - Global index score (total)	-.064	.138	.109	.138
SCIP - Verbal Learning - Immediate	-.213	.162	.167	.059
SCIP - Working memory test	.089	-.186	-.157	-.157
SCIP - Verbal Fluency test	-.130	.213	.202	.133
SCIP - Verbal Learning - Delayed	-.200	.206	.213	.049
SCIP - Psychomotor Speed test	.322	.006	-.136	.388
PANSS - Total score	.417	-.157	-.300	.327
PANSS - Positive score	.320	-.170	-.268	.189
PANSS - Negative score	.308	-.099	-.185	.211
PANSS - General Symptoms score	.414	-.135	-.290	.370
Personal and Social Performance score	-.508*	.451	.491*	.063
Hamilton Anxiety Score	.199	-.084	-.152	.166
Calgary Depression Score	.505*	-.293	-.415	.214
Cannabis cumulative lifetime (grams)	.218	-.019	-.074	.142
Cannabis use - Number of occasions	.477*	-.337	-.395	.027
Cannabis age onset (years)	.443	-.014	-.193	.544
THC_onset_rank	-.282	.279	.302	.065
EtOH cumulative lifetime (drinks)	-.242	.061	.141	-.229
EtOH age onset regular use (>1x/week)	.700**	-.494	-.623*	.123
EtOH_onset_rank	.203	-.147	-.171	.025
Lifetime # of cigarettes	-.072	.053	.079	-.050
Mean daily antipsychotic use (mg/day) - CPZ Equivalents	-.193	.012	.084	-.182
Duration of antipsychotic use (months)	-.376	.156	.316	-.371

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Figures

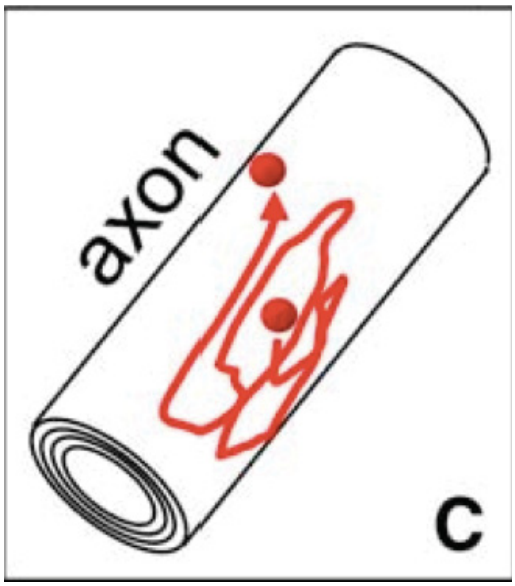

Figure 1: Illustration of water molecule (represented by red dot) randomly moving within an axon. Notice that more movement is permitted along the length of the axon rather than orthogonal to it.

This image was published in *Principles of Diffusion Tensor Imaging and Its Applications to Basic Neuroscience Research*, Neuron, 51 (5), p. 529, Copyright Elsevier 2006 (Mori and J. Zhang, 2006).

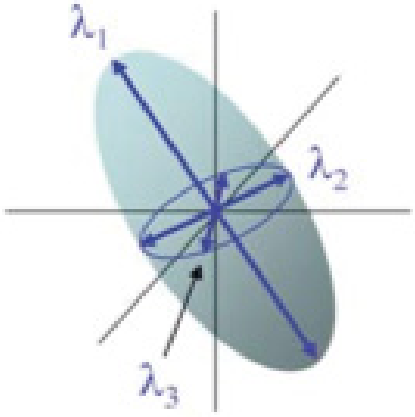

Figure 2: Illustration of the football-shaped diffusion tensor.
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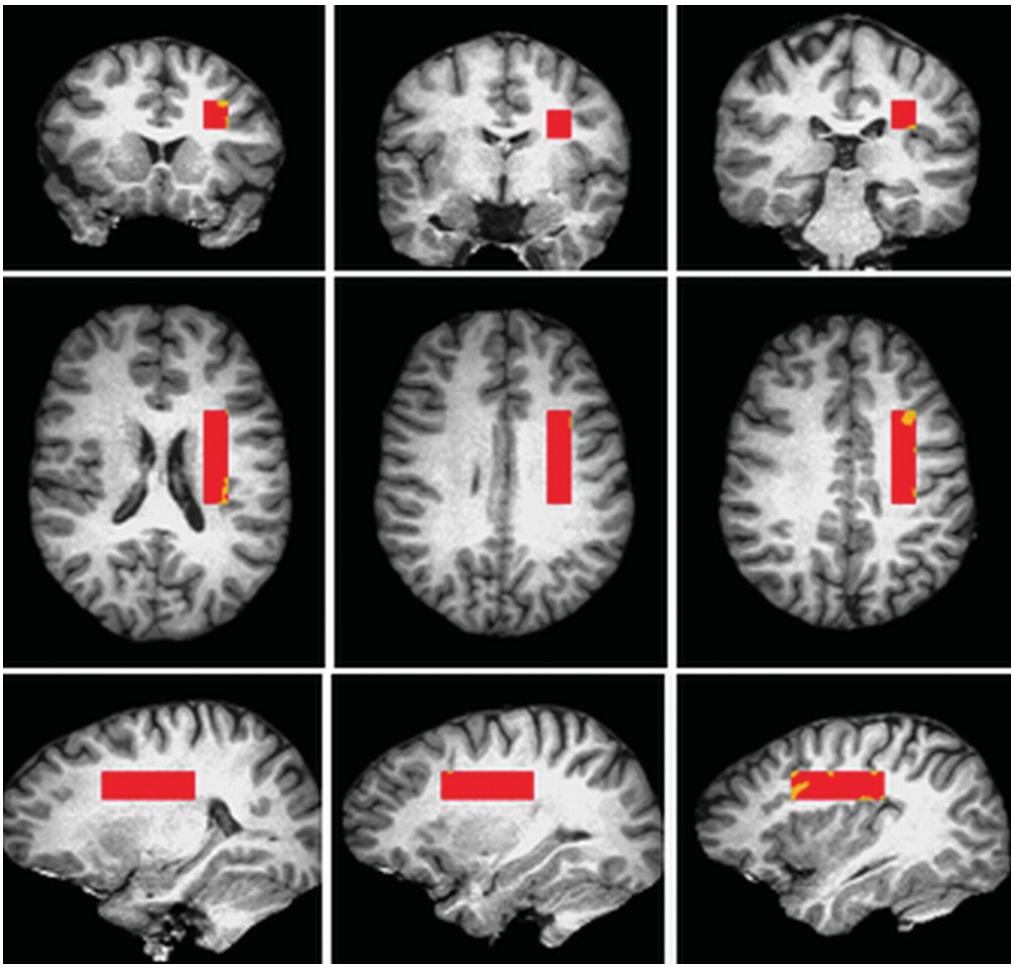

Figure 3: Voxel Of Interest – left Superior Longitudinal Fasciculus.

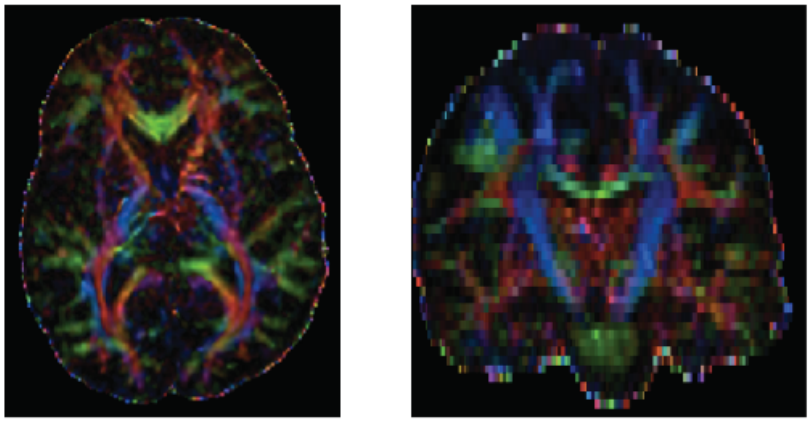

Figure 4: DTI FA maps of primary diffusion vector (V1) – color coded for 3D diffusion directionality.

References

Adamson, P., 2013. Child Well-being in Rich Countries: A comparative overview. Innocenti Report Card.

Addington, D., Addington, J., Schissel, B., 1990. A depression rating scale for schizophrenics. Schizophr. Res. 3, 247–251.

Afifi, A.K., Bergman, R.A., 2005. Functional neuroanatomy, second. ed. McGraw-Hill Medical, New York.

Alexander, A.L., Lee, J.E., Lazar, M., Field, A.S., 2007. Diffusion tensor imaging of the brain. Neurotherapeutics 4, 316–329. doi:10.1016/j.nurt.2007.05.011

Andréasson, S., Engström, A., Allebeck, P., Rydberg, U., 1987. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet. 330 (8574), 1483–1486. doi:10.1016/S0140-6736(87)92620-1

APA, 2000. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. American Psychiatric Association, Washington, DC.

APA, 2013. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). American Psychiatric Publishing, Incorporated, Arlington, VA.

Arseneault, L., 2002. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ 325 (7374), 1212–1213. doi:10.1136/bmj.325.7374.1212

Ashton, C.H., 2001. Pharmacology and effects of cannabis: a brief review. Br. J. Psychiatry 178, 101–106.

Baker, S.T.E., Yücel, M., Fornito, A., Allen, N.B., Lubman, D.I., 2013. A systematic review of diffusion weighted MRI studies of white matter microstructure in adolescent substance users. Neurosci. Biobehav. Rev. 37 (8), 1713–1723. doi:10.1016/j.neubiorev.2013.06.015

Basser, P.J., 1995. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed. 8, 333–344.

Basser, P.J., Mattiello, J., LeBihan, D., 1994. MR diffusion tensor spectroscopy and imaging. Biophys. J. 66 (1), 259–267. doi:10.1016/S0006-3495(94)80775-1

Bava, S., Frank, L.R., McQueeny, T., Schweinsburg, B.C., Schweinsburg, A.D., Tapert, S.F., 2009. Altered white matter microstructure in adolescent substance users. Psychiatry Res. 173, 228–237. doi:10.1016/j.pscychresns.2009.04.005

Bava, S., Jacobus, J., Thayer, R.E., Tapert, S.F., 2013. Longitudinal changes in white matter integrity among adolescent substance users. Alcohol. Clin. Exp. Res. 37 Suppl 1, E181–9. doi:10.1111/j.1530-0277.2012.01920.x

Beaulieu, C., 2002. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed. 15 (7-8), 435–455. doi:10.1002/nbm.782

Bersani, G., Orlandi, V., Kotzalidis, G.D., Pancheri, P., 2002. Cannabis and schizophrenia: impact on onset, course, psychopathology and outcomes. Eur. Arch. Psychiatry Clin. Neurosci. 252 (2), 86–92. doi:10.1007/s00406-002-0366-5

Bilder, R.M., Goldman, R.S., Robinson, D., Reiter, G., Bell, L., Bates, J.A., Pappadopulos, E., Willson, D.F., Alvir, J.M., Woerner, M.G., Geisler, S., Kane, J.M., Lieberman, J.A., 2000. Neuropsychology of first-episode schizophrenia: initial characterization and clinical correlates. Am. J. Psychiatry 157, 549–559.

Birchwood, M., 2000. Early intervention and sustaining the management of vulnerability. Aust N Z J Psychiatry 34 Suppl, S181–4.

Birchwood, M., Todd, P., Jackson, C., 1998. Early intervention in psychosis. The critical period hypothesis. Br J Psychiatry Suppl 172, 53–59.

Blanchard, J.J., Brown, S.A., Horan, W.P., Sherwood, A.R., 2000. Substance use disorders in schizophrenia: review, integration, and a proposed model. Clin Psychol Rev 20, 207–234.

Bleuler, E., 1950. Dementia praecox or the Group of Schizophrenias. International Universities Press, New York.

Bossong, M.G., Jansma, J.M., Bhattacharyya, S., Ramsey, N.F., 2014. Role of the endocannabinoid system in brain functions relevant for schizophrenia: An overview of human challenge studies with cannabis or Δ9-tetrahydrocannabinol (THC). Prog. Neuropsychopharmacol. Biol. Psychiatry 52C, 53–69. doi:10.1016/j.pnpbp.2013.11.017

Brown, A.S., Derkits, E.J., 2010. Prenatal Infection and Schizophrenia: A Review of Epidemiologic and Translational Studies. Am J Psychiatry 167, 261–280. doi:10.1176/appi.ajp.2009.09030361

Cannon, M., 2002. Obstetric Complications and Schizophrenia: Historical and Meta-Analytic Review. Am. J. Psychiatry 159, 1080–1092. doi:10.1176/appi.ajp.159.7.1080

Cannon, T.D., Thompson, P.M., van Erp, T.G.M., Toga, A.W., Poutanen, V.P., Huttunen, M., Lönnqvist, J., Standerskjold-Nordenstam, C.G., Narr, K.L., Khaledy, M., Zoumalan, C.I., Dail, R., Kaprio, J., 2002. Cortex mapping reveals

regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia. Proc. Natl. Acad. Sci. U.S.A. 99, 3228–3233. doi:10.1073/pnas.052023499

Casadio, P., Fernandes, C., Murray, R.M., Di Forti, M., 2011. Cannabis use in young people: The risk for schizophrenia. Neurosci. Biobehav. Rev. 35 (8), 1779–1787. doi:10.1016/j.neubiorev.2011.04.007

Chan, W.-Y., Yang, G.-L., Chia, M.Y., Lau, I.-Y., Sitoh, Y.-Y., Nowinski, W.L., Sim, K., 2010. White matter abnormalities in first-episode schizophrenia: a combined structural MRI and DTI study. Schizophr. Res. 119 (1-3), 52–60. doi:10.1016/j.schres.2009.12.012

Cheung, V., Cheung, C., McAlonan, G.M., Deng, Y., Wong, J.G., Yip, L., Tai, K.S., Khong, P.L., Sham, P., Chua, S.E., 2008. A diffusion tensor imaging study of structural dysconnectivity in never-medicated, first-episode schizophrenia. Psychol. Med. 38 (6), 877–885. doi:10.1017/S0033291707001808

Cookey, J., Bernier, D., Tibbo, P.G., 2014. White matter changes in early phase schizophrenia and cannabis use: An update and systematic review of diffusion tensor imaging studies. Schizophr. Res. doi:10.1016/j.schres.2014.04.026

Cornblatt, B.A., Lencz, T., Smith, C.W., Correll, C.U., Auther, A.M., Nakayama, E., 2003. The schizophrenia prodrome revisited: a neurodevelopmental perspective. Schizophr. Bull. 29 (4), 633–651.

Creese, I., Burt, D.R., Snyder, S.H., 1996. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. J. Neuropsychiatry Clin. Neurosci. 8, 223–226.

Crow, T.J., 1998. Schizophrenia as a transcallosal misconnection syndrome. Schizophr. Res. 30 (2), 111–114.

Danckert, J., Rossetti, Y., d'Amato, T., Dalery, J., Saoud, M., 2002. Exploring imagined movements in patients with schizophrenia. Neuroreport 13 (5), 605–609.

Davis, K.L., Kahn, R.S., Ko, G., Davidson, M., 1991. Dopamine in schizophrenia: a review and reconceptualization. Am. J. Psychiatry 148, 1474–1486.

Davis, K.L., Stewart, D.G., Friedman, J.I., Buchsbaum, M., Harvey, P.D., Hof, P.R., Buxbaum, J., Haroutunian, V., 2003. White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch. Gen. Psychiatry 60 (5), 443–456. doi:10.1001/archpsyc.60.5.443

Dekker, N., Schmitz, N., Peters, B.D., van Amelsvoort, T.A., Linszen, D.H., de Haan,

L., 2010. Cannabis use and callosal white matter structure and integrity in recent-onset schizophrenia. Psychiatry Res. 181 (1), 51–56. doi:10.1016/j.pscychresns.2009.06.003

Ehrenreich, H., Rinn, T., Kunert, H.J., Moeller, M.R., Poser, W., Schilling, L., Gigerenzer, G., Hoehe, M.R., 1999. Specific attentional dysfunction in adults following early start of cannabis use. Psychopharmacology (Berl). 142 (3), 295–301.

Ettinger, U., Schmechtig, A., Toulopoulou, T., Borg, C., Orrells, C., Owens, S., Matsumoto, K., van Haren, N.E., Hall, M.H., Kumari, V., McGuire, P.K., Murray, R.M., Picchioni, M., 2011. Prefrontal and Striatal Volumes in Monozygotic Twins Concordant and Discordant for Schizophrenia. Schizophr. Bull. 38, 192–203. doi:10.1093/schbul/sbq060

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 39, 175–191. doi:10.3758/BF03193146

Fields, R.D., 2008a. White matter in learning, cognition and psychiatric disorders. Trends Neurosci. 31, 361–370. doi:10.1016/j.tins.2008.04.001

Fields, R.D., 2008b. White matter matters. Sci. Am. 298, 54–61.

First, M.B., Gibbon, M., Spitzer, R.L., Williams, J.B.W., 2002. Structured clinical interview for DSM-IV-TR axis I disorders - research version. Biometrics Research Department, New York.

Franck, N., 2001. Defective Recognition of One's Own Actions in Patients With Schizophrenia. Am. J. Psychiatry 158 (3), 454–459. doi:10.1176/appi.ajp.158.3.454

Friedman, J.I., Tang, C., Carpenter, D., Buchsbaum, M., Schmeidler, J., Flanagan, L., Golembo, S., Kanellopoulou, I., Ng, J., Hof, P.R., Harvey, P.D., Tsopelas, N.D., Stewart, D., Davis, K.L., 2008. Diffusion tensor imaging findings in first-episode and chronic schizophrenia patients. Am. J. Psychiatry 165 (8), 1024–1032. doi:10.1176/appi.ajp.2008.07101640

Friston, K.J., Frith, C.D., 1995. Schizophrenia: a disconnection syndrome? Clin. Neurosci. 3, 89–97.

Fujiwara, H., Namiki, C., Hirao, K., Miyata, J., Shimizu, M., Fukuyama, H., Sawamoto, N., Hayashi, T., Murai, T., 2007. Anterior and posterior cingulum abnormalities and their association with psychopathology in schizophrenia: a diffusion tensor imaging study. Schizophr. Res. 95, 215–222. doi:10.1016/j.schres.2007.05.044

Gasparotti, R., Valsecchi, P., Carletti, F., Galluzzo, A., Liserre, R., Cesana, B., Sacchetti, E., 2009. Reduced fractional anisotropy of corpus callosum in first-contact, antipsychotic drug-naive patients with schizophrenia. Schizophr. Res. 108 (1-3), 41–48. doi:10.1016/j.schres.2008.11.015

Geddes, A.E., Huang, X.-F., Newell, K.A., 2011. Reciprocal signalling between NR2 subunits of the NMDA receptor and neuregulin1 and their role in schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 896–904. doi:10.1016/j.pnpbp.2011.02.017

Glass, M., Dragunow, M., Faull, R.L., 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77 (2), 299–318.

Goldman-Rakic, P.S., 1994. Working memory dysfunction in schizophrenia. J. Neuropsychiatry Clin. Neurosci. 6 (4), 348–357.

Greenstein, D., Lerch, J., Shaw, P., Clasen, L., Giedd, J., Gochman, P., Rapoport, J., Gogtay, N., 2006. Childhood onset schizophrenia: cortical brain abnormalities as young adults. Journal of child psychology and psychiatry, and allied disciplines 47, 1003–1012. doi:10.1111/j.1469-7610.2006.01658.x

Gruber, S.A., Dahlgren, M.K., Sagar, K.A., Gönenç, A., Lukas, S.E., 2013. Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. Psychopharmacology (Berl). 231 (8), 1455–65. Epub. doi:10.1007/s00213-013-3326-z

Gruber, S.A., Silveri, M.M., Dahlgren, M.K., Yurgelun-Todd, D., 2011. Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers. Exp Clin Psychopharmacol 19 (3), 231–242. doi:10.1037/a0023034

Gruzelier, J., Seymour, K., Wilson, L., Jolley, A., Hirsch, S., 1988. Impairments on neuropsychologic tests of temporohippocampal and frontohippocampal functions and word fluency in remitting schizophrenia and affective disorders. Arch. Gen. Psychiatry 45 (7), 623–629.

Guo, W., Liu, F., Liu, Z., Gao, K., Xiao, C., Chen, H., Zhao, J., 2012. Right lateralized white matter abnormalities in first-episode, drug-naive paranoid schizophrenia. Neurosci. Lett. 531 (1), 5–9. doi:10.1016/j.neulet.2012.09.033

Hao, Y., Liu, Z., Jiang, T., Gong, G., Liu, H., Tan, L., Kuang, F., Xu, L., Yi, Y., Zhang, Z., 2006. White matter integrity of the whole brain is disrupted in first-episode schizophrenia. Neuroreport 17 (1), 23–26.

Hof, P.R., Haroutunian, V., Friedrich, V.L., Byne, W., Buitron, C., Perl, D.P., Davis,

K.L., 2003. Loss and altered spatial distribution of oligodendrocytes in the superior frontal gyrus in schizophrenia. Biol. Psychiatry 53, 1075–1085.

Hollister, L.E., 1986. Health aspects of cannabis. Pharmacological reviews.

Hornak, J.P., 2010. The Basics of MRI. Interactive Learning Software.

Hulshoff Pol, H.E., Schnack, H.G., Mandl, R.C.W., Cahn, W., Collins, D.L., Evans, A.C., Kahn, R.S., 2004. Focal white matter density changes in schizophrenia: reduced inter-hemispheric connectivity. Neuroimage 21 (1), 27–35.

James, A., Hough, M., James, S., Winmill, L., Burge, L., Nijhawan, S., Matthews, P.M., Zarei, M., 2011. Greater white and grey matter changes associated with early cannabis use in adolescent-onset schizophrenia (AOS). Schizophr. Res. 128 (1-3), 91–97. doi:10.1016/j.schres.2011.02.014

Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148, 1301–1308.

Johansen-Berg, H., Behrens, T.E.J., 2009. Diffusion MRI. Academic Press.

Karlsgodt, K.H., Jacobson, S.C., Seal, M., Fusar-Poli, P., 2012. The relationship of developmental changes in white matter to the onset of psychosis. Curr. Pharm. Des. 18 (4), 422–433.

Karlsgodt, K.H., van Erp, T.G.M., Poldrack, R.A., Bearden, C.E., Nuechterlein, K.H., Cannon, T.D., 2008. Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol. Psychiatry 63 (5), 512–518. doi:10.1016/j.biopsych.2007.06.017

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13, 261–276.

Khantzian, E.J., 1997. The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. Harv Rev Psychiatry 4, 231–244. doi:10.3109/10673229709030550

Konrad, A., Winterer, G., 2008. Disturbed structural connectivity in schizophrenia -- primary factor in pathology or epiphenomenon? Schizophr. Bull. 34 (1), 72–92. doi:10.1093/schbul/sbm034

Kovasznay, B., Fleischer, J., Tanenberg-Karant, M., Jandorf, L., Miller, A.D., Bromet, E., 1997. Substance use disorder and the early course of illness in schizophrenia and affective psychosis. Schizophr. Bull. 23, 195–201.

Kraepelin, E., 1919. Dementia praecox and paraphrenia. Livingstone, Edinburgh.

Kringlen, E., 2000. Twin studies in schizophrenia with special emphasis on concordance figures. Am. J. Med. Genet. 97, 4–11.

Kumra, S., Ashtari, M., Cervellione, K.L., Henderson, I., Kester, H., Roofeh, D., Wu, J., Clarke, T., Thaden, E., Kane, J.M., Rhinewine, J., Lencz, T., Diamond, A., Ardekani, B.A., Szeszko, P.R., 2005. White matter abnormalities in early-onset schizophrenia: a voxel-based diffusion tensor imaging study. J Am Acad Child Adolesc Psychiatry 44 (9), 934–941. doi:10.1097/01.chi.0000170553.15798.94

Kyriakopoulos, M., Bargiotas, T., Barker, G.J., Frangou, S., 2008. Diffusion tensor imaging in schizophrenia. Eur Psychiatry 23 (4), 255–273. doi:10.1016/j.eurpsy.2007.12.004

Kyriakopoulos, M., Perez-Iglesias, R., Woolley, J.B., Kanaan, R.A.A., Vyas, N.S., Barker, G.J., Frangou, S., McGuire, P.K., 2009. Effect of age at onset of schizophrenia on white matter abnormalities. Br. J. Psychiatry 195 (4), 346–353. doi:10.1192/bjp.bp.108.055376

Lahti, A.C., Weiler, M.A., Tamara Michaelidis, B.A., Parwani, A., Tamminga, C.A., 2001. Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology 25, 455–467. doi:10.1016/S0893-133X(01)00243-3

Leggett, T., 2006. A review of the world cannabis situation. Bull Narc 58, 1–155.

Leucht, S., Kane, J.M., Kissling, W., Hamann, J., Etschel, E., Engel, R.R., 2005. What does the PANSS mean? Schizophr. Res. 79, 231–238. doi:10.1016/j.schres.2005.04.008

Lieberman, J.A., Perkins, D., Belger, A., Chakos, M., Jarskog, F., Boteva, K., Gilmore, J., 2001. The early stages of schizophrenia: speculations on pathogenesis, pathophysiology, and therapeutic approaches. Biol. Psychiatry 50, 884–897.

Lopez, A.D., Murray, C.C., 1998. The global burden of disease, 1990-2020. Nat. Med. 4, 1241–1243. doi:10.1038/3218

Lu, L.H., Zhou, X.J., Keedy, S.K., Reilly, J.L., Sweeney, J.A., 2011. White matter microstructure in untreated first episode bipolar disorder with psychosis: comparison with schizophrenia. Bipolar Disord. 13 (7-8), 604–613. doi:10.1111/j.1399-5618.2011.00958.x

Luck, D., Buchy, L., Czechowska, Y., Bodnar, M., Pike, G.B., Campbell, J.S.W., Achim, A., Malla, A., Joober, R., Lepage, M., 2011. Fronto-temporal disconnectivity and clinical short-term outcome in first episode psychosis: a DTI-tractography study. J. Psychiatr. Res. 45 (3), 369–377. doi:10.1016/j.jpsychires.2010.07.007

Maier, W., Buller, R., Philipp, M., Heuser, I., 1988. The Hamilton Anxiety Scale: reliability, validity and sensitivity to change in anxiety and depressive disorders. Journal of Affective Disorders 14, 61–68. doi:10.1016/0165-0327(88)90072-9

Makris, N., Kennedy, D.N., McInerney, S., Sorensen, A.G., Wang, R., Caviness, V.S., Pandya, D.N., 2005. Segmentation of subcomponents within the superior longitudinal fascicle in humans: a quantitative, in vivo, DT-MRI study. Cereb. Cortex 15 (6), 854–869. doi:10.1093/cercor/bhh186

Malone, D.T., Hill, M.N., Rubino, T., 2010. Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. Br. J. Pharmacol. 160 (3), 511–522. doi:10.1111/j.1476-5381.2010.00721.x

Maynard, T.M., Sikich, L., Lieberman, J.A., LaMantia, A.S., 2001. Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia. Schizophr. Bull. 27 (3), 457–476.

McGlashan, T.H., Johannessen, J.O., 1996. Early detection and intervention with schizophrenia: rationale. Schizophr. Bull. 22, 201–222.

McGrath, J., Saha, S., Chant, D., Welham, J., 2008. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiologic Reviews 30, 67–76. doi:10.1093/epirev/mxn001

Meador-Woodruff, J.H., Healy, D.J., 2000. Glutamate receptor expression in schizophrenic brain. Brain Res. Brain Res. Rev. 31, 288–294.

Mechoulam, R., Gaoni, Y., 1965. A Total Synthesis of dl-Δ 1-Tetrahydrocannabinol, the Active Constituent of Hashish 1. J. Am. Chem. Soc. 87, 3273–3275. doi:10.1021/ja01092a065

Mohr, B., Pulvermüller, F., Cohen, R., Rockstroh, B., 2000. Interhemispheric cooperation during word processing: evidence for callosal transfer dysfunction in schizophrenic patients. Schizophr. Res. 46 (2-3), 231–239.

Molina-Holgado, E., Vela, J.M., Arévalo-Martín, A., Almazán, G., Molina-Holgado, F., Borrell, J., Guaza, C., 2002. Cannabinoids Promote Oligodendrocyte Progenitor Survival: Involvement of Cannabinoid Receptors and Phosphatidylinositol-3 Kinase/Akt Signaling. The Journal of ….

Morgan, C., Fisher, H., 2007. Environment and schizophrenia: environmental factors in schizophrenia: childhood trauma--a critical review. Schizophr. Bull. 33, 3–10. doi:10.1093/schbul/sbl053

Mori, S., Zhang, J., 2006. Principles of Diffusion Tensor Imaging and Its Applications to Basic Neuroscience Research. Neuron 51 (5), 527–539.

doi:10.1016/j.neuron.2006.08.012

Morosini, P.L., Magliano, L., Brambilla, L., Ugolini, S., Pioli, R., 2000. Development, reliability and acceptability of a new version of the DSM?IV Social and Occupational Functioning Assessment Scale (SOFAS) to assess routine social funtioning. Acta Psychiatr Scand 101, 323–329. doi:10.1034/j.1600-0447.2000.101004323.x

Mulert, C., Kirsch, V., Whitford, T.J., Alvarado, J., Pelavin, P., McCarley, R.W., Kubicki, M., Salisbury, D.F., Shenton, M.E., 2012. Hearing voices: a role of interhemispheric auditory connectivity? World J Biol Psychiatry 13, 153–158. doi:10.3109/15622975.2011.570789

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.

Olney, J.W., Farber, N.B., 1995. Glutamate receptor dysfunction and schizophrenia. Arch. Gen. Psychiatry 52, 998–1007.

Park, S., Holzman, P.S., 1992. Schizophrenics show spatial working memory deficits. Arch. Gen. Psychiatry 49 (12), 975–982.

Patel, S., Mahon, K., Wellington, R., Zhang, J., Chaplin, W., Szeszko, P.R., 2011. A meta-analysis of diffusion tensor imaging studies of the corpus callosum in schizophrenia. Schizophr. Res. 129 (2-3), 149–155. doi:10.1016/j.schres.2011.03.014

Pearson, C., Janz, T., Ali, J., 2013. Health at a Glance: Mental and Substance Use Disorders in Canada.

Perälä, J., Suvisaari, J., Saarni, S.I., Kuoppasalmi, K., Isometsä, E., Pirkola, S., Partonen, T., Tuulio-Henriksson, A., Hintikka, J., Kieseppä, T., Härkänen, T., Koskinen, S., Lönnqvist, J., 2007. Lifetime prevalence of psychotic and bipolar I disorders in a general population. Arch. Gen. Psychiatry 64, 19–28. doi:10.1001/archpsyc.64.1.19

Perkins, D.O., Gu, H., Boteva, K., Lieberman, J.A., 2005. Relationship between duration of untreated psychosis and outcome in first-episode schizophrenia: a critical review and meta-analysis. Am J Psychiatry 162, 1785–1804. doi:10.1176/appi.ajp.162.10.1785

Peters, B.D., Blaas, J., de Haan, L., 2010. Diffusion tensor imaging in the early phase of schizophrenia: What have we learned? J. Psychiatr. Res. 44 (15), 993–1004. doi:10.1016/j.jpsychires.2010.05.003

Peters, B.D., de Haan, L., Vlieger, E.-J., Majoie, C.B., Heeten, den, G.J., Linszen,

D.H., 2009. Recent-onset schizophrenia and adolescent cannabis use: MRI evidence for structural hyperconnectivity? Psychopharmacol Bull 42, 75–88.

Peters, B.D., Szeszko, P.R., Radua, J., Ikuta, T., Gruner, P., Derosse, P., Zhang, J.-P., Giorgio, A., Qiu, D., Tapert, S.F., Brauer, J., Asato, M.R., Khong, P.L., James, A.C., Gallego, J.A., Malhotra, A.K., 2012. White Matter Development in Adolescence: Diffusion Tensor Imaging and Meta-Analytic Results. Schizophr. Bull. 38 (6), 1308–1317. doi:10.1093/schbul/sbs054

Pérez-Iglesias, R., Tordesillas-Gutiérrez, D., Barker, G.J., McGuire, P.K., Roiz-Santiañez, R., Mata, I., de Lucas, E.M., Quintana, F., Vazquez-Barquero, J.L., Crespo-Facorro, B., 2010. White matter defects in first episode psychosis patients: a voxelwise analysis of diffusion tensor imaging. Neuroimage 49 (1), 199–204. doi:10.1016/j.neuroimage.2009.07.016

Pino, O., G, G., J, G., JE, R., J, V., SE, P., 2006. A brief scale to assess cognitive impairment in psychiatric patients. Psicothema 18, 447–452.

Pope, H.G., Gruber, A.J., Hudson, J.I., Cohane, G., Huestis, M.A., Yurgelun-Todd, D., 2003. Early-onset cannabis use and cognitive deficits: what is the nature of the association? Drug Alcohol Depend. 69 (3), 303–310.

Price, G., Bagary, M.S., Cercignani, M., Altmann, D.R., Ron, M.A., 2005. The corpus callosum in first episode schizophrenia: a diffusion tensor imaging study. J. Neurol. Neurosurg. Psychiatry 76 (4), 585–587. doi:10.1136/jnnp.2004.042952

Purdon, S., 2005. The Screen for Cognitive Impairment in Psychiatry: Instructions and three alternate forms. P. Inc.

Rapoport, J.L., Addington, A.M., Frangou, S., Psych, M.R.C., 2005. The neurodevelopmental model of schizophrenia: update 2005. Mol. Psychiatry 10, 434–449. doi:10.1038/sj.mp.4001642

Rapoport, J.L., Giedd, J.N., Gogtay, N., 2012. Neurodevelopmental model of schizophrenia: update 2012. Mol. Psychiatry 17, 1228–1238. doi:10.1038/mp.2012.23

Romero, J., Garcia-Palomero, E., Berrendero, F., Garcia-Gil, L., Hernandez, M.L., Ramos, J.A., Fernández-Ruiz, J.J., 1997. Atypical location of cannabinoid receptors in white matter areas during rat brain development. Synapse 26 (3), 317–323. doi:10.1002/(SICI)1098-2396(199707)26:3<317::AID-SYN12>3.0.CO;2-S

Ruest, T., Holmes, W.M., Barrie, J.A., Griffiths, I.R., Anderson, T.J., Dewar, D., Edgar, J.M., 2011. High-resolution diffusion tensor imaging of fixed brain in a mouse model of Pelizaeus-Merzbacher disease: comparison with quantitative

measures of white matter pathology. NMR Biomed. 24 (10), 1369–1379. doi:10.1002/nbm.1700

Sadock, B.J., Kaplan, H.I., Sadock, V.A., 2007. Kaplan & Sadock's Synopsis of Psychiatry. Lippincott Williams & Wilkins.

Sadock, B.J., Sadock, V.A., Ruiz, P., 2009. Kaplan and Sadock's Comprehensive Textbook of Psychiatry, 9 ed. Lippincott Williams & Wilkins.

Schmahmann, J.D., Pandya, D.N., 2007. The complex history of the fronto-occipital fasciculus. J. Hist. Neurosci. 16 (4), 362–377. doi:10.1080/09647040600620468

Sexton, C.E., Mackay, C.E., Ebmeier, K.P., 2009. A systematic review of diffusion tensor imaging studies in affective disorders. Biol. Psychiatry 66, 814–823. doi:10.1016/j.biopsych.2009.05.024

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I): The Development and Validation of a Structured Diagnostic Psychiatric Interview for DSM-IV and ICD-10. Journal of clinical … 59 (Suppl 20).

Shergill, S.S., Kanaan, R.A., Chitnis, X.A., O'Daly, O., Jones, D.K., Frangou, S., Williams, S.C.R., Howard, R.J., Barker, G.J., Murray, R.M., McGuire, P., 2007. A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164, 467–473. doi:10.1176/appi.ajp.164.3.467

Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E.J., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23 Suppl 1, S208–S219. doi:10.1016/j.neuroimage.2004.07.051

Sobell, L.C., Sobell, M.B., Buchan, G., Cleland, P.A., 1996. The reliability of the Timeline Followback method applied to drug, cigarette, and cannabis use. 30th Annual Meeting of the Association for Advancement of Behavior Therapy. New York, NY.

Song, S.-K., Sun, S.-W., Ramsbottom, M.J., Chang, C., Russell, J., Cross, A.H., 2002. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 17, 1429–1436.

Szeszko, P.R., Robinson, D.G., Ashtari, M., Vogel, J., Betensky, J., Sevy, S., Ardekani, B.A., Lencz, T., Malhotra, A.K., McCormack, J., Miller, R., Lim, K.O., Gunduz-Bruce, H., Kane, J.M., Bilder, R.M., 2008. Clinical and neuropsychological correlates of white matter abnormalities in recent onset

schizophrenia. Neuropsychopharmacology 33 (5), 976–984. doi:10.1038/sj.npp.1301480

Tang, J., Liao, Y., Zhou, B., Tan, C., Liu, T., Hao, W., 2010. Abnormal anterior cingulum integrity in first episode, early-onset schizophrenia: A diffusion tensor imaging study. Brain Res. 1343, 199–205.

Uranova, N., Orlovskaya, D., Vikhreva, O., Zimina, I., Kolomeets, N., Vostrikov, V., Rachmanova, V., 2001. Electron microscopy of oligodendroglia in severe mental illness. Brain Res. Bull. 55, 597–610.

Uranova, N.A., Vostrikov, V.M., Vikhreva, O.V., Zimina, I.S., Kolomeets, N.S., Orlovskaya, D.D., 2007. The role of oligodendrocyte pathology in schizophrenia. Int. J. Neuropsychopharmacol. 10, 537–545. doi:10.1017/S1461145707007626

Van den Oord, E.J., Rujescu, D., Robles, J.R., Giegling, I., Birrell, C., Bukszár, J., Murelle, L., Möller, H.J., Middleton, L., Muglia, P., 2006. Factor structure and external validity of the PANSS revisited. Schizophr. Res. 82, 213–223.

van Os, J., Kenis, G., Rutten, B.P.F., 2010. The environment and schizophrenia. Nature 468, 203–212. doi:doi:10.1038/nature09563

Veen, N.D., Selten, J.-P., van der Tweel, I., Feller, W.G., HOEK, H.W., Kahn, R.S., 2004. Cannabis use and age at onset of schizophrenia. Am. J. Psychiatry 161, 501–506.

Wakana, S., Jiang, H., Nagae-Poetscher, L.M., van Zijl, P.C.M., Mori, S., 2004. Fiber tract-based atlas of human white matter anatomy. Radiology 230 (1), 77–87. doi:10.1148/radiol.2301021640

Wang, Q., Deng, W., Huang, C., Li, M., Ma, X., Wang, Y., Jiang, L., Lui, S., Huang, X., Chua, S.E., Cheung, C., McAlonan, G.M., Sham, P.C., Murray, R.M., Collier, D.A., Gong, Q., Li, T., 2011. Abnormalities in connectivity of white-matter tracts in patients with familial and non-familial schizophrenia. Psychol. Med. 41 (8), 1691–1700. doi:10.1017/S0033291710002412

Weinberger, D.R., 1987. Implications of normal brain development for the pathogenesis of schizophrenia. Arch. Gen. Psychiatry 44, 660–669.

Westerhausen, R., Kreuder, F., Santos Sequeira, Dos, S., Walter, C., Woerner, W., Wittling, R.A., Schweiger, E., Wittling, W., 2004. Effects of handedness and gender on macro- and microstructure of the corpus callosum and its subregions: a combined high-resolution and diffusion-tensor MRI study. Brain Res Cogn Brain Res 21, 418–426. doi:10.1016/j.cogbrainres.2004.07.002

White, T., Magnotta, V.A., Bockholt, H.J., Williams, S., Wallace, S., Ehrlich, S.,

Mueller, B.A., Ho, B.-C., Jung, R.E., Clark, V.P., Lauriello, J., Bustillo, J.R., Schulz, S.C., Gollub, R.L., Andreasen, N.C., Calhoun, V.D., Lim, K.O., 2011. Global white matter abnormalities in schizophrenia: a multisite diffusion tensor imaging study. Schizophr. Bull. 37 (1), 222–232. doi:10.1093/schbul/sbp088

White, T., Nelson, M., Lim, K.O., 2008. Diffusion tensor imaging in psychiatric disorders. Top. Magn. Reson. Imaging 19 (2), 97–109. doi:10.1097/RMR.0b013e3181809f1e

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 (2), 110–115. doi:10.1016/j.pscychresns.2009.04.014

Whitford, T.J., Kubicki, M., Shenton, M.E., 2011. Diffusion tensor imaging, structural connectivity, and schizophrenia. Schizophr Res Treatment 2011, 1–7. doi:10.1155/2011/709523

Wilson, W., Mathew, R., Turkington, T., Hawk, T., Coleman, R.E., Provenzale, J., 2000. Brain morphological changes and early marijuana use: a magnetic resonance and positron emission tomography study. J. Addict. Dis. 19 (1), 1–22. doi:10.1300/J069v19n01_01

Wyatt, R.J., 1991. Neuroleptics and the natural course of schizophrenia. Schizophr. Bull. 17, 325–351.

Zalesky, A., Solowij, N., Yücel, M., Lubman, D.I., Takagi, M., Harding, I.H., Lorenzetti, V., Wang, R., Searle, K., Pantelis, C., Seal, M., 2012. Effect of long-term cannabis use on axonal fibre connectivity. Brain 135 (7), 2245–2255. doi:10.1093/brain/aws136

Zhang, Y., Brady, M., Smith, S., 2001. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 20, 45–57. doi:10.1109/42.906424

Zou, L.-Q., Xie, J.-X., Yuan, H.-S., Pei, X.-L., Dong, W.-T., Liu, P.-C., 2008. Diffusion tensor imaging study of the anterior limb of internal capsules in neuroleptic-naive schizophrenia. Acad. Radiol. 15 (3), 285–289. doi:10.1016/j.acra.2007.09.026

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