UNIVERSITÀ DEGLI STUDI DI PADOVA

# UNIVERSITÀ DEGLI STUDI DI PADOVA

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# DTI STUDY OF THE FRONTAL LOBES, HIPPOCAMPUS, AMYGDALA AND NEUROCOGNITIVE ASSESSMENT IN PATIENTS WITH BIPOLAR-SCHIZOPHRENIC SPECTRUM DISORDERS

**Direttore della Scuola:** Ch.mo Prof. Gaetano Thiene

**Coordinatore d'Indirizzo:** Ch.ma Prof.ssa Elena Pegoraro

**Supervisore:** Ch.ma Prof.ssa Giulia Ida Perini

**Dottorando**: Dr.ssa Halima Follador

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## ABSTRACT

BACKGROUND: schizophrenic and bipolar disorders are complex and disabling psychiatric diseases whose recent neurobiological, neuropsychological and genetic findings is in contrast with the traditional categorical approach of psychiatric nosography.

The multiple threshold model describes the complex relationship between the shared genetic background and the wide phenotypic expression in the various disorders ascribed to the bipolar schizophrenic spectrum. This model assumes that common genes are involved in a spectrum of disorders ranging from major depression, to bipolar and schizophrenia and that their effect is additive along a continuum of risk: when a certain threshold is exceeded the quantitative difference becomes a qualitative difference that manifests itself as a different disorder (eg. switching from major depression to bipolar disorder to schizo-affective disorder than schizophrenic) (Kelsoe 2003).

A field of great interest in neuroscience and psychiatric research is finding evidence of shared clinical features and pathophysiological pathways between these disorders. Genetics, histopathological and MRI in vivo studies have consistently revealed abnormalities in brain neural networks among these disorders.

The Diffusion tensor imaging (DTI) is a fundamental brain imaging technique to investigate white matter‘s structural connectivity, despite its relative recently introduction in clinical practice and research.

AIM OF THE STUDY: to investigate the DTI measures of WM integrity in specific brain regions and the cognitive performances in a group of patients with the bipolar-schizophrenic spectrum disorders and a group of healthy control subjects. In order to verify or exclude specific diagnosis-related differences, we performed cross-sectional comparisons between the sub group of bipolar patients, schizophrenic patients and healthy controls.

METHODS: 64 patients -32 schizophrenic (SZ), 25 bipolars (BP)-, and 31 healthy controls underwent 1,5 T MRI scanning, comprehending DTI acquisitions and volumetric T1 3D with a specifically designed acquisition protocol, at the Neuroradiology Unit of Conegliano Hospital. Then we calculate DTI indices of bilateral frontal lobes, hippocampus and amygdala using ANALYZE 10.0 software, all recruited subjects underwent clinical and standardized, thorough neurocognitive assessment (ENB, Mondini et al; WCST).

RESULTS: we found statistically significant alterations of the DTI indicies for the regions of interest (ROIs), that pointed out shared abnormalities among the patients with bipolar schizophrenic-spectrum disorder regarding frontal lobes with respect to healthy control subjects; more interesting, we find a complex pattern of alterations among the hippocampal region and amygdala between the patients and the control group and also comparing the schizophrenic with the bipolar patients. Moreover we found out a significant impairment on the performances during the neurocognitive and neuro psychological assessment across all tests in the patients opposed to healthy controls.

We also pointed out some interesting correlations between the scores of the battery test administrated (ENB, Mondini et al; WCST) and the FA and ADC indices for the frontal lobes, as expected from the abundant current literature, but also for the hippocampus and amygdala. This approach could help to the understand some aspects of the complexity of the Bipolar-schizophrenic spectrum disorders

CONCLUSION: in this study we highlighted shared tracts among the spectrum disorders such as the common neurocognitive and neuropsychological impairment, the compromised structural integrity of the white matter in the frontal regions and probably, in some degree, even of the right hippocampus, implying that these two disorders may share some common pathophysiological mechanisms, further demonstrating how alterations in the cerebral white matter networks, involving the frontal regions and also subcortical structures, such as the hippocampus and amygdala, contribute to the pathophysiological process of schizophrenia and bipolar disorder.

Our findings also bring out differences among the two groups of patients, with the bipolars showing a most prominent alteration of the left amygdala and the schizophrenics a predominant deficit on the right hippocampus and amygdala.

In the bipolar-schizophrenic spectrum disorders it might be speculated that the alteration and disruption of white matter connectivity, and their correlation with neurocognitive performances, could be interpreted as a possible “biological marker”. This might help to specifically define the common and the different aspects of these disorders in order to better understand their complex pathophysiological mechanisms, jet to be clearly defined.

# RIASSUNTO

INTRODUZIONE: schizofrenia e disturbo bipolare sono malattie psichiatriche complesse e invalidanti, nelle quali il tradizionale approccio categoriale della nosografia psichiatrica entra in continuo dibattito in relazione alle più recenti scoperte nei campi della neurobiologia, della genetica, della neuropsicologia e del brain imaging.

Il modello delle soglie multiple descrive le complesse relazioni tra l'assetto genetico e l'ampia espressione fenotipica nei vari disturbi appartenenti allo spettro. Esso parte dal presupposto che i geni comuni siano coinvolti in tali disturbi lungo uno spettro che va da dalla depressione maggiore al disturbo bipolare alla schizofrenia e che il loro effetto sia additivo lungo un continuum di rischio: quando una determinata soglia è superata, la differenza 'quantitativa' diventa 'qualitativa' e si manifesta come un disturbo diverso.

Un campo di grande interesse nell'ambito delle neuroscienze e della ricerca in psichiatria è quello di scoprire caratteristiche cliniche e pattern psicopatologici condivisi tra questi disturbi.

Studi istopatologici, di genetica e di neuroimaging hanno evidenziato in modo rilevante alterazioni a livello dei network neuronali di questi disturbi, tramite diverse metodiche.

In particolare tra gli studi di brain imaging in risonanza magnetica, la tecnica non convenzionale di DTI si è rilevata, fin dalla sua introduzione, uno strumento estremamente promettente per gettare luce in particolare sulla complesse proprietà della sostanza bianca cerebrale e l'analisi dell'integrità dei fasci assonali.

SCOPO DELLO STUDIO: investigare, tramite metodica DTI e calcolo degli indici di diffusione l'integrità della sostanza bianca in specifiche regioni cerebrali, e valutare le alterazioni neurocognitive in due gruppi di pazienti appartenenti allo spettro bipolare-schizofrenico (rispettivamente schizofrenici e bipolari) e un gruppo di soggetti sani.

MATERIALI E METODI: 64 pazienti (32 SZ-25 BP) e 31 controlli sani sono stati sottoposti ad una procedura di Risonanza Magnetica cerebrale ad 1,5 Tesla, secondo un protocollo di acquisizione di acquisizione dedicato, comprendente sequenze T1 3D volumetriche e DTI, presso l'Unità Operativa di Neuroradiologia del Presidio Ospedaliero di Conegliano. Mediante l'utilizzo del Software ANALYZE 10.0, sono stati calcolati gli indici di diffusione DTI, in specifiche regioni regioni cerebrali con metodo di delineamento delle ROIs, in particolare soffermandoci sui lobi frontali e sul complesso amigdala-ippocampo. Tutti i soggetti sono stati sottoposti a valutazione neurocognitiva tramite la somministrazioone di una batteria di test tratti dall'esame neuropsicologico breve (ENB) e al Wisconsin Card Sorting Test (WSCT)

RISULTATI: sono state riscontrate alterazioni statisticamente significative degli indici di diffusione per le regioni di interesse (ROIs) che hanno evidenziato anormalità degli indici di diffusione nelle regioni frontali nei pazienti dello spettro (e condivise tra bipolari e schizofrenici) rispetto ai controlli sani. È stato evidenziato un pattern complesso di alterazioni degli indici di diffusione a livello dell'ippocampo e dell'amigdala tra i due gruppi di pazienti e i controlli sani. Oltre a questo, è stato riscontrato un peggiore funzionamento cognitivo e delle prestazioni al WSCT nei pazienti rispetto ai controlli sani. Si sono inoltre evidenziate alcune correlazioni tra i punteggi ottenuti ai test e gli indici di diffusione, in particolare per le regioni frontali condivise tra pazienti schizofrenici e bipolari.

CONCLUSIONI: I pazienti dello spettro schizofrenico-bipolare condividono le ridotte prestazioni ai test neuropsicologici, le alterazioni degli indici FA e ADC a livello dei lobi frontali e di ADC dell'ippocampo di sinistra, ma gli schizofrenici, rispetto ai bipolari hanno un pattern differente di alterazioni a livello della formazione ippocampale di destra dell'amigdala sinistra. La disconnettività appare quindi avere un ruolo centrale nella patogenesi dei disturbi dello spettro. L'identificazione di alterazioni degli indici di diffusione di specifiche regioni cerebrali potrebbe rappresentare un passo cruciale nell'individuare un correlato neurobiologico presente nei disturbi dello spettro bipolare-schizofrenico.

# FIRST PART: INTRODUCTION

## THE BIPOLAR-SCHIZOPHRENIC SPECTRUM DISORDERS

When considering a particular psychopathological phenomenon, the concept of *spectrum* is referred to a variety of syndromes that have in common the same etiologic determinants or, despite an apparent heterogeneity of clinical manifestations. This concept, in a dimensional point of view, is applied to those types of disorders, which share the same kind of psychopathological phenomena though in a continuum or gradation of severity.

Schizophrenic and bipolar disorders are disabling and complex psychiatric diseases. Their classical nosography and classification are nowadays under challenging debate.

The most recent neurobiological, neuropsychological and genetic findings in affective and schizophrenic disorders is in contrast with the traditional categorical approach to psychiatric nosography.

The multiple threshold model describes the complex relationship between the shared genetic background and the wide phenotypic expression in the various disorders ascribed to the bipolar schizophrenic spectrum. This model assumes that common genes are involved in a spectrum of disorders ranging from major depression, to bipolar and schizophrenia and that their effect is additive along a continuum of risk: when a certain threshold is exceeded the quantitative difference becomes a qualitative difference that manifests itself as a different disorder (eg. switching from major depression to bipolar disorder to schizo-affective disorder than schizophrenic) (Kelsoe 2003).

A field of great interest in neuroscience and psychiatric research is finding evidence of shared clinical features and pathophysiological pathways between these disorders .

The evidence for phenomenological, biological and genetic overlap between schizophrenia and bipolar disorders (Potash 2006; Potash and Bienvenu 2009; Purcell et al. 2009) is more and more increasing and has been gradually accumulating over the past 10-20 years.

Currently schizophrenia is considered to be a multifactorial disease, whose genetic component is due to genetic polymorphism of many genes, each of which contributes to the susceptibility of the disease with a small effect. On this heterogeneous genetic background should be acting environmental and epigenetic factors, determining first a neurodevelopment alteration and then the clinically evident expression of the disease.

The genes associated with the disease that find more agreement are DTNBP1 (dystrobrevin binding protein 1 or dysbindina), neuroregulin (NRG1), DISC-1 (disrupted in schizophrenia 1), COMT (catechol-O-methyl transferase), 4 RGS (regulator of G protein signaling 4), BDNF (brain-derived neurotrophic factor), GRM3 (metabotropic glutamate receptor).

It should be noted that for neurodevelopment is a process that is expressed in the course of life, involving processes typical of an early stage, such as training, neuronal migration and myelination, processes that occur primarily during adolescence, such as enhanced myelination and synaptic pruning, finally processes, such as synaptic plasticity (formation/maintenance of synapses), which occur throughout life (Andreasen et al., 2013).

In the end, it should be clarified that even for the most promising genes there is a significant inconsistency between the studies and the ability to replicate exactly the same results, in relation to the involvement of particular alleles in the liability for schizophrenia.

The neurodevelopmental hypothesis, formulated initially is based on the idea that schizophrenia results from an alteration in the level of development of the central nervous system. In its simplest enunciation this hypothesis argues that schizophrenia is the result of a compromise of neurodevelopmental processes that begins long before the appearance of symptoms of the disease and that culminates with the clinical onset of the same. The evidence in favor of this

model of altered neurodevelopment come from a large number of epidemiological studies, genetic and neuroimaging that have made this assumption the dominant paradigm in research on schizophrenia in the last twenty years.

The neurodevelopmental hypothesis predicts the existence of genes which confer a disease susceptibility and environmental factors acting early on the subject genetically predisposed, would be able to produce a dysregulation of the development of the central nervous system. This dysregulation, after producing structural and functional alterations before the clinical onset, would culminate in the complete expression of the disease, at the late teens or early adulthood.

Candidate endophenotypes in bipolar disorder mainly include neuroanatomical abnormalities such white matter hyperintensities, volume reduction of the anterior cingulate cortex and ventral striatum; and cognitive alterations such as deficits of attention, verbal learning, working memory, executive functions, planning ability, and inhibitory control.

It was therefore recognized the close correlation of the disease with the genetic constitution, although it is not yet defined a clear mode of transmission of the disorder.

The prevailing hypothesis outlines a complex mode of inheritance, not strictly linked to Mendelian laws but to specific interactions between one or more genes and environmental factors (Kendler, 2006).

It has been repeatedly observed that patients who subsequently develop the disease frequently present, even before the onset of psychosis, a large pattern of cognitive alterations (often already detectable during childhood), neurological signs (focal and not focal) (Keshavan et al., 2008), neurophysiological alterations (eg alteration of sensory gating): all alterations that would seem to be consistent with a neurodevelopmental disorder. With regard to neuropathological aspects of the disease, in schizophrenia would typically be present an increase in the neuronal density, a reduction in the dendritic tree and a cortical thinning.

It should be noted, however, that the term neurodevelopmental should be understood in a broad sense as the set of a series of processes: some typically

take place or only at a early stage (such as the formation and neuronal migration and the formation of the cortical plate), others occur primarily during adolescence (synaptic pruning and increased myelination), others may take place throughout the course of life, even in old age (synaptic plasticity). All these processes are regulated by genes, probably a very large number, which interact in a complex manner among themselves, with epigenetic factors and environmental factors. It may therefore occur in schizophrenic patients who experience a progression of the damage, a prolonged action of one or more of these deregulated processes (Andreasen et al., 2013).

Structural and functional alterations that are placed in an intermediate position between susceptibility genes and clinical manifestations of the disease are the so-called intermediate phenotypes or endophenotypes. For endophenotypes means stable biological markers (ie present even when the disease is not active), inheritable, quantifiable, that co-segregate with the disease, which are present in unaffected first-degree relatives with a higher frequency in the general population (Gottesman et al., 2003).

Meta-analysis based on the comparison between the results of cross-sectional studies of patients with a first psychotic episode and chronic patients, follow-up longitudinal studies of patients with a first psychotic episode (Heaton at al, 2001) shows that first psychotic episode are similar, both in type and severity to those in chronic patients. Since the alterations would be largely equivalent among patients with a first untreated episode and chronic patients in treatment, the cognitive impairment in schizophrenic patients would be characterized by a substantial stability during the natural history of the disease, without suffering the chronicity of course implies that inevitably relapse in symptoms and prolonged exposure to therapy.

However, it should be remembered that there are discrepant results about the stability of cognitive impairment.

The relationship between symptom severity and extent of neurocognitive impairment has been studied extensively in the literature. Most of the currently available studies has unanimously highlighted, as regards the magnitude of

positive symptoms, such as delusions hallucinations, a 'no correlation or a minimum correlation inverse type with cognitive performance (in particular with the impairment of' look out). As for the negative symptoms is instead present a correlation inverse type more consistent, although characteristically modest, between the intensity measured with an appropriate scale and cognitive performance (Palmer at al., 2009) negative symptoms are especially inversely correlated with IQ and performance in the following areas: attention, learning and memory, verbal fluency, processing speed, problem solving. Fluctuations in the symptoms level do not seem to be a determining factor in the test performance: the symptoms of schizophrenia themselves do not cause cognitive impairment.

The neurocognitive deficits are present almost invariably in the group of symptoms of the disease (Palmer et al., 2009) and influence the functional outcome of the patient to a greater extent than psychopathological manifestations do.

Impaired verbal declarative memory has been well documented in patients with schizophrenia and, together with the working memory, seems to be among the cognitive deficits that most correlates with functional outcome and the life quality of the patient.

The Bipolar Disorder presents, like other psychiatric disorders such as schizophrenia, cognitive symptoms whose severity is evaluated with specific neuropsychological tests that assess certain cognitive functions. Generally, the 'intensity' of these symptoms is less than in Schizophrenia; however, their constant presence, even during euthymic phase (Heaton, 2001) contributes to the absence of a complete functional recovery between episodes with important psychosocial difficulties of these patients even during periods of clinical remission.

The presence of cognitive deficits during the acute phases of a given disease is widely recognized in the literature. Manic / mixed state dominated by alterations in executive functions, episodic memory and spatial span performance, verbal memory, attention and language.

The demonstration that the presence of neurocognitive deficits persists even during clinical stability was fundamental in reconsidering their role in the pathogenesis of the disease. They could be considered potential endophenotypes of the disease, in close relationship with the 'genetic makeup', other than a generic state-related epiphenomena.

In a recent review (Tamminga et al., 2014) has been proposed an interesting model regarding the crucial role played by the hippocampus in the functioning of verbal declarative and visuospatial memory in healthy subjects as well as in the occurrence of cognitive deficits, delusions and hallucinations in schizophrenic patients that demonstrate that the integrity of the hippocampal formation is central to the proper functioning of the declarative memory.

On the hippocampus depend: 1) the rapid connection between inputs from various cortical regions, the so-called coding connection (conjunctive encoding), such that the number of features that make up an event is tied in a memory trace integrated, manageable and flexible; 2) subsequent recovery of patterns of interconnected information previously learned. The representations of connection (conjunctive representations) allow recognition by association, the inferential reasoning and memory retrieval of the event. The mechanism "complete pattern," refers to the recovery of a wide representation from an partial input.

The encoding of the episode requires a synthesis between the functions of the neocortical fronto-temporal network (which process information relating to various aspects of the event) and the medial temporal lobe (responsible for the formation of permanent representations of the individual characteristics of the event and the creation of an integrated representation in which the individual characteristics of the event are interconnected).

The involvement of the hippocampus in schizophrenia is something repeatedly replicated in studies of various kinds. MRI studies, both with ROI with VBM approaches, showed a reduction of hippocampal volume bilaterally, with reductions found most often for the volume of the hippocampus than in other brain areas. These volumetric reductions not only appear in chronic

schizophrenic patients but also in patients in their first psychotic episode (in the course of the disease there would be only a modest degree of progression of the volume reduction).

Review and meta-analysis of structural MRI studies with ROI approach have repeatedly concluded that in patients with schizophrenia structural brain abnormalities are more stably detectable reduction of VCT, an increase in the lateral ventricular volume and reductions in the volume level of the following structures: the hippocampus, amygdala, superior temporal gyrus, anterior cingulate gyrus, corpus callosum, prefrontal cortex and thalamus (Shenton et al., 2001; Steen et al., 2006).

It should be added that the landscape is widening in longitudinal studies of subjects in the so-called ultra-high risk for schizophrenia (ie in the premorbid phase of the disease), are intended to identify the one hand the presence of structural alterations before clinical onset of the same (candidates as a marker of vulnerability to disease) and other possible damage progression between premorbid phase, clinical onset and chronic phase. Although the results can not only be preliminary, most agree that the alteration volumetric MRI studies with ROI approach so far conducted have identified in these subjects is a reduction of hippocampal volume of gray matter, especially those affecting left hippocampus, in the period between premorbid phase and the early stages of the disease after the first psychotic episode (Gur et al., 2007).

Studies conducted over the past thirty years, using various methods of structural and functional neuroimaging have allowed the definition of pathophysiological theoretical models of the Bipolar Disorder based on research of specific anatomofunctional alterations mainly located in the Limbic System, Prefrontal Cortex and in the basal ganglia.

Recent reviews and meta-analyzes (Kempton et al 2008; McDonald 2009; Arnone 2009; Hallahan et al., 2011) show a wide variability in the results obtained by NMR structural studies to date, mainly due to the heterogeneity of samples examined, and also indicate how the findings more consistent and validated

evenly, although nonspecific, are dilatation of the lateral ventricles and the white matter hyperintensities.

The most recent meta-analysis reported functional alterations in agreement with the hypothesis that the hyper-activation of the limbic system and the simultaneous reduced prefrontal activity are the basis of altered regulation of emotions and cognitive deficits present in Bipolar Disorder, investigated by specific task activation (Drevets et al., 2008).

There is also suggestestion that the abnormal functioning prefrontal could be responsible for an altered modulation, and failure to inhibition of limbic structures related.

Currently, according to some authors the spectrum of cognitive affective and behavioral symptoms in the Bipolar Disorder result from dysfunction of a large neural circuit, the “the anterior Limbic network" (ALN) that consists of cortical and subcortical structures such as the amygdala, ventral striatum, subgenual prefrontal cortex, ventromedial prefrontal cortex, anterior hippocampus, anterior insula, cerebellar vermis (Adler; 2006).

The critical role of the hippocampus in bipolar disorder, has been confirmed by numerous studies, made mostly in the last two decades, which have brought to light, but not completely understood, the complex and early neurobiological changes detectable in this structure.

The most significant, quantitatively, come from structural neuroimaging studies (MRI) and functional (fMRI, PET), which, however, reported results often conflicting. Interesting data, and more and more, come from studies of neurochemistry spectroscopy (MRS) and by postmortem neuropathological studies, both histological and molecular, through which it was possible to detect significant changes in hippocampal cytoarchitectonic.

Strasser et al. (2005) have suggested the presence, in bipolar psychotic patients, alterations most closely related, and therefore more pronounced, to the schizophrenic spectrum compared to non-psychotic bipolar; however they have described only a trend in the reduction in the left hippocampus psychotic bipolar patients compared to normal controls.

In general, although it is clear from the clinical, cognitive and prognostic point of view that psychotic bipolar patients are more similar to the schizophrenic patients than to the non psychotic bipolar, both ROI that VBM studies in bipolar mania or psychotic depression do not report volumetric abnormalities compared to schizophrenics and healthy controls. Only a few studies found significant differences, mainly at the level of the left temporal lobe (Kasai et al., 2003)

Differences and similarities in insular and temporal pole MRI gray and white matter abnormalities in first-episode schizophrenia and affective psychosis have been found (Hirayasu et al., 2009).

## DIFFUSION TENSOR IMAGING

It is an MRI technique introduced in 1990 (Basser) that exploits the diffusive properties of the water to describe the structural characteristics of the tissues and has an enormous potential in the context of brain imaging, especially for the study of cerebral white matter and characterized by invasiveness, repeatability, rapid processing. It provides exquisite details on tissue microstructures

DTI INDICIES

FA (fractional anisotropy) measures the degree of anisotropy of the diffusion, it is an index of structural integrity of the white matter and coherence of fibre orientation.

- ✓ reduction of FA may involve the loss of coherence of fibre tracts and demyelination probably secondary to Wallerian degeneration.
- ✓ often associated with changes of ADC, although they measure different physical phenomena
- ✓ Loss of fibre coherence, architectural disorganization
- ✓ Transverse diffusion limited by the presence of debris (limited isotropic diffusion)
- ✓ Analytical problems related to crossing-fibres

ADC (apparent diffusion coefficient) is an index of the magnitude of the diffusion process (but not its direction)

- ✓ essentially correlated with the tissue structure and with the volume of the extracellular spaces that restrict the movement of water molecules

- ✓ an increase of the ADC is due to a reduction in tissue organization, which represents a microstructural barrier to water diffusion in the interstitium
- ✓ damage of the grey or white matter, even at the microstructural level results in an increase of ADC
- ✓ Acute phenomena, there is no axonal impairment yet.

FA maps and patterns of water self-diffusion in the brain as estimated by DTI have a high sensitivity and specificity and have been revealed useful to reliably distinguish between patients with schizophrenia and normal control Patterns of water self-diffusion in the brain as estimated by DTI (Ardekani, 2011).

Reduced FA, associated with an increase in radial diffusivity and a decrease in mode of anisotropy have been reported (Kumar; 2014) for several brain regions (callosal, posterior thalamic/optic, paralimbic, fronto-occipital) in psychosis.

In schizophrenia DTI revealed severe white matter disruption in the temporolimbic region, including the cingulum, as well as in the frontotemporal region (uncinate fasciculus), parietotemporal region (arcuate fasciculus), and corpus callosum region. decrease of FA in the prefrontal white matter,

Increased FA in the midline of the genu of the corpus callosum, and an increase of ADC in the bilateral orbitofrontal white matter have been reported in Bipolar disorder (Shizukuishi, 2013).

FA and ADC changes have been used to evaluate the integrity of white matter tracts in pathological conditions and in healthy brains. Difference of the diffusion measures have been found in several white matter diseases, but it is known that age affects both FA and ADC values, and small changes occur across the lifespan (Hakulinen,2014).

# SECOND PART: EXPERIMENTAL DATA

## 1. AIM OF THE STUDY

This study is part of a wider research funded by Regione Veneto and entitled *"Neural and cognitive endophenotypes in complex early-onset psychiatric diseases: a research on "common genes" in mood disorders and schizophrenia"*.

The aim of the present study was to investigate diffusion tensor imaging (DTI) measures of WM integrity in specific brain regions (frontal lobes, bilateral hippocampus and amygdala) and the cognitive performances in a group of patients with the bipolar-schizophrenic spectrum disorders and a group of healthy control subjects.

Furthermore, all recruited subjects underwent clinical and standardized, thorough neurocognitive assessment (ENB, Mondini et al; WCST).

In order to verify or exclude specific diagnosis-related differences, we performed cross-sectional comparisons between bipolar patients, schizophrenic patients and healthy controls.

In the current study we investigated the hypothesis that potential shared cognitive symptoms in SZ and BD are directly associated with structural alterations in the white matter within the frontal lobes, hippocampal brain region and amygdala.

# 2. METHODS

## 2.1. SUBJECTS ENROLLMENT

The global research aimed to recruit patients affected by early-onset disorders throughout the *bipolar-schizophrenic spectrum*. Additionally, we enrolled a sample of group of healthy control subjects (CTRL) to perform the data comparison.

Patients were eligible in the research if they met the following inclusion criteria:

- ✓ age 18-55
- ✓ specific Axis I disorders, according to DSM-IV TR criteria, within the bipolar-schizophrenic spectrum
- ✓ illness onset before the age of 35
- ✓ duration of illness over 3 years

Control subjects were enrolled from the surrounding community and acquaintances or from staff, and matched to the combined group of patients on the basis of age and gender. None of the comparison subjects had a personal or family history of major psychiatric disorders or actual use of psychotropic medications.

Exclusion criteria for all the groups of participants were:

- ✓ contraindications to magnetic resonance scanning
- ✓ presence of concomitant medical conditions
- ✓ head trauma leading to loss of consciousness for more than 5 minutes or lifetime history of neurological illness
- ✓ secondary diagnosis of Delirium, Dementia, Amnestic or other Cognitive Disorders according to DSM-IV TR
- ✓ DSM-IV Substance Abuse or Substance Dependence disorder within the previous 12 months

Study participation has been proposed to inpatients or outpatients referring to Psychiatry Unit of Conegliano and Psychiatry Clinic of Padova University.

A total of 109 patients were eligible and screened, and 103 of them (64 from Conegliano and 39 from Padova) joined the study.

In addition, a little group of 12 unaffected first-degree relatives (8 from Conegliano and 2 from Padova) was recruited, but was not included in the analysis of the present study.

With regard to healthy controls, 64 were proposed to participate and 57 of them accepted to follow the study.

103 subjects underwent RMN brain scan: 64 patients, 31 healthy controls and 8 unaffected relatives, for 95 of them (57 patients-32 SZ, 25 BP-, 30 controls and 8 unaffected relatives) the acquisition was eligible for the DTI study of the frontal regions and for 57 of them (37patients- 20 SZ, 17 BP- and 20 healthy subjects) also for the hippocampal and amygdala DTI study.

The psychological assessment was performed for 164 subjects (103 patients, 47 healthy controls and 12 unaffected relatives).

The local ethics committees (Padua and Treviso) approved this research and all the participants were enrolled after receiving an exhaustive description of the study procedures and they provided written informed consent.

## 2.2. CLINICAL ASSESSMENT

Psychiatric assessment was carried out by conducting a structured Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al. 1998) to determine specific diagnoses according to DSM-IV TR criteria, performing a mental status examination and obtaining psychiatric and medical history. Also the M.I.N.I. was administered in the control group of heathy subjects and relatives to rule out the presence of current or past psychiatric conditions.

We assessed symptoms severity with the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962; Ventura et al. 1993), the Global Assessment of

Functioning (GAF) (from DSM-IV-TR, page 34), the Young Mania Rating Scale (YMRS) for manic symptoms (Young et al. 1978), the Positive and Negative Syndrome Scale (PANSS) to rate severity of psychotic symptoms (Kay et al. 1987), the 17-item version of the Hamilton Depression Rating Scale (HDRS) to rate depressive symptoms (Hamilton 1960) and the 14-item Hamilton Rating Scale for Anxiety (HAS) (Hamilton 1959).

All patients were evaluated if considered clinically stable (inpatients were assessed next to discharge). All the scales were administered near the time that they underwent MRI. Age at onset and length of illness (expressed in years) were recorded during the clinical assessment.

Table 1 and 2 illustrate the demographic and clinical characteristics of the subsample of subjects, which were recruited for the brain imaging study.

**Table 2.1:** Demographic Data

| Subsamples | | | AGE (yr) | EDUCATION (yr) |
|---|---|---|---|---|
| Bipolars | N 25<br>Gender<br>M 16<br>F 15 | Mean | 40,06 | 12,74 |
| | | Std. Deviation | 9,571 | 3,172 |
| Schizophrenics | N 32<br>Gender<br>M 18<br>F 15 | Mean | 37,27 | 12,21 |
| | | Std. Deviation | 8,737 | 3,110 |
| CTRL | N 30<br>Gender<br>M15<br>F 16 | Mean | 36,61 | 16,16 |
| | | Std. Deviation | 10,701 | 2,794 |
| Total | | 87 | | |

**Table 2.2.:** Clinical data

| Subsample | | Age of onset | Ilness duration | GAF | BPRS | HAM-D | HAS | PANNS POS | PANNS NEG | PANNS GEN | YMRS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B 25 | | 25 | 25 | 25 | 25 | 25 | 25 | | | | 25 |
| | Mean | 25,48 | 14,58 | 51,32 | 34,48 | 8,97 | 9,45 | | | | 7,97 |
| | Std. Deviation | 9,685 | 8,330 | 13,474 | 8,334 | 5,218 | 4,581 | | | | 4,601 |
| S | N 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | |
| | Mean | 24,88 | 12,67 | 53,24 | 42,24 | 8,06 | 10,18 | 19,58 | 21,91 | 36,21 | |
| | Std. Deviation | 7,461 | 6,575 | 13,553 | 13,638 | 3,766 | 4,558 | 6,548 | 6,857 | 13,207 | |
| Tot | N 57 | 57 | 57 | 57 | 57 | 57 | 57 | 32 | 32 | 32 | 25 |
| | Mean | 25,17 | 13,59 | 52,09 | 38,48 | 8,50 | 9,83 | 19,58 | 21,34 | 36,21 | 7,97 |
| | Std. Deviation | 8,546 | 7,478 | 14,724 | 11,951 | 4,515 | 4,548 | 6,548 | 6,857 | 13,207 | 4,601 |

## 2.3. NEURO PSYCHOLOGICAL ASSESSMENT

The study participants underwent a thorough assessment with a comprehensive neuropsychological battery (ENB; Mondini et al., 2003) that included:

1. Digit span
2. Memory for a story in both immediate and delayed recall,
3. Memory with interference subtests (10 s version and 30 s version),
4. Trail making test (A and B)
5. Cognitive estimation
6. Overlapping figures test
7. Verbal fluency

**Digit span**

- ✓ Digits Forward (repetition of digits forward)
- ✓ Digits Backward (repetition of digits backward)

Evaluates the potential capacity of short-term memory, has also been linked with IQ tests, with reading skills and problem solving

**Memory for a story in both immediate and delayed recall (MSI, MSD)**

This test evaluates the ability to memorize a short story, the mechanisms of integration of information provided to allow the verbal comprehension and planning mechanisms for structuring the stored information.

**Memory with interference subtests (MI 10 s version and MI 30 s version),**

The purpose of the test is to assess working memory. Attention plays an important role as it acts on the learning stage, when the input information is sent to the working memory

**Trail making test (TMT A and TMT B)**

The aim of the test A is to assess the capabilities in visual-spatial research, selective attention and psychomotor skills of the subject. Test B assesses psychomotor speed, the visual-spatial ability, working memory, and especially of selective attention, split and alternated. For both these test the higher the score, the lower the performance.

**Cognitive estimation (Cog E)**

The test assess the response capability of the subject in front of requests that do not necessarily require a clear and precise answer, but instead need a valuation and an assessment of general knowledge of the world

**Overlapping figures test (OF)**

This test (adapted from Rey, 1966) verifies the ability of figure segmentation, the control and inhibition on the answers already provided.

**Verbal fluency**

The test checks subject's ability to retrieve words from the lexicon, selecting them on the basis of the initial phoneme, the ability to lexical access and retrieval, as well as the ability to select an appropriate search strategy (ie executive functions).

We considered the attribution of specific cognitive abilities test in accordance with the proposal by Mondini (Mondini et al., 2003), in order to assess executive functions, verbal working memory and attention (Digit Span, Memory with interference subtests) visual spatial memory, selective attention and psychomotor speed (TMT A, B), verbal learning and memory (Memory for a story in both immediate and delayed recall), vocabulary skills and executive functions (verbal fluency), visual-spatial skills and inhibitory control (Overlapping figures

**Wisconsin Card Sorting Test** (**WCST**) provides a versatile measure of neuropsychological functioning, assessing abstract thinking, cognitive flexibility, executive function and impairment of "set-shifting", i.e. the ability to display flexibility in the face of changing schedules of reinforcement. (Heaton R K, 1981 and 1993).

This Test has been asserted to measure:

- ✓ conceptual skills and executive function, scored by the number of categories achieved (CA);
- ✓ set shifting and the inability to maintain a strategy is measured by Failure to maintain set (FTMS) and CA
- ✓ task switching by the score of perseverative errors and perseverative response (respectively PE and PR)
- ✓ Insight into sorting principal scored by the conceptual level responses (CLR).

Several studies reported the sensitivity of the WCST performance to frontal lobe lesions (Nyhus E, 2009; Demakis, G. J., 2003; Mukhopadhyay P, 2008)

Most neuroimaging studies on WCST performance report a significant increase in metabolic or neural activity within frontal or prefrontal cortical regions (Barceló & Knight, 2002).

Furthermore, some authors show that damage in non-frontal or diffuse damage in frontal and non-frontal regions both affect WCST performance, specifically studies have reported that damage to temporal (Giovagnoli, 2001; Nyhus E, 2009), subcortical (Mukhopadhyay et al., 2008), hippocampal (Igarashi et al., 2002, Corcoran and Upton, 1993) can cause similar impairments on WCST performance as those subsequent to frontal lobe lesions.

## 2.4. IMAGING STUDY

**IMAGE ACQUISITION**

All subject went through a standard acquisition protocol of the whole-brain (sagittal T13D) using T1-weighted three-dimensional sequences (magnetization-prepared rapidly acquired gradient-echo, MPRAGE) in the sagittal plane for volumetric measurements (using the following protocol: TR = 10 msec, TE = 4 msec, TI = 300 msec, flip angle = 8°, slice thickness = 1.25 mm, matrix size = 256×256×192, voxel resolution 1×1×1,25 mm).

Diffusion MRI was used to quantify the diffusion of water molecules along multiple directions to infer white matter tissue architecture.

For this DTI study spin echo-sequences were acquired using the following parameters: TR = 10000 msec; TE = 68 msec; TI = 2400 msec; flip angle 90°, encoded as Reg-DTI FLAIR enc 15 dir SENSE. Acquisition matrix 112x112, 17 volumes resulted, 2 of which were baseline volumes (*b0*) and 15 diffusion weighted volumes (each quantifying water diffusion in a unique direction) with non collinear intensity gradients of field oriented along the three axes (Gx, Gy, Gz) and *b value*=1000 s/mm$^{2}$, slice thickness = 2mm.

Every acquisition produced 50-60 axial slices parallel to the anterior-posterior commissure.

In addition, axial proton density and T2-weighted images were obtained to exclude the presence of cerebral structural abnormalities on the MRI scan. A board-certified neuro radiologist reviewed all scans.

A total of 103 subjects (64 patients: 33 SZ, 31 BP; 8 unaffected first-degree relatives, and 31 healthy controls) underwent MRI scan .

MRI acquisitions have been achieved using a 1.5 T MRI scanner (Achieva XR; Philips Medical Systems) used at Neuroradiology Unit of Conegliano Hospital (ULSS7 Pieve di Soligo, TV).

The procedure was well tolerated by all subjects and no sedation was necessary.

**PROCESSING**

The imaging data (standard DICOM) were transferred from the MRI unit to the workstation and analysed using ANALYZE® 10.0 software (Analyzedirect Inc., Biomedical Imaging Resource, Mayo Foundation, Rochester, Minnesota USA), caring out the following steps:

- Definition of Region of Interest (ROI)
- DTI maps
- Tractography
- Diffusion index calculation

## ROI Definition

The different steps applied for the preprocessing of the Diffusion MRI data included motion correction, correction for eddy-current distortion.

First for every subject a three-dimensional spatial registration (*3-D Voxel Registration*) with the T13D sagittal acquisition was performed, in order to obtain a better image definition to allow an easier identification of the anatomical landmarks ROI were manually traced for the right and left Hippocampus (HR; HL), for the right and left amygdala (AR, AL) and for both the frontal lobes (FR, FL) using the specific software module *Region of Interest*. The coronal plane was chosen for the delimitations, according with the referring literature (Mayhew, 1992). Subsequently we verify the tracing on the other orientation planes to improve accuracy and precision, this method provides greater clarity of anatomic localization. Anatomical boundaries, according to brain atlases (Duvernoy 1988, Mai 2003) and neuroimaging literature (Pantel, 2000; Rademacher, 1992), were defined as follows:

*Hippocampal formation* (Fig.2.1-2.2-2.3): For the anterior boundary the alveus was used as a border between amygdala and hippocampus (Pantel et al. 2000; Rametti et al. 2007). Posteriorly, the tail of the hippocampus continues as the indusium griseum, a thin strip of gray matter overlying the surface of the corpus

callosum. For purposes of measurement, the posterior-most slice was defined as the slice where the hippocampus first appeared adjacent to the trigone of the lateral ventricle (Sheline et al. 1996; Sheline et al. 2012). The superior border followed the fornix-fimbria white matter junction, inferiorly by parahippocampal gyrus white matter, medially by the subarachnoid spaces of various cisterns (e.g., ambient cistern), and laterally by the cerebrospinal fluid-filled lateral ventricle. Included tissues were the gray matter complex, the cornu ammonis, dentate gyrus, and subiculum. The vertical digitation of the head of the hippocampus, which curves up and medial to the amygdala in coronal sections, the fornix-fimbria white matter complex and the alveus was included. Excluded tissues were various fluid-filled spaces including ventricles, subarachnoid spaces and sporadic fluid-density spaces in the hippocampus complex.

*Amygdala* (Fig.2.1-2.2-2.3).: to trace the anterior and posterior boundaries the axial plane was chosen firstly, as this represent the orientation in which they are most clear. The anterior limit is the thin layer that separates the amygdala from the white matter, the entorhinal cortex, the gyrus ambiens and uncus. The posterior boundary is defined as the portion where the most posterior nuclei of amygdala juxtapose to the ventral horn of the most anterior part of the lateral ventricles. Subsequently we transpose on the coronal plan to complete the tracing proceeding along the posterior-anterior direction and following the margins of the hippocampus and the optical tract. Particular care was taken not to include the peduncle of the lentiform nucleus, hippocampal tissue, and periamygdaloid tissue between lateral amygdala and white matter of the temporal lobe.

*Frontal Lobes (*Fig 2.3): these regions were traced on the coronal plane, starting from the first slice upon which the brain tissue can be seen proceeding until the slice where the external surface of the genu of corpus callosum can be seen, being that considered the posterior boundary.

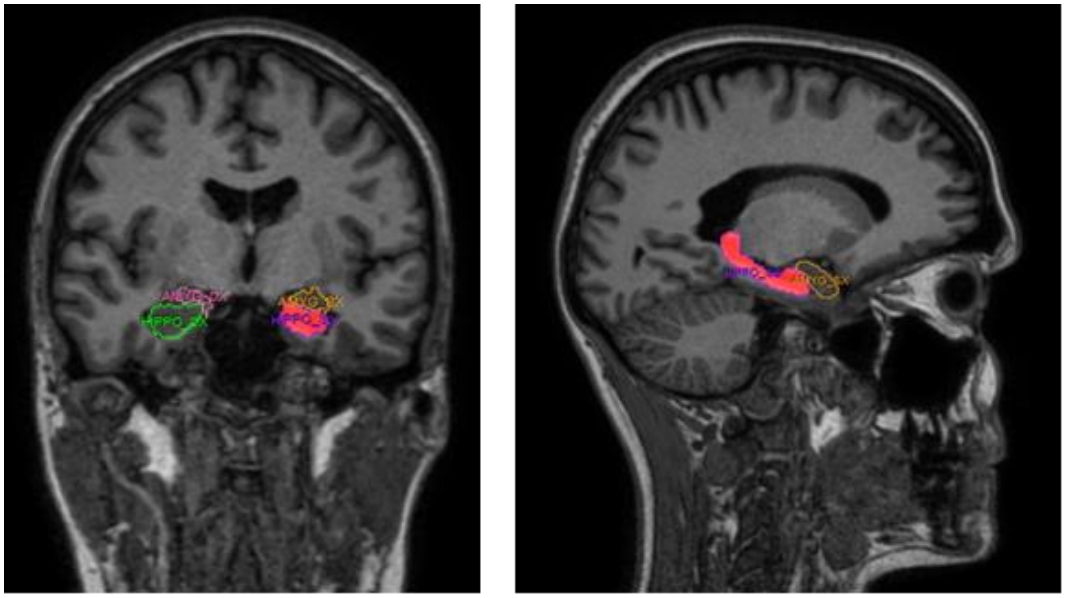

**Figure 2.1.** Hippocampal formation and amygdala in the coronal and sagittal plane

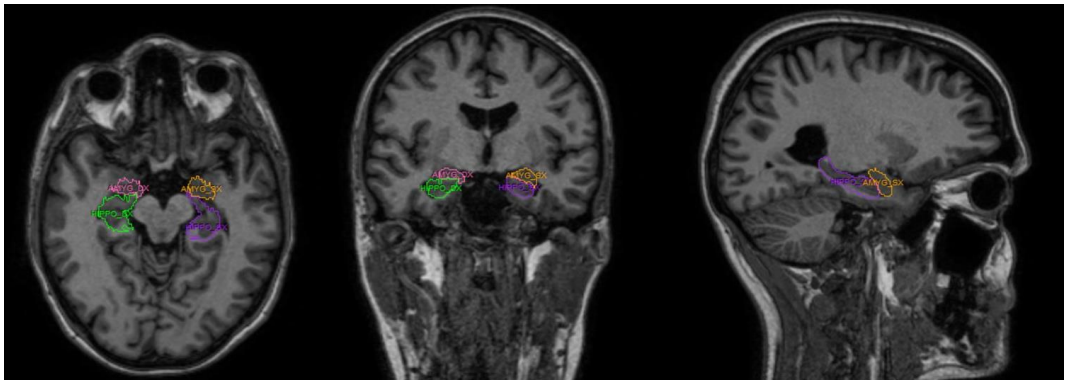

**Figure 2.2** Hippocampal formation and amygdala in the three orthogonal planes

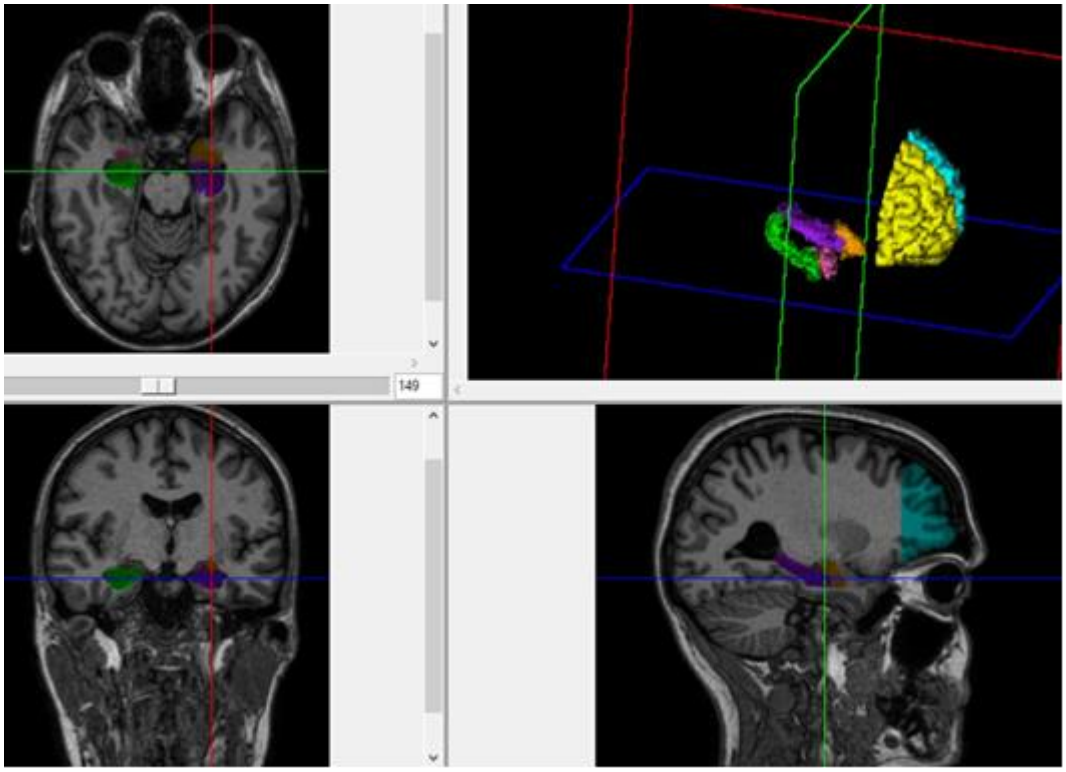


**Figure 2.3** ROI of Hippocampal formation and amygdala; 3D rendering of the and frontal lobes

From the tracing of the ROIs is possible to obtain an "Object Map" that the "DTI" module of the software Analyze® will use to perform the analysis of the white matter for every region of interest.

## DTI MAPS

The DTI module allows to create maps for the different diffusion indices and to perform tractography analysis by using the Object Maps. We computed five DTI standard maps weighed on precise diffusion indices: Fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), relative anisotropy (RA), volume ratio (VR) (FA, RA, VR, AD, RD) and the map of the apparent diffusion coefficient (ADC) and a color map defined by the eigenvalues of the tensor were obtained.

The intensity on the gray scale is directly proportional to the corresponding diffusion index or ADC. Anisotropy images only show the magnitude of the anisotropy. Because the direction of the principal eigenvector is also important,

color mapping is widely used, in which information regarding orientation (color) is combined with information on anisotropy (image intensity). For color coding, the principal eigenvector is projected into three different directions in the image reference frame (to different color components assigned to each voxel) using the RGB model. Red is associate to the sagittal direction (right-left), blue to the axial (up-down) and green to the coronal (back-forward) Fig 2.4. 2.5.

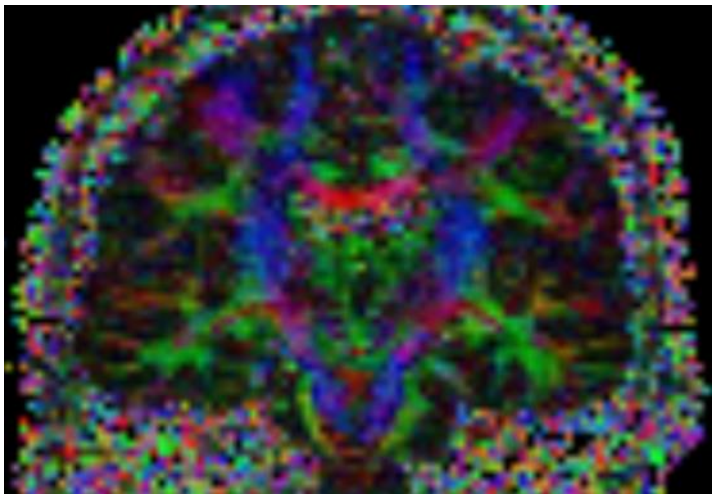

**Figure 2.4:** color map

## TRACTOGRAPHY & DIFFUSION INDEX CALCULATION

Once obtained, the DTI maps allow to estimate the diffusion indices of the selected regions through *fiber-tracking*; this application of the software is based on the Fiber Assignment by Continuous Tracking (FACT) algorithm. This deterministic model implies a user-defined threshold of FA for the start point of origin of the fibers (*seed point*) and for the point of termination (*end point)*. In this study the following parameters were used: *FA start thresh* = 0.2 e *FA stop thresh* = 0.2; *FA angle stop thresh* = 50°, *fiber length thresh* = 5mm, according to recent literature (Zhang, 2014) these values are more likely to discriminate between gray and white matter in brain tissue and to prevent crossing fibers artifacts. ( Fig 2.6-2,7).

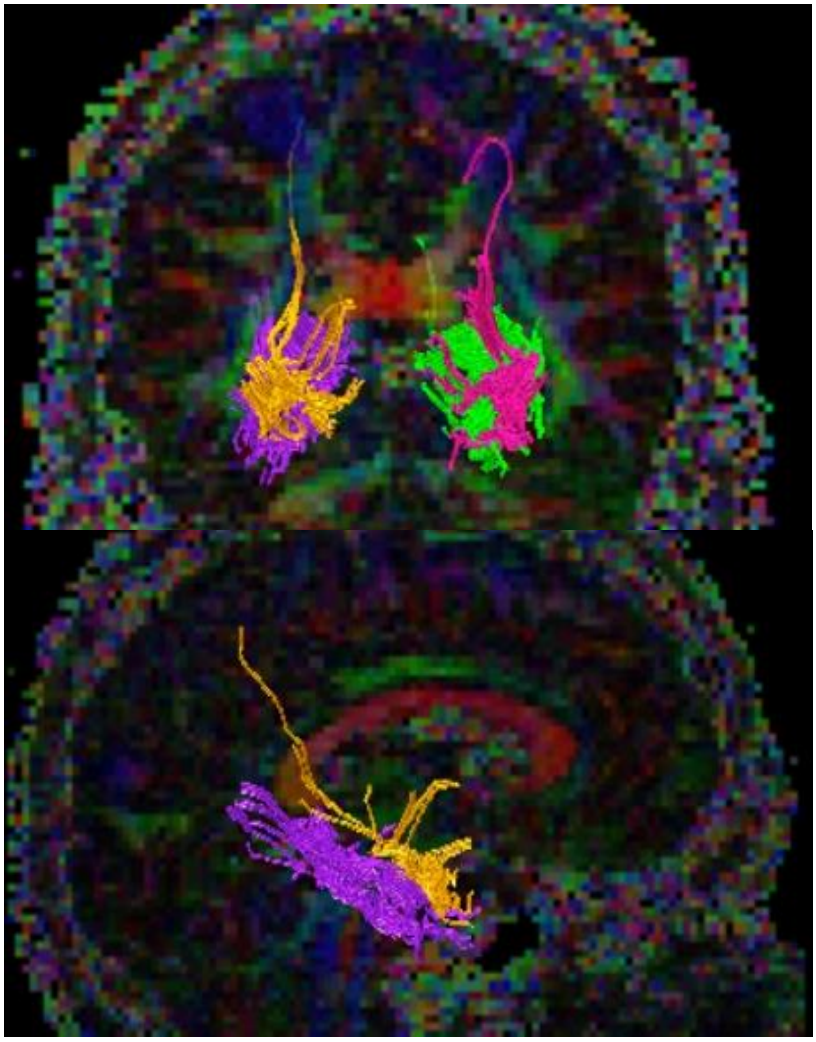

**Figure 2.5** Hippocampal formation on a color map, coronal and sagittal view.

Furthermore the software allows to define the tracts of white matter pertaining to a specific ROI or intercepted by other ROIs using the boolean operators AND/OR.

Based on this approach for each region of interest (ROI) the diffusion indices and their standard deviation are then calculated applying the specific tool of software *fiber metrics.*

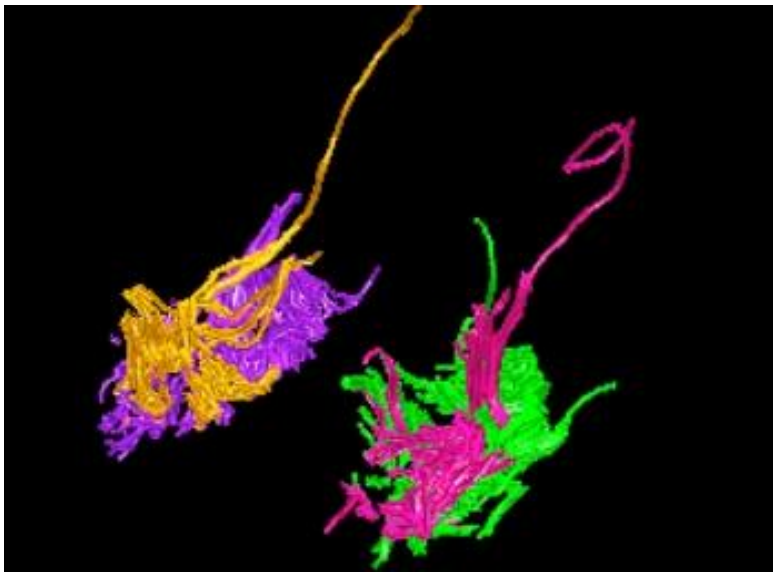

**Figure 2.6** : fiber tracking: bilateral Hippocampi and amygdales

For the purpose of this study we focused our interest on FA and mean ADC for the frontal lobes, right and left hippocampus, right and left amygdala and the connection between hippocampus and amygdala bilaterally (Right H-A, Left H-A),( Fig 2.6-2,7).

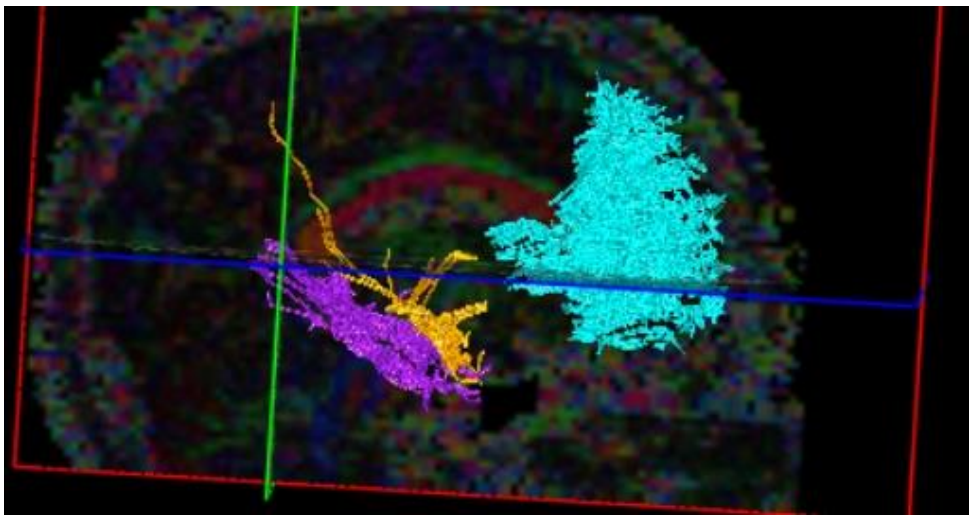

**Figure 2.7** : fiber tracking: frontal lobe and Hippocampal formation

## 2.4. STATISTICAL ANALYSIS

To verify the normal distribution of our variables we applied the Kolmogorov-Smirnov test or the Shapiro-Wilk test, whereas the Levene's test was performed to evaluate the homogeneity of variances in the different samples. With respect to DTI measures, all the data demonstrated a normal distribution.

To compare mean values between groups we used Student's t-test for independent samples if normality of distributions and equality of variances were confirmed, and Welch's t-test variant when homoscedasticity was not assumed. For comparison between non-normal variables, we applied the non-parametric Mann-Whitney U test.

An inter-rater and intra-rater reliability study was carried out by the two raters who calculated the specific ROIs (hippocampus, amygdala and frontal lobes) and Intraclass Correlation Coefficients (ICC) were then calculated. For the inter-rater reliability, ICC resulted 0.95 for frontal lobes, 0.92 and 0.90 for hippocampus and amygdala respectively; intra-rater correlation coefficients were calculated for frontal lobes (for the 2 raters respectively 0.96 and 0.96) and hippocampus (0.96 and 0.97) and for amygdala (0.94 and 0.94).

Analysis of covariance (ANCOVA) was used to assess DTI indicies, in order to reduce within group error and for the elimination of confounds.

Moreover, we performed group comparisons of cognitive measures (BVMT-R, HVLT-R) using two *independent ANOVAs* with group being a fixed factor and the test scores defined as independent variables. Group comparisons of BDI II and BRMAS were conducted using *t*-tests to compare the two groups (BD, CON). All comparisons of cognitive and neurobehavioral measures were conducted with the SPSS 21.0 software package.

Descriptive statistics and comparison analysis were performed by using SPSS software, version 16.0: all the tests were two-tailed and the level of significance was established at P equal or inferior to 0.05.

We used Pearson product-moment coefficient to assess the correlation between the neurocognitive scores and the DTI indicies.

Descriptive statistics and comparison analysis were performed by using SPSS software, version 16.0 and R software: all the tests were two-tailed and the level of significance was established at P equal or inferior to 0.05.

# 3. RESULTS

## 3.1. DEMOGRAPHIC AND CLINICAL DATA

No statistically significant differences were noticed regarding age between Schizophrenics (t=-,32; p=,84) or Bipolars (t=1,21; p=,32) and controls, neither between schizophrenic and bipolar patients (t=-1,04; p=,70).

Our analysis did not found any statistically significant difference with respect to education level between the two patient groups (Z=-1,14; p=,30), instead education level differed significantly between the schizophrenic group (Z=-3,14; p<,05) or the bipolar group (Z=-4,83; p<,05) compared to the healthy controls.

Patients' between-group comparison did not reveal statistically significant differences with respect to age at onset (t=,49; p=,72) and total duration of illness (t=,76; p=,53).

With regard to symptoms severity, the respective scores, when directly comparable, did not differ between schizophrenic and bipolar patients (for HDRS: t=,97; p=,33; for HAS: t=1,02; p=,32; for BPRS: Z=-,58; p=,63).

All the subjects in the study were right handed.

## 3.2. DTI DATA

In this study we measured the DTI indices, FA and ADC, for the hippocampal formation, amygdala and frontal region (bilaterally) of the two patient groups (Schizophrenics and Bipolars) in comparison with the healthy controls. Results from the analysis between the different groups are reported in the following tables:

**Table 3.1:** FA index of the ROIs, **Schizophrenic** patients vs healthy controls

| | **FA** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | SCHIZOPHRENICS N 32 | CONTROLS N 30 | *p* |
| RIGHT FRONTAL LOBE | 0,394 ± 0,017 | 0,407 ± 0,016 | <0,01 |
| LEFT FRONTAL LOBE | 0,404 ± 0,017 | 0,417 ± 0,019 | <0,01 |
| df=59 Covariates: age and education<br>a. Computed using alpha = ,01 | | | |

**Table 3.2:** FA index of the ROIs**, Schizophrenic** patients vs healthy controls

| | **FA** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | SCHIZOPHRENICS N 20 | CONTROLS N 20 | *p* |
| RIGHT HIPPOCAMPUS | 0,411 ± 0,028 | 0,445 ± 0,026 | <0,01 |
| RIGHT AMYGDALA | 0,360 ± 0,038 | 0,395 ± 0,027 | <0,01 |
| RIGHT H-A | 0,347 ± 0,046 | 0,369 ± 0,045 | ,072 |
| LEFT HIPPOCAMPUS | 0,431 ± 0,027 | 0,442 ± 0,027 | ,090 |
| LEFT AMYGDALA | 0,386 ± 0,051 | 0,402± 0,025 | ,113 |
| LEFT H-A | 0,369 ± 0,069 | 0,379 ± 0,061 | ,341 |
| df=37; Covariates: age and education<br>a. Computed using alpha = ,01 | | | |

**Table 3.3:** ADC index of the ROIs, **Schizophrenic** patients vs healthy controls

| | **ADC** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | SCHIZOPHRENICS N 32 | CONTROLS N 30 | *p* |
| RIGHT FRONTAL LOBE | 0,787 ± 0,02 | 0,752 ± 0,035 | <0,01 |
| LEFT FRONTAL LOBE | 0,780 ± 0,028 | 0,748 ± 0,042 | <0,01 |
| df=59 Covariates: age and education<br>a. Computed using alpha = ,01<br>ADC are expressed in $(mm^2/s)*10^3$ | | | |

**Table 3.4:** ADC index of the ROIs, **Schizophrenic** patients vs healthy controls

| | **ADC** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | SCHIZOPHRENICS N 20 | CONTROLS N 20 | *p* |
| RIGHT HIPPOCAMPUS | 0,825 ± 0,023 | 0,806 ± 0,023 | ,147 |
| RIGHT AMYGDALA | 0,832 ± 0,035 | 0,791 ± 0,031 | <0,01 |
| RIGHT H-A | 0,808 ± 0,043 | 0,793 ± 0,046 | ,131 |
| LEFT HIPPOCAMPUS | 0,839 ± 0,028 | 0,803 ± 0,035 | <0,01 |
| LEFT AMYGDALA | 0,821 ± 0,042 | 0,788 ± 0,033 | ,133 |
| LEFT H-A | 0,809 ± 0,051 | 0,808 ± 0,055 | ,160 |
| df=37; Covariates: age and education<br>a. Computed using alpha = ,01<br>ADC are expressed in $(mm^2/s)*10^3$ | | | |

**Table 3.5:** FA index of the ROIs, **Bipolar** patients vs healthy controls

| | **FA** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | BIPOLARS<br>25 | CONTROLS<br>30 | *p* |
| RIGHT FRONTAL LOBE | 0,391 ± 0,129 | 0,407 ± 0,016 | <0,01 |
| LEFT FRONTAL LOBE | 0,402 ± 0,017 | 0,417 ± 0,019 | <0,01 |
| df=52; Covariates: age and education<br>a. Computed using alpha = ,01 | | | |

**Table 3.6**:FA index of the ROIs**, Bipolar** patients vs healthy control**s**

| | **FA** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | BIPOLARS<br>17 | CONTROLS<br>20 | *p* |
| RIGHT HIPPOCAMPUS | 0,425 ± 0,034 | 0,445 ± 0,026 | ,080 |
| RIGHT AMYGDALA | 0,375 ± 0,038 | 0,395 ± 0,027 | ,070 |
| RIGHT H-A | 0,345 ± 0,036 | 0,369 ± 0,045 | ,042 |
| LEFT HIPPOCAMPUS | 0,436 ± 0,024 | 0,442 ± 0,027 | ,095 |
| LEFT AMYGDALA | 0,364 ± 0,035 | 0,402± 0,025 | <0,01 |
| LEFT H-A | 0,332 ± 0,045 | 0,379 ± 0,061 | ,018 |
| df=35; Covariates: age and education<br>a. Computed using alpha = ,01 | | | |

**Table 3.7:** ADC index of the ROIs, **Bipolar** patients vs healthy controls

| | **ADC** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | BIPOLARS N 25 | CONTROLS N 30 | *p* |
| RIGHT FRONTAL LOBE | 0,780 ± 0,021 | 0,752 ± 0,035 | <0,01 |
| LEFT FRONTAL LOBE | 0,775± 0,023 | 0,748 ± 0,042 | <0,01 |
| df=52; Covariates: age and education<br>a. Computed using alpha = ,01<br>ADC are expressed in $(mm^2/s)*10^3$ | | | |

**Table 3.8:** ADC index of the ROIs, **Bipolar** patients vs healthy controls

| | **ADC** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | BIPOLARS N 17 | CONTROLS N 20 | *p* |
| RIGHT HIPPOCAMPUS | 0,824 ± 0,024 | 0,806 ± 0,023 | ,145 |
| RIGHT AMYGDALA | 0,819 ± 0,033 | 0,791 ± 0,031 | 0,07 |
| RIGHT H-A | 0,816 ± 0,036 | 0,793 ± 0,046 | ,168 |
| LEFT HIPPOCAMPUS | 0,840 ± 0,014 | 0,803 ± 0,035 | <0,01 |
| LEFT AMYGDALA | 0,834 ± 0,027 | 0,788 ± 0,033 | <0,01 |
| LEFT H-A | 0,814 ± 0,034 | 0,808 ± 0,055 | ,169 |
| df=35; Covariates: age and education<br>a. Computed using alpha = ,01<br>ADC are expressed in $(mm^2/s)*10^3$ | | | |

The Right and left Frontal lobes demonstrated a statistically significant reduction in FA and a significant increase of ADC in both the group of patients suffering from Schizophrenia and from Bipolar disorder when compared to the control group.

Also we isolated a subsample of patients upon which it was possible to study the alterations of the DTI indices for the hippocampal formation, the amygdala and their interconnections.

The sub-group of patients with Schizophrenia, compared to the control group, showed a statistically a significant reduction of FA in the right hippocampus and an increase of mean ADC in the left hippocampus.

Right amygdala demonstrated a significant alteration of both DTI indices FA and ADC among these patients. With respect to the other brain region investigated, schizophrenic patients didn't reach a statistically significant reduction, compared with healthy subjects.

The Bipolar patients showed a significant increase of mean ADC of the left hippocampus and the left amygdala revealed significant reduction of FA and increase of mean ADC.

With respect to the other brain region investigated, the patients affected from Bipolar Disorder didn't reach any other statistically significant variation.

Then we also compared the two groups of patients between each other, in order to better understand the differences among them. We did not find any significant variation for the frontal lobe regions between the two groups, but bipolars and schizophrenics revealed significant difference among the amygdala-hippocampal complex.

The results are illustrated in the following tables:

**Table 3.9**:FA index of the ROIs**, Schizophrenics vs Bipolar** patients

| | **FA** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | SCHIZOPHRENICS 20 | BIPOLARS 17 | *p* |
| RIGHT HIPPOCAMPUS | 0,411 ± 0,028 | 0,425 ± 0,034 | <0,01 |
| RIGHT AMYGDALA | 0,360 ± 0,038 | 0,375 ± 0,038 | <0,01 |
| RIGHT H-A | 0,347 ± 0,046 | 0,345 ± 0,036 | ,329 |
| LEFT HIPPOCAMPUS | 0,431 ± 0,027 | 0,436 ± 0,024 | ,434 |
| LEFT AMYGDALA | 0,386 ± 0,051 | 0,364 ± 0,035 | <0,01 |
| LEFT H-A | 0,369 ± 0,069 | 0,332 ± 0,045 | ,164 |
| df=35; Covariates: age and education;<br>Computed using alpha = ,01 | | | |

**Table 3.10:** ADC index of the ROIs, **Schizophrenic** vs **Bipolar** patients

| | **ADC** (mean ± SD) | | **ANCOVA** |
|---|---|---|---|
| | SCHIZOPHRENICS N 20 | BIPOLARS N 17 | *p* |
| RIGHT HIPPOCAMPUS | 0,825 ± 0,023 | 0,824 ± 0,024 | ,105 |
| RIGHT AMYGDALA | 0,832 ± 0,035 | 0,819 ± 0,033 | ,096 |
| RIGHT H-A | 0,808 ± 0,043 | 0,816 ± 0,036 | ,458 |
| LEFT HIPPOCAMPUS | 0,839 ± 0,028 | 0,840 ± 0,014 | ,590 |
| LEFT AMYGDALA | 0,821 ± 0,042 | 0,834 ± 0,027 | <0,01 |
| LEFT H-A | 0,809 ± 0,051 | 0,814 ± 0,034 | ,458 |
| df=35; Covariates: age and education<br>Computed using alpha = ,01<br>ADC are expressed in $(mm^2/s)*10^3$ | | | |

To briefly summarize DTI data indices results between the three samples we can say that both groups of patients share the alteration of both FA and ADC (respectively reduction and increase) for the frontal regions and, when compared with the control group.

With respect to the hippocampus and amygdala the pattern is a little more complicated.

The patients also both present, when compared to controls, an increase of ADC for the left hippocampus, that is share among the patients group, but schizophrenics also demonstrated alterations in the right hippocampus (FA) and for the right amygdala (FA and ADC), while the bipolars showed a compromised the left amygdala for both FA and ADC.

When groups of patients are compared to each other, the schizophrenics show a significant statistically difference for FA of the right hippocampus and the FA of the right amygdala which is not shared with the Bipolars. Bipolars demonstrate alteration for the Left amygdala in both FA and ADC which is not present in the schizophrenics group. For the ADC of the right amygdala the bipolar patients show no difference with the Schizophrenics, that could mean that the increase of ADC is also present in some level, but not sufficient to reach significance when compared with the control group.

## 3.3. NEURO PSYCHOLOGICAL DATA

The results are illustrated in the following tables (see below):

**Table 3.11**: Neurocognitive performances, Schizophrenics vs healthy controls

| | **SCHIZOPHRENICS** (n=32) | | **HEALTHY CONTROLS** (n=30) | | **COMPARISON** | | |
|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **t** | **df** | **p** |
| **TMT-A** | 66,12 | 32,39 | 35,10 | 16,32 | 2,2 | 50 | *<0,01* |
| **TMT-B** | 142,56 | 80,13 | 72,70 | 20,19 | 3,4 | 52 | *<0,01* |
| **Digit span** | 3,69 | ,998 | 6,58 | 1,08 | 4,7 | 60 | *<0,01* |
| **MSI** | 6,00 | 2,06 | 21,13 | 3,83 | 5,54 | 57 | *<0,01* |
| **MSD** | 13,47 | 4,15 | 22,68 | 1,99 | 4,3 | 60 | *<0,01* |
| **M int. 10 sec** | 6,00 | 2,06 | 8,65 | ,661 | 4,1 | 60 | *<0,01* |
| **M int. 30 sec** | 4,53 | 2,38 | 8,45 | ,925 | 6,1 | 55 | *<0,01* |
| **VERBAL FLUENCY** | 9,84 | 3,19 | 17,19 | 3,15 | 4,8 | 61 | *<0,01* |
| **Cog E** | 4,38 | ,83 | 4,90 | ,301 | 5,9 | 54 | *<0,01* |
| **OVERL. FIGURES** | 17,94 | 4,45 | 26,71 | 5,78 | 10 | 53 | *<0,01* |

The Schizophrenics patients showed worse performances, compared to healthy subjects, for all the test administrated ($p<0,01$).

**Table 3.12:** Neurocognitive performances: Bipolars vs healthy controls

| | BIPOLARS (n=25) | | HEALTHY CONTROLS (n=30) | | COMPARISON | | |
|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **t** | **df** | **p** |
| **TMT-A** | 58,12 | 31,35 | 35,10 | 16,32 | 3,0 | 50 | *<0,01* |
| **TMT-B** | 130,56 | 79,23 | 72,70 | 20,19 | 3,2 | 54 | *<0,01* |
| **Digit span** | 3,99 | ,888 | 6,58 | 1,08 | 5,7 | 53 | *<0,01* |
| **MSI** | 5,80 | 1,05 | 21,13 | 3,83 | 4,5 | 54 | *<0,01* |
| **MSD** | 12,47 | 4,75 | 22,68 | 1,99 | 3,9 | 54 | *<0,01* |
| **M int. 10 sec** | 6,20 | 2,08 | 8,65 | ,661 | 4,9 | 45 | *<0,01* |
| **M int. 30 sec** | 4,03 | 1,37 | 8,45 | ,925 | 6,7 | 53 | *<0,01* |
| **VERBAL FLUENCY** | 10,45 | 2,66 | 17,19 | 3,15 | 5,0 | 52 | *<0,01* |
| **Cog E** | 4,44 | ,99 | 4,90 | ,301 | 6,1 | 53 | *<0,01* |
| **OVERL. FIGURES** | 18,94 | 4,30 | 26,71 | 5,78 | 11 | 51 | *<0,01* |

The Bipolar patients demonstrated a worse performance, compared to healthy subjects, in all the neurocognitive evaluation ($p<0,01$).

Neuropsychological scores were not significantly correlated with age of onset or illness duration.

The bipolars group, on the other end, shows less lower scores in the administrated test, in respect to the schizophrenic group, but not enough to reach statistical significance: the both groups are markedly compromised by cognitive point of view, even in state of clinical stability.

**Correlations between DTI indices and neurocognitive assessment**

In order to observe a possible association between DTI indices and neurocognitive test scores, we performed a correlation between the measurements in each test and the FA and ADC indices for all the ROIs.

We were not able to find significant correlations between test scores and FA and ADC among the frontal regions, but we have highlighted significant associations between specific test scores and FA and ADC indices of the hippocampal region and amygdala. The results are illustrated in the following tables:

**Table 3.14**: Correlation analysis between neurocognitive scores and DTI indices

| | FA R HIPPOCAMPUS | | ADC R HIPPOCAMPUS | | FA L HIPPOCAMPUS | | ADC L HIPPOCAMPUS | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| TMT-A | -0,20 | 0,26 | 0,08 | 0,07 | -0,25 | 0,09 | 0,07 | 0,66 |
| TMT-B | -0,65 | ***<0,05*** | 0,31 | 0,06 | -0,45 | 0,09 | 0,68 | 0,08 |
| Digit span | 0,67 | ***<0,05*** | -0,28 | 0,14 | 0,40 | 0,07 | -0,65 | ***<0,05*** |
| MSI | 0,74 | 0,08 | -0,49 | 0,08 | 0,20 | 0,15 | -0,90 | ***<0,05*** |
| MSD | 0,67 | ***<0,05*** | -0,61 | 0,16 | 0,12 | 0,25 | -0,93 | 0,07 |
| MI 10 sec | 0,67 | 0,08 | -0,39 | 0,08 | 0,50 | 0,08 | -0,80 | ***<0,05*** |
| MI 30 sec | 0,54 | 0,07 | -0,50 | 0,07 | 0,20 | 0,26 | -0,88 | ***<0,05*** |
| Verbal fluency | 0,63 | <0,05 | -0,44 | 0,08 | 0,16 | 0,37 | -0,90 | 0,08 |
| Cog E | 0,28 | 0,34 | -0,25 | 0,17 | 0,34 | 0,87 | -0,44 | 0,12 |
| Overl. Figures | 0,26 | 0,07 | -0,11 | 0,08 | 0,11 | 0,14 | -0,13 | 0,14 |

**Table 3.15**: Correlation analysis between neurocognitive scores and DTI indices

| | FA R AMYGDALA | | ADC R AMYGDALA | | FA L AMYGDALA | | ADC L AMYGADLA | |
|---|---|---|---|---|---|---|---|---|
| | **r** | **p** | **r** | **p** | **r** | **p** | **r** | **p** |
| TMT-A | -0,20 | 0,26 | 0,06 | 0,65 | -0,25 | 0,17 | 0,07 | 0,66 |
| TMT-B | -0,45 | 0,41 | 0,07 | 0,12 | -0,41 | 0,10 | 0,68 | 0,80 |
| Digit span | 0,67 | **<0,05** | -0,07 | 0,13 | 0,66 | **<0,05** | -0,66 | **<0,05** |
| MSI | 0,64 | 0,12 | -0,39 | 0,60 | 0,40 | 0,06 | -0,90 | 0,14 |
| MSD | 0,61 | 0,30 | -0,40 | 0,20 | 0,16 | 0,36 | -0,91 | 0,13 |
| MI 10 sec | 0,65 | 0,50 | -0,39 | 0,09 | 0,10 | 0,58 | -0,80 | 0,08 |
| MI 30 sec | 0,51 | 0,09 | -0,50 | 0,08 | 0,20 | 0,26 | -0,88 | 0,28 |
| Verbal fluency | 0,60 | 0,30 | -,641 | **<0,05** | 0,16 | 0,37 | -0,90 | 0,80 |
| Cog E | 0,18 | 0,32 | -0,25 | 0,17 | 0,03 | 0,87 | -0,04 | 0,82 |
| Overl. Figures | 0,21 | 0,25 | -0,11 | 0,54 | 0,21 | 0,24 | -0,14 | 0,44 |

We found coherent correlation between TMT B, Digit span, MSD scores and FA of right hippocampus, between digit span and FA of the right amygdala and ADC of right amygdala and verbal fluency, that, in our study, is specifically impaired in schizophrenic patients, we also observed a correlation between digit span and FA and ADC of left amygdala which is more impaired in the bipolar patients.

More important we pointed out a significant correlation between Digit span, MSI, MI 10 and MI 30 and ADC of the left hippocampus which is impaired in both group of patients.

The main resulting data derived from the administration of the WCST are illustrated in the following tables:

**Table 3.16**: WCST scores comparison : Schizophrenics (S ) vs healthy controls (CTRL)

| | GROUP | Mean | SD | t | df | p |
|---|---|---|---|---|---|---|
| WCST Tot | S N=31 | 115,30 | 19,122 | 7,510 | 61 | <0,01 |
| | CTRL N=30 | 82,61 | 15,357 | | | |
| TOTAL ERRORS | S | 42,88 | 26,664 | 5,703 | 54 | <0,01 |
| | CTRL | 13,97 | 7,351 | | | |
| PERSEVERATIVE RESPONSES | S | 29,11 | 22,265 | 5,088 | 56 | <0,01 |
| | CTRL | 8,00 | 5,837 | | | |
| PERSEVERATIVE ERRORS | S | 23,14 | 20,189 | 4,331 | 57 | <0,01 |
| | CTRL | 7,10 | 4,110 | | | |
| NON-PERSEVERATIVE ERRORS | S | 18,59 | 10,474 | 5,698 | 55 | <0,01 |
| | CTRL | 6,90 | 3,889 | | | |
| CONCEPTUAL LEVEL RESPONSES | S | 39,46 | 26,371 | -4,360 | 56 | <0,01 |
| | CTRL | 62,67 | 12,030 | | | |
| CATEGORIES ACHIEVED | S | 3,77 | 2,431 | -5,320 | 58 | <0,01 |
| | CTRL | 6,00 | ,500 | | | |
| FAILURES TO MAINTAIN SET | S | 1,37 | 1,334 | 2,732 | 55 | <0,01 |
| | CTRL | ,50 | 1,042 | | | |

**Table 3.17**: WCST scores comparison : Bipolars (BP) vs healthy controls (CTRL)

| | GROUP | Mean | SD | t | df | p |
|---|---|---|---|---|---|---|
| WCST Tot | BP N=25 | 111,84 | 17,049 | 7,092 | 54 | <0,01 |
| | CTRL N=30 | 82,61 | 15,357 | | | |
| TOTAL ERRORS | BP | 40,69 | 21,705 | 6,345 | 54 | <0,01 |
| | CTRL | 13,97 | 7,351 | | | |
| PERSEVERATIVE RESPONSES | BP | 30,28 | 18,187 | 6,476 | 53 | <0,01 |
| | CTRL | 8,00 | 5,837 | | | |
| PERSEVERATIVE ERRORS | BP | 25,41 | 15,555 | 6,328 | 53 | <0,01 |
| | CTRL | 7,10 | 4,110 | | | |
| NON-PERSEVERATIVE ERRORS | BP | 18,00 | 14,009 | 4,164 | 54 | <0,01 |
| | CTRL | 6,90 | 3,889 | | | |
| CONCEPTUAL LEVEL RESPONSES | BP | 47,57 | 24,259 | -3,033 | 54 | <0,01 |
| | CTRL | 62,67 | 12,030 | | | |
| CATEGORIES ACHIEVED | BP | 4,74 | 1,723 | -4,007 | 54 | <0,01 |
| | CTRL | 6,00 | ,500 | | | |
| FAILURES TO MAINTAIN SET | BP | ,88 | ,711 | 1,587 | 53 | ,080 |
| | CTRL | ,50 | 1,042 | | | |

Both groups of patients showed a significant impairment on the main items of the WCST, when compared with the healthy subjects; although Bipolar patients did not showed a significant difference in one item of WCST with respect to the control group, when we performed a comparison between the Bipolars and the Schizophrenics we did not find any statistically significant difference between the two groups. Scores were not significantly correlated with age of onset or illness duration.

**Correlations between DTI indices and WSCT scores**

The results are illustrated in the following tables (see below):

**Table 3.18**: Correlation analysis between WCST scores and DTI indices

| | | FA R FRONTAL LOBE | ADC R FRONTAL LOBE | FA L FRONTAL LOBE | ADC L FRONTAL LOBE |
|---|---|---|---|---|---|
| WSCT tot | *r* | -,783** | ,692** | -,740** | ,642* |
| | *p* | <0,01 | <0,01 | <0,01 | <0,05 |
| TOT E | *r* | -,642** | ,588* | -,777* | ,737* |
| | *p* | <0,01 | <0,05 | <0,05 | <0,05 |
| PR | *r* | -,699** | ,617* | -,632* | ,629* |
| | *p* | <0,01 | <0,05 | <0,05 | <0,05 |
| PE | *r* | -,690** | ,146 | -,578* | ,177 |
| | *p* | <0,01 | ,130 | ,162 | ,134 |
| NP E | *r* | -,620** | ,603* | -,582** | ,176 |
| | *p* | <0,01 | <0,05 | <0,01 | ,070 |
| CLR | *r* | ,591* | -,085 | ,191 | -,711* |
| | *p* | <0,05 | 0,153 | 0,136 | <0,05 |
| CA | *r* | ,693** | -,168 | ,691** | -,697* |
| | *p* | <0,01 | ,092 | <0,01 | <0,05 |
| FTMS | *r* | -,138 | ,157 | -,107 | ,061* |
| | *p* | 0,083 | ,156 | ,091 | <0,05 |

***Correlation is significant at the 0.01 level*
**Correlation is significant at the 0.05 level*

**Table 3.19**: Correlation analysis between WCST scores and DTI indices

| | | FA R HIPPOCAMPUS | ADC R HIPPOCAMPUS | FA L HIPPOCAMPUS | ADC L HIPPOCAMPUS |
|---|---|---|---|---|---|
| WSCT tot | *r* | -,154 | ,221 | -,155 | ,694** |
| | *p* | ,244 | ,092 | ,241 | <0,01 |
| TOT E | *r* | -,070 | ,230 | -,173 | ,697** |
| | *p* | ,091 | 0,140 | 0,130 | <0,01 |
| PR | *r* | -,130 | ,609* | -,187 | ,717** |
| | *p* | ,080 | <0,05 | 0,125 | <0,01 |
| PE | *r* | -,079 | ,690* | -,164 | ,682** |
| | *p* | 0,078 | <0,05 | 0,128 | <0,01 |
| NP E | *r* | -,008 | ,049 | -,120 | ,775** |
| | *p* | 0,103 | 0,210 | 0,239 | <0,01 |
| CLR | *r* | ,085 | -,145 | ,181 | -,742** |
| | *p* | 0,130 | 0,91 | 0,086 | <0,01 |
| CA | *r* | ,052 | -,173 | ,117 | -,679** |
| | *p* | 0,126 | 0,104 | 0,150 | <0,01 |
| FTMS | *r* | -,015 | ,076 | ,169 | ,775 |
| | *p* | 0,070 | 0,126 | 0,090 | 0,080 |

***Correlation is significant at the 0.01 level*
**Correlation is significant at the 0.05 level*

We found strong correlations between the most part of the WSCT scores and both the DTI indices of the frontal lobes, as expected; furthermore our results showed a significant association between the majority of the WCST scores (WSCT tot, TOT E, PR, PE,NP E,CLR, CA) analyzed and ADC of left the hippocampus, there was also a trend for the ADC of the right hippocampus and PR and PE.

Moreover we found a significant correlation between WSCT total score and FA of the right amygdala (r-,604, p<0,01), no further correlations were revealed by our study among other regions.

# 4. DISCUSSION AND CONCLUSIONS

In the present study DTI acquisitions have been used to analyze, in a group of patients with bipolar-schizophrenic spectrum disorders and a group of healthy controls, the structural properties of white matter fibers, highlighted by the tractography study at the hippocampal formation, amygdala, the frontal regions and interconnections of the hippocampus-amygdala complex. This assessment was based on the calculation of the diffusion indices FA and ADC.

In this study, the alteration pattern of these indices has resulted variably, in the different structures considered, both from the qualitative and quantitative point of view. This also appears to be a phenomenon often observed at the level of the brain parenchyma of healthy subjects, with a greater tendency to the heterogeneity of the FA values than those of ADC (Ardekani, 2014; Brander, 2014). The above indices, infact represent phenomena that are not always overlapping, presenting a consistent trend in some cases and incongruent in others. In the study population, we observed a homogeneous and marked alteration at the level of the frontal lobes, while the FA and ADC values of the fibers located in other regions did not show an equally concordant variability.

The heterogeneity of the result can be explained by the fact that the DTI indices are indeterminate by nature: they describe the properties of the diffusion phenomenon and are used for obtaining information on the integrity of white matter fibers, only indirectly.

The FA index reflects the degree of restriction of the diffusion phenomenon of water molecules along a certain direction or the orientation and consistency of the fibers. Often, to simplify, the FA is represented as an expression of axonal integrity.

On the contrary the ADC coefficient measures the cell density (Sotak, 2004) and is a very sensitive index to even subtle changes at the level of the plasma membrane. These measures, resulting in an increase of interstitial spaces,

amplify the magnitude of the diffusion phenomenon, regardless of the spatial orientation that this assumes. Several studies, also in diseases other than bipolar-schizophrenic spectrum disorders, indeed show how the ADC may increase in the absence of a concomitant reduction of the FA: for example this occurs in those areas, apparently non-pathological, which are subjected to a diffuse damage or in lesions caused by acute events (Hakulinen,2014). We emphasize, however, that the ADC can also be increased in chronic injuries. A further characteristic of the ADC is not to assume different values if calculated in the white or the grey matter: this reflects the fact that this coefficient measures alterations of the essential properties of the cell rather than those only at the axonal level.

Another fundamental element of the indeterminate indices of diffusion is inherent to the DTI study that, as previously described, has a resolution at the voxel level. The calculated DTI data is a summary of all the diffusion phenomena that occur within each voxel, so these values should always be treated with caution. Although in the fiber-tracking algorithm, threshold values are imposed to discriminate between white and grey matter, the features defined are almost never completely consisting of axons, especially if detected at particularly small and complex anatomical structures. The data will be further altered in the case where such structures are to be found in the vicinity of the cerebral ventricles, where the presence of cerebrospinal fluid may cause important changes in the values of the diffusion indices.

On this basis we can formulate some hypotheses to explain those situations which occurred in the present study, in which the FA and ADC did not vary consistently.

In the case where, in patients, FA decreases but ADC does not show a statistically significant increase, it is possible that, at the level of white matter fibers, the alteration of the FA is mainly attributable to a phenomenon of architectural disorganization of axons that occurs without significant alterations of the entity of the diffusion phenomenon of water molecules (Kolasa, 2014). The hypothesis

is that the data must be attributed to the presence of fibers which do not describe clearly oriented bundles and are no longer consistent with each other. Is still possible that degradation products, caused by the cell damage, create random barriers to the diffusion thereby determining a limited isotropic trend. The ADC will therefore be reduced, without altering the degree of anisotropy. This phenomenon has been specifically observed during the acute phases of demyelinating diseases, in tumors with high cellularity, in various metabolic diseases and exposure to toxic agents (Mukherjee, 2008).

Finally, it is possible that they have been identified regions characterized by a strong multi-directionality, in which fibers are intersecting and overlapping within the same voxel. This would cause analytical problems determining the reduction of FA values, even if there is no real compromise of the structural integrity of the axons. In the literature, it is estimated that more than 90% of the voxels containing white matter fibers present with intersecting trajectories (Reijmer, 2012). Therefore this suggests that in some cases, the DTI method is highly sensitive to macroscopic factors more so than those of microscopic nature.

In the case where, in patients, the ADC increases but the FA does not decrease in a statistically significant manner: it is possible that the highlighted lesions are particularly subtle (edema, inflammation, interstitial damage etc.), such as not to cause gross alterations of the axonal integrity (therefore without causing a loss, at least partial, of the oriented component of the diffusion of water molecules in the axons on which depends the FA); or the alteration of the ADC value is the effect of acute phenomena (e.g. excitotoxic glutamate) that do not cause a frank impairment of the axonal integrity;

We can also speculate that the increase of the ADC is due to a reduction of the grey matter density in the region under examination (a phenomenon which could still determine, indirectly, alterations of cerebral connectivity with a consequent increase of space available for diffusion phenomena (Poot, 2013).

Finally, it is possible that this alteration can also be related to neuroleptic therapy, which seems to affect the number or the diameter of the axons (Hakulinen, 2013).

In the case in which the FA values diminish and concomitantly the ADC increases, the damage at the level of the white matter fibers seems to be more consistent: it can be the loss of axonal integrity which determines a reduction of the diffusion orientation along the axon and also the net increase in the magnitude of the diffusion phenomenon (regardless of its directionality). Other alterations that may occur with the same trend of variation of the diffusion indices are those involving the myelin sheath. However, it must be emphasized that the available indices are not sufficient to uniquely determine what is the pathophysiological process at the basis of the obtained data. All of the above described changes and further variations of the fiber density, their consistency and diameter, cannot be excluded from the critical analysis of the results obtained. It's also likely that the data obtained is the synthesis of more phenomena that act concomitantly.

In light of the above we can say that, in the regions where statistically significant differences were found for both indices, they were coherent (in patients the FA value is reduced while the ADC is increased) and consistent with widespread disruption of white matter integrity.

Analysis of the results showed, in schizophrenic and bipolar patients, a significant reduction in FA and increase of ADC values of the frontal regions on both right and left, compared to healthy controls. These results replicate one of the most established findings in the literature. Review and meta-analysis identified areas at the level of the deep white matter of the frontal and temporal regions as those where we observe the greatest reductions of FA (Shizukuishi, 2014; Sigmudsson, 2001; Serafini G, 2014). This fact is significant because it has been even replicated by studies based on different methodologies such as the voxel-based and the region of interest tractography. In particular ROI studies have identified significant decreases of FA of the cingulate gyrus (Rosenberger et al., 2008), of the left inferior longitudinal fasciculus (Friedman et al., 2008) and the

inferior fronto-occipital fasciculus (Rosenberger et al., 2008), which are among the most important bundles of connection with the frontal and temporal regions.

More important, decreases of FA and/or increase of ADC were recorded, in our study, in the hippocampal and amygdala regions.

The manual ROI studies in the literature that focus their analysis on these regions in schizophrenic and bipolar patients are not numerous. This is because the anatomical structures concerned are small and difficult to isolate manually. Currently the most common technique is the voxel-based one in which acquisitions is adapted to a standardized space on which masks of regions of interest obtained from specific atlases are further applied. Although the number of studies in this regard is again reduced, clearer alterations seem to affect the fornix structures, which constitute the main connection tract of the hippocampus to other brain regions; at this level were observed a decrease of FA and an increase of the ADC, with the possibility of correlating the data with the volume reduction of hippocampi and with decreased performance in neuropsychological tests (in particular memory organization).

Although the publications of studies that use DTI to investigate the integrity of the fibers in different brain regions are still limited, further evidence for the disconnection theory can be found in other types of studies: functional MRI showed patterns of abnormal activation of fronto-temporal areas in patients with schizophrenia, whereas studies of stereological MRI rather frequently identified a strong correlation between volumetric reductions of grey matter in the frontal and prefrontal cortex and reduced volume of grey matter in other brain regions such as the amygdala-hippocampal complex and the superior temporal gyrus. This suggests a possible involvement of the white matter, which is the connecting element between different brain areas

Although in the literature there are limited DTI studies to which compare our data, one can find evidence of correlation between reduced hippocampal volumes and compromised structural integrity of the white matter in other brain

areas: for example, an interesting fact is that reduced volumes of the amygdala-hippocampal complex correlate with a decrease in the prefrontal cortex white matter; stressing the importance of the alterations made to the fronto-temporal connectivity in the genesis of schizophrenia (Breier 1992).

Interpreting the results of our study, we should take into account some limitations and confounding factors.

The first limitation is related to the number of subjects involved. Although in the literature is not uncommon to find DTI studies with similar number of subjects to ours, the statistical power could be affected by the small size of the selected groups.

The second limitation is given by the number of orientations of the diffusion gradients available. Although just six oriented gradients are sufficient for the calculation of the DTI indices, it is known that the more there are, the better the estimate of the diffusion tensor and consequently the calculation of the derived indices. This is especially desirable in the case where calculating the values of the DTI indices for regions of reduced dimensions, in which the average value is not weighted on a large number of voxels. However, the acquisition of large values of diffusion gradient orientation is often not feasible using an MRI scanner with magnetic field of 1.5 Tesla as important acquisitions would lead to very long processing times.

Also, the mode of ROI segmentation of the structures involved has been criticized.
The literature shows how this system could lead to some variability in the FA and ADC values, especially those calculated on the margins of the anatomical structures. (Marenco, 2006) However, in our study, a manual methodology was chosen which, although not immediate and quite time-consuming, allows the selection of the regions of interest with high detail even for small and structurally complex regions and allows the detection of small differences that occur between subjects. The evaluations that derive are more targeted and the

results are more accurate. This identifies the procedure we used as the gold-standard currently available for selection and analysis of structures. Due to the laboriousness of the method, in several studies, it was preferred to make use of the semiautomatic analysis: this determines a certain uncertainty in many of the data present in the literature.
A further limitation is the fact that patients considered in this study were, at the time of the DTI study, in the compensated clinical stage, precluding the possibility to fully define the contribution determined by the state of illness. There is also the inability to determine the weight of antipsychotic therapy on DTI data, since the study population is not drug-naive.

However the DTI approach has resulted to be an extremely interesting resource for the in vivo study of the white matter structural properties, which otherwise could only be studied with histological investigations. The fact that the DTI is a still relatively new technique implies the possibility of further important implementations that could solve the problems identified in this study. There are already in development new systems based on the direct analysis of the spins of hydrogen protons, capable of displaying more trajectories within the same voxel, also in the case of crossing-fibers, and able to study the characteristics of areas of high uncertainty as those formed by the grey matter (Ozcan, 2010). These systems certainly allow greater accuracy of the data produced and a more specific analysis of the structural properties of brain tissue.

To briefly summarize DTI data indices and neurocognitive assessment results between the three samples we can say that the two groups of patients share the alteration of both FA and ADC (respectively reduction and increase) for the frontal regions, when compared with the control group.

With respect to the hippocampus and amygdala the pattern is a little more complicated.

The patients also both present, when compared to controls, an increase of ADC for the left hippocampus, that is share among the patients group, but schizophrenics also demonstrated alterations in the right hippocampus (FA) and for the right amygdala (FA and ADC), while the bipolars showed a compromised the left amygdala for both FA and ADC.

When groups of patients are compared to each other, the schizophrenics show a significant statistically difference for FA of the right hippocampus and the FA of the right amygdala which is not shared with the Bipolars, and the Bipolars demonstrated alteration for the Left amygdala in both FA and ADC which is not present in the schizophrenics group. For the ADC of the right amygdala the bipolar patients show no difference with the Schizophrenics, that could mean that the increase of ADC is also present in some level, but not sufficient to reach significance when compared with the control group.

While in the case of the frontal regions (in both groups of patients), right amygdala (only for the Schizophrenics) and left amygdala (only in the bipolars) we found that FA values diminish and concomitantly the ADC increases, the damage seem to be more consistent with widespread disruption of white matter integrity, in the case only of FA reduction, as in the right hippocampus of schizophrenics we could speculate that a phenomenon of architectural disorganization of axons has occurred, involving the loss of coherence of fiber tracts and demyelination.

In the case of the left hippocampus (in both groups of patient) a significant increase of the ADC was observed, for which it can be assumed that, at this level, may have occurred a broader injury process, determining not specifically a reduction of the diffuse axonal injury, but also a more generic cellular alteration or this could be interpreted as an expression of early structural damage which has not progressed in a more consistent manner and therefore doesn't show a decrease of the FA index.

Our sample showed a statistically significant reduction in a wide range of cognitive skills attention (TMT-A and TMT-B), working memory (TMT-B, memory test with interference 10:30 sec, digit span), processing speed (TMT-A and TMT-B), executive functions (phonemic fluency), verbal declarative memory (memory test prose immediate and delayed).

We found coherent correlation between TMT B, Digit span, MSD scores and FA of right hippocampus, between digit span and FA of the right amygdala and ADC of right amygdala and verbal fluency, that, in our study, is specifically impaired in schizophrenic patients, we also observed a correlation between digit span and FA and ADC of left amygdala which is more impaired in the bipolar patients.

More important we pointed out a significant correlation between Digit span, MSI, MI 10 and MI 30 and ADC of the left hippocampus which is impaired in both group of patients.

Both group of patients revealed a substantial impairment on the WCST, with respect to healthy controls, in line with the indications from other studies.

As expected we find coherent correlation between the most of the scores and DTI indices of bilateral frontal regions with a prevalence for FA, but also significant correlations for ADC, less expected we pointed out an association between almost all WCST scores (with the exception of FTMS) and ADC of left hippocampus, which is, along with the frontal lobes, a common altered region in both group of patients. Moreover we found a significant correlation between WSCT total score and FA of the right amygdala, that is especially impaired in the schizophrenic group.

It should be noted that the overall picture of the structure-function relationship in bipolar schizophrenic spectrum patients, is greatly complex, in consideration of the relative difficulty in being able to isolate, in a highly specific way, individual cognitive abilities through neuropsychological assessments, using the currently available tests.

The results of this study highlight shared tracts among the spectrum disorders such as the common neurocognitive and neuropsychological impairment, the compromised structural integrity of the white matter in the frontal regions and probably in some degree even of the right hippocampus, implying that these two disorders may share some common pathophysiological mechanisms. Furthermore they suggest that alterations in the cerebral white matter networks, involving the frontal regions and also subcortical structures, such as the hippocampus and amygdala, contribute to the pathophysiological process of schizophrenia and bipolar disorder.

Our results also bring out differences among the two groups of patients, with the bipolars showing a most prominent alteration of the left amygdala and the schizophrenics a predominant deficit on the right hippocampus and amygdala: these alterations of the white matter tracts may be specific to the different disorder and candidate as structural markers.

These findings suggest a graded expression and a complex pattern of susceptibility and vulnerability among the spectrum and can be interpreted as partly contradictory to Kraepelin's distinction of two entirely different and independent disease entities. However, our findings are in line with a large body of recent imaging and genetic research showing that bipolars and schizophrenic patients share some core molecular and pathophysiological mechanisms (Craddock and Owen, 2005; Craddock and Owen, 2010) and thereby cannot be conceptualized as two entirely distinct classes of disorders. We may assume that they may have in common some pathophysiological pathways but that there might be also distinct alterations across multimodal measures. Such an interpretation would conform to the findings from recent genome-wide association studies, which reveal partial but not complete overlap of the genetic risk profiles of these disorders (Thompson et al., 2014).

The results could thus help to further advance and integrate current pathophysiological models of the bipolar-schizophrenic spectrum disorders. The next implementations in the technical and methodological aspects of DTI will likely reduce the degree of heterogeneity of the results, thus allowing investigation of the elements that are at the origin of these changes and also the effects to which they lead.

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# REFERENCES


Alvarado-Alanis P, León-Ortiz P, Reyes-Madrigal F, Favila R, Rodríguez-Mayoral O, Nicolini H, Azcárraga M, Graff-Guerrero A, Rowland LM, de la Fuente-Sandoval C. Abnormal white matter integrity in antipsychotic-naïve first-episode psychosis patients assessed by a DTI principal component analysis. Schizophr Res. 2015 Jan 22. pii: S0920-9964(15)00023-7

Andreasen NC, Liu D, Ziebell S, Vora A, Ho BC. Relapse duration, treatment intensity, and brain tissue loss in schizophrenia: a prospective longitudinal MRI study. Am J Psychiatry. 2013 Jun;170(6):609-15.

Andreasen, Nancy C, Peg Nopoulos, Vincent Magnotta, Ronald Pierson, Steven Ziebell, and Beng-Choon Ho. 2011. "Progressive Brain Change in Schizophrenia: a Prospective Longitudinal Study of First-episode Schizophrenia." *Biological Psychiatry* 70 (7) (October 1): 672–9. Arat HE, Chouinard VA, Cohen BM, Lewandowski KE, Öngür D. Diffusion tensor imaging in first degree relatives of schizophrenia and bipolar disorder patients. Schizophr Res. 2014 Dec 23. pii: S0920-9964

Ardekani BA, Tabesh A, Sevy S, Robinson DG, Bilder RM, Szeszko PR. Diffusion tensor imaging reliably differentiates patients with schizophrenia from healthy volunteers. Hum Brain Mapp. 2011 Jan;32(1):1-9.

Arnone, D, J Cavanagh, D Gerber, SM Lawrie, KP Ebmeier, and AM Mcintosh. 2009. "Magnetic Resonance Imaging Studies in Bipolar Disorder and Schizophrenia: Meta-analysis." *The British Journal of Psychiatry* 195: 194–201. Barysheva M, Jahanshad N, Foland-Ross L, Altshuler LL, Thompson PM. White matter microstructural abnormalities in bipolar disorder: A whole brain diffusion tensor imaging study. Neuroimage Clin. 2013 Apr 5;2:558-68.

Bellani M, Perlini C, Ferro A, Cerruti S, Rambaldelli G, Isola M, Cerini R, Dusi N, Andreone N, Balestrieri M, Pozzi Mucelli R, Tansella M, Brambilla P. White matter microstructure alterations in bipolar disorder. Funct Neurol. 2012 Jan-Mar;27(1):29-34. PubMed PMID: 22687164; PubMed Central PMCID: PMC3812760.

Berg, E. A. A simple, objective technique for measuring flexibility in thinking . Journal of General Psychology, 1948 39, 15-22 .

Beyer JL, Taylor WD, MacFall JR, Kuchibhatla M, Payne ME, Provenzale JM, Cassidy F, Krishnan KR. Cortical white matter microstructural abnormalities inbipolar disorder. Neuropsychopharmacology. 2005 Dec;30(12):2225-9.

Bohlken MM, Mandl RC, Brouwer RM, van den Heuvel MP, Hedman AM, Kahn RS, Hulshoff Pol HE. Heritability of structural brain network topology: a DTI study of 156 twins. Hum Brain Mapp. 2014 Oct;35(10):5295-305.

Bollettini I, Poletti S, Locatelli C, Vai B, Smeraldi E, Colombo C, Benedetti F. Disruption of white matter integrity marks poor antidepressant response in bipolar disorder. J Affect Disord. 2014 Nov 15;174C:233-240

Brander A, Koskinen E, Luoto TM, Hakulinen U, Helminen M, Savilahti S, Ryymin P, Dastidar P, Ohman J. Diffusion tensor imaging of the cervical spinal cord inhealthy adult population: normative values and measurement reproducibility at 3T MRI. Acta Radiol. 2014 May

Breier A, Buchanan RW, Elkashef A, Munson RC, Kirkpatrick B, Gellad F. Brain morphology and schizophrenia. A magnetic resonance imaging study of limbic, prefrontal cortex, and caudate structures. Arch Gen Psychiatry. 1992Dec;49(12):921-6.

Canales-Rodríguez EJ, Pomarol-Clotet E, Radua J, Sarró S, Alonso-Lana S, Del Mar Bonnín C, Goikolea JM, Maristany T, García-Álvarez R, Vieta E, McKenna P, Salvador R. Structural abnormalities in bipolar euthymia: a multicontrast molecular diffusion imaging study. Biol Psychiatry. 2014 Aug 1;76(3):239-48.

Corcoran, R., & Upton, D. (1993). A role for the hippocampus in card sorting? Cortex, 29, 293–304

Craddock, N, and MJ Owen. 2007. "Rethinking Psychosis: The Disadvantages of a Dichotomous Classification Now Outweigh the Advantages." *World Psychiatry* 6: 84–91.

Craddock, N, M C O'Donovan, and M J Owen. 2005. "The Genetics of Schizophrenia and Bipolar Disorder: Dissecting Psychosis." *Journal of Medical Genetics* 42 (3) (March): 193–204.

Craddock, Nick, and Michael J Owen. 2005. "The Beginning of the End for the Kraepelinian Dichotomy." *The British Journal of Psychiatry* 186 (May): 364–6.

Craddock, Nick, and MJ Owen. 2010. "The Kraepelinian Dichotomy - Going, Going ... but Still Not Gone." *The British Journal of Psychiatry* (196): 92–95.

Demakis, G. J. (2003). A meta-analytic review of the sensitivity of the Wisconsin Card Sorting Test to frontal and lateralized frontal brain damage. Neuropsychology, 17, 255–264.

DeRosse P, Nitzburg GC, Ikuta T, Peters BD, Malhotra AK, Szeszko PR. Evidence from structural and diffusion tensor imaging for frontotemporal deficits in psychometric schizotypy. Schizophr Bull. 2015 Jan;41(1):104-14.

Duvernoy HM. 1988. "Human Hippocampus: an Atlas of Applied Anatomy." Bergmann, Munich.

Emsell L, Chaddock C, Forde N, Van Hecke W, Barker GJ, Leemans A, Sunaert S, Walshe M, Bramon E, Cannon D, Murray R, McDonald C. White matter microstructural abnormalities in families multiply affected with bipolar I disorder: a diffusion tensor tractography study. Psychol Med. 2013 Nov 27:1-12.

Emsell L, Langan C, Van Hecke W, Barker GJ, Leemans A, Sunaert S, McCarthy P, Nolan R, Cannon DM, McDonald C. White matter differences in euthymic bipolar I disorder: a combined magnetic resonance imaging and diffusion tensor imaging voxel-based study. Bipolar Disord. 2013 Jun;15(4):365-76.

Friedman JI, Tang C, Carpenter D, Buchsbaum M, Schmeidler J, Flanagan L, Golembo S, Kanellopoulou I, Ng J, Hof PR, Harvey PD, Tsopelas ND, Stewart D, Davis KL. Diffusion tensor imaging findings in first-episode and chronic schizophrenia patients. Am J Psychiatry. 2008 Aug102(1-3):181-8.

Giovagnoli, A. R. (2001). Relation of sorting impairment to hippocampal damage in temporal lobe epilepsy. Neuropsychologia, 39, 140–150.

Gottesman, II, and TD Gould. 2003. “The Endophenotype Concept in Psychiatry: Etymology and Strategic Intentions.” *American Journal of Psychiatry* 160 (April): 636–645.

Green, E K, D Grozeva, I Jones, L Jones, G Kirov, S Caesar, K Gordon-Smith, et al. 2010. “The Bipolar Disorder Risk Allele at CACNA1C Also Confers Risk of Recurrent Major Depression and of Schizophrenia.” *Molecular Psychiatry* 15 (10) (October): 1016–22. doi:10.1038/mp.2009.49.

Gur, Raquel E, Matcheri S Keshavan, and Stephen M Lawrie. 2007. “Deconstructing Psychosis with Human Brain Imaging.” *Schizophrenia Bulletin* 33 (4) (July): 921–31. doi:10.1093/schbul/sbm045.

Ha TH, Her JY, Kim JH, Chang JS, Cho HS, Ha K. Similarities and differences of white matter connectivity and water diffusivity in bipolar I and II disorder. Neurosci Lett. 2011 Nov 14;505(2):150-4. doi: 10.1016/j.neulet.2011.10.009. Epub 2011 Oct 12. PubMed PMID: 22008503.

Hahn C, Lim HK, Lee CU. Neuroimaging findings in late-onset schizophrenia and bipolar disorder. J Geriatr Psychiatry Neurol. 2014 Mar;27(1):56-62. Review.

Hakulinen U, Brander A, Ryymin P, Öhman J, Soimakallio S, Helminen M, Dastidar P, Eskola H. Repeatability and variation of region-of-interest methods using quantitative diffusion tensor MR imaging of the brain. BMC Med Imaging. 2012 Oct

Hamilton M. 1959. “The assessment of anxiety states by rating.” *Brit J Med Psychol* 32:50.

Hamilton M. 1960. “A rating scale for depression.” *Journal of neurology, neurosurgery, and psychiatry* 23:56-62.

Harms MP, Akhter KD, Csernansky JG, Mori S, Barch DM. Fractional anisotropy in individuals with schizophrenia and their nonpsychotic siblings. Psychiatry Res. 2015 Jan 30;231(1):87-91.

Heaton RK, Golshan S, Cadenhead KS. Course of neurocognitive deficits in the prodrome and first episode of schizophrenia. Neuropsychology. 2010 Jan;24(1):109-20.

Heaton, R. K. . The Wisconsin Card Sorting Test manual. 1981,Odessa: Psychological Assessment Resources Inc

Heaton, R. K., Chelune, G. J., Talley, J. L., Kay, G. G., & Curtis, G. (1993). Wisconsin Card Sorting Test (WCST). manual revised and expanded. Odessa: Psychological Assessment Resources Inc.

Hirayasu, Y, M E Shenton, D F Salisbury, J S Kwon, C G Wible, I A Fischer, D Yurgelun-Todd, et al. 2009. “Subgenual Cingulate Cortex Volume in First-episode Psychosis.” *The American Journal of Psychiatry* 156 (7) (July): 1091–3.

Igarashi, K., Oguni, H., Osawa, M., Awaya, Y., Kato, M., Mimura, M., et al. (2002). Wisconsin card sorting test in children with temporal lobe epilepsy. Brain &Development, 24, 174–178.

Ikuta T, Peters BD, Guha S, John M, Karlsgodt KH, Lencz T, Szeszko PR, Malhotra AK. A schizophrenia risk gene, ZNF804A, is associated with brain white matter microstructure. Schizophr Res. 2014 May;155(1-3):15-20.

Kafantaris V, Kingsley P, Ardekani B, Saito E, Lencz T, Lim K, Szeszko P. Lower orbital frontal white matter integrity in adolescents with bipolar I disorder. J Am Acad Child Adolesc Psychiatry. 2009

Karbasforoushan H, Duffy B, Blackford JU, Woodward ND. Processing speed impairment in schizophrenia is mediated by white matter integrity. Psychol Med. 2015 Jan;45(1):109-20.

Kay SR, Fiszbein A, Opler LA. 1987. "The positive and negative syndrome scale (PANSS) for schizophrenia." *Schizophrenia Bulletin* 13:261-276.

Kelsoe, John R. 2003. "Arguments for the Genetic Basis of the Bipolar Spectrum." *Journal of Affective Disorders* 73 (1-2) (January): 183–97.

Kempton, Matthew J, John R Geddes, Ulrich Ettinger, Steven C R Williams, and Paul M Grasby. 2008. "Meta-analysis, Database, and Meta-regression of 98 Structural Imaging Studies in Bipolar Disorder." *Archives of General Psychiatry* 65 (9) (September): 1017–32. doi:10.1001/archpsyc.65.9.1017.

Kendler, Kenneth S. 2006. "Reflections on the Relationship Between Psychiatric Genetics and Psychiatric Nosology." *The American Journal of Psychiatry* 163 (7) (July): 1138–46. doi:10.1176/appi.ajp.163.7.1138.

Kenneth Martin A, Robinson G, Reutens D, Mowry B. Cognitive and structural neuroimaging characteristics of schizophrenia patients with large, rare copy number deletions. Psychiatry Res. 2014 Dec 30;224(3):311-8.

Keshavan, M S, S Anderson, C Beckwith, K Nash, J W Pettegrew, and K R Krishnan. 1995. "A Comparison of Stereology and Segmentation Techniques for Volumetric Measurements of Lateral Ventricles in Magnetic Resonance Imaging." *Psychiatry Research* 61 (1) (May 31): 53–60.

Kochunov P, Chiappelli J, Wright SN, Rowland LM, Patel B, Wijtenburg SA, Nugent K, McMahon RP, Carpenter WT, Muellerklein F, Sampath H, Hong LE. Multimodal white matter imaging to investigate reduced fractional anisotropy and its age-related decline in schizophrenia. Psychiatry Res. 2014 Aug 30;223(2):148-56.

Kolasa M, Hakulinen U, Helminen M, Hagman S, Raunio M, Rossi M, Brander A, Dastidar P, Elovaara I. Longitudinal assessment of clinically isolated syndrome with diffusion tensor imaging and volumetric MRI. Clin Imaging. 2014 Oct 23. pii:

Koskinen EA, Hakulinen U, Brander AE, Luoto TM, Ylinen A, Ohman JE. Clinical correlates of cerebral diffusion tensor imaging findings in chronic traumaticspinal cord injury. Spinal Cord. 2014 Mar;52(3):202-8

Kumar J, Iwabuchi S, Oowise S, Balain V, Palaniyappan L, Liddle PF. Shared white-matter dysconnectivity in schizophrenia and bipolar disorder with psychosis. Psychol Med. 2014 Aug 4:1-12.

Kumar J, Iwabuchi S, Oowise S, Balain V, Palaniyappan L, Liddle PF. Shared white-matter dysconnectivity in schizophrenia and bipolar disorder with psychosis. Psychol Med. 2014 Aug 4:1-12.

Lener MS, Wong E, Tang CY, Byne W, Goldstein KE, Blair NJ, Haznedar MM, New AS, Chemerinski E, Chu KW, Rimsky LS, Siever LJ, Koenigsberg HW, Hazlett EA. White matter abnormalities in schizophrenia and schizotypal personality disorder. Schizophr Bull. 2015 Jan;41(1):300-10

Leroux E, Delcroix N, Dollfus S. Left fronto-temporal dysconnectivity within the language network in schizophrenia: an fMRI and DTI study. Psychiatry Res. 2014 Sep 30;223(3):261-7.

Li J, Kale Edmiston E, Chen K, Tang Y, Ouyang X, Jiang Y, Fan G, Ren L, Liu J, and psychosis. Schizophr Res. 2015 Jan;161(1):85-93.Review.

Linke J, King AV, Poupon C, Hennerici MG, Gass A, Wessa M. Impaired anatomical connectivity and related executive functions: differentiating vulnerability and disease marker in bipolar disorder. Biol Psychiatry. 2013 Dec 15;74(12):908-16.

Marenco S, Rawlings R, Rohde GK, Barnett AS, Honea RA, Pierpaoli C, Weinberger DR. Regional distribution of measurement error in diffusion tensor imaging. Psychiatry Res. 2006 Jun 30

Mayhew TM. A review of recent advances in stereology for quantifying neural structure. J Neurocytol. 1992 May;21(5):313-28. Review.

McDonald, C, J Zanelli, S Rabe-Hesketh, I Ellison-Wright, P Sham, S Kalidindi, R M Murray, and N Kennedy. 2004. “Meta-analysis of Magnetic Resonance Imaging Brain Morphometry Studies in Bipolar Disorder.” *Biological Psychiatry* 56: 411– 417. doi:10.1016/j.biopsych.2004.06.021.

McDonald, C, N Marshall, ET Bullmore, K Schulze, B Chapple, and E Bramon. 2006. “Regional Brain Morphometry in Patients With Schizophrenia or Bipolar Disorder and Their Unaffected Relatives.” *The American Journal of Psychiatry* 163 (March): 478–487.

Mukherjee D. Antithrombotics for PCI: an indigenous direct thrombin inhibitor (DTI) makes the cut. Indian Heart J. 2008

Mukhopadhyay, P., Dutt, A., Kumar Das, S., Basu, A., Hazra, A., Dhibar, T., et al. (2008). Identification of neuroanatomical substrates of set-shifting ability: Evidence from patients with focal brain lesions. Progress in Brain Research, 168, 95–104

Natarajan R, Hagman S, Wu X, Hakulinen U, Raunio M, Helminen M, Rossi M, Dastidar P, Elovaara I. Diffusion Tensor Imaging in NAWM and NADGM in MS and CIS: Association with Candidate Biomarkers in Sera. Mult Scler Int. 2013

Nortje G, Stein DJ, Radua J, Mataix-Cols D, Horn N. Systematic review and voxel-based meta-analysis of diffusion tensor imaging studies in bipolar disorder. J Affect Disord. 2013 Sep 5;150(2):192-200.

Nyhus E, Barceló F. The Wisconsin Card Sorting Test and the cognitive assessment of prefrontal executive functions: a critical update. Brain Cogn. 2009 Dec;71(3):437-51.

O’Donovan, Michael C, Nicholas Craddock, Nadine Norton, Hywel Williams, Timothy Peirce, Valentina Moskvina, Ivan Nikolov, et al. 2008. “Identification of Loci Associated with Schizophrenia by Genome-wide Association and Follow-up.” *Nature Genetics* 40 (9) (September): 1053–5. doi:10.1038/ng.201.

Oertel-Knöchel V, Reinke B, Alves G, Jurcoane A, Wenzler S, Prvulovic D, Linden D, Knöchel C. Frontal white matter alterations are associated with executive cognitive function in euthymic bipolar patients. J Affect Disord. 2014 Feb; (5):313-28.

Oertel-Knöchel V, Reinke B, Feddern R, Knake A, Knöchel C, Prvulovic D, Pantel J, Linden DE. Episodic memory impairments in bipolar disorder are associated with functional and structural brain changes. Bipolar Disord. 2014 Dec;16(8):830-45

Ohmuro N, Matsumoto K, Katsura M, Obara C, Kikuchi T, Hamaie Y, Sakuma A, Iizuka K, Ito F, Matsuoka H. The association between cognitive deficits and depressive symptoms in at-risk mental state: A comparison with first-episode psychosis. Schizophr Res. 2015 Jan 21. pii: S0920-9964(15)00012-2.

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M. Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: a DTI study. Schizophr Res. 2014 Aug;157(1-3):190-7.

Overall JE, Gorham DR. 1962. "The Brief Psychiatric Rating Scale." *Psycol Rep* 10:799.

Owen, Michael J, Nick Craddock, and Assen Jablensky. 2007. "The Genetic Deconstruction of Psychosis." *Schizophrenia Bulletin* 33 (4) (July): 905–11. doi:10.1093/schbul/sbm053.

Owen, MJ, and Nick Craddock. 2009. "Diagnosis of Functional Psychoses: Time to Face the Future." *The Lancet* 373 (9659): 190–191. doi:10.1016/S0140-6736(09)60053-2.

Özcan A. Characterization of imaging gradients in diffusion tensor imaging. J Magn Reson. 2010 Nov;207(1):24-33

Özcan A. Noise and nonlinear estimation with optimal schemes in DTI. MagnReson Imaging. 2010 Nov):24-33.

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK. Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res. 2014 Sep 30;223(3):226-35.

Pantel, J, D S O'Leary, K Cretsinger, H J Bockholt, H Keefe, V A Magnotta, and N C Andreasen. 2000. "A New Method for the in Vivo Volumetric Measurement of the Human Hippocampus with High Neuroanatomical Accuracy." *Hippocampus* 10 (6) (January): 752–8.

Pantelis, Christos, Murat Yücel, Stephen J Wood, Dennis Velakoulis, Daqiang Sun, Gregor Berger, Geoff W Stuart, Alison Yung, Lisa Phillips, and Patrick D McGorry. 2005. "Structural Brain Imaging Evidence for Multiple Pathological Processes at Different Stages of Brain Development in Schizophrenia." *Schizophrenia Bulletin* 31 (3) (July): 672–96.

Park JY, Park HJ, Kim DJ, Kim JJ. Positive symptoms and water diffusivity of the prefrontal and temporal cortices in schizophrenia patients: a pilot study. Psychiatry Res. 2014 Oct 30;224(1):49-57.

Patil, Chirag G., Shivanand P. Lad, Laurence Katznelson, and Edward R. Laws. 2007. "Brain Atrophy and Cognitive Deficits in Cushing's Disease." *Neurosurgical FOCUS* 23 (3) (September): 1–4.

Phelps, James, Jules Angst, Jacob Katzow, and John Sadler. 2008. "Validity and Utility of Bipolar Spectrum Models." *Bipolar Disorders* 10 (1 Pt 2) (February): 179–93.

Phillips, Mary L, Michael J Travis, Andrea Fagiolini, and David J Kupfer. 2008. "Medication Effects in Neuroimaging Studies of Bipolar Disorder." *The American Journal of Psychiatry* 165 (3) (March): 313–20. doi:10.1176/appi.ajp.2007.07071066.

Poletti S, Bollettini I, Mazza E, Locatelli C, Radaelli D, Vai B, Smeraldi E, Colombo C, Benedetti F. Cognitive performances associate with measures of white matter integrity in bipolar disorder. J Affect Disord. 2014 Dec 18;174C:342-352.

Poot DH, Jeurissen B, Bastiaensen Y, Veraart J, Van Hecke W, Parizel PM, Sijbers J. Super-resolution for multislice diffusion tensor imaging. Magn Reson Med. 2013 Jan

Potash, James B, and O Joseph Bienvenu. 2009. “Neuropsychiatric Disorders: Shared Genetics of Bipolar Disorder and Schizophrenia.” *Nature Reviews. Neurology* 5 (6) (June): 299–300. doi:10.1038/nrneurol.2009.71.

Potash, James B. 2006. “Carving Chaos: Genetics and the Classification of Mood and Psychotic Syndromes.” *Harvard Review of Psychiatry* 14 (2) (January): 47–63. Prasad KM, Upton CH, Nimgaonkar VL, Keshavan MS. Differential susceptibility of white matter tracts to inflammatory mediators in schizophrenia: An integrated DTI study. Schizophr Res. 2015 Jan;161(1):119-25

Purcell, Shaun M, Naomi R Wray, Jennifer L Stone, Peter M Visscher, Michael C O’Donovan, Patrick F Sullivan, and Pamela Sklar. 2009. “Common Polygenic Variation Contributes to Risk of Schizophrenia and Bipolar Disorder.” *Nature* 460 (7256) (August 6): 748–52. doi:10.1038/nature08185.

Rademacher J, Galaburda AM, Kennedy DN, Filipek PA, Caviness VS Jr. Human cerebral cortex: localization, parcellation, and morphometry with magnetic resonance imaging. J Cogn Neurosci. 1992 Fall;4(4):352-74.

Rametti, Giuseppina, Nuria Segarra, Carme Junqué, and Nuria Bargalló. 2007. “Left Posterior Hippocampal Density Reduction Using VBM and Stereological MRI Procedures in Schizophrenia.” *Schizophrenia Research* 96: 62–71. doi:10.1016/j.schres.2007.04.034.

Reijmer YD, Leemans A, Heringa SM, Wielaard I, Jeurissen B, Koek HL, Biessels GJ; Vascular Cognitive Impairment Study group. Improved sensitivity to cerebral white matter abnormalities in Alzheimer's disease with spherical deconvolution PLoS One. 2012

Roalf DR, Gur RE, Verma R, Parker WA, Quarmley M, Ruparel K, Gur RC. White matter microstructure in schizophrenia: Associations to neurocognition and clinical symptomatology. Schizophr Res. 2015 Jan;161(1):42-9.

Rosenberger G, Kubicki M, Nestor PG, Connor E, Bushell GB, Markant D, Niznikiewicz M, Westin CF, Kikinis R, J Saykin A, McCarley RW, Shenton ME. Age-related deficits in fronto-temporal connections in schizophrenia: a diffusion tensor imaging study. Schizophr Res. 2008 Jul;102(1-3):181-8.

Serafini G, Pompili M, Borgwardt S, Houenou J, Geoffroy PA, Jardri R, Girardi P, Amore M. Brain changes in early-onset bipolar and unipolar depressive disorders: a systematic review in children and adolescents. Eur Child Adolesc Psychiatry. 2014 Nov;23(11):1023-41. Review.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Weiller E, Herqueta T, et al. 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 Psychiatry 59(20):22-33.

Sheline, Yvette I, Brianne M Disabato, Jennifer Hranilovich, Carrie Morris, Gina D D’Angelo, Carl Pieper, Tommaso Toffanin, et al. 2012. “Treatment Course With Antidepressant Therapy in Late-Life Depression.” *American Journal of Psychiatry* 169 (November): 1185–1193.

Sheline, Yvette I, Po W Wang, Mokhtar H Gado, John G Csernansky, and Michael W Vannier. 1996. “Hippocampal Atrophy in Recurrent Major Depression.” *Proc. Natl. Acad. Sci. USA* 93 (April): 3908–3913.

Shenton, M E, C C Dickey, M Frumin, and R W McCarley. 2001. “A Review of MRI Findings in Schizophrenia.” *Schizophrenia Research* 49 (1-2) (April 15): 1–52.

Shizukuishi T, Abe O, Aoki S. Diffusion tensor imaging analysis for psychiatric disorders. Magn Reson Med Sci. 2013;12(3):153-9. Epub 2013 Jul 12. Review.

Siever, LJ, and KL Davis. 2004. “The Pathophysiology of Schizophrenia Disorders: Perspectives From the Spectrum.” *American Journal of Psychiatry* 161 (March): 398–413.

Sigmundsson T, Suckling J, Maier M, Williams S, Bullmore E, Greenwood K, Fukuda R, Ron M, Toone B. Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. Am J Psychiatry. 2001 Feb;158(2):234-43.

Situ W, Liao H, Zhou B, Xia X, Tan C. Application of diffusion tensor imaging for detecting structural changes in the brain of schizophrenic patients. Int J Psychiatry Clin Pract. 2014 Dec 22:1-5

Sotak CH. The role of diffusion tensor imaging in the evaluation of ischemic brain injury - a review. NMR Biomed. 2002 Nov-Dec

Steen, R Grant, Courtney Mull, Robert Mcclure, Robert M Hamer, and Jeffrey A Lieberman. 2006. “Brain Volume in First-episode Schizophrenia.” *The British Journal of Psychiatry* 188: 510–518.

Tamminga CA, Pearlson G, Keshavan M, Sweeney J, Clementz B, Thaker G. Bipolar and schizophrenia network for intermediate phenotypes: outcomes across thepsychosis continuum. Schizophr Bull. 2014 Mar;40 Suppl 2:S131-7.

Thompson et al;The ENIGMA Consortium: large-scale collaborative analyses of neuroimaging and genetic data. Brain Imaging Behav. 2014 Jun;8(2):153-82. doi: 10.1007/s11682-013-9269-5. Review.

Velakoulis, Dennis, Christos Pantelis, Patrick D Mcgorry, Paul Dudgeon, Warrick Brewer, Mark Cook, Patricia Desmond, Nicola Bridle, and Paul Tierney. 1999. “Hippocampal Volume in First-Episode Psychoses and Chronic Schizophrenia.” *Archives of General Psychiatry* 56: 133–141.

Ventura J, Green M et al. 1993. “Training and quality assurance with the Brief Psychiatric Rating Scale: the drift busters.” *Int J Method Psychiat Res* 3:221.

Voineskos AN. Genetic underpinnings of white matter 'connectivity': Heritability, risk, and heterogeneity in schizophrenia. Schizophr Res. 2015 Jan;161(1):50-60.

Young RC, Biggs JT et al. 1978. “A rating scale for mania: reliability, validity and sensitivity.” *The British Journal of Psychiatry* 133:429.

Zhang B, Xu Y, Zhu B, Kantarci K. The role of diffusion tensor imaging in detecting microstructural changes in prodromal Alzheimer's disease. CNS Neurosci Ther. 2014 Jan;20(1):3-9.

Zhang F, Qiu L, Yuan L, Ma H, Ye R, Yu F, Hu P, Dong Y, Wang K. Evidence for progressive brain abnormalities in early schizophrenia: a cross-sectional structural and functional connectivity study. Schizophr Res. 2014 Oct;159(1):31-5.

Zhou Y, Jiang W, Liu Z, Xu K, Wang F. A comparative diffusion tensor imaging study of corpus callosum subregion integrity in bipolar disorder and schizophrenia. Psychiatry Res. 2014 Jan 30;221(1):58-62.

Zhu J, Zhuo C, Qin W, Wang D, Ma X, Zhou Y, Yu C. Performances of diffusion kurtosis imaging and diffusion tensor imaging in detecting white matter abnormality in schizophrenia. Neuroimage Clin. 2014 Dec 9;7:170-6.