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HIPPOCAMPAL VOLUMES IN PATIENTS WITH BIPOLAR-SCHIZOPHRENIC SPECTRUM DISORDERS AND THEIR UNAFFECTED FIRST-DEGREE RELATIVES

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to my family

to my girlfriend,

up now in Hong Kong.

“...The so-called ‘psychotically depressed’ person who tries to kill herself doesn't do so out of quote ‘hopelessness’ or any abstract conviction that life's assets and debits do not square. And surely not because death seems suddenly appealing. The person in whom its invisible agony reaches a certain unendurable level will kill herself the same way a trapped person will eventually jump from the window of a burning high-rise. Make no mistake about people who leap from burning windows. Their terror of falling from a great height is still just as great as it would be for you or me standing speculatively at the same window just checking out the view; i.e. the fear of falling remains a constant. The variable here is the other terror, the fire's flames: when the flames get close enough, falling to death becomes the slightly less terrible of two terrors. It's not desiring the fall; it's terror of the flame yet nobody down on the sidewalk, looking up and yelling ‘Don't!’ and ‘Hang on!’, can understand the jump. Not really. You'd have to have personally been trapped and felt flames to really understand a terror way beyond falling...”

Infinite Jest – DFW

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ABSTRACT

BACKGROUND: schizophrenic and bipolar disorders are complex and disabling psychiatric diseases whose classical nosography and classification are still under challenging debate aiming to overcome the traditional “Kraepelinian Dichotomy”. For the past hundred years most clinical work and research in psychiatry has proceeded under the assumption that schizophrenia and bipolar disorder are distinct entities with separate underlying disease processes and treatments. In more recent years there has been increasing evidence for phenomenological, biological and genetic overlap between the two disorders (Potash and Bienvenu 2009). Nowadays, the categorical approach to psychiatric nosography is in contrast with the recent neurobiological, neuropsychological and genetic findings in affective and schizophrenic disorders. Further, symptoms and signs constituting bipolar and schizophrenic disorders are continuously, not dichotomously, distributed; there may be no point of “real cleavage” (Phelps et al. 2008). This recognition has led some clinicians and researchers to call for a diagnostic model that, moving to a “dimensional perspective”, formally recognizes a continuous spectrum from schizophrenic to bipolar (and recurrent depressive) disorders. Kelsoe argued that the existing data coming from various fields of research in bipolar and schizophrenic disorders may best fit a model in which different set of genes predispose to overlapping phenotypes in a continuum. Given the apparent overlap of regions of the genome implicated in bipolar disorder with those for schizophrenia (Kelsoe 1999; Berrettini 2000), the data suggest the possibility that a common polygenic background predisposes to both bipolar disorder and schizophrenia, according to the so-called “multiple threshold model” (Kelsoe 2003). As highlighted by Craddock and Owen, the recent findings are compatible with a model of functional psychosis in which susceptibility to a spectrum of clinical phenotypes is under the influence of

overlapping sets of genes, which, together with environmental and epigenetic factors, determine an individual's expression of illness (Craddock and Owen 2005). A lot of interest is focusing on brain structural abnormalities in patients suffering from schizophrenia and bipolar disorder. A huge amount of neuroimaging studies has been published so far, however the literature is heterogeneous and there is still some degree of uncertainty concerning what key regions are involved in the pathogenesis of such disorders. Schizophrenia and Bipolar Disorder have a number of overlapping symptoms and risk factors, but it is not yet clear if the disorders are characterized by similar deviations in brain morphometry or whether any such deviations reflect the impact of shared susceptibility genes on brain structure. To date there is no consensus about whether, and to what extent, gray matter loss in Schizophrenia is mirrored in Bipolar Disorder and what is the effect of medication or other confounding factors. Studies in family members of patients, who share the risk of the disease but not the confounding factors, may help elucidate whether abnormalities in brain structures are shared by both illnesses.

AIM OF THE STUDY: to investigate hippocampal gray matter volume differences in a group of patients with bipolar-schizophrenic spectrum disorders, a group of their unaffected first-degree relatives, and a group of healthy control subjects.

METHODS: a total of 104 subjects - 36 schizophrenic or schizoaffective (SZ), 27 bipolar (BP), 2 major depression, 8 unaffected relatives (UR), and 31 healthy controls (HC) - underwent 1,5 T MRI scanning, with volumetric T1 3D acquisition protocol, at the Neuroradiology Unit of Conegliano Hospital. We calculate bilateral hippocampal gray matter volume (HV) and total cerebral volume (TCV) in a sample of 31 SZ, 27 BP, 8 UR and 26 HC, with a stereological method using ANALYZE 10.0 software.

RESULTS: we found statistically significant reductions in bilateral HV in the BP-SZ patients compared to HC; the direct comparison between patient groups

identified statistically significant reduction in the right HV of SZ, but no significant differences for left HV or TCV (however statistical significance was lost after normalization); statistically significant reduction in the left HV and a trend towards statistical significance for right HV in the UR compared to HC (a trend towards statistically significant reduction in bilateral HV persisted after normalization).

CONCLUSION: it might be speculated that the alterations of the gray matter volume in the hippocampus highlighted in our study could be interpreted as a possible structural “biological marker” in the bipolar-schizophrenic spectrum.

RIASSUNTO

INTRODUZIONE: schizofrenia e disturbo bipolare sono malattie psichiatriche complesse e invalidanti, il cui inquadramento nosografico è oggetto di continuo dibattito nel superamento della classica “dicotomia Kraepeliniana” tra Dementia Praecox e Malattia Maniaco-Depressiva. Negli ultimi cento anni, buona parte della pratica clinica e della ricerca in psichiatria sono state basate sull’assunto che schizofrenia e disturbo bipolare fossero entità categorialmente distinte, separate da distinti meccanismi patologici e trattamenti. In anni più recenti invece, si sono accumulate numerose evidenze a supporto di una parziale sovrapposizione fenomenologica, biologica e genetica tra questi disturbi (Potash e Bienvenu 2009). Attualmente, l’approccio nosografico “categoriale” nei disturbi affettivi e schizofrenici è in contrasto con le più recenti scoperte in ambito neurobiologico, neuropsicologico e genetico. Inoltre è stato evidenziato come, nemmeno dal punto di vista clinico vi sia un reale punto di “separazione” tra i due disturbi, che presentano segni e sintomi comuni e sovrapponibili (Phelps et al. 2008). Tale consapevolezza ha portato clinici e ricercatori a orientarsi verso un modello diagnostico che, spostandosi in una prospettiva “dimensionale”, formalmente riconosce l’esistenza di uno spettro tra disturbi schizofrenici e bipolari. Kelsoe afferma che i dati provenienti dai vari filoni di ricerca nei disturbi bipolari e schizofrenici potrebbero essere meglio spiegati da un modello in cui differenti set di geni predispongono a fenotipi clinici che si sovrappongono in un continuum. Data la documentata sovrapposizione fra regioni genomiche implicate nel disturbo bipolare con quelle della schizofrenia (Kelsoe 1999; Berrettini 2000), le evidenze suggeriscono la possibilità che un substrato poligenico comune possa conferire una predisposizione a entrambi i disturbi, secondo il cosiddetto modello delle “soglie multiple” (Kelsoe 2003). Come sottolineato da Craddock e Owen, le più recenti scoperte in tale ambito sono compatibili con un modello di psicosi funzionale, nel quale la suscettibilità ad uno spettro di fenotipi clinici è sotto l’influenza di un set di geni condivisi, che,

insieme a fattori ambientali ed epigenetici, determina l'espressione di malattia in ciascun individuo (Craddock e Owen 2005). Notevole interesse si sta inoltre focalizzando sulle alterazioni strutturali cerebrali in pazienti affetti da schizofrenia e disturbo bipolare. Nonostante l'ingente mole di studi di neuroimaging finora pubblicati, la letteratura sull'argomento è molto eterogenea ed esiste ancora notevole incertezza su quali siano le specifiche regioni cerebrali coinvolte nella patogenesi di tali disturbi. Schizofrenia e Disturbo Bipolare condividono una serie di sintomi e fattori di rischio, ma non è ancora stato chiarito se questi disturbi siano caratterizzati da comuni modificazioni morfometriche cerebrali e se tali alterazioni riflettano l'impatto di geni comuni di suscettibilità sulla morfologia del cervello. Ad oggi, non è stato definitivamente chiarito se, e fino a che punto, la documentata perdita di sostanza grigia nella Schizofrenia si rifletta anche nel Disturbo Bipolare e su quali siano gli effetti della farmacoterapia o di altri fattori di confondimento. Gli studi sui membri non affetti di pazienti schizofrenici e bipolari, che condividono la predisposizione genetica ai disturbi, ma non i fattori di confondimento, possono rivelarsi utili nel verificare se le varie anomalie cerebrali siano condivise nelle due patologie.

SCOPO DELLO STUDIO: analizzare eventuali differenze volumetriche nella sostanza grigia ippocampale in un gruppo di pazienti dello spettro bipolare-schizofrenico, un gruppo di familiari di primo grado non affetti e un gruppo di soggetti sani di controllo.

MATERIALI E METODI: un totale di 104 soggetti - 36 pazienti con disturbo schizofrenico o schizoaffettivo (SZ), 27 pazienti con disturbo bipolare (BP), 2 pazienti affetti da depressione maggiore ricorrente, 8 familiari di primo grado non affetti (UR) e 31 controlli sani (HC) sono stati sottoposti ad una procedura di Risonanza Magnetica cerebrale ad 1,5 Tesla, secondo un protocollo di acquisizione di sequenze T1 3D volumetriche, presso l'Unità Operativa di Neuroradiologia del Presidio Ospedaliero di Conegliano. Mediante l'utilizzo del Software ANALYZE 10.0, sono stati calcolati, con un metodo stereologico, i

volumi bilaterali della sostanza grigia ippocampale (HV) ed il volume cerebrale totale (TCV) in un campione di 31 SZ, 27 BP, 8 UR e 26 HC.

RISULTATI: sono state riscontrate riduzioni volumetriche statisticamente significative della sostanza grigia di ippocampo destro e sinistro tra i gruppi di pazienti dello spettro bipolare-schizofrenico rispetto ai controlli; nel confronto diretto tra il gruppo di pazienti schizofrenici e quello dei bipolari è stata identificata una riduzione statisticamente significativa del volume della sostanza grigia dell'ippocampo destro (tale significatività non persiste in seguito a normalizzazione) e nessuna significativa differenza nei volumi della sostanza grigia dell'ippocampo sinistro o nel volume cerebrale totale; nel confronto tra il gruppo di familiari di primo grado non affetti rispetto al gruppo di soggetti sani di controllo è stata evidenziata una significativa riduzione volumetrica della sostanza grigia dell'ippocampo sinistro e un trend verso la significatività statistica per l'ippocampo destro (tali riduzioni volumetriche della grigia ippocampale mantenevano bilateralmente tale trend verso la significatività statistica anche dopo la normalizzazione).

CONCLUSIONE: l'alterazione volumetrica della sostanza grigia ippocampale evidenziata nel nostro studio potrebbe essere interpretata come un possibile "marker biologico" strutturale nei disturbi dello spettro bipolare-schizofrenico.

FIRST PART: INTRODUCTION

1. BEYOND THE KRAEPELINIAN DICHOTOMY

Schizophrenic and affective disorders are complex and disabling psychiatric diseases whose classical nosography and classification are still under challenging debate aiming to overcome the traditional “Kraepelinian Dichotomy” between *Dementia Praecox* and *manisch-depressive Irresein* (Kraepelin E 1899).

More than 100 years ago, Emil Kraepelin split the non-organic (so-called *functional*) psychoses into two disorders and thereby created a dichotomous approach to classification that persists to this day. For the past hundred years most clinical work and research in psychiatry has proceeded under the assumption that schizophrenia and bipolar affective disorder are distinct entities with separate underlying disease processes and treatments. Although Kraepelin himself came to question the validity of this dichotomy, it has become reified in the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders (DSM), as well as WHO’s International Classification of Diseases (ICD). In the absence of ‘laboratory’ tests based on a solid understanding of pathogenesis, the criteria available to psychiatry for validating nosological categories have been restricted to clinical features, outcome and family history (Robins and Guze 1970). It is widely, but erroneously, assumed that the two disorders are discrete, natural disease entities with distinct pathogenesis, which can be identified by current operational diagnostic conventions.

This belief has survived despite the fact that, although typical schizophrenia and bipolar disorder are often seen, many patients have both psychotic and affective symptoms over the course of their illnesses and it is not uncommon for patients to receive both diagnoses at different times (Owen and Craddock 2009).

Moreover, many individuals with severe psychiatric illness have both prominent mood and psychotic symptoms, raising the possibility, indeed the likelihood, that there is not a neat biological distinction between schizophrenia and bipolar affective disorder (Craddock and Owen 2005).

There is increasing evidence for phenomenological, biological and genetic overlap between schizophrenia and bipolar disorders (Potash 2006; Potash and Bienvenu 2009; Purcell et al. 2009). Evidence has been gradually accumulating over 10-20 years from genetic epidemiology that is inconsistent with the dichotomous view. Recent molecular genetic findings are most persuasive. In *The Lancet*, Paul Lichtenstein and colleagues (Lichtenstein et al. 2009) report the largest family study of schizophrenia and bipolar disorder ever undertaken. They studied more than 2 million nuclear families, which were identified from the Swedish population and hospital-discharge registers. Craddock and Owen say that these results are clear: first-degree relatives of probands with schizophrenia or bipolar disorder have an increased risk of *both* disorders (Craddock and Owen 2010). Moreover, there is evidence from half-siblings and adopted-away relatives that this is due substantially to genetic factors. Classical family studies (Berrettini 2000; Bramon and Sham 2001), twin studies (Cardno et al. 2002) and recent molecular genetic studies (Purcell et al. 2009; Owen et al 2007; Craddock et al 2005) support the hypothesis of overlapping susceptibility between the disorders (Craddock and Owen 2007). Additionally, genome-wide association studies (GWAS) have demonstrated the existence of common DNA variants (single nucleotide polymorphisms, SNPs) that influence risk of both schizophrenia and bipolar disorder. There is direct molecular genetic support for a substantial genetic overlap between schizophrenia and bipolar disorder from the recent large-scale GWAS of bipolar disorder and schizophrenia in which thousands of individuals have been studied for hundreds of thousands of common DNA variants spread across the genome (Badner and Gershon 2002; Vazza et al. 2007; O'Donovan et al. 2008; Moskvina et al. 2009; Green et al. 2010; Purcell et al. 2009).

The studies described above indicate that schizophrenia and bipolar disorder (and recurrent depression) do not “breed true”, but have an overlap in genetic risk and are therefore likely to share some aspects of pathogenesis.

2. THE BIPOLAR-SCHIZOPHRENIC “SPECTRUM”

The DSM and ICD systems, so-called “categorical” models, have proven useful, facilitating, considerable gains in research, since the DSM initiated this system of dichotomous (present/absent) decision making, thus improving comprehension, communication and sharing of clinical information between clinicians from different parts of the world (Jablensky 1999). The “dichotomy” also formed the basis of the operational diagnostic criteria that brought a degree of rigour and reproducibility to psychiatric research (Craddock and Owen 2005). Any psychiatrist with experience of functional psychotic illness knows that many patients do not have disorders that conform to either prototypical dichotomous category. Many individuals receive one diagnosis at one time or from one team and the alternative diagnosis at a different time or from another team. Further, findings emerging from many fields of psychiatric research, such as neuroimaging, neuropathology and neuropsychology, do not fit well with the traditional dichotomous model (Murray et al. 2004).

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

Emil Kraepelin himself, in his latter years, was close to revising his own celebrated dichotomy between manic-depressive insanity and dementia praecox in order to take account of a large group of intermediate psychoses. He continued to develop and refine his ideas about psychiatric diagnoses, and his thinking had in many ways moved on from the dichotomous classification by the end of his life (Jablensky 1999; Jablensky 2010; Craddock and Owen 2007).

Kraepelin conceptualized the individual course of affective disorders over a lifetime as a movement along the continuum from normal fluctuations in mood (including basic states or temperaments) via full-blown mania and depression to psychotic syndromes: he described a continuum from syntonetic, mood-congruent psychotic affective disorders to parathymic, mood-incongruent psychotic disorders (Angst and Gamma 2008).

Although the dichotomous view has dominated clinical psychiatry for over 100 years, there has been a long history of dissent. Many nosologists have developed their own models and approaches: important recent examples include Crow's continuum model (Crow 2008), the spectrum models of bipolarity of Angst (Angst 2007; Phelps et al. 2008) and Akiskal (Akiskal and Pinto 1999; Evans et al. 2005; Akiskal 2007), and Marneros' focus on schizoaffective disorder (Marneros 2006).

When considering complex psychiatric infirmities, such as schizophrenia and affective disorders, a categorical approach is limiting (Frank 2011): it is important to take a "longitudinal" approach to diagnosis and to consider the nature and occurrence of psychotic and affective symptoms across the patient's illness history. It is also of fundamental importance to consider mixed or subthreshold symptomatology (Cassano, Mantua, and Fagiolini 2011). Besides, the reliability and stability of the DSM-IV schizoaffective diagnosis is poor (Forrester, Owens, and Johnstone 2001; Kane 2010). The relative proportions of psychotic and affective symptoms as well as the type (bipolar type, depressive type) of the affective syndrome therefore are key components in its diagnosis and subtyping, generating a *de-facto* dimensional concept of schizoaffective disorder. The nosographic heterogeneity of schizoaffective disorder reflects the possibility that this disorder may straddle the middle of a continuum between schizophrenia and bipolar disorder (Keshavan et al. 2011). One of the criticisms of schizoaffective disorder by clinical and research psychiatrists is the lack of reliability and temporal stability that has been reported using current definitions (Maj et al. 2000). However, this is an almost inevitable consequence of the overly restrictive nature of current definitions of schizoaffective disorder, together with

the tendency of clinicians to make diagnoses “cross-sectionally” rather than “longitudinally” (Craddock and Owen 2007).

Intermediate cases suggest that the symptoms and signs constituting bipolar and schizophrenic disorders may be continuously, not dichotomously, distributed; there may be no point of “real cleavage” (Phelps et al. 2008). This recognition has led some clinicians and researchers to call for a diagnostic model that, moving to a “dimensional perspective”, formally recognizes a continuous spectrum from schizophrenic to bipolar (and recurrent depressive) disorders.

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

As pointed out by Jules Angst (Angst 2007), for the first time in psychiatric history Kretschmer in 1921 proposed a dimensional concept (from normal to pathological) for schizophrenia (schizothymic – schizoid – schizophrenic) and for affective disorders (cyclothymic temperament – cycloid “psychopathy” – manic-depressive disorder), a few years later carried on by Bleuler in 1922.

The term “spectrum” was first used in psychiatry in 1968 for the schizophrenia spectrum, which integrated schizoid personalities (Kety et al. 1968). In more recent years, the hypothesis of a continuum or spectrum of schizophrenia-related phenotypes is supported by frequent observation that apparently different psychotic disorders tend to aggregate in first degrees relatives of schizophrenia-diagnosed patients (Siever and Davis 2004). Epidemiological studies suggest that genetic susceptibility to schizophrenia is shared with genetic predisposition to other related syndromes (for example, schizotypal or paranoid personality disorder) (Baron and Risch 1987; Kety et al. 1994).

With regard to mood or affective disorders, in 1977 Akiskal proposed a cyclothymic-bipolar spectrum and in 1981 Klerman suggested a mania spectrum (Akiskal et al. 1977; Klerman 1981). According to Phelps and colleagues (Phelps et al. 2008) Kraepelin emphasized not mania but rather a high degree of recurrence as the principal identifying feature of manic-depressive course and Klerman included in his spectrum a bipolar category (could be considered bipolar V type) in which individuals had only depression, no hypomania or mania, but a high familial incidence of bipolar disorder. Later, the concept of bipolar spectrum was expanded in order to encompass all kind of disturbances that demonstrate a mood alteration as the principal psychopathological element, from subthreshold to full psychotic manic disorders, in a continuum of symptom severity: affective personalities - dysthymia/cyclothymia - recurrent major depressive disorder - bipolar disorder II and I (Akiskal 1983; Akiskal and Pinto 1999; Akiskal 2007).

JR Kelsoe (Kelsoe 2003) argued that the existing data coming from various fields of research in bipolar and schizophrenic disorders may best fit a model in which different set of genes predispose to overlapping phenotypes in a continuum. Schizophrenia and affective disorders do not follow a Mendelian form of transmission, rather they demonstrate a complex pattern of genetic transmission (Kendler 2006). According to Kelsoe, the problem with the bipolar spectrum described above is that it does not include a number of diagnostic and syndromic entities that also occur in the families and co-twins of bipolar patients. Specifically, this should include unipolar depression, dysthymia, as well as, psychotic mood disorder, schizoaffective disorder and possibly schizophrenia (Kelsoe 1999; Kelsoe 2003). Given the apparent overlap of regions of the genome implicated in bipolar disorder with those for schizophrenia (Kelsoe 1999; Berrettini 2000), these data suggest the possibility that the same gene in each of these regions predisposes to both bipolar disorder and schizophrenia. This will not be conclusively known until the genes are definitively identified. It is possible that each region contains separate genes for each disorder that are coincidentally near each other. However, if a substantial portion of the genes for

these two disorders is common, it will raise the challenging question of how the same genes can lead to two different phenotypes.

Kelsoe proposed a “multiple threshold model” (Fig. 1) to combine a quantitative trait model of transmission (polygenic transmission) with qualitatively different phenotypes, lying on the spectrum: the more polygenic susceptibility alleles one person has, the higher is the value of the quantitative trait; qualitatively different traits result when a critical threshold is exceeded and different thresholds lead to different disorders (unipolar depression - bipolar disorder - schizoaffective disorder - schizophrenia).

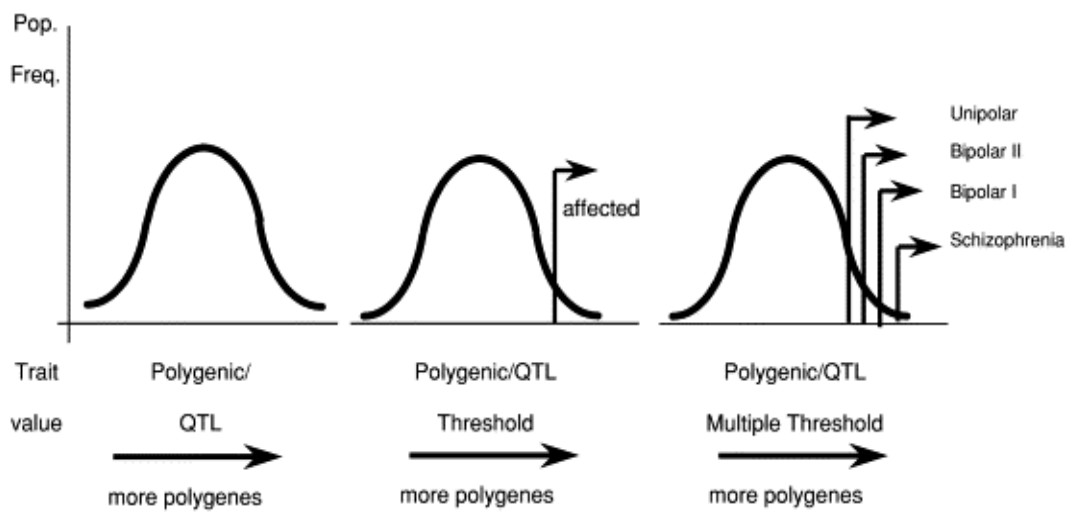


Figure 1. Multiple Threshold model (from Kelsoe 2003).

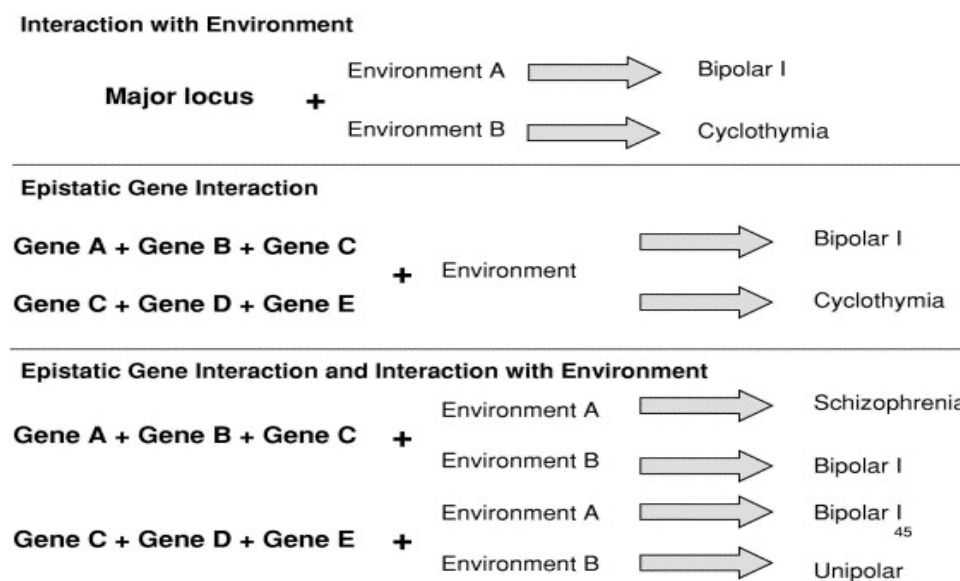


Figure 2. Gene x Environment interactions (from Kelsoe 2003).

In addition, allelic heterogeneity, the presence of different mutations within the same susceptibility gene, epistatic gene-gene interaction and different gene-environment interactions (Fig. 2) may produce the variety of phenotypes observed (Kelsoe 2003).

In conclusion, as highlighted by Nicholas Craddock and Michael Owen, the recent findings are compatible with a model of functional psychosis in which susceptibility to a spectrum of clinical phenotypes is under the influence of overlapping sets of genes which, together with environmental factors, determine an individual's expression of illness (Fig. 3).

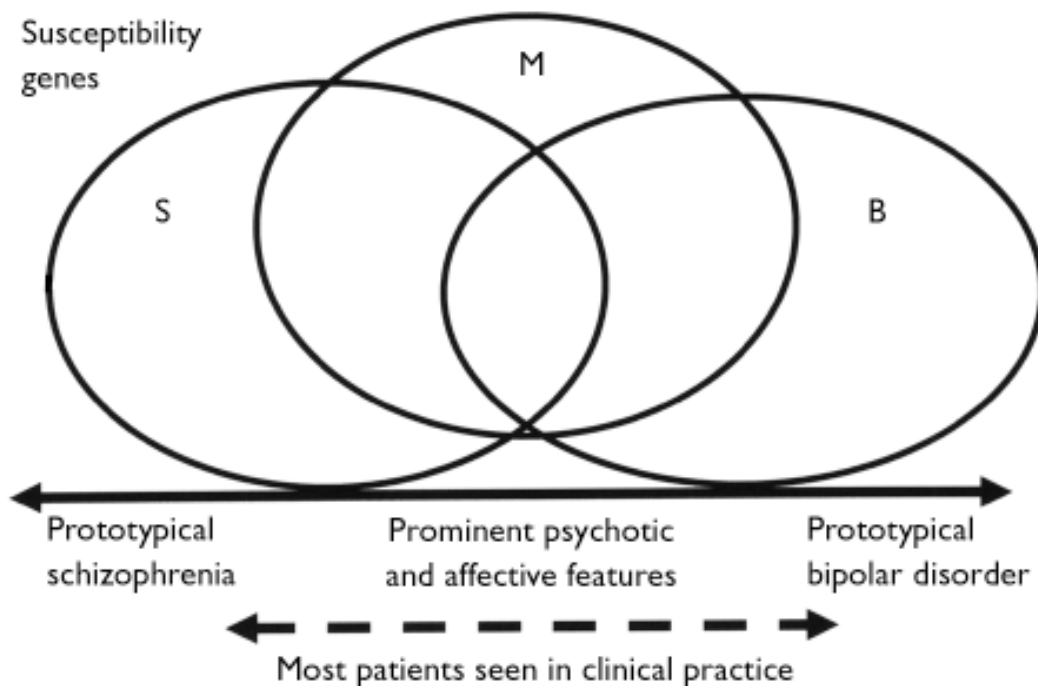


Figure 3. Possible relationship between susceptibility genes, environment and clinical phenotypes (from Craddock and Owen, 2005).

In addition to the interface between bipolar disorder and schizophrenia, there is genetic overlap between the functional psychoses and major depressive disorder with extension into subclinical (or normal) variation.

According to Craddock & Owen (2005), most patients want to be given an unambiguous and accurate diagnosis, but psychiatrists are understandably reluctant to be too dogmatic in the early stages of psychotic illness, recognizing that the cross-sectional picture may change longitudinally, often frustrating patients, leading to diagnostic revisions between categories and creating an impression that psychiatrists are indecisive or incompetent.

Moving to a spectrum concept with recognition of overlapping pathogenetic factors and varying expression (dependent upon both genetic risk and environmental exposure) would allow a confident and clear diagnosis to be offered (perhaps 'psychosis-spectrum illness' or 'mood– reality disorder'), with a clear explanation that some specific tests and a period of observation will help to clarify the likely course of illness and response to treatment.

Data from the ongoing large scale molecular genetic studies (particularly, but not exclusively, whole genome association studies), together with data from other areas of neuroscience, offer the opportunity of starting to put psychiatric classification on a robust framework that has biological validity (Craddock and Owen 2007).

It seems likely that sets of overlapping genes that confer risks along different domains of psychopathology, will be gradually identified, corresponding to the disruption of different brain systems. Unravelling the neurobiology underlying these overlaps will shed light on the bewildering degree of "comorbidity" observed across disorders and the widespread non-specificity of treatments.

Finally, we now have at our disposal powerful molecular genetic tools that should allow us to identify the biological systems that are involved in disease pathogenesis. These techniques allow us to study biological systems in large numbers of individuals whilst they are alive. For the first time in psychiatry, this provides the opportunity to validate our diagnostic concepts and procedures against biologically relevant criteria that in many cases will relate to the effectiveness of treatments (Craddock and Owen 2007).

3. STRUCTURAL MRI IN THE SPECTRUM

The impressive developments in neuroimaging are likely to provide us with the power to study the functioning of specific, relevant brain systems *in vivo* in individuals during differing phases of illness and in response to varying environmental situations. Craddock and Owen (2007) imagine that these approaches will be complemented by developments in many other fields, and this will facilitate the bringing together of diverse domains of research evidence that can be synthesized into models of brain function and dysfunction and their relationship with psychopathology.

Neuroimaging has been embraced by investigators applying diverse methods to examine brain structure and function in psychiatric disorders. *In vivo* measurement is afforded by magnetic resonance imaging (MRI) examining neuroanatomy through structural MRI (sMRI), connectivity through diffusion tensor imaging (DTI), and neurochemistry through magnetic resonance spectroscopy (MRS). Magnetic resonance also enables examination of brain physiology using functional MRI (fMRI) methods (Gur, Keshavan, and Lawrie 2007). In the absence of quantitative analysis, routine brain imaging cannot aid in the differential diagnosis of psychiatric diseases without considering the clinical presentation. Thus far, studies using imaging techniques to determine prognosis or treatment response have not generated sufficiently replicated findings. There are, however, encouraging results from several studies evaluating these technologies as possible predictors of diagnosis (Agarwal et al. 2010).

In addition, heterogeneous structural MRI findings can be influenced by various factors including scan acquisition parameters, image quality, hardware/software employed in the tracing procedure and type of segmentation technique (manual or automatic) (Geuze, Vermetten, and Bremner 2005).

Currently, the “*gold standard*” for determining the volumes of specific brain areas (Region of Interest, ROI) is the “manual tracing” method (Tae et al. 2008; Doring et al. 2011): however, this technique has the disadvantage of being time-consuming and operator-dependent.

Semi-automated and automated segmentation techniques have generated intense interest given the wide range of clinical applications for MRI volumetry in the last decades. In recent years, the ROI analytic manual approach initially applied has been replaced by semi automated methods, such as *design-based Stereology* (Keshavan et al. 1995; Sheline et al. 1996; Schmitz and Hof 2005) or by automated methods for regional parcellation and *voxel-based morphometry* (VBM) (Ashburner and Friston 2000), that can efficiently yield information on the entire brain, permitting validation of reported findings and new discovery of other affected regions.

A lot of interest is focusing on brain structural abnormalities in patients suffering from schizophrenia and bipolar disorder.

A huge amount of neuroimaging studies has been published so far, however the literature is heterogeneous and there is still some degree of uncertainty concerning what key regions are involved in the pathogenesis of such disorders. Schizophrenia and Bipolar disorder have a number of overlapping symptoms and risk factors, but it is not yet clear if the disorders are characterized by similar deviations in brain morphometry or whether any such deviations reflect the impact of shared susceptibility genes on brain structure.

One of the best-characterized brain abnormalities in Schizophrenia is gray matter reduction, consistently reported by numerous morphometric studies using ROI or VBM analyses. In this disorder, brain volumes have consistently been found to be decreased, particularly in the frontal and temporal lobes, including the anterior cingulate (Baiano et al. 2007), superior temporal gyrus (Sun et al. 2009), hippocampus (Steen et al. 2006), thalamus (Konick and Friedman 2001), and striatum, with increases in the lateral and third ventricles (Vita et al. 2006).

These findings are variously replicated and reported in reviews or meta-analyses including patients with first-episode or chronic schizophrenia (Wright et al. 2000; Shenton et al. 2001; Honea et al. 2005; Pantelis et al. 2005; Olabi et al. 2011) and also in studies with individuals at high risk for schizophrenia (Chan et al. 2011).

On the other hand, the majority of structural neuroimaging studies in Bipolar Disorder are ROI-based and the literature consists of a small number of studies examining a limited number of structures, for example amygdala (Strakowski, Adler, and DelBello 2002) or anterior cingulate (Konarski et al. 2008), with small sample sizes and contradicting evidences (McDonald et al. 2004; Kempton et al. 2008). Although VBM studies in bipolar disorder are increasing in number in recent years, findings remain contradictory; however two recent meta-analyses revealed gray matter reduction in the insula and anterior cingulate cortex (Bora et al. 2010; Ellison-Wright and Bullmore 2010). Ventricular enlargement and the presence of white matter hyper-intensities are among the most consistently reported abnormalities (Kempton et al. 2008; Strakowski et al. 2002).

With regard to hippocampal volumes, data emerging from ROI-based or VBM studies are extremely variable: the majority of them failed to detect significant volume differences between bipolar patients and healthy controls (Hauser et al. 2000; Altshuler et al. 2000; Brambilla et al. 2003; Strasser et al. 2005; Adler et al. 2007); however a few studies report significant hippocampal reductions in patients compared to control subjects (Blumberg et al. 2003).

Recently, Chepenik and colleagues reported hippocampal decrease in two different studies comparing bipolar patients vs. healthy controls. They found that hippocampal volumes were significantly smaller in bipolar compared to control subjects and, analyzing the *BDNF val66met polymorphism*, the presence of the BDNF met allele was associated with smaller hippocampal volumes in both diagnostic groups: moreover the bipolar subgroup who carried the BDNF met allele had the smallest hippocampi (Chepenik et al. 2009); furthermore, bilateral hippocampal reduction in bipolar patients correlated with verbal memory impairment (Chepenik et al. 2012).

According to Bearden and colleagues (Bearden et al. 2008), localized hippocampal decreases in patients with bipolar disorder are subtle and not easily detectable with conventional volumetric measures. In a different study they found out decreased volume in the CA1 region of right hippocampus in unmedicated bipolar patients, thus demonstrating the sensitivity of 3D anatomic

mapping methods for detecting subtle alterations in hippocampal structure in bipolar disorder (Bearden et al. 2008).

There are few morphometric studies comparing *psychotic* bipolar patients to healthy controls, and these are mainly ROI-based. Majority of these studies report no volumetric abnormalities in the examined regions including the amygdalo-hippocampal complex, thalamus, superior temporal gyrus and insula (Bora et al. 2008). Volume reductions were occasionally reported only in the subgenual cingulate cortex (Hirayasu et al. 1999), left hippocampus (Velakoulis et al. 1999), while one study reported increased striatal volume (Getz et al. 2002).

Two meta-analyses have sought to clarify the neuroanatomical differences between bipolar and schizophrenia patients and to identify differences between each patient group and healthy controls.

A meta-analysis of VBM studies, that encompassed thousands of schizophrenia and hundreds of bipolar patients, identified widespread gray matter reductions in the schizophrenia group compared with healthy controls that included the insula bilaterally, dorsolateral prefrontal cortex, superior temporal cortex, bilateral hippocampal-amygdala region, thalamus, anterior cingulate, medial frontal gyrus and posterior cingulate; smaller gray matter volumes were found in the right and left insula, perigenual anterior cingulate, and subgenual anterior cingulate in the bipolar studies, though not in the bilateral hippocampal-amygdala regions. The areas of reduced gray matter observed in studies of bipolar patients overlapped substantially with areas of reduced gray matter in schizophrenia patients, though the reductions seen bilaterally in the hippocampal-amygdala area among schizophrenia patients were not observed in bipolar patients. An area of the perigenual anterior cingulate cortex was selectively smaller among bipolar patients than healthy controls, a region where no differences between schizophrenia patients and healthy controls have been found (Ellison-Wright and Bullmore 2010). Arnone and colleagues carried out a meta-analysis of volumetric studies, using a variety of methods to segment magnetic resonance images, and found smaller volumes in the right amygdala

among schizophrenia patients when they were directly compared with bipolar patients (Arnone et al. 2009).

In literature, there are relatively few studies *directly* comparing brain volumes in patients with schizophrenia vs. bipolar disorder: one of the aims of the direct comparison between patients group is to provide critical tests of hypotheses derived from meta-analysis in a controlled study.

Brown and colleagues (Brown et al. 2011) performed a cross-sectional VBM study comparing 17 chronic schizophrenia and 15 chronic bipolar I patients and 21 healthy subjects matched for age, gender and duration of illness. Whole brain gray matter volume of both the schizophrenia and bipolar groups was smaller than among healthy control subjects. Regional voxel-wise comparisons showed that gray matter volume was smallest within frontal and temporal regions of both patient groups; a second ROI analyses found moderately large to large differences between schizophrenia and healthy subjects in the amygdala and hippocampus, but hippocampal volume in the bipolar group did not differ significantly from the healthy control volume. Unlike Ellison-Wright and Bullmore's study, there were no group differences in the perigenual anterior cingulate. When schizophrenia and bipolar groups were directly compared, the schizophrenia group showed smaller gray matter volumes in right subcortical regions involving the right hippocampus, putamen, and amygdala.

A Croatian study aiming to compare hippocampal volumes in separate groups of patients with schizophrenia, schizoaffective and bipolar disorder, reported no significant difference in volume between bipolar patients and healthy controls, whereas hippocampal volume was statistically significantly reduced in the group of patients with schizophrenia and schizoaffective disorder, compared to either bipolar disorder or control group (Radonić et al. 2011).

In a recent 3 Tesla VBM study, comparing schizophrenic and psychotic bipolar individuals, Yüksel and colleagues reported that schizophrenic patients demonstrate gray matter reductions in multiple frontal and temporal regions compared to healthy controls and in the subgenual cortex compared to psychotic bipolar patients; gray matter volume was increased in the right posterior

cerebellum in schizophrenia patients compared to controls; however, psychotic bipolar patients did not show significant gray matter deficits compared to healthy controls or schizophrenic patients. They claim that it is currently not clear whether the negative findings in the bipolar disorder literature are a result of sample selection, medication biases, technical issues or represent a true biological pattern (Yüksel et al. 2012).

Considering what has been described above, to date there is no consensus about whether, and to what extent, gray matter loss in Schizophrenia is mirrored in Bipolar Disorder and what is the effect of medication or other confounding factors. Studies in family members of patients, who share the *risk* of the disease but not the confounding factors, may help elucidate whether abnormalities in brain structures are shared by both illnesses.

In family members of patients with schizophrenia have been found reductions in whole brain and hippocampus volume, as reported in a meta-analysis (Boos et al. 2007). Decreases in gray and white matter and increases in caudate nucleus volumes have been reported in relatives of patients with bipolar disorder (Noga, Vldar, and Torrey 2001; van der Schot et al. 2010).

Again, a few studies have directly compared patients with schizophrenia, bipolar disorder and their first- and second-degree relatives. Increased familial risk of schizophrenia and bipolar disorder was reported for white matter volume reductions in the left frontal and temporoparietal regions (McDonald et al. 2004). Increased familial risk of schizophrenia but not of bipolar disorder was associated with lateral end third ventricle enlargement (McDonald et al. 2006), loss of gray and white matter in the dorsolateral and ventrolateral prefrontal cortices (McIntosh et al. 2006) and thalamus (McIntosh et al. 2004). Increased familial risk of bipolar disorder but not of schizophrenia was associated with gray matter loss in the right anterior cingulate gyrus and ventral striatum (McDonald et al. 2004). Finally, an abnormal shape of the hippocampus was reported in schizophrenic patients but not in their family members, neither in bipolar patients nor in their unaffected relatives (Connor et al. 2004).

In a recent paper, Hulshoff Pol and colleagues set out to study *twins* concordant and discordant for Schizophrenia and Bipolar Disorder to examine whether the genetic risk of these disorders is reflected in brain volumes and cortical thickness. A total of 310 individuals from 158 twin pairs (discordant for schizophrenia, concordant and discordant for bipolar disorder and healthy twin pairs) were included. They found that overlapping genetic liabilities for the two disorders are reflected in shared abnormalities in cortical gray and white matter. Specifically, the elevated genetic risk of schizophrenia and bipolar disorder is most prominently reflected in a global decrease in white matter volume, in specific thinning of parahippocampal and orbitofrontal cortices and thickening of temporoparietal and superior motor cortices; furthermore, increased genetic liability for schizophrenia but not for bipolar disorder was associated with a thicker right parietal cortex, whereas increased genetic liability for bipolar disorder but not for schizophrenia was associated with enlarged intracranial volume (Hulshoff Pol et al. 2012).

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 hippocampal gray matter volume differences between a group of patients with bipolar-schizophrenic spectrum disorders and a group of healthy control subjects, by using volumetric magnetic resonance imaging. In order to verify or exclude specific diagnosis-related volumetric differences, we performed cross-sectional comparisons between bipolar patients, schizophrenic patients and healthy controls.

Moreover, we identified a little sample of unaffected first-degrees relatives of the recruited patients and compared their hippocampal volumes with the same control group, aiming to detect (or speculate) a putative *structural marker* in common with the patient group.

2. METHODS

2.1. SUBJECTS ENROLLMENT

The global research aimed to recruit patients suffering from different early-onset disorders throughout the *bipolar-schizophrenic spectrum*, comprising bipolar type I and II disorder (BD), recurrent major depressive disorder (MDD), schizoaffective disorder (SA) and schizophrenia (SZ). Furthermore, we aimed to enroll a sample of unaffected first-degree relatives (UR) of the recruited patients and a group of healthy control subjects (HC).

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 (APA, 2000), within the bipolar-schizophrenic spectrum (as listed above)
- illness onset before the age of 35
- duration of illness over 3 years

Control subjects were recruited from the community of friends and acquaintances or from staff, and were 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 current use of psychotropic medications.

Exclusion criteria for all the groups of participants were:

- presence of concomitant medical conditions
- lifetime history of neurological illness or head trauma leading to loss of consciousness for more than 5 minutes
- 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
- contraindications to magnetic resonance scanning

The research was approved by the relevant local ethics committees (Padova and Treviso) and all the subject were enrolled after receiving a complete description of the study procedures and providing written informed consent.

Participation in the research has been proposed to outpatients and inpatients referring to Psychiatry Unit of Conegliano and Psychiatry Clinic of Padova University. A total of 101 patients were eligible and screened, and 96 of them (70 from Conegliano and 26 from Padova) joined the study.

In addition, a little group of 10 unaffected first-degree relatives (8 from Conegliano and 2 from Padova) was recruited.

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

2.2. CLINICAL ASSESSMENT

Psychiatric assessment was carried out by obtaining psychiatric and medical history, performing a mental status examination and 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. The M.I.N.I. was also administered in the control group to rule out the presence of current or past psychiatric conditions.

The patient group, basing on DSM-IV TR criteria, is divided in: 49 patients with Schizophrenia or Schizoaffective Disorder, 6 patients with Psychotic Disorders Not Otherwise Specified, 38 patients with Bipolar type I or II Disorder, and 3 patients with Major Depressive Disorder.

Symptoms severity was assessed with the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962; Ventura et al. 1993), the 17-item version of the Hamilton Depression Rating Scale (HDRS) to rate depressive symptoms (Hamilton 1960), the 14-item Hamilton Rating Scale for Anxiety (HAS) (Hamilton 1959), 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), and the Global Assessment of Functioning (GAF) (from DSM-IV-TR, page 34).

All patients were evaluated if considered clinically stable (inpatients were assessed next to discharge). All the scales were administered on the day that subjects underwent MRI. The data were used to analyse the relationships between volumetric data and concurrent symptom state and severity. Age at onset and length of illness (expressed in years) were recorded during the clinical assessment.

Table 1 and 2 report the demographic and clinical characteristics of the subsample of subjects, which were recruited for the imaging study and whose ROI volumetry was accomplished (see below).

Table 1. Demographic data (see below).

		SCHIZOPHRENICS	BIPOLARS	CONTROLS	RELATIVES
total number		31	27	26	8
sex	M	17	13	13	4
	F	14	14	13	4
age, yr (m ± sd)		37,4 ± 8,9	39,8 ± 9,1	36,6 ± 10,6	37,6 ± 7,8
edu, yr (m ± sd)		12 ± 3,3	12,8 ± 3,2	15,3 ± 2,8	13,6 ± 3,3

Table 2. Clinical data (all values are reported as mean \pm standard deviation).

		SCHIZOPHRENICS	BIPOLARS
age at onset (years)		24,5 \pm 6,9	25,4 \pm 7,1
Illness duration (years)		12,9 \pm 6,7	14,3 \pm 8,5
BPRS		35,6 \pm 6,2	30,2 \pm 3,1
HDRS		9,8 \pm 3,2	8,7 \pm 5,2
HAS		10,9 \pm 5,1	9,2 \pm 4,6
YMRS			7,9 \pm 5,3
PANSS	POS	19,5 \pm 5,4	
	NEG	23,4 \pm 8,3	
	GEN	46,1 \pm 12,8	
GAF		45,4 \pm 18,3	56,8 \pm 13,2

2.3. MAGNETIC RESONANCE IMAGING

IMAGE ACQUISITION

A total of 104 subjects (64 patients: 36 SZ or SA, 27 BP, 2MDD; 8 unaffected first-degree relatives, and 31 healthy controls) underwent MRI scanning during the study.

MRI acquisitions have been performed using a 1.5 T MRI scanner (Achieva XR; Philips Medical Systems) in the Neuroradiology Unit of Conegliano Hospital (ULSS7 Pieve di Soligo, TV). Whole-brain T1-weighted three-dimensional images (magnetization-prepared rapidly acquired gradient-echo, MPRAGE) were acquired in the sagittal plane for volumetric measurements (using the following

acquisition 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). Precautions were taken to minimize subjects' motion during the MRI scan by instructing them to remain as still as possible. However, to correct for head tilt or positioning, sagittal images were aligned approximately along the anterior commissure-posterior commissure line.

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 neuroradiologist reviewed all scans.

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

PROCESSING

The imaging data were transferred from the MRI unit to a PC workstation and analysed using the semi-automated ANALYZE® 10.0 software (Analyzedirect Inc., Biomedical Imaging Resource, Mayo Foundation, Rochester, Minnesota USA).

We carried out a stereological analysis to measure bilateral hippocampal gray matter volume (HV) and total cerebral volume (TCV): *stereology* is a semi-automated method whose accuracy and validity for volume estimation has been reported in several studies and stereological measurements yield high repeatability and precision (Ronan et al. 2006).

Our sampling and counting protocol has been derived from previous reference protocols and research articles (see, Sheline et al. 1996; Rametti et al. 2007).

Coronal slices were sampled and a rigid grid of points, with random starting position and angle of deviation from horizontal, was then superimposed on the images. Selecting the optimal numbers of slices and grid sizes are essential for assessing structurally complex objects whose profiles change significantly from slice to slice. Inter-slice distance and grid size were chosen to yield a Coefficient of Error (CE) in the 0.01-0.04 range. For hippocampal gray matter detection, a 2×2 mm² rigid grid was superimposed on every third coronal slice; for total cerebral volume a 10×10 rigid grid was superimposed every ten coronal slice.

Grid points falling within the hippocampal gray matter or total cerebral volume were counted.

The *Orthogonal* tool of the software allows three orthogonal views (sagittal, axial, and coronal) of each grid at the same time, thus enabling the raters to decide more accurately which cross is included in the hippocampal or cerebral structure: (see Fig. 5, 7, 9 and 11). This provides greater clarity of anatomic localization. Two raters, who were blind to subject identity and clinical characteristics, performed all the measurement procedures for the selected regions of interest.

Anatomical boundaries, according to brain atlases (Duvernoy 1988) and neuroimaging literature, were defined as follows:

- *Total Cerebral volume* (Fig. 4 and 5). All brain tissue of the cerebral hemispheres (both gray and white matter), including the midbrain superior to the pons. The superior border of the pons was chosen as the point of demarcation because it is easily recognizable (Sheline et al. 1996). Cerebellum, optic nerve, tracts and chiasm, and cerebrospinal fluid were excluded.

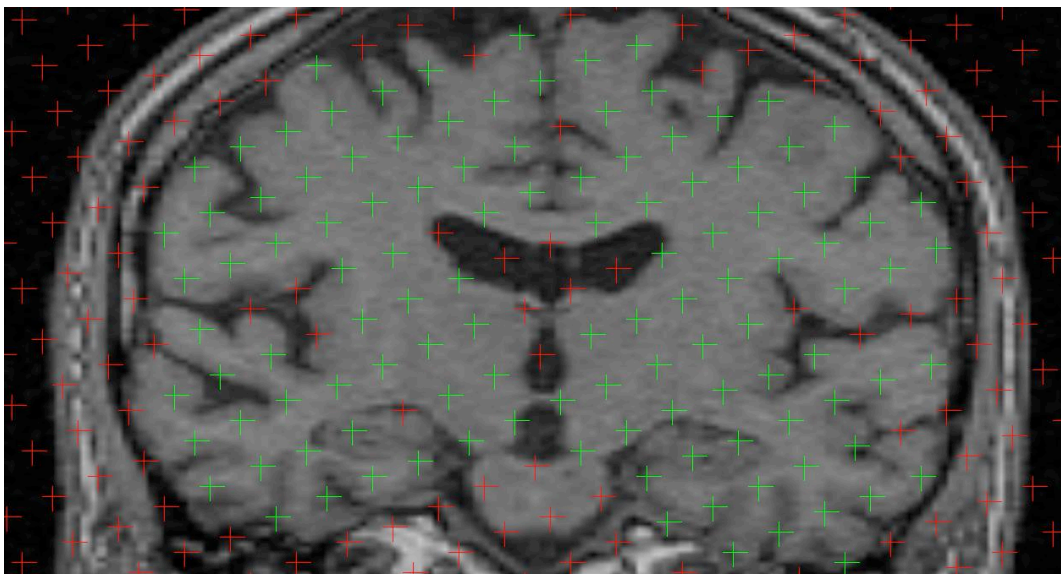


Figure 4. Total cerebral volume, in the coronal plane.

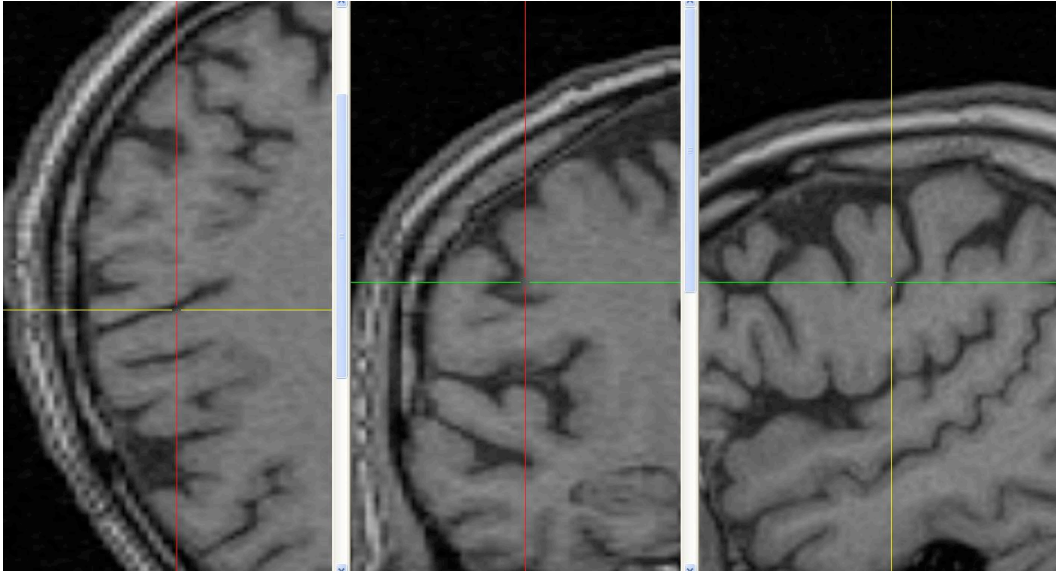


Figure 5. Same cerebral portion, in the 3 orthogonal axes.

- *Hippocampal formation* (Fig. 6-11). 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 for volumetry 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). Volumetrically included tissues were an elongated gray matter complex bordered superiorly by 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. The gray matter complex included 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, was included. Volumetrically excluded tissues were the fornix-fimbria white matter complex, the alveus, the white matter of the parahippocampal gyrus, various fluid-filled spaces including ventricles, subarachnoid spaces and sporadic fluid-density spaces in the hippocampus complex, and the amygdala proper and the white matter border with it (Sheline et al. 1996).

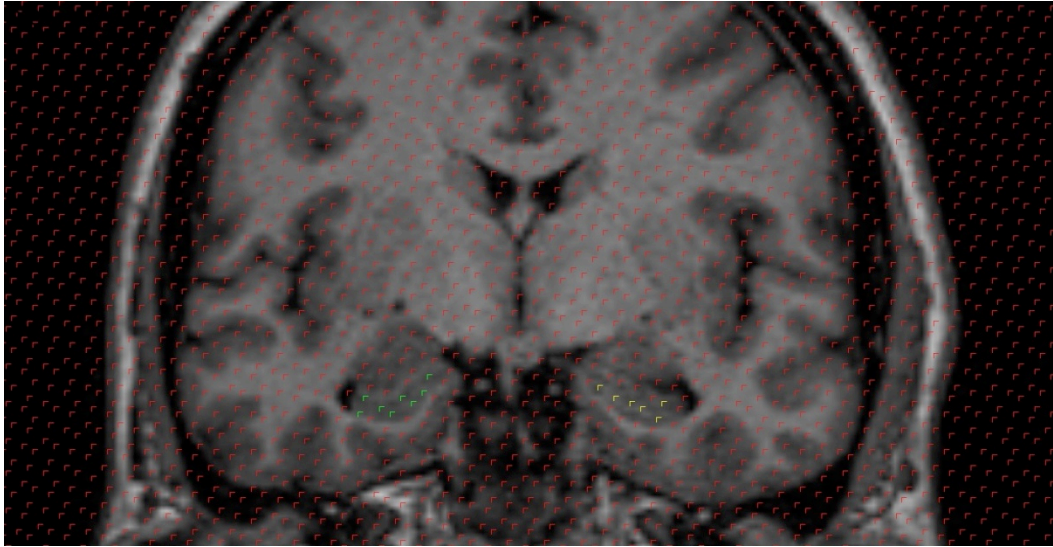


Figure 6. Hippocampal formation (head), in the coronal plane.

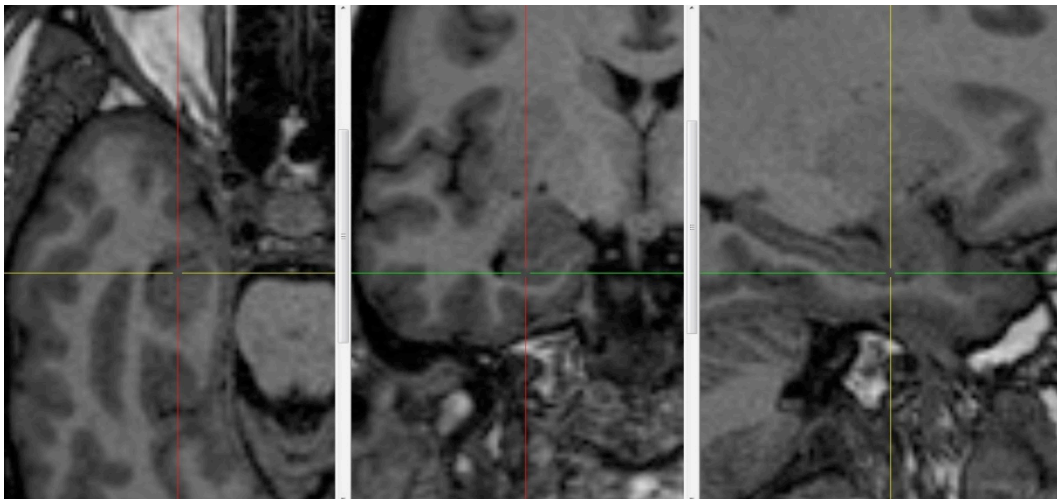


Figure 7. Hippocampal formation (head), in the 3 orthogonal axes.

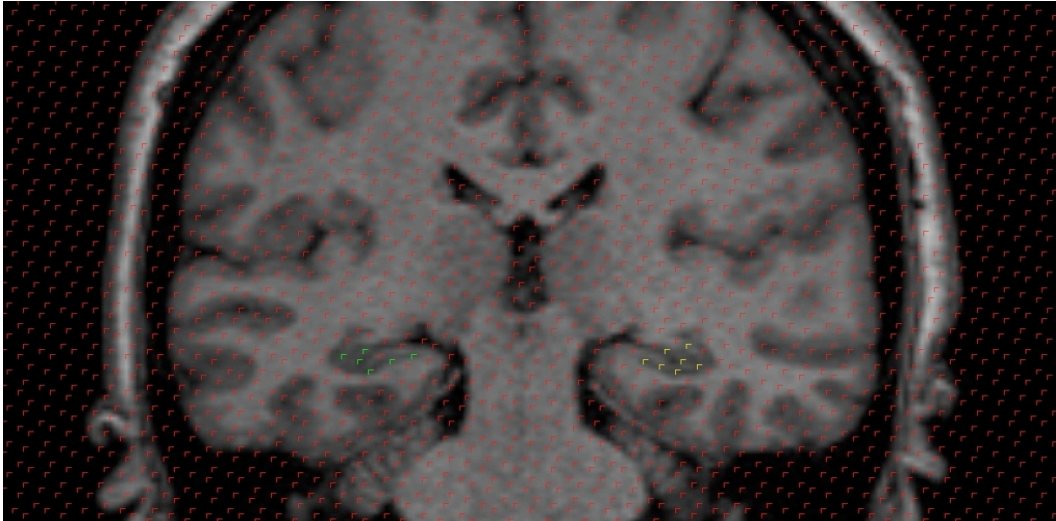


Figure 8. Hippocampal formation (body), in the coronal plane.

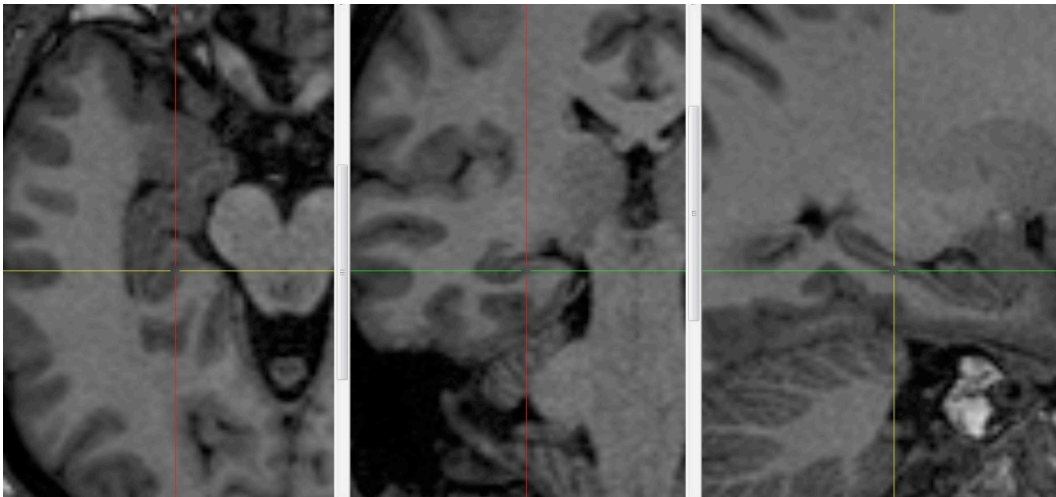


Figure 9. Hippocampal formation (body), in the 3 orthogonal axes.

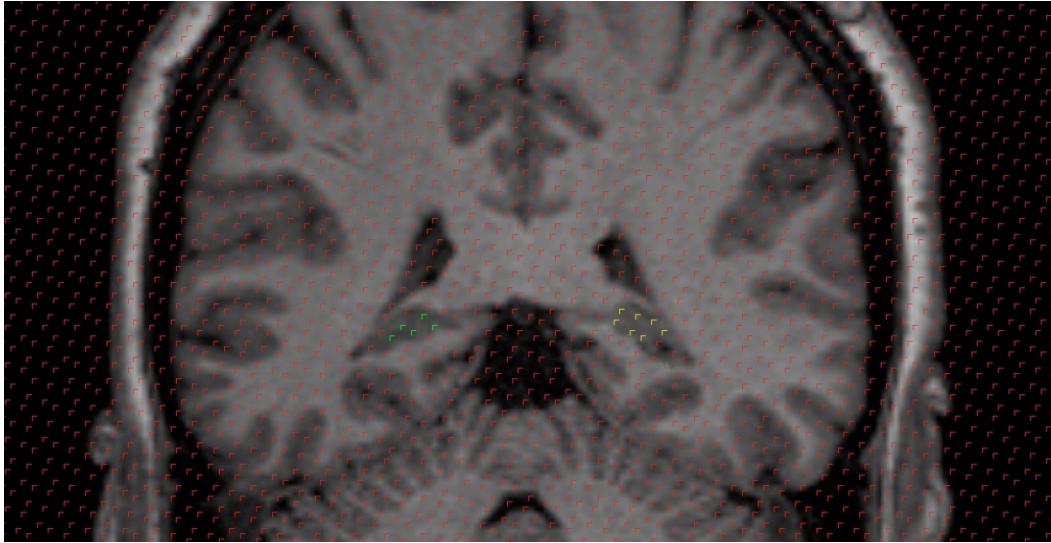


Figure 10. Hippocampal formation (tail), in the coronal plane.

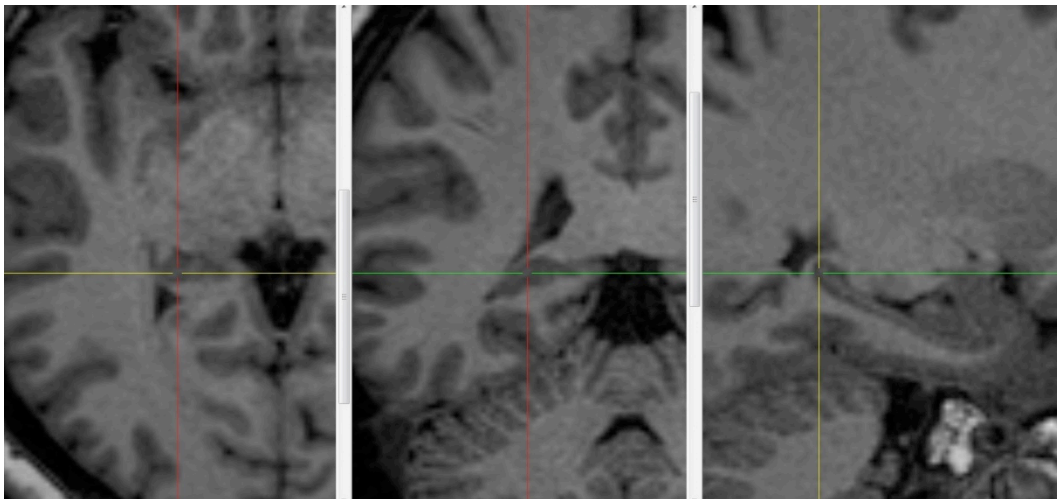


Figure 11. Hippocampal formation (tail), in the 3 orthogonal axes.

VOLUME CALCULATION

Based on the number of selected grid points, a volume estimate is extrapolated by the software. Each region of interest (ROI) volume is then calculated applying the specific *Statistics* tool of ANALYZE software: the resulting values are expressed in mm³.

We calculate bilateral hippocampal volume (HV) and total cerebral volume (TCV) in a sample of 31 schizophrenic patients, 27 bipolar patients, 8 unaffected first-degree relatives and 26 healthy controls.

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 ROI volumes of patients and controls, all the data demonstrated a normal distribution (see, as examples, Fig. 12 and 13).

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, and to compare data with the small sample of unaffected first-degree relatives, we applied the non-parametric Mann-Whitney U test.

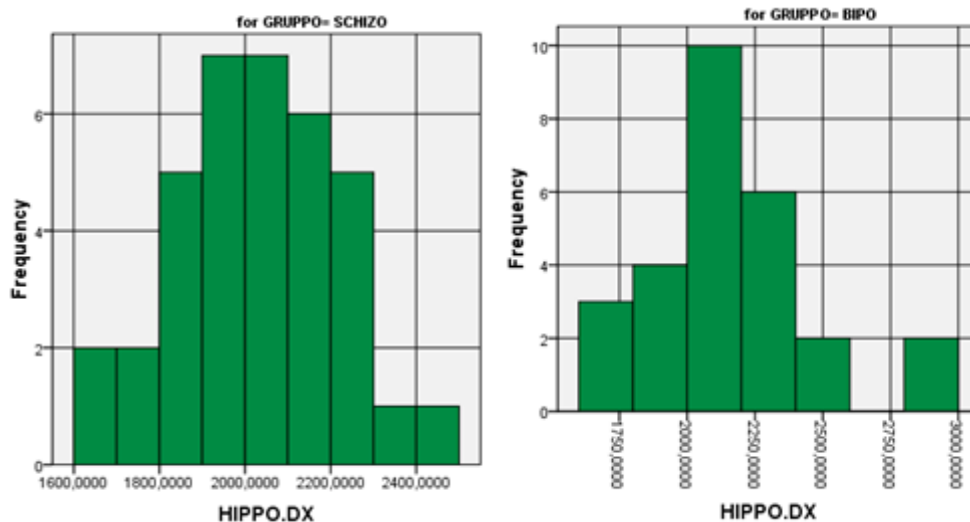


Figure 12. Right Hippocampus (HIPPO.DX in the picture) in the Schizophrenic and Bipolar group.

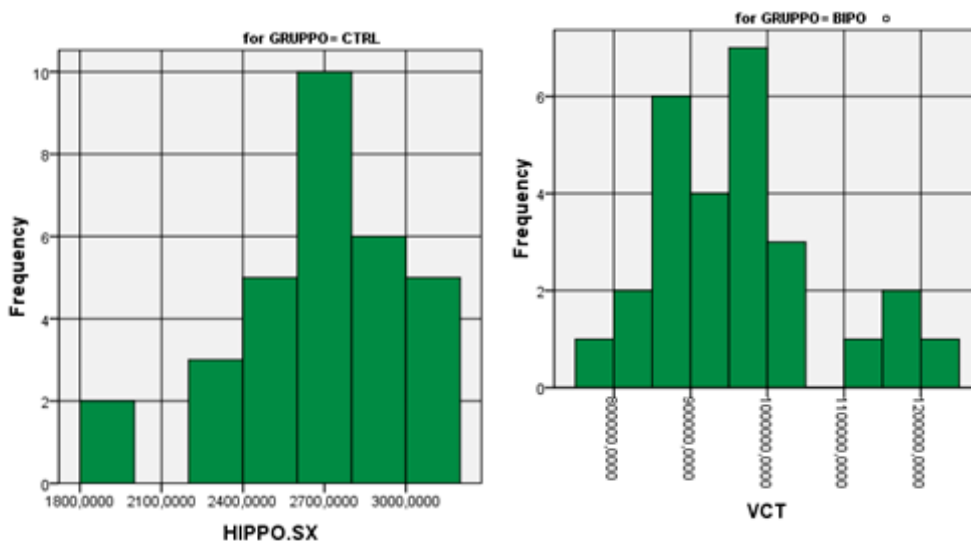


Figure 13. Left Hippocampus (HIPPO.SX in the picture) in the Control group and Total Cerebral Volume (VCT in the picture) in the Bipolar group.

An inter-rater and intra-rater reliability study was carried out by the two raters who calculated hippocampal gray matter (HV) and total cerebral volumes (TCV) and Intraclass Correlation Coefficients (ICC) were then calculated. For the inter-rater reliability, ICC resulted 0.94 for TCV, 0.90 and 0.91 for right and left HV respectively; intra-rater correlation coefficients were calculated for right (for the 2 raters respectively 0.94 and 0.96) and left (0.96 and 0.97) hippocampal gray matter volumes and for total cerebral volumes (0.96 and 0.96). The overall coefficient of error (CE) was 0.02.

To correct for individual variations in head size, we normalized the calculated hippocampal volumes by performing a ratio with total cerebral volumes ($HV \text{ in } mm^3 / TCV \text{ in } mm^3 \times 100$), as described in literature (Free et al. 1995; Whitwell et al. 2001; Rametti et al. 2007).

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.

3. RESULTS

3.1. DEMOGRAPHIC AND CLINICAL DATA

Our analysis did not report any statistically significant difference with respect to age between Schizophrenics ($t=-,33$; $p=,73$) or Bipolars ($t=-1,21$; $p=,22$) and controls, neither between schizophrenic and bipolar patients ($t=1,04$; $p=,30$), nor between the unaffected relatives and the control group ($Z=-0,38$; $p=,70$).

No statistically significant differences were noticed in education level between the two patient groups ($Z=-1,14$; $p=,30$) or between the unaffected relatives and the healthy controls ($Z=-1,45$; $p=,14$); education level differed significantly between the schizophrenic group ($Z=-4,12$; $p<,05$) or the bipolar group ($Z=-2,93$; $p<,05$) compared to the healthy controls.

Patients' between-group comparison did not reveal statistically significant differences with respect to age at onset ($t=,49$; $p=,62$) and total duration of illness ($t=,78$; $p=,43$).

With regard to symptoms severity, the respective scores, when directly comparable, did not differ between schizophrenic and bipolar patients (for HDRS: $t=,96$; $p=,35$; for HAS: $t=1,03$; $p=,31$; for BPRS: $Z=-,59$; $p=,60$).

3.2. VOLUMETRIC DATA

Results from the volumetric comparison analysis between the different groups are reported in the following tables (see also Fig. 14-16).

Right and left hippocampi demonstrated a statistically significant reduction in gray matter volume in the two patient groups compared to the control group. With respect to total cerebral volume, schizophrenic patients reached statistically significant reduction, whereas bipolar patients' total cerebral volumes did not differ significantly from the controls' ones (Tab. 3 and 4).

Table 3. Volumetric (mean \pm sd) comparison between Schizophrenics and Controls.

	VOLUMES (mm ³)		t-test	
	SCHIZO (n=31)	CONTROLS (n=26)	t	p
TCV	907417,13 \pm 103775,20	1011718,87 \pm 112704,08	3,94	< ,00
right HIPPO	2021,02 \pm 191,47	2769,20 \pm 336,90	10,9	< ,00
left HIPPO	1970,78 \pm 243,09	2696,28 \pm 312,51	10,6	< ,00
right HIPPO/TCV	0,2246 \pm 0,026	0,2756 \pm 0,036	6,58	< ,00
left HIPPO/TCV	0,2184 \pm 0,026	0,2684 \pm 0,035	6,65	< ,00

Table 4. Volumetric (mean \pm sd) comparison between Bipolars and Controls.

	VOLUMES (mm ³)		t-test	
	BIPO (n=27)	CONTROLS (n=26)	t	p
TCV	961419,38 \pm 113480,78	1011718,87 \pm 112704,08	1,69	,09
right HIPPO	2143,42 \pm 286,17	2769,20 \pm 336,90	7,56	< ,00
left HIPPO	1978,89 \pm 184,75	2696,28 \pm 312,51	10,4	< ,00
right HIPPO/TCV	0,2250 \pm 0,034	0,2756 \pm 0,036	5,37	< ,00
left HIPPO/TCV	0,2077 \pm 0,025	0,2684 \pm 0,035	7,46	< ,00

In the direct comparison between schizophrenic and bipolar patients, we identified statistically significant gray matter volume reduction in the schizophrenics' right hippocampus, but no significant between-group differences for left hippocampus or total cerebral volume. However statistical significance was lost after normalization (Tab. 5).

Table 5. Volumetric (mean \pm sd) comparison between Schizophrenics and Bipolars.

	VOLUMES (mm ³)		t-test	
	SCHIZO (n=31)	BIPO (n=27)	t	p
TCV	907417,13 \pm 103775,20	961419,38 \pm 113480,78	1,96	,054
right HIPPO	2021,02 \pm 191,47	2143,42 \pm 286,17	2,03	,046
left HIPPO	1970,78 \pm 243,09	1978,89 \pm 184,75	0,14	,885
right HIPPO/TCV	0,2246 \pm 0,026	0,2250 \pm 0,034	0,04	,962
left HIPPO/TCV	0,2184 \pm 0,026	0,2077 \pm 0,025	-1,64	,105

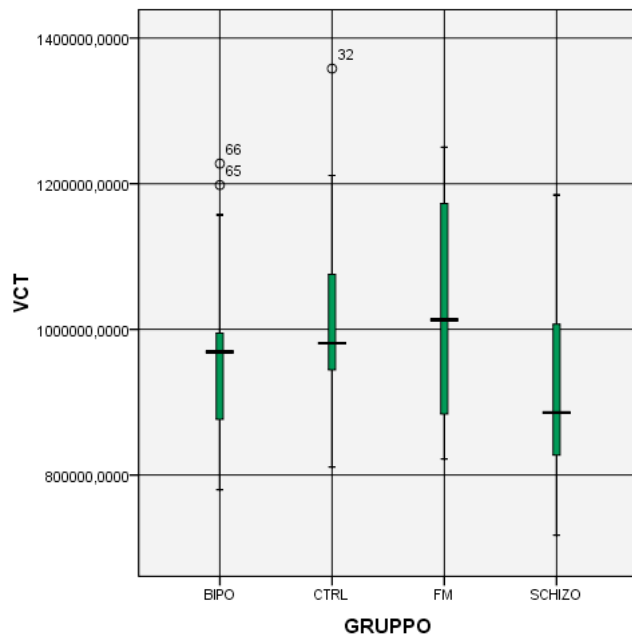


Figure 14. Total cerebral volumes (VCT in the picture) in the different groups (in the picture FM=unaffected relatives, CTRL=healthy controls).

Table 6. Volumetric (mean \pm sd) comparison between Unaffected Relatives and Controls.

	VOLUMES (mm³)		Mann-Whitney	
	RELATIVES (n=8)	CONTROLS (n=26)	Z	p
TCV	1026479,96 \pm 166361,13	1011718,87 \pm 112704,08	-0,10	,91
right HIPPO	2550,92 \pm 251,51	2769,20 \pm 336,90	-1,80	,07
left HIPPO	2450,37 \pm 173,36	2696,28 \pm 312,51	-2,57	,01
right HIPPO/TCV	0,2519 \pm 0,030	0,2756 \pm 0,036	-1,70	,08
left HIPPO/TCV	0,2427 \pm 0,032	0,2684 \pm 0,035	-1,80	,07

Comparison analysis between the unaffected first-degree relatives and the same control group (Tab. 6) revealed a statistically significant gray matter volume reduction in the left hippocampus and a trend towards statistical significance for right hippocampus; furthermore a trend towards statistically significant bilateral reduction in hippocampal gray matter volumes persisted after normalization.

No statistically significant differences in total cerebral volumes were found between the groups.

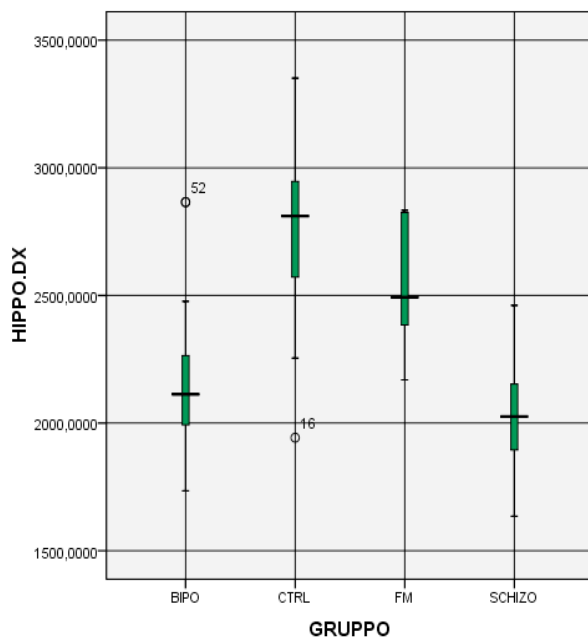


Figure 15. Right hippocampal volumes (HIPPO.DX in the picture) in the different groups (in the picture FM=unaffected relatives, CTRL=healthy controls).

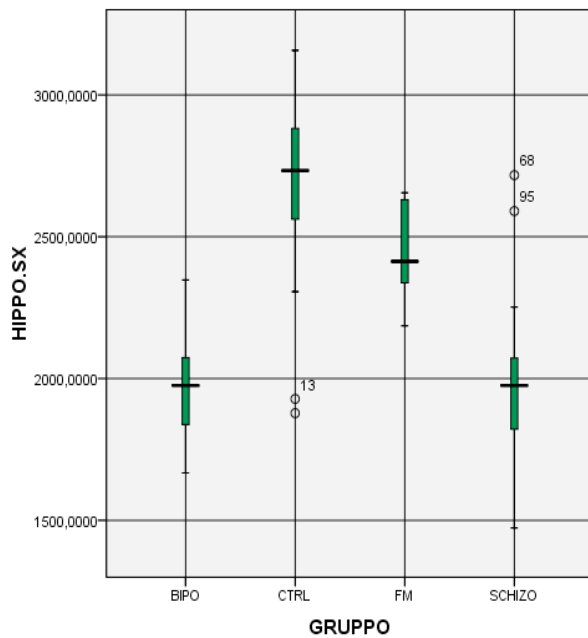


Figure 16. Left hippocampal volumes (HIPPO.SX in the picture) in the different groups (in the picture FM=unaffected relatives, CTRL=healthy controls).

4. DISCUSSION AND CONCLUSIONS

In the present study we aimed to investigate the presence of volumetric differences in gray matter hippocampal volumes and total cerebral volumes in a group of patients suffering from bipolar-schizophrenic spectrum disorders and in a little group of unaffected first-degree relatives, in comparison with matched healthy control subjects.

We identified statistically significant reductions in bilateral gray matter hippocampal volumes and total cerebral volumes in the schizophrenic patients compared to healthy subjects. Our data are in line with the majority of research papers, reviews and meta-analysis, which demonstrate significant reductions in hippocampal and total cerebral volumes in first-episode or chronic schizophrenia, and also individuals at high risk for schizophrenia (Steen et al. 2006; Wright et al. 2000; Shenton et al. 2001; Pantelis et al. 2005; Honea et al. 2005; Olabi et al. 2011; Chan et al. 2011).

In literature, there is a lot of interest concerning brain anomalies in Schizophrenia and, particularly, if such alterations in morphology and connectivity are expressions of neurodevelopmental or neurodegenerative phenomena (or possibly both of them).

It has been postulated that early (pre- and perinatal) neurodevelopmental lesions (e.g., viral infections during pregnancy or obstetric complications) (McNeil, Cantor-Graae, and Ismail 2000) render the brain vulnerable to anomalous late (particularly post-pubertal) neurodevelopmental processes (for example, the so-called "extended pruning"), and these anomalous neurodevelopmental processes interact with other causative factors associated with the onset of psychosis (e.g., substance use, stress, and dysregulation of the HPA axis function), which together have neuroprogressive sequelae involving medial temporal and prefrontal regions (Pantelis et al. 2005).

Excess glucocorticoids cause retraction and simplification of dendrites in the hippocampus and this morphological change probably accounts for the hippocampal volume loss. Mechanisms by which glucocorticoids affect the brain

include decreased neurogenesis and synthesis of neurotrophic factors (BDNF), impaired glucose utilization (given the Dentate Gyrus sensitivity to hypoglycemia), and increased actions of excitatory amino acids (glutamate) in CA3 pyramidal neurons (Yusim et al. 2000; Szeszko et al. 2006; Patil et al. 2007; Toffanin et al. 2011).

According to Andreasen and colleagues, some schizophrenic patients are undergoing a process of progressive brain change, defined as a decrement in brain tissue volume that is occurring at a more rapid rate in patients than in control subjects. Thus, schizophrenia has a “neuroprogressive” component, defined as tissue volume decrease occurring after onset. The gray matter loss is consistent with the known neuropathology of the illness, which involves cortical thinning, due primarily to shrinkage in neuropil, and without either the neuronal loss or gliosis that characterize neurodegenerative processes. Another mechanism that may explain the gray matter decreases occurring after the onset of schizophrenia is diminished neuroplasticity, an impairment in activity-dependent neuroplasticity that affects spines and synapses, leading to a shrinkage of neuropil (Andreasen et al. 2011).

Unexpectedly, our study found bilateral hippocampal gray matter reduction also in the bipolar group, compared to healthy controls.

In literature, with regard to hippocampal volumes, data emerging from ROI-based or VBM studies are extremely variable: the majority of them failed to detect significant volume differences between bipolar patients and healthy controls (Hauser et al. 2000; Altshuler et al. 2000; Brambilla et al. 2003; Strasser et al. 2005; Adler et al. 2007; Lim et al. 2013). According to Frey and colleagues, the possibility that only specific subtypes of bipolar disorder are associated with structural brain abnormalities might, at least to some extent, explain the lack of positive findings of hippocampal volume changes in the disease (Frey et al. 2007). Nevertheless, some studies support the existence of volumetric changes in the hippocampus of BD patients. For instance, Swayze and colleagues, comparing 48 bipolar patients to 47 healthy controls, found that the right hippocampus was

significantly smaller in patients, particularly in men (Swayze et al. 1992). Differences in hippocampal volume were also found in studies focusing on pediatric, as well as on older, bipolar patients. Blumberg et al. (2003) studied 36 bipolar type I patients (14 adolescents and 22 adults) and 56 healthy controls (23 adolescents and 33 adults). Considering adolescents and adults together, there was a decrease of 5.3% in bilateral hippocampal volumes in bipolar patients, although this difference did not reach statistical significance. An age-group comparison (BD adolescents versus HC adolescents), nevertheless, showed that hippocampal volume was significantly decreased in bipolar adolescents, whereas this difference was not observed between adult bipolars versus adult controls. Frazier and colleagues compared 43 adolescents suffering from bipolar disorder with 20 healthy controls and found that bipolar patients had smaller hippocampal volumes, and this effect was predominantly driven by female gender (Frazier et al. 2005).

Recently, Chepenik and colleagues reported hippocampal decrease in two different studies comparing bipolar patients and healthy controls. They found that hippocampal volumes were significantly smaller in bipolar compared to control subjects and, analyzing the *BDNF val66met polymorphism*, the presence of the BDNF met allele was associated with smaller hippocampal volumes in both diagnostic groups: moreover the bipolar subgroup who carried the BDNF met allele had the smallest hippocampi (Chepenik et al. 2009; 2012).

In a recent study, comparing patients with borderline personality disorder, patients with bipolar disorder and healthy controls, Rossi and colleagues reported that the bipolar group showed significantly smaller right hippocampal volume compared to the control group: specifically, using 3D surface mapping, alterations were localized in the right dentate gyrus (Rossi et al. 2012).

In line with the above findings, it could be speculated that hippocampal volume reduction in bipolar disorder — likewise in recurrent major depression (Nifosì et al. 2010) — may be due to chronic stress response. Actually, dentate gyrus-dependent inhibition of the stress response plays an important role in mood disorders. During stress, hippocampal projections traversing the fimbria inhibit

the HPA axis. A very recent study, aiming to measure the volumes of the Dentate Gyrus, Cornu Ammonis and fimbria in patients with Bipolar Disorder type II and healthy controls, reported a smaller left CA2-3 volume in bipolar subjects and a reduced left fimbria volume in unmedicated patients compared to medicated patients and controls (Elvsåshagen et al. 2013).

Various neuropathological post mortem studies support the thesis of a specific hippocampal involvement in Bipolar Disorder. Benes and colleagues found decreased number, density, and size of nonpyramidal neurons in CA2 and a similar trend in the CA3 region (Benes et al. 1998); Liu et al. stained hippocampal tissue sections from CA1 area, and found that BD patients had a significant 12% reduction in pyramidal somal size (Liu et al. 2007). Other morphological studies suggest that GABAergic and glutamatergic abnormalities might take place in discrete subregions of the hippocampal formation in individuals with bipolar disorder (Frey et al. 2007). Heckers and colleagues found decreased density of GAD (glutamic acid decarboxylase) 65 and GAD 67 mRNA-positive neurons in the hippocampus of bipolars, suggesting changes in GABA synthesis or loss of GABAergic cells (Heckers et al. 2002). Physiologically, hippocampal GABAergic interneurons play a critical role in feedback and feedforward inhibitory mechanisms, thereby controlling the excitability of pyramidal neurons, to coordinate neuronal transmission and avoid excessive firing (Buzsáki 1997). Disruption of hippocampal GABAergic and glutamatergic neurotransmission might lead to abnormal brain responses to stress (McEwen 1999). Conversely, the hippocampus is particularly vulnerable to damage after repeated stress (Sapolsky 1996).

These above described studies further indicate that bipolar disorder might be associated with abnormal glutamatergic transmission and synaptic plasticity in the hippocampus and its closely related regions. In each of the four cornu ammonis sectors (CA1-4) of the hippocampus, GABAergic interneurons are interspersed with a much larger number of glutamatergic principal neurons, but a single interneuron provides inhibition through 1000 to 2000 synapses with principal neurons. Hippocampal GABAergic neurons are classified basing on the

expression of calcium-binding proteins, such as parvalbumin, calbindin, and calretinin, and neuromodulators, such as somatostatin, neuropeptide Y, vasoactive intestinal peptide, and nitric oxide synthase. These “markers” identify subtypes of hippocampal interneurons with distinct morphological, physiological, and molecular properties. Konradi and colleagues demonstrate that there is strong evidence for a marked reduction of somatostatin- and parvalbumin-positive interneurons in bipolar disorder. They claim that the finding of significantly reduced nonpyramidal cell layer volume in bipolar disorder provides compelling evidence for a subtle volume difference of the hippocampus, beyond the resolution of current imaging studies. Given the reduced number of immunopositive interneurons they interpret the pattern of volume change observed in the subjects with bipolar disorder as further support for hippocampal interneuron pathology in bipolar disorder (Konradi et al. 2011).

Rajkowska pointed out that the neuropathological changes observed in Bipolar Disorder resemble neurodevelopmental rather than neurodegenerative pathology (Rajkowska 2003). This assumption is in accordance with recent reports showing reduced hippocampal reelin, a protein that is involved in neuronal migration during brain development (Knable et al. 2004).

Konradi et al. (2011) did also observe a significant decrease in the size of hippocampal neurons, most pronounced in sector CA2-3, in line with a similar report of decreased pyramidal cell size in CA1 in bipolar disorder. They argue that the somal size of adult hippocampal neurons could be a distal read-out of neurodevelopmental abnormalities or a more proximal consequence of malfunction of trophic factors and synaptic remodeling during adulthood. Risk genes associated with psychotic disorders, including DISC1 and neuregulin, have been associated with regulation of neuronal size (Duan et al. 2007; Krivosheya et al. 2008). In line with the previously described evidences in relation to schizophrenia, there is converging evidence demonstrating that hippocampal impairment is present early in the course of Bipolar disorder, suggesting that such changes are more likely to be related to constitutive — genetic or neurodevelopmental — rather than to chronicity or to treatment effects.

In contrast with our preliminary data, when directly comparing the two separate groups of patients, we only found a moderate statistically significant difference in schizophrenics' right hippocampus, and such significance was lost after normalization. Even if both schizophrenic and bipolar patients demonstrated statistically significant reductions in bilateral hippocampal gray matter when compared to healthy control subjects, they did not reveal marked specific differences when compared to each other.

Although it's quite recognized that Schizophrenia and Bipolar disorder have a number of overlapping symptoms and risk factors, it is not yet clear if the disorders are characterized by similar deviations in brain morphometry or whether any such deviations reflect the impact of shared susceptibility genes on brain structure. Different meta-analysis reported that the areas of reduced gray matter observed in studies of bipolar patients overlapped substantially with areas of reduced gray matter in schizophrenia patients, though the reductions seen bilaterally in the hippocampal-amygdala area among schizophrenia patients were not observed in bipolar patients (Ellison-Wright and Bullmore 2010) and the latter demonstrated enlarged amygdala volumes (Arnone et al. 2009). Studies in literature may be contradictory because of between-study heterogeneity in the patient and control groups in terms of medication use and demographic and clinical variables.

There is good evidence to suggest medication may affect brain structure: cross-sectional and longitudinal studies have suggested lithium increased gray matter volume (particularly in the hippocampus) possibly through its neurotrophic effects (Kempton et al. 2008). The impact of drugs on brain structures is a matter of debate (Germaná et al. 2010). However, it is not clear whether the potential effect on brain morphology is stable over time or reversible after switching or discontinuing medications. In neuroimaging studies, the potential confounding effect of medications is a major issue and this is especially problematic for studies of complex forms of pathology such as Schizophrenia or Bipolar Disorder, in which the majority of individuals may be receiving psychotropic medication (Phillips et al. 2008; Hafeman et al. 2012; Hajek et al. 2012). We could speculate

that negative findings in literature may be also due to the confounding factor of lithium or anticonvulsants medication.

Similarly to Brown and colleagues' study (Brown et al. 2011), our schizophrenic patients showed smaller gray matter volumes in the right hippocampus when compared to bipolar patients; on the contrary, Brown et al. did not report any hippocampal differences when bipolar subjects were compared to controls.

The significant results in our bipolar patients compared to controls might be explained considering the patients' demographical and clinical variables.

First, global disease severity and functioning: as highlighted by the GAF scoring, also the bipolar group suffered from moderate to serious illness with regard to psychological, social and occupational functioning. Clinically, even if they were assessed in a stable phase, most of them presented multiple mood episodes and in some cases psychotic symptoms along the course of the disease; furthermore, given the quite early age at onset, they presented sustained a long duration of illness.

Some morphometric studies comparing psychotic bipolar patients to healthy controls occasionally reported volumetric reductions in the subgenual cingulate cortex (Hirayasu et al. 1999) and left hippocampus (Velakoulis et al. 1999).

In a longitudinal study, Moorhead and colleagues found that patients with bipolar disorder showed a greater decline in hippocampal gray matter density over 4 years than control subjects. Reductions in temporal lobe gray matter correlated with the number of intervening mood episodes over the follow-up period (Moorhead et al. 2007). These results imply that the decrease in hippocampal volume in bipolar disorder may be depending on the severity of the disease. The previously cited article by Hajek et al. (2012) provide indirect support for neuroprotective effects of Lithium but negative effects of illness burden on hippocampal volumes in bipolar disorders.

Another possible confounder is education: the two patient groups had comparable levels of education, but they showed significantly lower levels of education than the healthy controls.

Moreover, contradicting evidences and heterogeneity in structural MRI findings in literature can be influenced by various factors including scan acquisition parameters, image quality, hardware/software employed in the tracing procedure and type of segmentation technique (manual or automatic) (Geuze, Vermetten, and Bremner 2005). It is currently not clear whether the negative findings in the bipolar disorder literature are a result of sample selection, medication biases, technical issues or represent a true biological pattern (Yüksel et al. 2012).

In the past years, given the difficulty to separate amygdala from the hippocampus, the majority of studies combined amygdala and hippocampus in the "amigdalo-hippocampal complex" and then perform an approximate separation, attributing the anterior part to amygdala and the rear part to the hippocampus (Hirayasu et al. 1998; Lawrie et al. 1999).

It is possible that, in our study, better spatial resolution in the acquisition of imaging data and the use of the stereological method are more likely to detect discrete hippocampal atrophy. In fact, we obtained high coefficients of inter-rater and intra-rater reliability, in the specific gray matter delimitation of Dentate Gyrus, Cornu Ammonis and Subiculum; moreover, by using the "orthogonal" tool we were able to work on three orthogonal perspectives simultaneously, thus facilitating the delineation of difficult boundaries (e.g., the transition of the hippocampus to the amygdale in the anterior slices).

The results from the comparison analysis between the unaffected first-degree relatives and the healthy control group are very interesting. We revealed a statistically significant gray matter volume reduction in the left hippocampus, a trend towards statistical significance for right hippocampus and no statistically significant differences in total cerebral volumes between the groups; moreover the trend towards statistically significant bilateral reduction in hippocampal gray matter volumes persisted after normalization.

To date, there is no consensus about whether, and to what extent, gray matter loss in Schizophrenia is mirrored in Bipolar Disorder and what is the effect of

medication or other confounding factors. Studies in family members of patients, who share the risk of the disease but not the confounding factors, may help elucidate whether abnormalities in brain structures are shared by both illnesses. In literature, hippocampal volume reductions and some other morphometric deviations associated with schizophrenia have also been noticed in unaffected first-degree relatives of patients (Seidman et al. 2002); a meta-analysis of studies comprising first-degree relatives of schizophrenia patients and healthy controls confirmed reductions in whole brain and hippocampus volume (Boos et al. 2007). By contrast, unaffected relatives of patients with bipolar disorder have been rarely studied (McDonald et al. 2006). A recent meta-analysis (Fusar-Poli et al. 2012) failed to detect significant differences in amygdala and hippocampal volumes in individuals at high risk for bipolar disorder. However, results of meta-analysis should be interpreted with caution since conflicting data might also be a consequence of heterogeneity across studies concerning different MRI scanners, different acquisition protocols, small sample sizes and different inclusion criteria for the high-risk cohorts.

The genetic inheritance of bipolar disorder and schizophrenia is complex: it is most likely to be polygenic rather than due to genes of major effect. Since unaffected relatives of patients with bipolar-schizophrenic spectrum disorders are likely to share some susceptibility genes with affected patients, without overt expression of the clinical phenotype, structural abnormalities detectable in high-risk individuals but not in controls may reflect genetically driven trait-related deficits for the disorder. Sometimes the same abnormalities may also be evident both in the unaffected and in the patient groups, suggesting the possibility of genetic risk factors shared by both groups (Fusar-Poli et al. 2012).

Studying unaffected first-degree relatives gives the advantage that the detected abnormalities cannot be confounded by medication or other illness-related factors; conversely, studies of the unaffected relatives are limited by the fact that they cannot discriminate between genetic and shared environmental influence. Anyway, examination of the unaffected relatives of patients is a valid

mean to study the relation between increased genetic risk and brain abnormalities, since these people carry the genetic risk for the disease but not the disease itself. In addition, studies in unaffected twins and first-degree relatives of probands might help to hypothesize putative endophenotypes for such complex multifactorial disorders. Following Gottesman and Gould's rationale, the reasonable criteria for valid endophenotypes are the following: 1) the endophenotype is associated with illness in the population; 2) the endophenotype is heritable; 3) the endophenotype is primarily state-independent (manifests in an individual whether or not illness is active); 4) within families, endophenotype and illness co-segregate; 5) the endophenotype found in affected family members is found in non-affected family members at a higher rate than in the general population (Gottesman and Gould 2003; Hasler et al. 2006; Braff et al. 2007).

Interpreting the results of our study, we should take into account some limitations and confounding factors. First, for the patient group, we could not be able to rule out the confounding factor of psychotropic medication, in particular lithium and first- or second-generation antipsychotics. Second, the small sample size of the unaffected relatives could limit the statistical power of our comparison.

In conclusion it might be speculated that the alterations of the gray matter volume in the hippocampus highlighted in our study could be interpreted as a possible structural "biological marker" in the schizophrenic-bipolar spectrum.

Our future perspectives aim to increase the sample of unaffected first-degree relatives and to investigate possible correlations between the identified structural alterations and polymorphisms of candidate genes (e.g. NTK3, BDNF, COMT, GSK3), in order to verify the endophenotype hypothesis and, therefore, the speculation of a common genetic substrate for the spectrum.

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