STRUCTURAL BRAIN IMAGING IN SCHIZOPHRENIA: CONTEMPORARY ISSUES

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Abstract

Our contemporary concept of schizophrenia represents an evolution of Kraepelin's 'dementia praecox'. Kraepelin believed that he had identified a natural disease entity reflecting a single underlying morbid process. However, the heterogeneity of symptoms and course, together with the absence of any pathognomonic biological markers for the disorder, has led critics to question the validity of schizophrenia as a scientific construct. In the first section of this thesis structural brain imaging is presented as a powerful tool for investigating the biological correlates of schizophrenia, thereby informing the ongoing evolution of the concept. The major pathophysiological hypotheses of schizophrenia are discussed from the perspective of structural brain imaging. The contemporary literature is then reviewed before three potential strategies for future structural imaging research are presented: (1) imaginative clinical study design employing carefully selected sub-groups from within the schizophrenic population; (2) adoption of newly developed approaches to MRI data acquisition and analysis; (3) utilisation of novel structural imaging techniques. In the second part of this thesis three studies are presented, each illustrating one of the strategies described above.

Study 1: Structural magnetic resonance imaging (MRI) of the brain in presumed carriers of gene(s) for schizophrenia, their affected and unaffected siblings.

The aim of this study was to establish if the gene(s) for schizophrenia are associated with specific abnormalities of brain structure. Six sib-ships from multiply affected families were recruited. Each sib-ship consisted of one patient with schizophrenia, one 'obligate carrier' without the disorder but with an affected child and one 'non-affected non-carrier'. MRI was conducted with a semi-automated region of interest analysis. Between-group comparisons were tested by repeated measures analysis of variance. Reductions in volumes of cortical structures and of whole brain were found only in schizophrenics and therefore appear to be associated with phenotype. In contrast, reduced volume of the amygdalo-hippocampal complex (AHC) was found in both schizophrenics and obligates and therefore appears to be associated with genetic risk for the disorder even in the absence of disease. Reduced AHC volume may therefore represent an endophenotypic marker for schizophrenia.

Study 2: A voxel based morphometry (VBM) and region of interest (ROI) analysis of the genotypic and phenotypic neuroanatomy of schizophrenia.

The aim of this study was to explore the likely impact of a new method of data analysis upon future structural brain imaging research. The MRI data set from study 1 (above) was analysed using a voxel-based statistical technique (VBM) and a conventional ROI approach. The results obtained by the two methods were then compared. Overall, the results obtained by VBM were compatible with those obtained by ROI. However, the extent of the overlap varied according to the statistical methods employed. Reassuringly, maximal agreement was found when the 'optimal', most methodologically appropriate, VBM analysis was compared with the 'optimal' ROI analysis (from study 1).

Study 3: Diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (^{1}H-MRS) in schizophrenic subjects and normal controls.

This study was included to illustrate the challenges inherent in the adoption of novel imaging techniques. The study was designed to identify anatomical correlates of functional dysconnectivity between the pre-frontal and temporal regions in schizophrenia. Ten patients with DSM-IV schizophrenia were compared with ten healthy controls. Diffusion anisotropy and NAA concentrations were measured in the same pre-frontal white matter region. DTI revealed no differences in diffusion anisotropy between schizophrenics and controls suggesting intact white matter cytoarchitecture. ^{1}H-MRS revealed non-significant but consistently reduced NAA concentrations (by 10-15%) in the pre-frontal white matter in schizophrenic subjects suggesting abnormal function of structurally intact neurons.

Declaration of Authorship

I, Dr Robby Steel hereby declare that:

(a) This thesis has been composed by me.

(b) None of the work in this thesis has been submitted in candidature for any other degree, diploma or professional qualification.

(c) The work it describes was conducted by me, in collaboration with a number of research workers from the University of Edinburgh, Department of Psychiatry and elsewhere. Those parts of the work which were conducted by other researchers are clearly identified in the acknowledgements section of this thesis.

Details of my contribution to each study are outlined below:

Study 1- review over 250 family-trees drawn up for Edinburgh High Risk Study for presence of obligate carriers; contact family members (with consent of general practitioner); obtain informed consent from subjects; complete structured interview incorporating PSE and SADS-L on all subjects; accompany all subjects to MRI scan; design and conduct appropriate statistical analyses of volumetric data (in collaboration with Patrick Miller);

write first and subsequent drafts of paper (incorporating contributions and comments from fellow authors); write chapter 2A of thesis.

Study 2 – advise on covariates to be used in optimised analysis; advise on anatomical location of SVC analysis; design and conduct appropriate statistical analyses of raw VBM data (in collaboration with Patrick Miller); write first draft of paper (incorporating contributions and comments from fellow authors); write chapter 2B of thesis.

Study 3 – contribute to study design; obtain ethical approval; identify and contact suitable subjects and controls; obtain informed consent from subjects and controls; complete structured interview incorporating SADS-L on all subjects; accompany all subjects and controls to MRI scan; design and conduct appropriate statistical analyses of volumetric data (in collaboration with Patrick Miller); write first and subsequent drafts of paper (incorporating contributions and comments from fellow authors); write chapter 2C of thesis.

Signed.....

Date.....10/6/06.....

Preface

This thesis would not have been possible without the good will of the volunteers who kindly agreed to answer innumerable questions and to lie in a noisy magnetic resonance imaging (MRI) scanner. I am greatly indebted to everyone who took part.

Prior to consenting, each potential subject was offered a comprehensive written and verbal explanation of the rationale behind the study and of the likely practicalities of participation. On signing the consent form, one of the volunteers asked "so this machine will take a picture of my brain which will show my schizophrenia – is that right?" To my eternal shame I answered "yes." The problem with my answer lies not in the description of an MRI scan as a "picture of the brain", but in the intimation that schizophrenia exists as a physical entity amenable to imaging. Schizophrenia is a concept, a collection of ideas and theories, which attempts to encapsulate a host of observations and subjective experiences. An MRI scan can no more "show" a person's schizophrenia than a DNA sequence can "show" his humanity.

This is not to say that "pictures of the brain" are irrelevant. In this thesis I outline the role that structural brain imaging has played in advancing our understanding of schizophrenia. I also describe three of my own studies that illustrate the use of imaging as a research tool. However, the utility of this body of research is inevitably limited by the unsatisfactory nature of the concept it seeks to elucidate. I shall therefore begin my thesis by outlining the origins and evolution of contemporary operational definitions of schizophrenia.

Acknowledgements

I would like to reiterate my deeply felt thanks for all the people who agreed to participate as subjects in my studies. I would also like to thank the representatives of the Theodore and Vada Stanley Foundation and of the Schizophrenia Research fund who agreed to provide financial support for this research. The studies would not have been possible without their help.

I must also acknowledge the considerable contributions made by all of my collaborators, most notably:

Heather Whalley for extracting the volumetric Region of Interest data presented in Chapter 2A.

Dr Emmanuel Stamatakis for conducting the Voxel-Based Morphometry analyses described in Chapter 2B.

Dr Mark Bastin and Dr Ian Marshall for devising the scanning potocols and conducting the complex mathematical analyses described in Chapter 2C.

A number of my colleagues not only contributed to the studies described in this thesis but also provided me with invaluable advice during the writing up stage. Professor Eve Johnstone acted as my formal advisor; Dr Stephen Lawrie played a central role in the design of the studies described in this thesis and acted as my informal advisor; Professor Jonathon Best was a valuable source of advice on radiological matters and provided a number of the images included in this thesis; and Dr Patrick Miller provided statistical advice.

Whilst composing this thesis, I have been fortunate to receive additional advice and support from friends and colleagues who were not directly involved in the studies it describes. Professor Robert Kendell lent the benefit of his considerable experience to chapters 1A and 1B. My father, Professor Michael Steel provided constructive comments from a (non-psychiatric) medical perspective and also did his best to prevent me from taking grammatical liberties with the English language.

Finally I must thank my long-suffering wife, Sarah, who has been an M.D. widow for the best part of a year. I do not know how to make it up to her but I shall begin by not asking her to read the thing.

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1A. Origins of the Contemporary Concept of 'Schizophrenia'

Kraepelin and Dementia Praecox

Although the term 'Schizophrenia' is conventionally credited to the Swiss psychiatrist Eugen Bleuler[1], modern operational definitions such as those listed in DSM-IV[2] and ICD-10[3] (see figures 1Avi and 1Avii) owe more to the work of Bleuler's German contemporary Emil Kraepelin. The modern concept of schizophrenia is an evolution of Kraepelin's concept of 'Dementia Praecox'[4]. Kraepelin asserted that a "tangible morbid process"[4] was responsible for the syndrome that he had described. He believed that, with time, the pathology underlying dementia praecox would be revealed. All subsequent research into the biological correlates of schizophrenia can be regarded as contributing to the search for Kraepelin's "tangible morbid process". An appreciation of the environment in which Kraepelin worked, the methods that he employed and the way in which his ideas evolved are therefore prerequisites for any meaningful interpretation of contemporary research in this field.

As we enter the 21st century, medical practice strives to be 'evidence-based'. However, 19th century medicine could more accurately be described as 'eminence-based'. It was de rigueur for the ambitious clinical academic to establish his reputation by describing a new syndrome (which was frequently given an eponymous epithet). The core clinical skills of history taking and examination were held in high regard and scientific papers consisted of detailed clinical case reports. The 'Medical Model' proposed that a group of patients with similar symptoms and signs were

likely to share a common underlying pathology. The first step in understanding any disease process was the accurate identification and description of the clinical syndrome.

By the second half of the 19th century the rigorous application of this medical model was proving highly productive. The relatively new sciences of microbiology and histopathology were making enormous advances. The accepted wisdom amongst medical academics decreed that signs and symptoms resulted from "disease entities" each of which resulted from a single necessary and sufficient causative agent. The discovery, by Robert Koch, of the Anthrax and Tubercle Bacilli reinforced this view. Also described during this period were the now familiar syndromes of 'Broca's aphasia', 'Wernicke's encephalopathy' and 'Korsakoff's psychosis'. In 1863, Rudolph Virchow, Professor of pathology in Berlin, stated confidently "all disturbances of function and structure in disease are due to cellular abnormalities . . . the phenomena of a particular disease are brought about by a series of cellular changes"[5].

Meanwhile psychiatry was still in the process of securing its position as a reputable medical specialty. Academic psychiatry was in its infancy. Although lecturing in psychiatry had begun with Johann Heinroth in 1811 in Leipzig and with Jean-Etienne Esquirol in 1817 in Paris, the first autonomous University Department was not established until 1832 at the Charité Hospital in Berlin[6]. What we would now recognise as the modern model for an academic department (a department situated close to other medical departments, dedicated to teaching and research where

professors could admit patients for those purposes) was first established by Wilhelm Griesinger, professor of psychiatry in Berlin from 1865-1868. In his preface to the first edition of *Archive for Psychiatry and Nervous Diseases* (the world's first biological psychiatry journal) in 1867, Griesinger wrote "psychiatry has undergone a transformation in its relationship to the rest of medicine, this transformation rests principally on the realisation that patients with so-called 'mental illnesses' are really individuals with illnesses of the nerves and the brain" [7].

Griesinger and his contemporaries such as Ludwig Meyer in Hamburg and Theodor Meynert in Vienna subjected post-mortem specimens of their deceased patients' brains to pathological and histopathological investigation. The primary aim of this research is clearly stated in Meynert's text of 1890 "the more that psychiatry seeks, and finds, its scientific basis in a deep and finely grained understanding of the anatomical structure of the brain, the more it elevates itself to the status of a science that deals with causes."[8] The early biological psychiatrists had some notable successes. The brain pathology associated with the syndrome of 'dementia paralytica'[9], first described by Esquirol in 1814, was gradually elucidated (although direct evidence that the syndrome, now called tertiary syphilis, was caused by syphilitic spirochetes in the brain was not published until almost 100 years later[10]). The gross and microscopic brain changes associated with senile dementia were also identified (although Alzheimer's seminal paper[11] was not published until 1906). Unfortunately most psychiatric patients did not appear to have any demonstrable brain pathology.

Academic psychiatry was faced with a dilemma: if as Griesinger stated "patients with so-called 'mental illnesses' are really individuals with illnesses of the nerves and the brain" then why were researchers unable to identify pathological changes in the brains of many sufferers? The majority of biological psychiatrists were presumably confident (as we are today) that technological progress would ultimately produce techniques capable of revealing the biological correlates of insanity. However, the first step in the successful application of the medical model is the identification of clinical syndromes, that is groups of patients with similar symptoms and signs. Rather than wait passively for advances in laboratory science, a number of 19th century psychiatrists set about the task of defining clinical syndromes and devising systems of classification. Many of the nomenclatures proposed were based upon highly speculative aetiological assumptions. The result was a plethora of imaginatively titled syndromes and classificatory chaos. Kraepelin's principle achievement was that he brought order to this chaos.

Kraepelin was something of a psychiatric polymath. After graduating from the University of Würzburg in 1878, he worked in Munich for four years as resident to the brain biologist Bernhard von Gudden. Here he trained in neuroanatomy and neuro-histopathology but was unable to participate fully in experimental work because an eye problem prevented him from spending long periods looking down microscopes[12] (unlike his friend Alois Alzheimer and fellow resident Franz Nissl). While in Munich, he became interested in the work of experimental psychologist Wilhelm Wundt and in 1882 he joined Wundt's newly founded psychological laboratory in Leipzig. In 1883, at the age of 27, he wrote his first textbook[13]

(apparently because he intended to marry and needed the money[14]). He then worked as an asylum physician for two years before being appointed to the chair of psychiatry at the University of Dorpat in Estonia. Kraepelin did not speak Estonian and was therefore not in a position to make detailed studies of patients' psychopathology. Instead, he developed an interest in physical signs (such as abnormal movements) and in the longitudinal course of psychiatric illness. In 1890 he was appointed professor of psychiatry in Heidelberg where he continued his work on illness trajectories. He was also instrumental in bringing Alzheimer and Nissl to Heidelberg and was an important collaborator on Alzheimer's studies into the histopathology of dementia[15]. From 1904 to1922 he was professor of psychiatry in Munich.

The principal tool that Kraepelin employed in his clinical studies was the 'data card'[16]. The clinical details of each patient were recorded on a card at the time of admission. The card was then placed in a 'diagnosis box'. Once Kraepelin and his colleagues had had time to study the patient, they would take the card from the box and add the revised diagnosis together with an explanation. This process would be repeated when the patient was discharged. Kraepelin also kept a separate list of the names and discharge diagnoses of all of his patients. Kraepelin explained the rationale behind his cards and lists "in this manner we were able to get an overview and see which diagnoses had been incorrect and the reasons that had led us to this false conception"[17]. The clinical observations contained on the cards included most of the components of a modern mental state examination such as abnormal appearance, movements, speech, affect, thoughts and perceptions as well as an

assessment of cognitive ability. In 1893 Kraepelin incorporated his observations into the fourth edition of his textbook. In the preface he explained that data gathered from patients underpinned every assertion that he made and that his ultimate aim was to "try to cut nature at the joints: to identify natural disease entities."[18] By 1919 when Kraepelin published the eighth edition of his textbook, he and his team had gathered longitudinal data from over a thousand patients[19].

Kraepelin's classification evolves with each successive edition of his Lehrbuch. Many of the concepts are borrowed from other eminent psychiatrists of the era, Kraepelin's contribution is that he synthesises them into a workable model. The term 'dementia praecox'[20] first appears in the fifth edition (1896), whilst the central tenant of his legacy (that the 'functional' psychoses can be divided into two disorders: manic-depressive psychosis and dementia praecox)[21] is first proposed in the sixth edition (1899).

Despite Kraepelin's stated reliance upon empirical clinical data, the systems of classification described in the third and fourth editions do not stand out from those proposed by his contemporaries. The focus is on symptoms and the groupings are arranged according to presumed brain pathology. Kraepelin does, intriguingly, introduce the concept of a "psychic process of degeneration"[22] leading to, amongst other things, "premature dementia". In the fifth edition the idea of degeneration is abandoned, Kraepelin describes dementia praecox as a "metabolic disorder". He subdivides dementia praecox into mild and severe forms, and 'hebephrenia' whilst classifying 'catatonia' and 'dementia paranoides' separately. This edition marks a

decisive shift in Kraepelin's method - "I have abandoned any effort to classify [psychosis] on the basis of the clinical presentation."[20] He also acknowledges the problems inherent in classifications based upon putative environmental causes (such as 'masturbatory insanity'[23] and 'wedding-night psychosis'[23]) - "as long as we are unable clinically to group illnesses on the basis of cause, and to separate dissimilar causes, our views about aetiology will necessarily remain unclear and contradictory."[20] Instead he argues that the "inner nature" of psychiatric disorders is "manifest in their course and outcome"[20].

In the sixth edition Kraepelin proposes a classification system dividing psychiatric illness into 13 major groups, each of which represents a distinct clinical picture unfolding and elaborating over time "each according to its own fundamental law"[4]. One group, called 'dementia praecox' incorporates the previously described syndromes of 'hebephrenia', 'catatonia' and 'dementia paranoides'. Kraepelin justifies this grouping on the basis that each syndrome typically affects patients in early life and that the course of each is inevitably chronic and deteriorating. He argues that these three syndromes are all manifestations of a common endogenous brain disorder (either metabolic or possibly endocrinological in nature)[4]. The symptoms themselves represent a "disruption of mental operations". Another group, called 'manic depressive psychosis' incorporates 'mania', 'melancholia', 'periodical insanity' and 'circular psychosis'. Kraepelin writes of this grouping, "in the course of the years I have become more and more convinced that all are really just manifestations of a single disease process."[4] As in previous editions of his Lehrbuch, Kraepelin cites evidence from his data cards to support his arguments.

It is clear from the sixth edition of his textbook that Kraepelin recommended making diagnoses according to the overall clinical picture. By way of illustration he presents detailed accounts of his patients' symptomatology together with precise definitions of key psychopathological terms. He suggests that symptoms are central to diagnosis and stresses that a diagnosis ought to have prognostic value, "the doctor's first task at the bedside is being able to form a judgement about the probable further course of the case. People always ask him this. The value of a diagnosis for the practical activity of the psychiatrist consists of letting him give a reliable look at the future."[4] The notion that Kraepelin differentiated his cases purely on the basis of course is a misconception. Rather he *observed* the differences in outcome and used this observation as evidence in support of his theory that the two psychoses reflect two distinct pathological processes, that each is a "natural disease entity".

Clearly Kraepelin did not work in a scientific vacuum. As noted above, many of his contemporaries proposed their own classification systems and Kraepelin incorporated many of their ideas into his own work. He continued to evolve his classification system in the seventh and eighth editions of his Lehrbuch (and the ninth which was published posthumously)[24,25]. Figure 1Ai below shows the symptoms of dementia praecox as listed in the eighth edition of Kraepelin's Lehbruch[19]. The enduring influence of his work can probably be attributed to the insights contained in the sixth edition, most notably the emphasis upon course and outcome as the keys to the "inner nature" of psychiatric disorders.

Figure 1Ai - **Symptoms described by Kraepelin as Characteristic of Dementia Praecox**

Hallucinations

- Unpleasant voices
- Voices that comment on the thoughts and doings of the patient
- Commanding voices
- Patient's own thoughts spoken aloud
- Somatic tactile hallucinations
- Sexual tactile hallucinations
- Olfactory hallucinations
- Gustatory hallucinations

Delusions

- Delusions of influence
- Delusions of persecution
- Grandiose delusions
- Sexual delusions
- Ideas of reference

Incoherence of thought and speech

- Stereotypy of speech
- Poverty of speech
- Mutism
- Neologisms

Catatonic symptoms

- Automatic obedience
- Echolalia and echopraxia
- Stereotypy of movement
- Catatonic excitement
- Mannerisms
- Negativism

Disordered attention

Disordered judgement

Emotional dullness

Avolition

Autism

The conceptual framework laid down by Kraepelin proposes a pathological process (possibly metabolic) leading to disruption of normal mental processes: This disruption taking a variety of forms (and thereby producing a variety of symptoms) but invariably beginning in adolescence or early adulthood and progressing to dementia[4]. His textbooks contain detailed case reports that serve to illustrate his main points and also act as a guide to readers attempting to replicate his diagnostic methods. He does not, however, attempt to operationalise his diagnostic criteria, preferring to weave clinical description into his theoretical framework. In the following extract Kraepelin deals with phenomena that we would now call 'negative symptoms': "There are apparently two principal groups of disorders that characterise the malady. On the one hand we observe a weakening to those emotional activities which permanently form the mainsprings of volition . . . Mental activity and instinct for occupation become mute. The result of this highly morbid process is emotional dullness, failure of mental activities, loss of mastery over volition, of endeavour, and ability for independent action . . . The second group of disorders consist in the loss of the inner unity of activities of intellect, emotion, and volition in themselves and among one another . . . The near connection between thinking and feeling, between deliberation and emotional activity on the one hand, and practical work on the other is more or less lost. Emotions do not correspond to ideas. The patient laughs and weeps without recognisable cause, without any relation to their circumstances and their experiences, smile as they narrate a tale of their attempted suicide . . ."[19]

In later editions of his Lehbuch, Kraepelin reiterates his belief that the tangible morbid process underlying dementia praecox will ultimately be revealed but his optimism is not shared by all of his contemporaries. In 1908 Nissl writes "it was a bad mistake not to realise that the findings of brain anatomy bore no relationship to psychiatric findings."[26] Eugen Bleuler shared Kraepelin's belief in the biological basis of symptoms (although he suggests the mechanism is toxic rather than metabolic)[1] but he argues that certain symptoms, rather than being the product of disrupted mental processes are a direct result of the morbid process. He suggests that these 'fundamental' symptoms hold the key to understanding the illness. Whilst other 'accessory' symptoms are secondary phenomena and therefore less important[27].

Bleuler's ideas are reflected in the name he chooses for the condition "I call dementia praecox schizophrenia because, as I hope to show, the splitting of the different psychic functions is one of its most important features"[26]. "In each case there is a more or less clear splitting of the psychological functions: as the disease becomes distinct, the personality loses its unity"[27]. Kraepelin does not appear to have put up much of a fight in defending his (borrowed) terminology. In the eighth edition of his textbook he acknowledges for the first time that 'dementia praecox' is a translation of the French 'démence précoce' proposed by Belgian psychiatrist Bénédict Morel in 1860[28]. Kraepelin then writes "it has since been found that the assumptions upon which the name chosen rested are at least doubtful . . . the possibility cannot in the present state of our knowledge be disputed that a certain number of cases of dementia praecox attain to complete and permanent recovery and also the relations to the period of youth do not appear to be without exception."[19]

Bleuler's concept of 'the group of schizophrenias' is broader than Kraepelin's dementia praecox. The most overt evidence of this widening of the concept is the incorporation of 'paraphrenia' and the introduction of the notion of 'simple' schizophrenia. Bleuler outlines his ideas in his famous monograph of 1911. Here is his introduction: "Certain symptoms of schizophrenia are present in every case and at every period of the illness even though, as with every other disease symptom, they must have attained a certain degree of intensity before they can be recognised with any certainty. Here, of course, we are discussing only large symptom-complexes as a whole. For example, the peculiar association disturbance is always present, but not each and every aspect of it. Sometimes the anomalies of association may manifest themselves in 'blocking', or in the splitting of ideas; at other times in different schizophrenic symptoms." "Besides these specific permanent or fundamental symptoms, we can find a host of other, more accessory manifestations such as delusions, hallucinations or catatonic symptoms. These may be completely lacking during certain periods, or even throughout the entire course of the disease; at other times, they alone may determine the clinical picture."[29] He goes on to outline his view of the fundamental symptoms of schizophrenia as alterations of the simple and compound functions of the brain. He illustrates his theory with clinical examples.

Bleuler's ideas attracted considerable support, particularly in North America[30]. However, the application of his theory to clinical practice is problematic. His fundamental symptoms (see figure 1Aii below) are poorly defined and by Bleuler's own admission "exist in varying degrees and shadings on the entire scale from

pathological to normal"[29]. Hence in Bleuler's model the boundaries between schizophrenia and normality are blurred. The degree of interpretation required in deciding whether or not Bleuler's fundamental symptoms are present leads to poor diagnostic precision. The inevitable consequence is that modern operational definitions of schizophrenia, whilst retaining Bleuler's term, make only indirect references to his concept.

Figure 1Aii - **Bleuler's "Fundamental" symptoms of Schizophrenia**

Loss of Continuity of Associations

Lack of purpose or goal in the speech; poverty of ideas
Thought condensations
Stereotypy; echolalia
Thought blocking
Pressure of thoughts; clang associations

Loss of Affective Responsiveness

Lack of depth to the affect; restricted affect
Lack of consistency of affective manifestation
Inappropriate or blunted affect

Loss of Attention

Lack of selectivity of attention
Impaired active attention

Ambivalence

Affective ambivalence: the same concept is accompanied simultaneously by pleasant and unpleasant feelings
Ambivalence of will: the patient wishes and does not wish the same thing at the same time
Intellectual ambivalence: the patient expresses contradictory thoughts in the same sentence

Autism

The German psychiatrist and philosopher Karl Jaspers explores the tension between theory and practice in his 1913 work "General Psychopathology"[31]. Jaspers makes the philosophical distinction between 'explanation' and 'understanding'. He argues

that theories and classifications are necessary for explanation but that in order to understand the uniquely human characteristics of his patient the psychiatrist must first adopt an empathic stance. Jaspers is highly critical of contemporary theories, which he dismisses as "theoretical transformations of anatomical cerebral structures into fantastic constructs of parallel psychic events which prove most uninformative"[32]. He warns that "theory has a limited usefulness but it is often superfluous and like some creeping plant it tends on the whole to suffocate real insight, lively observation and all scientific progress"[33].

Jaspers suggests that the clinician who views mental illness from his own perspective will only have access to the external manifestations, the objective 'pathology'. This clinician may be able to *explain* what he sees in terms of symptoms, syndromes and diagnosis but he will not *understand* his patient. In contrast, the clinician who empathises (places himself in the subjective scene of the patient's experience) will have access to the patient's state of mind, the subjective 'phenomenology'. The encompassing phenomenological description obtained through empathy offers a degree of understanding of the patient as an individual, the essential features of his existence ('existenz'). Jaspers argues that, from a phenomenological perspective, the defining characteristic of psychosis is that the patient's experiences are 'nonunderstandable', that is the empathic clinician is unable to share in them because he can not readily imagine experiencing them himself.

The empathic approach to psychiatric assessment is now regarded as standard psychiatric practice and modern classifications are largely based upon

phenomenology. It is ironic that the man who stressed the importance of understanding (vis-à-vis explanation) in psychiatry should have contributed so much to subsequent attempts at explanation. As discussed below, one of the criticisms of operational classifications is their inability to cope with in-between cases (Jaspers referred to these as 'psychoses in combination'). Jaspers warns of the dangers of rigid application of structures derived from explanatory theories "there should be no obscuring of what is still unknown. Contradictions should come clearly to light. It is preferable to have a decisiveness which provokes discontent than satisfaction with a pseudo-knowledge won by approximations and a purely logical arrangement."[34]

Amongst the many academic psychiatrists influenced by Jaspers was Kurt Schneider. Schneider shared Bleuler's desire to identify symptoms that are fundamental to schizophrenia but adopted Jasper's empathic approach in his search for 'comprehension by interaction'. His 'symptomatic comprehension' of the disorder is based upon the idea that the non-understandable components of schizophrenia relate to an inability to recognise boundaries between self and not-self. Schneider's theory culminates in 1959 with the publication of his list of eleven 'symptoms of the first rank'[35] each of which is characterised by a loss of autonomy (see figure 1Aiii below). Schneider argues that because these symptoms reflect the subjective experience that is central to schizophrenia, they are likely to be pathognomic of the condition in the absence of overt brain disease. In contrast to the fundamental symptoms proposed by Bleuler, Schneider's first rank symptoms are essentially all-or-none phenomena. A trained interviewer adopting an empathic stance ought to be capable of making a confident judgement as to their presence or absence. They therefore lend themselves

to incorporation into operational definitions of schizophrenia as they have the potential to allow a group of patients to be identified reliably and with precision. (Subsequent to their incorporation into standardised diagnostic instruments, Schneider's first rank symptoms have been repeatedly misrepresented as entirely empirical, with no theoretical basis.)

Figure 1Aiii - **Schneider's 'First Rank' symptoms of Schizophrenia**

Auditory Hallucinations
- Patient's own thoughts spoken aloud
- Voices discussing or arguing about patient
- Voices describing patient's activity as it takes place

Delusional Perception

Passivity of somatic sensation

Thought insertion

Thought withdrawal

Thought broadcasting

Passivity of emotions and feelings

Passivity of desires or impulses

Passivity of movement

By the 1970's Kraepelin's original concept of Dementia Praecox had evolved in a number of different directions. The term schizophrenia was being used in different ways in different countries. Various 'schools' of psychiatrists were emerging each following its own favoured theory. Attempts to produce a universally accepted nomenclature had been unsuccessful. This caused particular problems for researchers who were finding it increasingly difficult to make comparisons between

studies that employed markedly different inclusion criteria. In 1972 researchers at Washington University published the first operational diagnostic criteria for schizophrenia[36] (see figure 1Aiv below). The 'Feighner Criteria' had been validated by follow-up and family studies, rather than by clinical judgement and experience and were designed primarily as inclusion criteria for researchers wishing to study schizophrenia. The emphasis upon chronicity and poor psychosocial outcome rather than specific symptoms suggests that Feighner's view of schizophrenia owes more to Kraepelin than to Bleuler or Schneider.

Figure 1Aiv - **Feighner criteria for Schizophrenia**

A - Both of the following are necessary:

1. A chronic illness with at least six months of symptoms prior to the index evaluation without return to the premorbid level of psychosocial adjustment.
2. Absence of a period of depressive or manic symptoms sufficient to qualify for affective disorder or probable affective disorder.

B - The patient must have at least one of the following:

1. Delusions or hallucinations without significant perplexity or disorientation associated with them.
2. Verbal production that makes communication difficult because of a lack of logical or understandable organization. (In the presence of muteness the diagnostic decision must be deferred.)

C - At least three of the following manifestations must be present for a diagnosis of "definite" schizophrenia, and two for a diagnosis of "probable" schizophrenia.

1. Single
2. Poor premorbid social adjustment or work history
3. Family history of schizophrenia
4. Absence of alcoholism or drug abuse within one year of onset of psychosis
5. Onset of illness prior to age 40

At this time it was not clear whether the different ways in which the term schizophrenia was being used in different countries was a consequence of genuine regional variations in symptomatology (or even entirely different diseases). The "US-UK Diagnostic Project"[37] and later "International Pilot Study of Schizophrenia"[38] (IPSS) were designed to shed light on this issue. The designers of these ambitious studies, recognising the necessity for high inter-rater reliability, developed a structured interview for use by the researchers in different countries. This structured interview, the "Present State Examination" (PSE)[39], incorporated Schneider's first rank symptoms. When Robert Spitzer and colleagues from the Columbia University in New York designed a shorter semi-structured interview, the "Schedule for Affective Disorders and Schizophrenia" (SADS)[40], they stuck to the successful formula of the PSE. Spitzer and colleagues also used the findings of the PSE-based IPSS to 'refine' the Feighner Criteria, publishing their "Research Diagnostic Criteria" (RDC)[41] (see figure 1Av below) in 1978. In reality the RDC bear little relation to the Feighner criteria. The emphasis on chronicity and poor psychosocial functioning are replaced by a list of psychotic phenomena closely resembling Schneider's list of first rank symptoms.

Figure 1Av – **Research Diagnostic Criteria for Schizophrenia**

A. During an active phase of the illness at least two of the following are required for definite and one for probable diagnosis of schizophrenia:

1. Thought broadcasting, insertion, or withdrawal
2. Delusions of being controlled or influenced, other bizarre delusions, or multiple delusions
3. Somatic, grandiose, religious, nihilistic, or other delusions without persecutory or jealous content lasting at least one week
4. Delusions of any type if accompanied by hallucinations of any type for at least one week
5. Auditory hallucinations in which either a voice keeps up a running commentary on the subject's behaviours or thoughts as they occur, or two or more voices converse with each other
6. Non-affective verbal hallucinations spoken to the subject
7. Hallucinations of any type throughout the day for several days or intermittently for at least one month
8. Definite instances of marked formal thought disorder (as defined in this manual) accompanied by either blunted or inappropriate affect, delusions or hallucinations of any type, or grossly disorganized behaviour

B. Signs of the illness have lasted at least two weeks from the onset of a noticeable change in the subject's usual condition.

C. At no time during the active period of illness being considered has the subject met the full criteria for either probable or definite manic or depressive syndrome to such a degree that it was a prominent part of the illness.

These rigid operational criteria for research stood in stark contrast to the looser descriptive nomenclatures published by the World Health Organisation[42] and the American Psychiatric Association[43]. As a highly respected figure in American psychiatry, Spitzer had been invited to head the American Psychiatric Association task force[44] commissioned to produce the third edition of the Diagnostic and Statistical Manual (DSM-III)[45]. The result was a ground-breaking document containing both detailed descriptions and operational diagnostic criteria for a

comprehensive range of psychiatric disorders. The DSM-III criteria for schizophrenia are essentially a combination of the Feighner criteria and the RDC. These have subsequently evolved through a revised version (DSM-III R)[46] to their current form in the fourth edition (DSM-IV)[47] and the latest "text revision" (DSM-IV TR)[2] (see figure 1Av below).

Figure 1Avi - **DSM-IV (TR) diagnostic criteria for Schizophrenia**

A. Characteristic symptoms: Two or more of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated)

1. Delusions
2. Hallucinations
3. Disorganized speech (e.g. frequent derailment or incoherence)
4. Grossly disorganized or catatonic behaviour
5. Negative symptoms (i.e. affective flattening, alogia or avolition)

Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behaviour or thoughts, or two or more voices are conversing with each other.

B. Social/occupational dysfunction: for a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or, when the onset is in childhood or adolescence, failure to achieve the expected level of interpersonal, academic or occupational achievement).

C. Duration: Continuous signs of the disturbance persist for at least 6 months, of which at least one month should be of symptoms that meet Criterion A. The 6 months may include periods of prodromal and residual symptoms. During these prodromal or residual periods, the signs of disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g. odd beliefs, unusual perceptual experiences).

D. Schizoaffective and mood disorder exclusion: Schizoaffective disorder and mood disorder with psychotic features have been ruled out because either no major depressive, manic, or mixed episodes have occurred concurrently with the active-phase symptoms, or if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the active and residual periods.

E. Substance/general medical condition exclusion: The disturbance is not due to the direct physiological effects of a substance or a general medical condition.

F. Relationship to a pervasive developmental disorder: if there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

When DSM-III was published in 1980, its contemporary rival, ICD-9[48] was effectively rendered redundant. The rivalry stretched back to the publication by the World Health Organisation in 1948 of the 6th revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD-6)[49] (itself a revision of the fifth International List of Causes of Death published by the International Statistical Institute)[50]. The American Psychiatric Association, rather than adopt ICD-6, had established its own "Committee on Naming"[51], which published the first edition of DSM[52] in 1952. The WHO-appointed committee preparing ICD-10 acknowledged the breakthrough that DSM-III represented and borrowed heavily from its format, structure and content (see figure 1Avii below). The APA-appointed team preparing DSM-IV returned the complement by borrowing from ICD-10. The two classifications are clearly not identical; however, in their current forms the similarities are striking.

Figure 1Avii - **ICD-10 diagnostic criteria for Schizophrenia**

A. Either at least one of the syndromes, symptoms, and signs listed under (1) below or at least two of the symptoms and signs listed under (2) should be present for most of the time during an episode of psychotic illness lasting for at least 1 month (or at some time during most of the days)

(1) At least one of the following must be present:

1. Thought echo, thought insertion or withdrawal, or thought broadcasting
2. Delusions of control, influence, or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensations; delusional perception
3. Hallucinatory voices giving a running commentary on the patient's behaviour, or discussing the patient among themselves, or other types of hallucinatory voices coming from some part of the body
4. Persistent delusions of other kinds that are culturally inappropriate and completely impossible

(2) Or at least two of the following:

1. Persistent hallucinations in any modality, when occurring every day for at least 1 month, when accompanied by delusions without clear affective content, or by persistent over-valued ideas
2. Neologisms, breaks, or interpolations in the train of thought, resulting in incoherence or irrelevant speech
3. Catatonic behaviour, such as excitement, posturing, or waxy flexibility, negativism, mutism, or stupor
4. "Negative" symptoms, such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses

B. Exclusion clauses:

1. If the patient also meets criteria for manic episode or depressive episode, the criteria listed under (1) and (2) above must have been met before the disturbance of mood developed
2. The disorder is not attributable to organic brain disease, or to alcohol- or drug-related intoxication, dependence, or withdrawal.

One interesting concept introduced in ICD-10 and carried over into DSM-IV is the description of symptoms as either 'positive' or 'negative'. These terms were probably first used by the 19th century English neurologist John Hughlings-Jackson who wrote "Disease is said to 'cause' the symptoms of insanity, I submit that disease only produces negative mental symptoms, answering to the dissolution, and that all elaborate positive mental symptoms (illusions, hallucinations, delusions, and extravagant conduct) are the outcome of activity of nervous elements untouched by any pathological process; that they arise during activity on the lower level of evolution remaining."[53] The way in which the terms 'positive' and 'negative' are used in current editions of ICD and DSM is not substantially different from the way in which Hughlings-Jackson used them. However, his theoretical model (in which symptoms are explained according to the Darwinian evolution of brain structures) has not been retained.

In time both the ICD-10 and DSM-IV-TR classifications will be superseded. However, for contemporary schizophrenia researchers they represent the current 'state of the art' in the attempt to provide a robust definition of an infuriatingly ethereal clinical entity. The divergent evolution of Kraepelin's concept that prompted Feighner and colleagues[36] to produce strict operational diagnostic criteria has been successfully reversed. Researchers across the globe can now be confident that the 'schizophrenia' they are studying is the same as the 'schizophrenia' they read about in journals. Unfortunately many of the questions faced by Kraepelin remain unanswered. In particular, his 'tangible morbid process' has not been identified. The

next chapter examine the challenges that 'schizophrenia' presents to contemporary researchers.

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1B. Problems 'Schizophrenia' Presents to Structural Imaging Researchers

The value of any scientific experiment is intrinsically limited by the reliability of the observations and the validity of the study design. Schizophrenia researchers are faced with fundamental issues relating to the reliability and validity of the very concept that they are attempting to expound. These problems are an inevitable consequence of the way in which the concept of schizophrenia has evolved.

The ultimate goal of medical classification is, as Kraepelin wrote, "to cut nature at the joints: to identify natural disease entities."[1] A natural disease entity ought to be amenable to study. Patients diagnosed with such a disorder would share specific, pathognomic disease markers. But no such marker has yet been identified for schizophrenia. Indeed much of the research into biological correlates of schizophrenia is characterised by heterogeneity[2]. To some this brings into question the validity of 'schizophrenia' as a scientific concept[3,4]. Certainly the "patchwork"[5] nature of the diagnostic criteria listed in ICD and DSM bring to mind a series of stabs at nature rather than a single decisive cut: the broad categorisations pay homage to Kraepelin; the recommended method for ascertaining the symptomatology is Jaspers' 'empathic interview'; the lists of 'defining features' are adaptations of those proposed by Schneider and, to a lesser, extent Bleuler; individual symptoms are labelled as 'positive' or 'negative' in accordance with Hughlings-Jackson. Furthermore, the basic premise underlying Kraepelin's diagnostic categories (an emphasis upon course and outcome) has been dropped; Jaspers saw empathic interviewing as a tool for promoting 'understanding' which he considered to be the antithesis of the 'explanation' offered by diagnosis; and the potpourri of defining

symptoms chosen from the works of Schneider, Bleuler and Hughlings-Jackson appear without reference to the (mutually incompatible) aetiological theories from which they were originally derived. As it is currently defined, 'schizophrenia' is the camel of medicine - a disorder designed by committee.

Reliability

Different researchers employing different diagnostic criteria for schizophrenia will inevitably disagree about the diagnosis in a proportion of cases. (Indeed even a single researcher applying different diagnostic criteria simultaneously will eventually disagree with himself!) For this reason, the mere existence of competing diagnostic criteria, results in a diagnosis that is less than perfectly reliable. To make matters worse, the criteria change periodically. In practice this represents less of a problem to contemporary schizophrenia researchers than one might think. There is a great deal of overlap between the various nomenclatures. For example, a study by McGlashan[6] in 1984, comparing various diagnostic systems, found a concordance rate between DSM-III and RDC of $\kappa = 0.88$. Although the author is not aware of an equivalent study specifically designed to measure the concordance between DSM-IV and ICD-10, both nomenclatures are evolutions of DSM-III and both were developed with the help of field trials to ensure good reliability. It seems likely that the concordance rate, if measured, would be very high. There is one important proviso to this statement: DSM-IV criteria specify a period of disturbed functioning of no less than six months; this is not replicated in ICD-10; hence concordance rates for first episode cases may be low. The danger of further divergence with each subsequent revision remains, but it is essential that the concepts evolve if they are to succeed in incorporating new research findings.

The subjective nature of the psychiatric interview brings with it the risk of poor inter-rater reliability. Kraepelin acknowledges this problem in the 8th edition of his textbook: "the statements of different observers can in the first place not be

compared at all"[7]. Much of the variation in assessment has been eliminated from modern schizophrenia research with the introduction of structured psychiatric interviews such as the PSE[8]. Indeed, any contemporary study of schizophrenia that fails to employ a structured interview will struggle to be accepted by a respected peer review journal and studies that employ a number of clinical interviewers routinely include calculations of inter-rater reliability. When piloting the PSE, Wing and colleagues found that psychiatrists trained in its use agreed upon the diagnosis of schizophrenia in 92% of cases[9]. Reliability of diagnosis is therefore no longer a major problem in well conducted studies of schizophrenia. As Kendell writes in the 'Companion': "In skilled hands, psychiatric diagnoses are now as reliable as the clinical judgements made in other branches of medicine, and sometimes more so."[10]

Validity

The ability reliably to diagnose schizophrenia may be a necessary prerequisite for meaningful scientific study but, as Kendell explains: "Reliability can be high while validity remains trivial and in such a situation high reliability is of very limited value."[11] Kendell distinguishes the two: "Reliability is concerned with the defining characteristics of a class, validity with the correlates of class membership."[11] Researchers involved in imaging studies are searching for correlations between the diagnosis of schizophrenia and brain structure. Their findings will therefore have a direct bearing upon the validity of the concept of schizophrenia (consistent, meaningful correlations will enhance it whilst inconsistent, heterogeneous findings will undermine it). Conversely the validity of structural imaging research is entirely dependent upon the validity of the concept under study. To put this another way, if Kraepelin was incorrect in his assertion that dementia praecox is a natural disease entity then the search for his tangible morbid process may be headed up a blind alley.

It is interesting to note that although Kraepelin proposed thirteen diagnostic categories, the only ones to have survived in any recognisable form are 'dementia praecox' and 'manic-depressive psychosis'. Whilst the durability of these two categories may reflect the validity of Kraepelin's original scientific concepts, one has to acknowledge alternative explanations for their continued acceptance (such as chance, professional convenience or even collective intellectual sloppiness). Kraepelin was undoubtedly more scientifically rigorous than many of his contemporaries. However, critics argue that when his methods are scrutinised, using modern scientific criteria, fundamental problems are revealed[3]. Some commonly

expressed criticisms of Kraepelin's work in developing his concept of dementia praecox are summarised below (Figure 1Bi).

Figure 1Bi – **Criticisms of Kraepelin's Work**

1. Little scientific method to observations
In his texts, Kraepelin does not present systematically gathered data but instead relies upon his own personal experience and beliefs. He reports the patterns that he has observed in his cards and lists but makes no attempt to demonstrate that the associations he identifies occur more frequently than would be expected by chance (i.e. reach statistical significance). Also Kraepelin knew many of the patients well and was therefore not a blind, impartial observer.

2. Pathological explanations proposed without evidence
Kraepelin repeatedly asserts that he is attempting to classify psychiatric patients according to underlying brain pathology. He argues that dementia praecox is a natural disease entity, probably a metabolic or endocrine disorder. However, at the time he was writing there was no empirical evidence of any metabolic, endocrine or other biological disturbance in these patients. Critics could argue that his explanations are personal declarations of faith rather than objective statements of fact.

3. Terms poorly defined
It is clear that Kraepelin used very different criteria for diagnosing dementia praecox in different patients. In his texts he argues that he is making the diagnosis upon the basis of the total clinical picture and provides detailed descriptions of individual cases. However, critics argue that he does not define his terms with sufficient clarity to enable readers to reliably replicate his observations[12].

4. Texts contain many internal inconsistencies
Kraepelin's concepts and nomenclature change with each edition of his text. Certain revisions directly contradict his previous writings, for example, 'catatonia', 'dementia paranoides' and 'dementia praecox' are presented as separate conditions in the 5th edition but are grouped together as "manifestations of a single disease process" in the 6th edition. The majority of these contradictions can be dismissed as necessary steps in the evolution of a sophisticated theory. However, occasionally Kraepelin proposes a revision that appears to undermine a central tenet of his theory. For

example, in the 8th edition he writes "a certain number of cases of dementia praecox attain to complete recovery and also the relations to the period of youth do not appear to be without exception."[7]

5. Population not comparable to modern populations
In Kraepelin's descriptions there is a greater emphasis upon physical abnormalities (e.g. gait, tremor, pupillary response) than is found in modern psychiatric texts. Kraepelin's neurology colleagues argued that a number of his patients were suffering from Encephalitis Lethargica[13]. One modern critic contends "the referents of 'schizophrenia' gradually changed until the term came to be applied to a population who bore only a slight and superficial resemblance to Kraepelin's and Bleuler's."[14]

Criticisms of Kraepelin's work are only immediately relevant if they help to highlight problems with our current concept of schizophrenia. As has already been demonstrated the problems of reliability that Kraepelin faced have largely been resolved. The problems of validity are more challenging. Towards the end of his life, Kraepelin himself expresses doubts about the validity of the diagnostic categories he had proposed. In 1920 he writes of dementia praecox (now renamed 'schizophrenia'): "There is no doubt that schizophrenic symptoms may also occur without any damage to cerebral tissue, . . . this fact is of great importance because it points to the possibility that other curable illnesses may in certain circumstances assume schizophrenic forms."[15] He even questions the validity of the core assumption upon which his entire system of classification (and arguably all modern psychiatry) is based: "we must seriously consider how far the phenomena on which we normally base our diagnosis really do afford insight into the basic pathological process."[15]

Establishing validity is not as straight forward as establishing reliability. The American Psychological Association distinguishes between four different types of validity: content, concurrent, predictive and construct[16]. Although their criteria were originally devised for assessing the validity of psychometric tests, they can be adapted for assessing the validity of diagnostic categories[17]. The criticisms of Kraepelin's work listed in Figure 1Bi incorporate challenges to all four types of validity (content in 2 & 5; concurrent in 3; predictive in 4; construct in 1 & 4). The contemporary construct of 'schizophrenia', including modern operational definitions, will now be evaluated in accordance with these four criteria.

Content Validity

The American Psychological Association defines the content validity of a psychometric test as follows: "If a test performance is to be interpreted as a sample of performance or a definition of performance in some universe of situations, the manual should indicate clearly what universe is represented and how adequate is the sampling."[18] Kendell adapts this definition for clinical diagnoses defining the content validity of a diagnosis as "the demonstration that the defining characteristics of a given disorder are indeed enquired into and elicited before that diagnosis is made."[11] The widespread adoption of standardised structured psychiatric interviews together with operational research diagnostic criteria guarantees a high level of content validity in schizophrenia research. Content validity is probably not so assured in the clinical setting.

Concurrent Validity

Establishing the concurrent validity of schizophrenia involves demonstrating that independent techniques for arriving at the diagnosis produce the same patient groupings. It is axiomatic that high concordance between different diagnostic criteria and high inter-rater reliability are prerequisites for concurrent validity. However such a demonstration of reliability of diagnosis is not in itself sufficient because the techniques employed to arrive at the diagnosis are closely related (i.e. not independent). A potentially more convincing method of establishing concurrent validity is 'cluster analysis'.

'Cluster analysis' is the generic term for a range of statistical techniques designed to separate a single heterogeneous population into a number of discrete, relatively homogenous 'clusters'. Each cluster is comprised of individuals who share certain characteristics. Critics of this approach argue that the clusters that emerge are determined by the rules laid down at the start of the analysis and point to the fact that different statistical rules produce very different clusters. Furthermore the clusters are only likely to be useful if the shared characteristics are clinically relevant (and recognisable). The cluster analysis literature relating to schizophrenia is probably best described as inconclusive. The largest and most robustly designed analysis to date is the transatlantic study conducted by Everitt, Gourlay and Kendell in 1971[19]. The authors interpret the results as supportive of the distinction between schizophrenia and manic-depression, pointing out that two different methods of analysis in two different populations of patients (giving four analyses altogether) all produced separate clusters clearly identifiable with the depressive and manic phases

of manic-depression, with acute paranoid schizophrenia and with chronic schizophrenia. However, critics argue that the relationship between clusters and diagnosis in their data is weak, pointing to the fact that over 60% of patients from every diagnostic group fell into two or three 'dustbin' clusters[4]. An intriguing addition to this literature is a retrospective study of almost 200 of Kraepelin's original patients. Jablensky and colleagues derived clinical symptoms (consistent with PSE definitions) from the original case material and performed a cluster analysis, which they interpreted as reproducing closely Kraepelin's dichotomous classification[20].

Predictive Validity

As noted in the previous chapter, Kraepelin himself considered predictive validity to be the most clinically important characteristic of a diagnosis: "The value of a diagnosis for the practical activity of the psychiatrist consists of letting him give a reliable look at the future."[21] Kendell shares this view: "In the last resort all diagnostic concepts stand or fall by the strength of the prognostic and therapeutic implications they embody."[11]

Unfortunately the literature suggests that a diagnosis of schizophrenia is of limited prognostic value. As the summary in DSM-IV TR concedes: "Most studies of course and outcome in schizophrenia suggest that the course may be variable, with some individuals displaying exacerbations and remissions, whereas others remain chronically ill. Because of variability in definition and ascertainment, an accurate summary of the long-term outcome of schizophrenia is not possible. Complete

remission (i.e. a return to full premorbid functioning) is probably not common in this disorder. Of those who remain ill, some appear to have a relatively stable course, whereas others show a progressive worsening associated with severe disability."[22]

The literature in relation to the therapeutic implications of a diagnosis of schizophrenia is also characterised by variability. A pragmatic observational study of the relationship between diagnosis and treatment in 1000 patients admitted to psychiatric hospitals in London in 1964 (admittedly pre-dating operational diagnostic criteria) concludes "the findings are not consistent with the notion that each particular diagnosis leads logically, or habitually, to a particular treatment. It suggests that variables other than diagnosis may be as, or more important than, diagnosis in predicting choice of treatment."[23] On an anecdotal level, the author recalls a teaching method employed by the professor of clinical pharmacology at his medical school (a physician). He would hand the students a series of medication 'kardexes' and invite us to deduce the patients' diagnoses. This task, whilst challenging to a medical student who had rarely opened the good professor's textbook, was theoretically achievable in the vast majority of cases. The same task would be impossible with a substantial proportion of psychiatric 'kardexes'. Whilst antipsychotic medication is almost universally accepted as the treatment of choice for schizophrenia, the evidence from randomised controlled trials suggests that only a proportion of patients (estimates range from 20-80%) benefit from such treatment[24,25,26]. The efficacy of antipsychotic medication is not specific to schizophrenia as treatment trials in mania[27], severe depression[28] and delirium[29] demonstrate. Furthermore, treatments more commonly used in affective disorders

such as ECT[30] and lithium[31] have been used successfully in patients with a diagnosis of schizophrenia as have benzodiazepines[32].

Construct Validity

In order to demonstrate the construct validity of a diagnosis, one must obtain objective evidence that the various components of the diagnosis are present in patients with the diagnosis but not in other patients. At its most basic level construct validity relates to the specificity and sensitivity of diagnostic criteria. However, the concept (or 'construct') of schizophrenia is far wider than that represented by the necessarily reductionist operational definitions contained in ICD and DSM. The symptoms and signs listed in these nomenclatures are considered to be manifestations of some underlying brain pathology, the characteristics of which are not yet fully understood. The validity of the construct ultimately depends upon robust and plausible research findings. All research into aetiological risk factors and biological markers (including all structural imaging work) can therefore be viewed as either enhancing or undermining the construct validity of schizophrenia.

The first step in establishing the construct validity of schizophrenia is to demonstrate that the characteristic, defining symptoms listed in ICD and DSM have high specificity and sensitivity. This task is far from straightforward because specificity and sensitivity are usually established by comparison with a gold standard (ideally a pathognomonic biological marker). However, no gold standard exists for the diagnosis of schizophrenia. In their comparative study of ICD-10 and DSM-IIIR criteria, Amin and colleagues[33] attempt to get around this problem by using the

temporal stability of diagnosis at three year follow-up as the gold standard. They report high specificity (ICD-10 89%, DSM-IIIR 93%) but low sensitivity (ICD-10 64%, DSM-IIIR 51%). Other authors have taken a different approach, examining individual components of the diagnostic criteria such as Schneiderian first rank symptoms. Nancy Andreasen reviewing this literature concludes "Schneider's first rank symptoms seem to carry diagnostic weight for schizophrenia when 'massively' present and when known organic psychoses are excluded. However, at least 10% of affectively ill probands, especially manics, have also been shown to have at least one first rank symptom. The main problem in their clinical application is their low sensitivity (on average less than 50%)"[34].

Andreasen's use of the phrase "massively present" reflects a problem common to all categorisations that rely upon subjective judgements: where to draw the boundaries. The inclusion in ICD-10 of the categories 'schizotypal disorder' and 'schizoaffective disorders' is a tacit acknowledgement of the arbitrary nature of the diagnostic boundaries for schizophrenia.

'Schizotypal disorder' is defined as "a disorder characterised by eccentric behaviour and anomalies of thinking and affect which resemble those seen in schizophrenia, although no definite and characteristic schizophrenic anomalies occur at any stage."[35] Its inclusion within the same group as schizophrenia is justified on the following grounds: "there is some evidence that it is related to schizophrenia (as one of the 'schizophrenic spectrum' disorders)."[35] The equivalent category in DSM-IV is called 'schizophreniform disorder' whilst an additional category of 'schizotypal

personality disorder' is listed elsewhere. The "evidence" to which the authors of ICD-10 refer stems largely from genetic research, which suggests that a genetic liability to schizophrenia may be associated with psychopathology resembling that seen in schizophrenia[36] only less severe (Andreasen might regard certain symptoms as being present but not 'massively' so). Evidence from structural imaging research suggests that these individuals, like schizophrenic patients, have reduced temporal lobe volume[37]. There is additional evidence from functional imaging[37] and psychometric studies[38,39] in support of the notion of a 'schizophrenic spectrum'. The presence of a large number of 'sub-threshold' cases apparently blending into normality undermines the validity of the construct of schizophrenia as a tightly defined, categorical disorder with a characteristic aetiology and neuropathology. To borrow from the language of classificatory theory, a number of the characteristic features of schizophrenia appear to be better suited to a 'dimensional' rather than the current 'categorical' classification.

The 'schizoaffective disorders' are defined in ICD-10 as "episodic disorders in which both affective and schizophrenic symptoms are prominent but which do not justify a diagnosis of either schizophrenia or depressive or manic episodes."[35] The unsatisfactory nature of this category is acknowledged in the diagnostic notes: "the relationship of this group of disorders with both schizophrenia and the affective disorders are uncertain. . . The diagnosis depends upon difficult clinical judgements about the approximate 'balance' between the number, severity and duration of the schizophrenic and the affective symptoms."[35] The existence of patients with both schizophrenic and affective symptoms is clearly a challenge to the Kraepelian view

that these are separate disorders. Kraepelin himself acknowledged "the difficulties which still prevent us from distinguishing reliably between manic-depressive insanity and dementia praecox."[15] He recognised the challenge presented by this group of patients: "No experienced psychiatrist will deny that there is an alarmingly large number of cases in which it seems impossible, in spite of most careful observation, to make a firm diagnosis."[15] As Kraepelin observed, the frequency with which affective and schizophrenic symptoms co-exist in the population is too high to be explained by chance. Family studies demonstrate that schizophrenia and bipolar affective disorder often co-exist within the same family[40,41] and molecular linkage studies reveal that some susceptibility loci may be common to both[42]. This raises the possibility of a shared genetic susceptibility and therefore a degree of overlap between the patho-physiological mechanisms underlying schizophrenia and bipolar disorder, a notion that is broadly supported by the post-mortem literature[43]. A number of the characteristic structural imaging findings are also common to both schizophrenia and bipolar disorder (see overview, chapter 1D).

If there is no unambiguous pathophysiological distinction between the two conditions, one has to question whether it is justifiable to regard them as separate. One statistical method for testing the validity of a diagnostic distinction is 'discriminant function analysis'. Kendell emphasises the potential importance of such an approach: "a bimodal distribution of scores on a discriminant function, obtained from an unselected population and cross validated on a second population, should be the accepted criterion of validity for all diagnostic distinctions."[44] A number of researchers, including Kendell himself, have applied this technique to the

functional psychoses in an attempt to demonstrate a 'point of rarity' between schizophrenia and manic depression but none has been successful[45,46]. One could interpret these results as powerful evidence against the Kraepelian division of psychosis into schizophrenia and bipolar disorder.

Conclusions

Our contemporary concept of schizophrenia owes not only its basic structure but also its limitations to the work of Emil Kraepelin. The problems of poor reliability and limited content validity have been successfully tackled by the adoption of tight operational definitions and structured psychiatric interviews. However, attempts to demonstrate the concurrent validity of the concept using cluster analysis have been inconclusive. The predictive validity of the diagnosis as defined by course, outcome and treatment response remains limited. Fundamental questions relating to the validity of Kraepelin's construct remain unanswered. His "tangible morbid process" has not yet been found. The evidence for a 'schizophrenic spectrum' suggests that, rather than being a "natural disease entity", schizophrenia may blend into normality. Most worrying of all for supporters of Kraepelin is the substantial body of evidence indicating an overlap between schizophrenia and bipolar disorder.

For contemporary schizophrenia researchers the legacy of uncertain validity remains something of a 'Catch 22'. Nancy Andreasen and William Carpenter Jr. describe the problem succinctly: "Although we can now define a particular construct of schizophrenia with reasonable agreement, the construct must be recognised as provisional and based on a need to achieve consensus about definitions rather than on

an understanding of pathophysiology and aetiology. The major challenge confronting the student of schizophrenia is to identify its mechanisms and causes"[47]. The 'catch' lies in the potentially self-defeating task of trying to identify the pathophysiological mechanisms and causes of a construct whose origins owe nothing to the science of pathophysiology. One would hope that provisional, incomplete insights into the biological mechanisms associated with schizophrenia might lead to an evolution of the construct into something less nebulous and more amenable to pathophysiological investigation. Daniel Weinberger, speaking at the 10th Biennial Winter Workshop on Schizophrenia expressed his frustration with the current situation "being a schizophrenia researcher in the year 2000 is like being the pilot of an aircraft circling above what you hope is an airstrip waiting for somebody to turn the landing lights on."[48]

Potential Solutions

When Kraepelin described dementia praecox, he was confident that he had identified a "natural disease entity" attributable to a "tangible morbid process" the nature of which would inevitably be revealed by scientific investigation. Yet more than a century later, two of the world's leading schizophrenia researchers acknowledge that little has changed: "The major challenge confronting the student of schizophrenia is to identify its mechanisms and causes"[47]. Clearly things have not turned out the way that Kraepelin had initially hoped. One possible reason for the relatively slow rate of progress is the limited validity of schizophrenia as a scientific concept (as outlined earlier in this chapter). The question of whether to work within the limitations of the concept or to abandon it altogether is as much a philosophical as a scientific one. The psychiatrist and philosopher Karl Jaspers commenting on the debate surrounding Kahlbaum's concept of a 'unitary psychosis' in his text 'General Psychopathology' writes: "The original question: are there only *stages and variants* of one unitary psychosis or is there *a series of disease-entities* which we can delineate, now finds its answer: *there are neither*. The latter view is right in so far that the idea of disease-entities has become a fruitful orientation for the investigations of special psychiatry. The former view is right in so far that no actual disease-entities exist in scientific psychiatry."[49] Of course Kahlbaum's concept has now been abandoned and certain 'actual disease-entities' have subsequently been identified within the field of psychiatry (e.g. tri-nucleotide repeats as the underlying molecular mechanism in Huntingdon's Chorea). However, when considered in the context of the contemporary debate surrounding the validity of schizophrenia as a scientific concept, Jasper's comments acquire a sobering resonance.

The arguments put forward both by supporters and by opponents of the contemporary concept of schizophrenia will now be examined. A compromise, or 'third way' alternative will then be presented.

'Status Quo' - Accept current concept as a useful starting point

Kraepelin's concept may be slightly over a century old but for the first three quarters of that century scientific investigation was seriously hindered by the absence of an agreed definition (and therefore poor reliability of diagnosis). In the twenty-five to thirty years since the introduction of operational criteria and standardised psychiatric interviews important scientific advances have been made. Perhaps the most fundamental of these has been the repeated demonstration of an association between a diagnosis of schizophrenia and structural brain abnormalities (as described in detail in chapter 1D). The central challenge of biological schizophrenia research has shifted from proving that the syndrome of schizophrenia has biological correlates in the brain to elucidating the precise biological mechanisms underlying the disorder[50]. Supporters of the current concept would argue that to abandon it now would equate to throwing the baby out with the bath water. To paraphrase Jaspers, the medical concept of schizophrenia as a 'disease' has become a fruitful orientation for researchers in biological psychiatry.

The strength of the current ICD and DSM approach lies in the periodic revision of diagnostic criteria. This allows the criteria to evolve in line with scientific advances. In theory this ought to enable a virtuous cycle to become established in which

research refines and validates the criteria, which in turn facilitate future research. Critics point to a number of problems with this approach. First the process is inevitably slow. The significance of research findings often takes decades to become apparent (as demonstrated by the delay between the publication of ground-breaking studies and the award of Nobel prizes). Past experience shows that even widely accepted research findings are not incorporated into diagnostic criteria (for example the ICD-10 criteria for Alzheimer's dementia make no reference to structural or functional imaging findings nor to genetic risk factors[35]). Second, the existence of competing nomenclatures introduces the risk of divergence of scientific concepts with damaging consequences. Finally, the widespread acceptance of narrow operational definitions prevents psychiatrists from thinking in broader terms. This inhibits the development of clinical skills and encourages premature closure on research questions that ought to remain open[51].

'Revolution' - Abandon current concept and start again

As Greisinger explained in 1867, the 'medical model' of insanity "rests principally on the realisation that patients with so-called 'mental illnesses' are really individuals with illnesses of the nerves and the brain"[52]. Alternative explanatory models have been proposed the majority based upon sociological[53], psychological[54] or psychodynamic[55] theories. The 20th century saw the rise and fall of the psychodynamic and anti-psychiatry movements. These challenges to the medical model of insanity were ultimately unsustainable against the weight of scientific evidence. Brain imaging provided incontrovertible evidence for abnormalities of brain structure and function whilst randomised controlled treatment trials proved the

efficacy of physical treatments such as psychotropic medication and electro-convulsive therapy. At the start of the 21st century the medical model is in an unassailable position. Sociological and psychological theories of insanity are now viewed as adjuncts rather than alternatives to biological theories.

Revolutionaries no longer challenge Greisinger's assertion that psychiatric symptoms relate to biological processes in the brain. Their attention has shifted to the conceptual framework that is currently employed to explore that relationship. A number of geneticists, no doubt frustrated with the slow rate of progress in psychiatric genetics, have proposed the abolition of the current classification of psychosis and its replacement with a system based upon "behavioural subtypes" determined by segregation data from molecular genetic studies of families[56]. Mary Boyle, a clinical psychologist, dismisses 'Schizophrenia' as "a scientific delusion"[3]. She argues that psychotic symptoms are not 'un-understandable' as suggested by Jaspers but are amenable to cognitive-behavioural formulation and management. Another psychologist, Richard Bentall questions the reliability, construct validity, predictive validity and aetiological specificity of the diagnosis concluding: "there would seem to be little reason to feel confident about the validity of the schizophrenia diagnosis. [The widespread acceptance of the concept as a valid foundation for scientific research] would appear to be little more than an article of faith. It may be more realistic to accept that 'schizophrenia' is not a useful scientific category and that for all these years researchers have been pursuing a ghost within the body of psychiatry."[4] Bentall and colleagues point out "the history of science is littered with examples in which progress has been impeded by the continued use of

invalid concepts leading to persistent asking of the wrong questions". They propose the abolition of the current classificatory system and its wholesale replacement by a system based upon the findings of "large-scale empirical research"[4]. They advocate exploration of hierarchical, dimensional and multidimensional approaches to classification and the adoption of multivariate and factor analytic techniques in order to differentiate patients on the basis of symptoms. Most critics of 'schizophrenia' point to the fact that no pathognomonic biological marker has yet been found for the condition despite decades of research. This assertion clearly echoes Jasper's earlier observation that "no actual disease-entities exist in scientific psychiatry"[49] (although Jasper's original observation has subsequently been overtaken by advances in medical science).

Whilst wiping the slate clean as Bentall and others suggest might appear to be intellectually rigorous, it is almost certainly unachievable. It is naïve to believe that the subjective component of psychiatric diagnosis will ever be eradicated by the adoption of empirical criteria. After all the current system is based upon (imperfect) empirical findings. Such a revolutionary approach would inevitably cause enormous scientific and clinical disruption. It is by no means certain that the resultant nomenclature would be superior to that which could be achieved through the current *evolutionary* approach (periodic revision of diagnostic criteria with the incorporation of contemporary research findings and results of field trials). It must be remembered that 'schizophrenia' is a clinical as well as a scientific concept and that any diagnostic system brought in to replace it would have to demonstrate clinical utility as well as scientific validity.

As well as proposing the wholesale abolition of the current classificatory system, Bentall et al. are amongst the advocates of a symptom-based approach to research. They identify five potential advantages: "(i) the avoidance of problems of diagnosis and classification; (ii) the focus on phenomena that are usually ignored; (iii) the facilitation of theoretical development; (iv) the recognition that clinical phenomena are related to normal behaviour; and (v) the potential improvement in classification which might follow from a better understanding of individual symptoms."[4] From Kraepelin's original descriptions to the present day, schizophrenia has been characterised by a wide range of symptoms and behaviours. The idea that individual symptoms might have specific biological correlates is very attractive. With the emergence of functional imaging techniques, symptom-based experimental design is enjoying something of a resurgence. Unfortunately the evidence from structural imaging research suggests that the relationship between individual schizophrenic symptoms and specific structural brain abnormalities is a weak one[57] (see chapter 1D).

'Third way' - Explore heterogeneity within current concept

As described in the previous chapter, Kraepelin's concept of 'dementia praecox' incorporated the previously described syndromes of 'dementia paranoides', 'hebephrenia' and 'catatonia'[21]. Each of these syndromes survives as a 'subtype' of schizophrenia in the DSM-IVTR classification (labelled 'Paranoid', 'Disorganised', and 'Catatonic' respectively). 'Undifferentiated' and 'Residual' subtypes are also listed. ICD-10 schizophrenia includes the five subtypes from DSM-IV and adds

'Simple' and 'Post-schizophrenic depression' together with categories for 'Other' and 'Unspecified'. The status of these subtypes within the broader context of psychiatric classification is ambiguous. Since Kraepelin's time it has been presumed that they are merely slightly different expressions of the same disease process. The vast majority of structural imaging research does not attempt to differentiate between them. Yet their endurance is testament to the symptomatic heterogeneity that characterises schizophrenia.

A number of attempts have been made to bring order to the highly varied and seemingly unrelated symptoms that are considered to be indicative of schizophrenia. Hughlings-Jackson's positive and negative dichotomy[58] is almost universally accepted and is reflected in both DSM-IVTR and ICD-10. More recently, Liddle's proposal of three core dimensions: psychomotor poverty; reality distortion; and disorganisation[59] has gained support. From the researcher's perspective, the various methods for subdividing schizophrenic subjects according to symptoms are potentially valuable. They offer a half-way house between accepting lock, stock and barrel, the poorly validated Kraepelian concept of a "natural disease entity" called 'schizophrenia' and abandoning it altogether by adopting an atheoretical, symptom-based approach.

Symptomatic diversity is only one of the many forms of heterogeneity in schizophrenia. As noted earlier in this chapter, there is considerable variation in the course and outcome of the illness. This is acknowledged in the ICD-10 classification in which a fifth character may be used to classify course: continuous; episodic with

progressive deficit; episodic with stable deficit etc. Kraepelin believed that course and outcome offer a clearer indication of underlying pathological processes than do symptoms. It would therefore seem logical for researchers attempting to elucidate the aetiology of schizophrenia to group patients according to course, long-term outcome or treatment response. In doing so they would be following the Kraepelian tradition.

A wide range of aetiological risk factors have been identified for schizophrenia, these include: family history; obstetric complications; maternal viral infection; cannabis use etc. It ought to be possible to explore the role of each individual risk factor by adopting suitable study designs (see chapter 2A). One could, for example, differentiate patients with a strong family history of schizophrenia from patients who are known to have no family history of psychosis. A small number of studies of this design have been published and are reviewed in chapter 1D.

Any study design that defines a sub-group of schizophrenic patients (whether on the basis of symptoms, course, risk factors or any other heterogeneous characteristic) is a direct challenge to the concept of schizophrenia as a "natural disease entity" reflecting a "single tangible morbid process". In acknowledging that the heterogeneity of the condition may be employed as a tool in the search for the aetiology, one is accepting that there may be heterogeneity in the causative mechanisms of schizophrenia. In recent years the Kraepelian view of schizophrenia as a single disorder with variations in clinical presentation has been challenged by the notion that it may be composed of multiple disorders with a common core

clinical syndrome[60] (as previously mentioned, Kraepelin himself acknowledged this possibility towards the end of his life[15]). Critics of the multiple aetiology theory argue that there is insufficient evidence to link particular symptoms or groups of symptoms directly to particular aetiological risk factors[61]. Meanwhile the theory's supporters argue that it is self-evident that the link between aetiology and symptoms is tenuous (this is why progress has been so slow) and that recognition of different risk factors in different cases (genetic predisposition, viral infection, obstetric complications etc.) is proof of heterogeneity in aetiology[62]. As Tsuang and Faraone contest, the debate should no longer be "Heterogeneity: yes or no?" but "Heterogeneity: how much?"[57] The role that structural imaging has played in informing this debate is discussed in chapter 1D.

Over the years a number of hypotheses have been proposed as candidates for Kraepelin's tangible morbid process, (a number of the more influential hypotheses are examined in Chapter 1C.) Although these have provided useful models for understanding certain aspects of the condition, they fall well short of a pathological explanation. The author shares the view of De Lisi, Tsuang, Faraone and others that schizophrenia probably has multiple aetiologies[60,62]. To the author's mind, the question of whether there is one 'schizophrenia' or several 'schizophrenias' appears to invite the Jasperian response "there are neither"[49]. Although the author acknowledges the limitations of schizophrenia as a scientific concept, he does not share Boyle and Bentall's view that it should be abandoned[3,4]. He believes that structural imaging research can continue to contribute to the understanding of the pathophysiological processes underlying the condition(s). Furthermore, he is

optimistic that technological advances including new imaging techniques (such as Diffusion Tensor Imaging utilised in the study described in Chapter 2C) and new methods of data analysis (such as Voxel Based Morphometry, illustrated by example in Chapter 2B) will provide new insights into the biological correlates of schizophrenia. In the author's opinion the various forms of heterogeneity that characterise the condition offer clues to the nature of the underlying aetiological mechanisms. Thoughtful study design, employing carefully selected sub-groups of affected individuals (such as the 'Obligates' design adopted in the study described in Chapter 2A) ought to be able to utilise the heterogeneity within the schizophrenic population as a tool in the search for the pathological mechanisms underlying the disorder. How this might be achieved is explored in Chapter 1E. But first, Chapter 1C will describe the role that structural brain imaging has played in schizophrenia research to date.

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1C. Role of Structural Brain Imaging in Schizophrenia Research

Historical overview of structural imaging methods

On Friday 8th November 1895, German physicist Wilhelm Roentgen exposed a photographic plate to the rays emitted from his 'Cooke's Tube'. The developed plate comprised the world's first radiograph[1]. Shortly afterwards he invited his wife Bertha to place her left hand on a photographic plate whilst he fired 'Roentgen's Rays' at it. The developed plate provided a clear image of the bones in her hand and the rings on her finger (see Figure 1Ci). Medical imaging was born. We do not know what Emil Kraepelin was doing on that day but it is tempting to imagine him working on the 5th edition of his textbook[2] (which was published the following year), perhaps pondering over a new chapter entitled "dementia praecox".

Given their near simultaneous conception, one might expect developments in medical imaging to be reflected in the evolution of Kraepelin's concept. However, the physical characteristic of X-rays (or 'Roentgen's Rays') that enabled Wilhelm to photograph the bones in Martha's hand is their ability to pass, virtually unhindered, through soft-tissue. This renders X-rays unsuitable for imaging soft-tissue structures such as the brain. The fact that the brain is surrounded by the bony structure of the skull compounds the problem (although the challenges are not insurmountable as subsequent developments in Computed Tomography demonstrate). Consequently, seven or eight decades would pass before medical imaging produced any meaningful insights into schizophrenia.

Figure 1Ci. Wilhelm Roentgen's 1895 radiograph of his wife's hand.

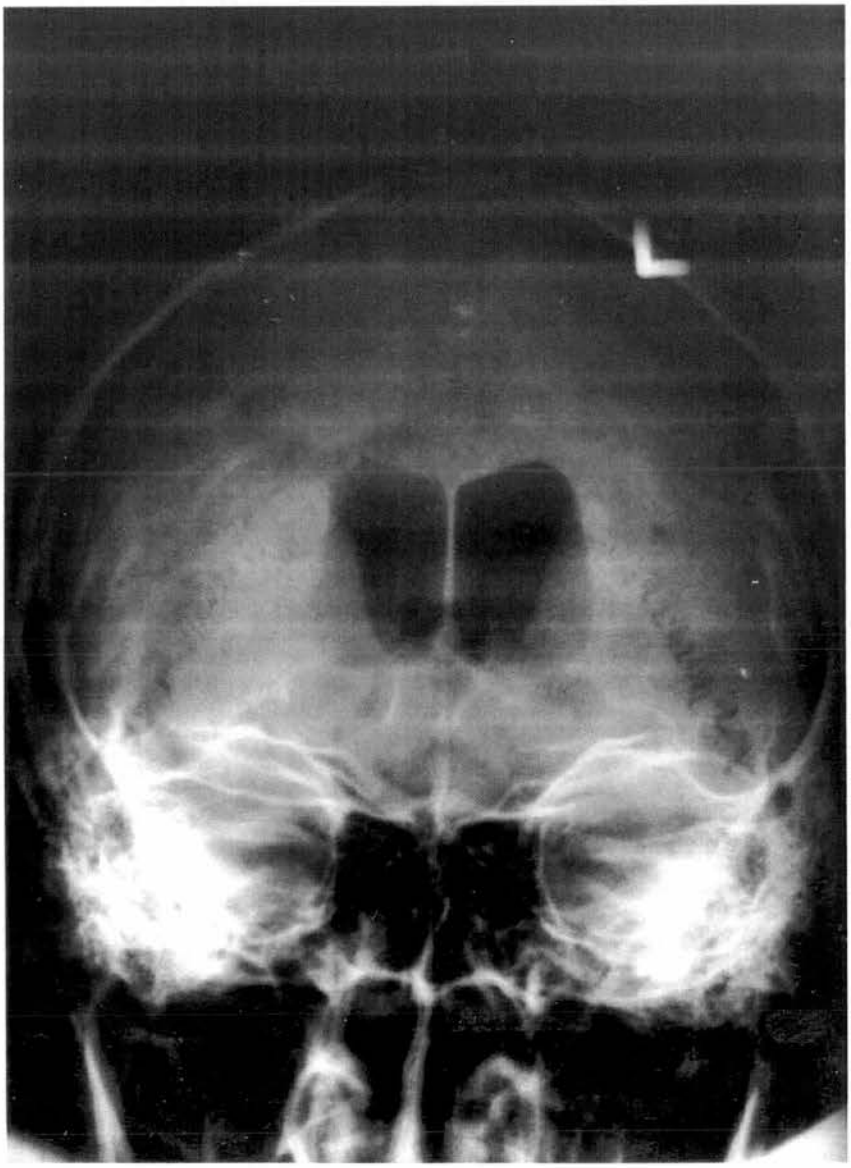
Pneumoencephalography

The first in-vivo structural imaging technique to be employed by schizophrenia researchers was pneumoencephalography. This involves performing a lumbar puncture, replacing a volume of cerebrospinal fluid with air, sitting the subject upright to allow the air to pass into the ventricles, then taking radiographs of the head in various different planes. The air shows up dark on the radiographs allowing estimates of ventricular size and shape to be made[3]. Pneumoencephalogropy is a dangerous, potentially fatal procedure. The ventriculograms produced are incomplete and of relatively poor spatial resolution (see figure 1Cii). As an imaging technique it is therefore of limited utility and was rendered obsolete by Computed Tomography (CT).

Despite these limitations, a number of uncontrolled pneumo-encephalographic studies demonstrating enlarged ventricles in association with schizophrenia can be found in the literature. The earliest is a study by German psychiatrists Jacobi and Winkler published in 1927[4]. Matthew Moore and colleagues in Philadelphia published the first study in English in 1933[5]. They employed pneumo-encephalography to provide in-vivo confirmation of their post-mortem work. Pneumo-encephalographic studies of mental disorders continued through the 1930s in Germany (with psychiatrists such as Lemke). However, its impact upon the wider psychiatric community was limited (possibly as a consequence of its association with the Nazi philosophy of eugenics). The technique re-emerged in the 1960s with German, Norweigian and Japanese studies. The largest single pneumo-encephalographic study of schizophrenia was published in 1964 by the German

psychiatrist Gerd Huber[6]. He imaged 212 chronically hospitalised schizophrenic patients aged less than 50 and found that 81.6% exhibited evidence of gross atrophy, most commonly enlargement of the third ventricle[6].

Figure 1Cii. An A-P view of a ventriculogram showing enlarged lateral ventricles.



Computed Tomography

In the late 1960's, British inventor Geoffrey Hounsfield developed a method of rotating an X-ray source and detector through 360° and using the data collected to generate a cross-sectional image or 'slice' through the body. His original experimental machine took nine days to acquire data and 2½ hours to reconstruct a single slice[1]. His technique was first put to clinical use in 1972 and in 1979 he was awarded the Nobel Prize (an honour bestowed upon Wilhelm Roentgen 78 years earlier).

Eve Johnstone and colleagues published the first X-ray CT study of schizophrenia in 1976. They compared data acquired from CT scans of 17 institutionalised schizophrenic patients with data from 8 age-matched controls. The results showed that schizophrenia is associated with increased ventricular size[7]. Robin Murray has described the publication of this study as marking "the beginning of the modern era of schizophrenia research"[8]. Daniel Weinberger has stated that reading Johnstone's 1976 Lancet paper inspired him to abandon his planned career in neurology in favour of a lifetime in schizophrenia research[9]. It is notable that his first major contribution to the schizophrenia literature was a successful replication of Johnstone's findings[10].

CT technology has moved on since 1976 (compare figures 1Ciii and 1Civ). Some modern CT scanners acquire data in a spiral, which can be reconstructed as a slice in any orientation or as a three-dimensional representation. The whole brain can now be imaged in a matter of seconds and spatial resolution of the order of a millimetre can be achieved[1]. Over 100 quantitative CT studies comparing the brains of

schizophrenic subjects with those of controls have now been published[11]. (The cumulative findings are presented in the following section.) However, CT is an X-ray technique and as such can not escape the physical limitations of X-rays. As a form of ionising radiation X-rays have the potential to produce mutations in both somatic cells and gametes. Exposing research subjects to a large number of scans is therefore undesirable, particularly where a safer alternative is available. As noted above, X-rays are relatively unaffected by soft-tissue hence CT does not discriminate particularly well between different types of soft-tissue (such as white and grey matter). It is also unreliable in parts of the brain that are close to bone such as the mid-brain and cerebellum (because of the tendency for artefacts to arise during the reconstruction phase). During the 1980s and '90s, Magnetic Resonance Imaging (MRI) gradually replaced CT as the imaging method of choice for schizophrenia research.

Figure 1Ciii. An X-ray CT image of the brain from an EMI 1010 machine. The image is in the axial plane at the level of the lateral ventricles. It is a clinical scan identifying a sub-arachnoid cyst in the right temporal region.

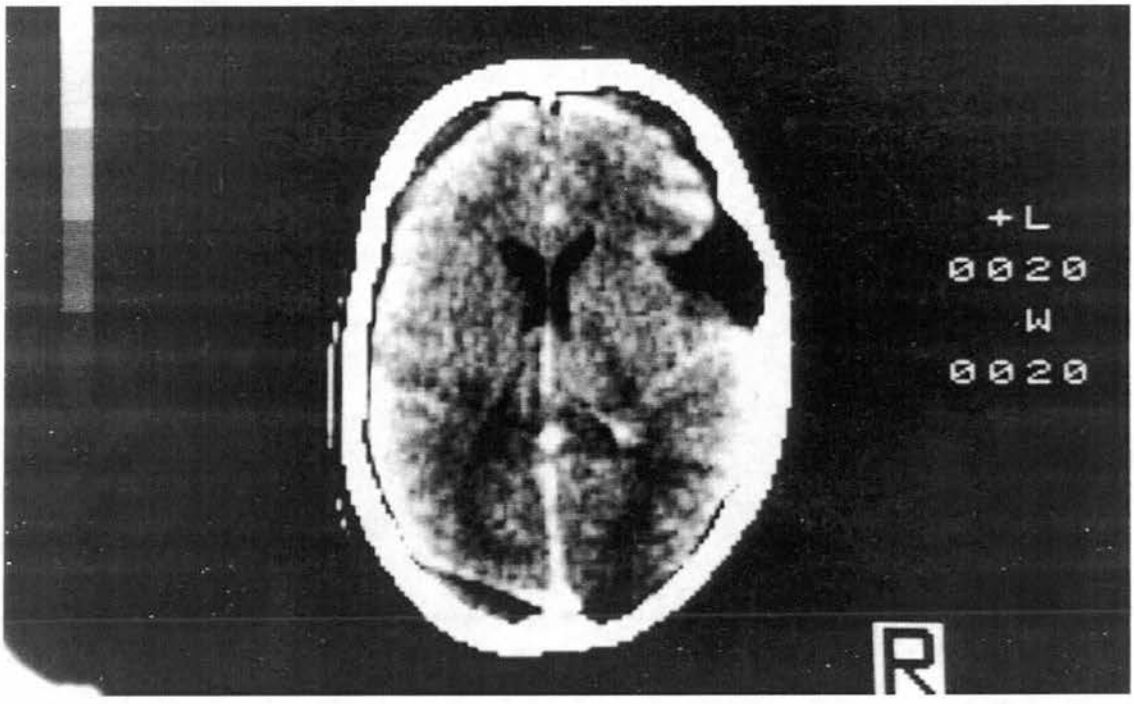


Figure 1Civ. An image of approximately the same axial slice shown in Figure 1Ciii but acquired using a modern X-ray CT machine. Note the superior spatial resolution.

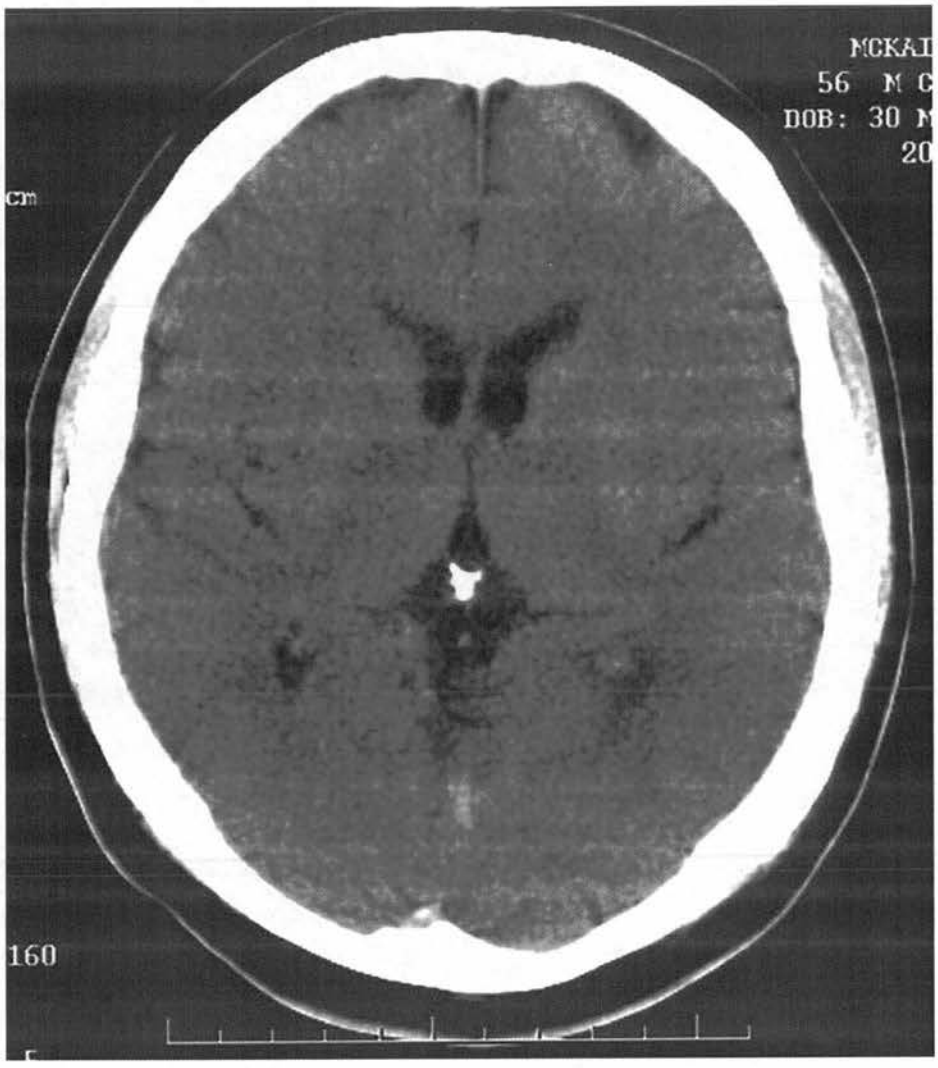
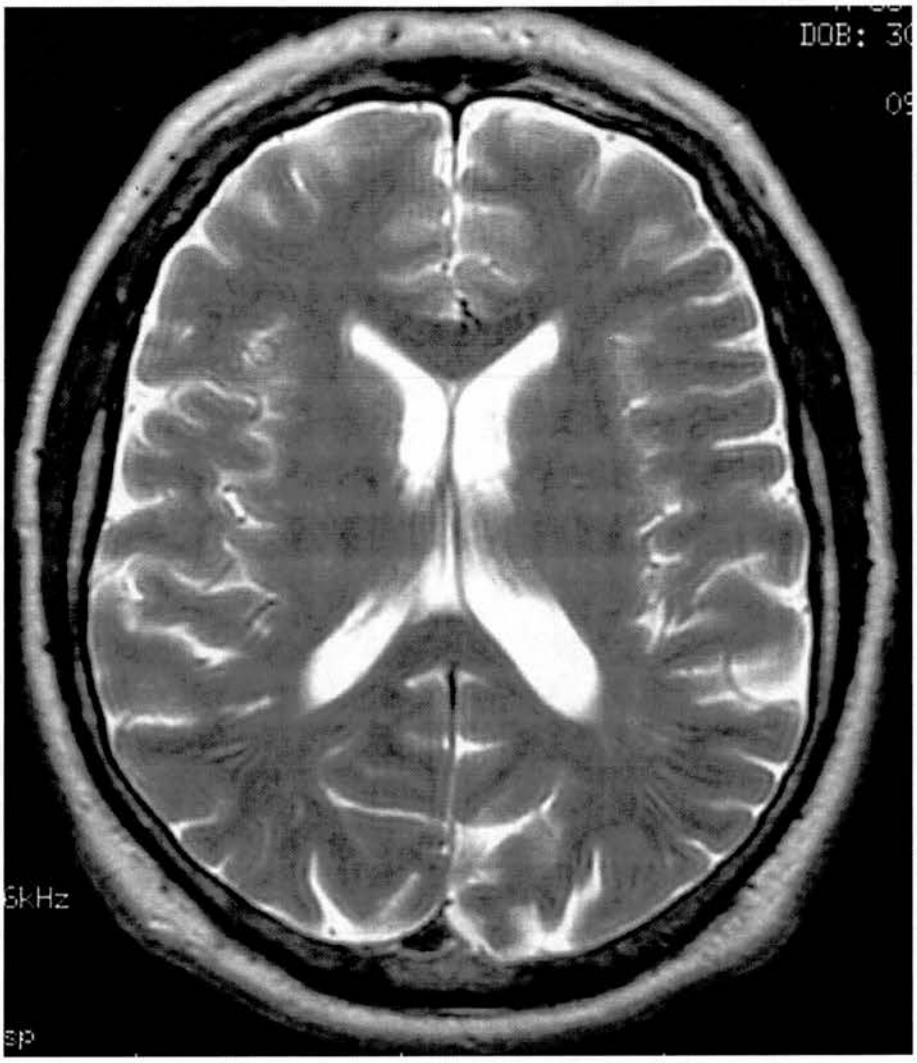


Figure 1Cv. A T_2 MRI image of approximately the same slice. Note the superior soft-tissue discrimination.



Magnetic Resonance Imaging

The phenomenon of Nuclear Magnetic Resonance (NMR) was first identified in the 1930s by American physicist Isidor Rabi. During the 1940s two teams of scientists working independently developed similar techniques for using NMR to examine the chemical composition of small volume samples. Felix Bloch and Edward Purcell were jointly awarded the Nobel Prize in Physics in 1952 for their work on Magnetic Resonance Spectroscopy (MRS)[12]. By the late 1960s, MRS was established in chemistry laboratories throughout the world as a powerful, non-destructive method for analysing samples (see Figure 1Cvi). In 1973, New York chemist Paul Lauterbur published a short paper in Nature describing the use of MRS to establish the relative position in space of two test-tubes containing different chemicals[13]. By taking NMR techniques from the single dimension of MRS into two dimensions, Lauterbur had laid the foundations of MRI. During the 1970s and '80s, a number of research groups developed Lauterbur's technique. Progress was rapid, partly because researchers were able to adapt a number of methods of data analysis that had previously been devised for CT imaging. The first commercial MRI scanners were installed in 1983[12].

MRI offers three major advantages over CT[13]. First it does not use ionising radiation and is therefore less likely to be mutagenic. Second, it is highly sensitive to small differences in soft-tissue composition hence one can readily differentiate between white and grey matter (see figure 1Cv). Third, it is less affected by proximity to bone. MRI does have certain disadvantages: it is slower than CT and is therefore more liable to movement artefact; patients with metal in their body (aneurysm clips,

cardiac pace-makers etc.) can not be imaged; it does not image cortical bone as well as CT; and certain MRI techniques are prone to distortion close to air-tissue boundaries. Also anyone who has conducted MRI-based research will know that not everyone is able to tolerate the confined space and loud noise associated with an MRI scanner. However, for the vast majority of structural brain imaging research applications, MRI is now the technique of choice[13].

Over the past two decades, MRI technology has advanced at pace. More powerful and smoothly graded magnetic fields together with increased computer processing power and new techniques such as echo planar imaging have reduced image acquisition times and improved spatial resolution and tissue discrimination[13]. The shorter scanning times and the adoption of strategies such as cardiac gating have reduced, but not eliminated, the problems associated with movement. To date, at least 200 case-control studies of schizophrenia employing structural MRI have been published[14] with numerous others currently in progress (this literature is discussed in chapter 1D). In recent years MRI has been employed to provide information about regional cerebral blood flow, an indicator of local neuronal activity. Although functional imaging is beyond the scope of this thesis, it is clear that fMRI offers a number of important advantages over PET and SPET and the technique is already offering valuable insights into the biological processes underlying schizophrenia[15,16,17].

Figure 1Cvi. An example of a Magnetic Resonance Spectrum. The peaks labelled correspond to choline (CHO), creatinine (CR) and N-acetyl aspatrate (NAA).

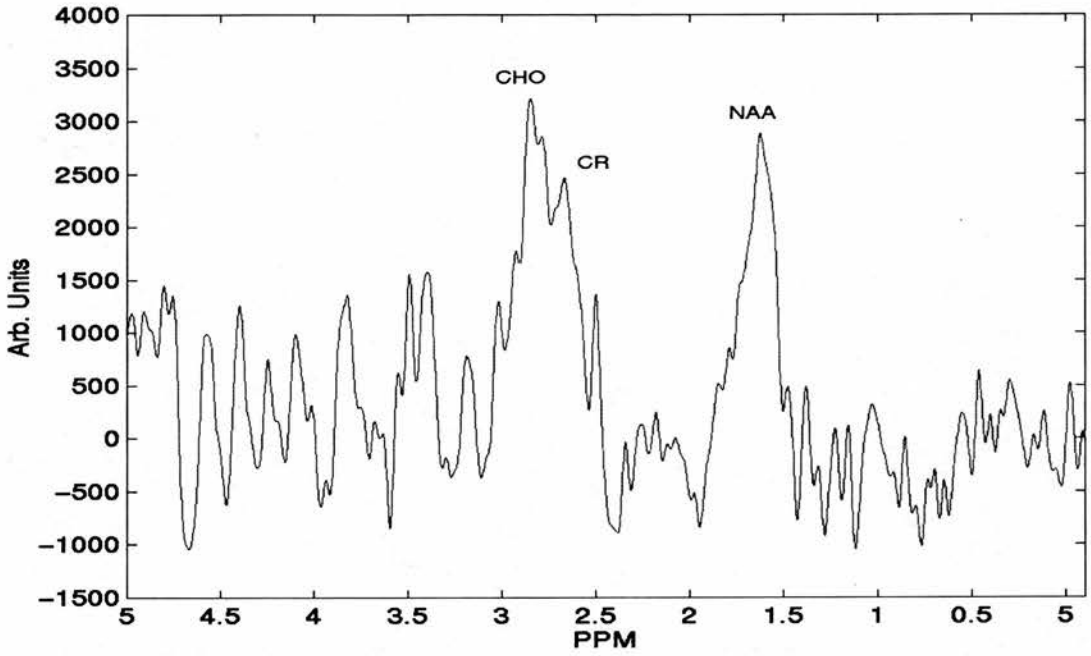
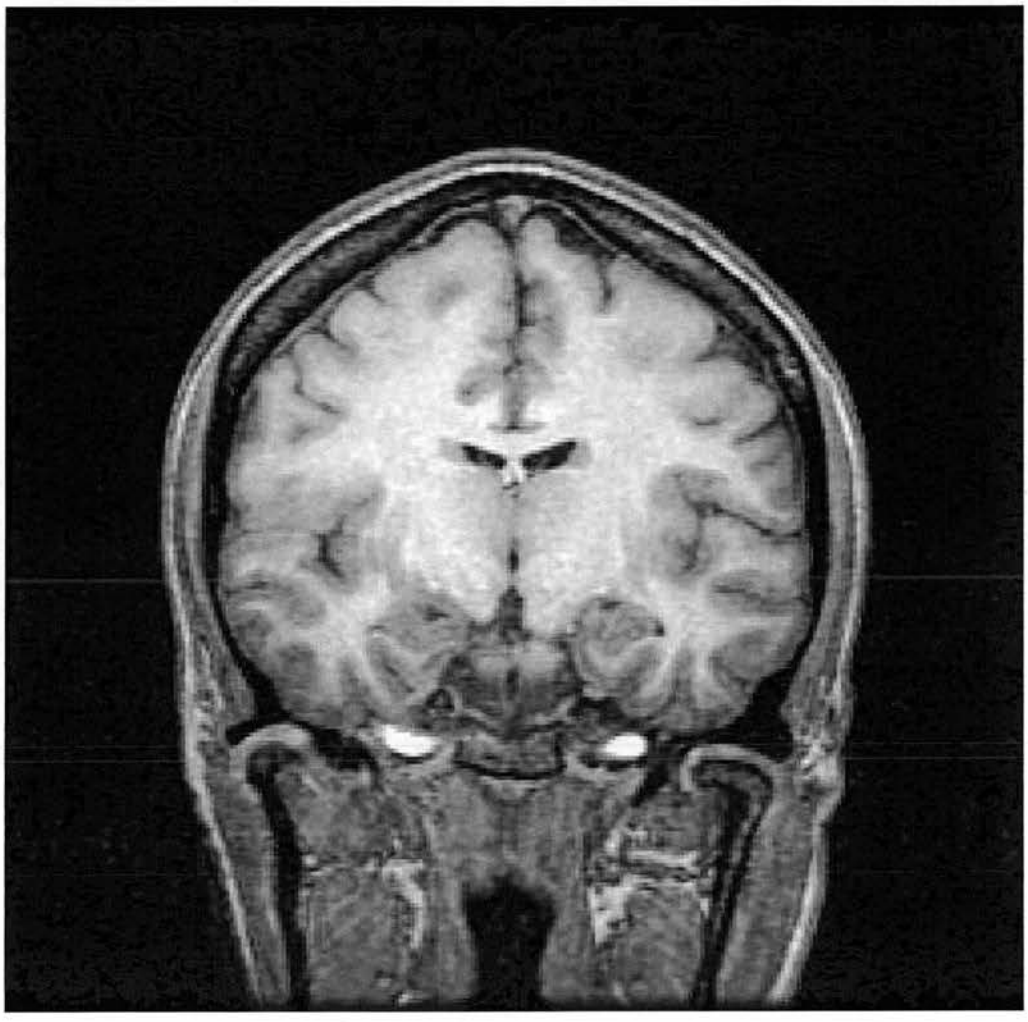


Figure 1Cvii. A T_1 MRI image in the coronal plane as used for volumetric analysis. This image is at the level of the thalamus, anterior to the 3rd ventricle. Isotropic voxels are obtained by using a slice thickness of only 1.8mm.



New structural MRI techniques such as Diffusion Tensor Imaging (DTI) (see chapter 2C for an example) and the development of new methods for analysing structural MRI data such as Voxel Based Morphometry (VBM) (see chapter 2B for an example) are discussed in Chapter 1E. But first, Chapter 1D described the contribution that structural imaging research has made to our understanding of schizophrenia.

Contribution of structural imaging findings to major patho-physiological hypotheses

Since the inception of Kraepelin's concept of dementia praecox, a variety of explanatory models have been proposed as candidates for the underlying "tangible morbid process"[18]. As discussed in chapter 1A, Kraepelin himself suggested that the pathology may be "either metabolic or possibly endocrinological in nature"[18]. In recent decades, during what might be called "the modern era of schizophrenia research"[8], three theories have been notable for the support they have attracted: the 'Dopamine Hypothesis'; the 'Neurodevelopmental Hypothesis'; and the 'Disconnection Hypothesis'. In this chapter the author addresses each of these theories from the perspective of structural brain imaging before describing an interesting recent attempt at unification: the 'Dysplastic Net Hypothesis'.

Of course not all of the models proposed for schizophrenia are rooted in neuropathology. There is a huge body of work examining schizophrenia from a less overtly 'biological' viewpoint. Sociological, psychological and even psychodynamic theories have been offered. Whilst none of these models could be considered germane to a thesis about structural brain imaging, their collective contribution to our understanding of schizophrenia is undeniably important. Furthermore the powerful 'biological/non-biological' dichotomy against which many of these theories arose has now been replaced by an acceptance that biological, psychological and sociological factors interact in a reciprocal fashion. Theories that were originally presented as *explanatory* are now considered *contributory*. The author will now

present a necessarily brief overview of some of the 'non-biological' theories that he feels are most relevant to contemporary structural imaging research.

Non-Biological Hypotheses

Childhood environment

Psychodynamic theory is based upon the principle that difficulties in adulthood are a consequence of early childhood experience, in particular early perceptions of the mother-child relationship. Although none of the psychodynamic formulations of psychosis (anger turned against the self, breakdown of the defence mechanisms in the face of overwhelming inner conflict etc.) would withstand scientific scrutiny, the notion that the relationship with one's parents may be important is evident in many of the early sociological theories. Bateson and colleagues suggested that schizophrenia results from "double bind" communication between parent and child [Bateson *et al.*, 1956]. Lidz and colleagues observed that parent's of schizophrenic children display "schism" and "skew" in their marriages and described their personalities as dominated by "narcissistic egocentricity" [Lidz *et al.*, 1957]. Wynne and Singer also focussed upon relationships within families and characterised the parenting styles of parents with schizophrenic children as "fragmented" and "amorphous" [Wynne & Singer, 1963 & 1965]. These theories lent credence to the once popular notion of the "schizophrenogenic mother", a term first coined in 1948 by Frieda Fromm-Reichmann.

One of the major criticisms of this body of work is that it fails to establish the direction of causality. It is perhaps hardly surprising that relationships within a

family are unusual when one of the family members is suffering from a severe mental disorder. More recent studies have tried to circumvent this problem by adopting a retrospective design. For example, retrospective analysis of a cohort of over 5,000 children born in the UK in a single week in March 1946 reveals a statistically significant deficit in the parenting experience of those individuals who subsequently develop schizophrenia. Health visitors' assessments conducted when the children were four years old were examined. Ratings of parenting skills of mothers of children who went on to develop schizophrenia were six times more likely to be "poor" than were those of other children [Jones *et al.*, 1994]. Whilst few would now argue that the development of schizophrenia in late adolescence or early adulthood is directly attributable to inconsistent early parenting, studies such as that described above suggest that the early family environment may be a contributory factor in some cases.

The current view that early family environment may contribute to the development of schizophrenia through a complex interaction with biological vulnerability factors is supported by evidence from adoption studies. For example, a Finnish study of over 140 children adopted away from their schizophrenic biological mother finds that those children reared in "disturbed" adoptive families are significantly more likely to develop severe mental illness in adulthood than those children reared in "healthy" adoptive families [Tienari, 1991].

Stress

Another aspect of the psychodynamic model reflected in sociological and psychological theories is the notion that symptoms arise as a consequence of external pressures. There are two major strands to this work: life events; and expressed emotion.

The study of life-events in schizophrenia was pioneered by George Brown and Jim Birley in the 1960s. They observed a two-fold increase in a wide range of life events experienced by 50 schizophrenic patients in the three weeks prior to admission to hospital [Brown & Birley, 1968]. Brown argues that life events may "trigger" psychotic breakdown in people with a strong pre-existing tendency to schizophrenia rather than having a "formative" role in the causation of the disorder [Brown *et al.*, 1973]. The initial findings have been largely supported by subsequent studies including a large multicentre trial conducted by the WHO incorporating almost 400 subjects [Day *et al.*, 1987]. However, the issue of direction of causality remains largely unresolved.

The notion of 'environmental stress' incorporates not only what happens to us (life events) but also how others interact with us (whether supportive or undermining etc.). Within the schizophrenia literature this issue has been addressed primarily through the concept of 'expressed emotion'. The level of expressed emotion within a relationship reflects the the number of critical comments that are made and the degree of hostility and emotional over-involvement that are displayed. Perhaps the most influential studies to employ this concept were those conducted in the 1970s by Julian Leff and Christine Vaughn. They analysed the results of studies involving 128

schizophrenic out-patients and found that patients who lived in a high expressed emotion environment were four times more likely to relapse than patients who lived in a low expressed emotion environment [Vaughn & Leff, 1976]. Furthermore, the time spent within the high expressed emotion environment had a significant impact upon relapse rates such that those patients who spent more than 35 hours per week exposed to high expressed emotions were more than twice as likely to relapse as those exposed for less than 35 hours. Subsequent studies not only support Leff and Vaughn's findings but suggest that interventions that reduce expressed emotion within families can be successful in reducing relapse rates in schizophrenia [Tarrier *et al.*, 1988].

Social Isolation

That people who suffer from schizophrenia are less likely to be married or to have a large social network than the general population is well established. It is easy to see how both the positive and negative symptoms of the disorder might contribute to this social isolation. A number of recent studies suggest that social isolation pre-dates the onset of symptoms and may in itself represent a risk factor for the disorder. For example van Os and colleagues found that not only were single people more likely to develop schizophrenia than were married people but that the risk increased still further if the single person lived in a neighbourhood where most people were living in partnerships [van Os *et al.*, 2000]. The authors suggest that being single in a predominantly 'couples' environment leads to increased social isolation (actual and/or perceived). Coming from the opposite direction, Jablensky and Cole found that marriage is protective against schizophrenia, particular for men, and that this

effect could not be fully accounted for by better-adjusted males being more likely to marry [Jablensky & Cole, 1997]. The 1946 cohort study described above also touches on the issue of social isolation with a preference for solitary play at the age of 4 or 6 conveying a two-fold increase in lifetime risk of schizophrenia [Jones *et al.*, 1994]. Of course the direction of causality is again difficult to establish, particularly if one accepts the concept of the 'schizophreniform spectrum' (see Chapter 1D).

Social isolation has also been implicated in the now well-establihed relationship between schizophrenia and migration [Harrison *et al.*, 1988; Sugarman & Craufurd 1994; Hutchison *et al.*, 1996]. Certainly the complex nature of the relationship suggests that powerful sociological factors, of which social isolation is likely to be one, are at work [Boydell *et al.*, 2001].

Substance Misuse

A large cohort study of over 45,000 Swedish army conscripts found that cannabis use before the age of eighteen was associated with a two-to-three-fold risk of subsequent schizophrenia [Andreasson *et al.*, 1987]. Moreover the effect appeared to be dose-related with heavy users at six times the risk of non-users. However, as with most studies involving recreational drug users the findings are complicated by multiple substance misuse (for example half of the cannabis users also used amphetamines) and skewed socio-demographic distribution. Some of the criticisms of the original paper have been addressed in more detailed follow-ups of the same cohort [Andreasson *et al.*, 1989; Zammit *et al.*, 2002]. Furthermore, a number of recent studies from various groups around the world have successfully replicated the main

finding [Arseneault *et al.*, 2002; Patton *et al.*, 2002; Fergusson *et al.*, 2003; Verdoux *et al.*, 2003].

The Dopamine Hypothesis

The phrase "The Dopamine Hypothesis" was probably first coined by Snyder in 1975[19,20]. However, Carlsson & Lindqvist[21] and Randrup & Munkvad[22] had proposed the central tenant of the theory - that the symptoms of schizophrenia are caused by an increase in dopaminergic transmission in the brain, a decade earlier. The dopamine hypothesis therefore pre-dates the introduction of CT and MRI and its conception owes nothing to structural imaging findings.

The dopamine hypothesis was born out of neuro-pharmacology research, which demonstrated that schizophrenia-like symptoms can be induced by dopamine agonists[22] and that most antipsychotic agents share a common denominator of dopamine blockade[23,24]. Although there is no direct role for structural imaging in this type of research, parallels between structural imaging and neuro-pharmacological findings could potentially lend indirect support to the hypothesis. However, the most robust structural imaging findings in schizophrenia relate to increases in ventricular volume (see chapter 1D). Clearly such widespread alterations in macroscopic brain structure are not readily accounted for by alterations in a neurotransmitter system that has very few projections to the cerebral cortex.

This is not to say that the two areas of research share no common ground whatsoever. Receptor binding studies demonstrate that the basal ganglia contain by

far the highest concentration of dopamine (D_2) receptors of any brain region[25]. This is reflected by the structural imaging finding of increased basal ganglia volume in first episode schizophrenic patients receiving antipsychotic medication (D_2 antagonists)[26]. Furthermore, knowledge of the major dopaminergic pathways (mesolimbic and mesocortical in particular) gleaned from neuro-pharmacology has, to some extent, informed the decision by many contemporary structural (and functional) imaging groups to focus attention upon limbic structures and prefrontal regions of the brain.

A series of functional brain imaging studies conducted in the early 1990s appeared to undermine the dopamine hypothesis. For example, in 1992, a group from the Institute of Psychiatry published a SPET study comparing six schizophrenic patients treated with conventional antipsychotics with ten patients treated with the atypical antipsychotic Clozapine. They found that Clozapine had greater efficacy in relieving psychotic symptoms despite very low D_2 occupancy [Pilowsky *et al.*, 1992]. The following year the same group published another SPET study of 18 schizophrenic patients receiving typical antipsychotic medication, 10 of whom had shown a good clinical response and eight of whom had shown a poor response. They found that "clinical improvement appears to occur in the responsive group, even at relatively low D_2 occupancy, whereas non-responders do not improve as maximal D_2 blockade." [Pilowsky *et al.*, 1993]. The notion of a simple relationship between D_2 occupancy and antipsychotic efficacy was no longer tenable.

In recent years dopamine has proved something of a "comeback kid" in schizophrenia research [Jones & Pilowsky 2002]. Functional imaging studies adopting dynamic challenge paradigms suggest that endogenous dopamine release may be hyper-sensitive and exaggerated in psychotic patients. For example, Laruelle and colleagues measured occupancy of striatal D_2 receptors following administration of amphetamine and found it to be doubled in schizophrenics compared with controls. Furthermore the increase in endogenous dopamine release was greatest in the most acutely ill patients [Laruelle *et al.*, 1996]. The growing body of research using PET and SPET to measure alterations in occupancy rates at specific receptors in response to various drugs has led to an updating of the dopamine hypothesis. The contemporary dopamine hypothesis states that overactive phasic dopamine transmission in limbic regions may be responsible for positive symptoms, whilst underactive tonic dopamine transmission in frontal and prefrontal regions may be responsible for negative symptoms [Moore et al., 1999]. Kapur states it eloquently as follows: "somewhere in their late teens patients develop an abnormality of the dopamine system such that there is an exaggerated release of dopamine, out of synchrony with the stimuli that usually induce them. This state does not lead to any physical feelings, but, leads to the assignment of inappropriate salience and motivational significance to external and internal stimuli." [Kapur, 2003].

Although functional imaging is beyond the scope of this thesis, the revised dopamine hypothesis is important as it offers a possible link between neurodevelopmental insult and later onset of acute psychosis. There is some evidence from lesion studies in animals [Lipska et al., 1993] and from SPET studies of infants with hypoxic-

ischaemic brain damage [Kapucu et al., 1998] to support the notion that early developmental insult might lead to "dopamine sensitisation" in later life through a mechanism of reverse tolerance [Murray et al., 2002].

The Neurodevelopmental Hypothesis

Medical historians might argue that the neurodevelopmental hypothesis of schizophrenia can be traced back to Thomas Clouston's proposal of 'adolescent insanity' expounded in his 1888 lecture entitled "The Neuroses of Development"[27]. However, the earliest coherent formulations of the hypothesis in the modern literature were written almost one hundred years later. Two papers published in 1987 outline the arguments for the role of neurodevelopmental factors in the aetiology of schizophrenia. Daniel Weinberger[28], writing in Archives of General Psychiatry, suggests that the epidemiological and histopathological data "are consistent with a neurodevelopmental model in which a fixed 'lesion' from early in life interacts with normal brain maturational events that occur much later"[28]. Although Weinberger cites evidence from CT studies, structural imaging findings are not central to his proposal. Meanwhile, in a short article in the British Medical Journal, Robin Murray and Shôn Lewis argue "a model that regards early neurodevelopmental deviance as one of several risk factors provides a unifying explanation for what until now have been regarded as curious epiphenomena of schizophrenia."[29] The first piece of evidence they cite stems directly from structural brain imaging: "cerebral ventricles are enlarged in many schizophrenics . . . such changes are present at the earliest stage of schizophrenia and are not progressive."[29]

Murray and Lewis also cite epidemiological findings such as excess winter births amongst schizophrenic patients and increased incidence of obstetric complications, left-handedness, soft neurological signs and cognitive impairment. They draw parallels between schizophrenia and conditions such as epilepsy and dyskinesias, which are recognised as being neurodevelopmental in origin. The putative pathological mechanism they advance is one of "interference with neuronal fallout" during the early development of the nervous system resulting in "impairment of the organisation of axonal connections, which leads to immature patterns of cells and their projections persisting . . . the lesion lies dormant until the brain matures sufficiently to call into operation the damaged systems."[29] They demonstrate the feasibility of a prolonged latent period between brain insult and subsequent disturbance of brain function by citing evidence from lesion studies in animals.

Since 1987, the collective evidence from over 100 structural brain imaging studies of first episode schizophrenic subjects[30] has provided considerable support for Murray and Lewis' assertion that cerebral ventricular enlargement is present "at the earliest stage of schizophrenia" (see chapter 1D). However, whilst these data demonstrate that the more substantial structural brain abnormalities associated with schizophrenia are not attributable to chronicity of illness nor to treatment, they do not *prove* that the abnormalities are neurodevelopmental in origin. Abnormalities identified in first episode patients could, theoretically, arise immediately prior to (or in conjunction with) the onset of symptoms. These possibilities, however unlikely, can not be entirely discounted until prospective data relating to healthy individuals who

subsequently develop schizophrenia become available. This is one of the aims of the Edinburgh High-Risk Study[31] (described in chapter 1D).

Murray and Lewis' second assertion, that the abnormalities "are not progressive" has not received unanimous support from subsequent CT and structural MRI findings. Whilst some follow-up imaging studies of schizophrenic cohorts report no evidence of progression[32,33], others suggest that certain structural brain abnormalities may show a degree of progression during the first few years of illness[30,34,35,36].

Other findings from structural brain imaging provide indirect support for the neurodevelopmental hypothesis. The cerebral asymmetry (or torque), which is a normal feature of brain morphology and is thought to arise as a result of normal development during the second trimester of intra-uterine life, appears to be reduced in schizophrenic subjects[37,38]. Similarly, the gyri of the cerebral cortex may be less convoluted in schizophrenic subjects[39]. Furthermore, cerebral ventricular enlargement has been found to correlate with premorbid events such as childhood social and educational adjustment and history of obstetric complications[40], albeit rather inconsistently.

The epidemiological evidence employed by Murray and Lewis in support of their hypothesis has, by-and-large, stood the test of time. The associations between schizophrenia and excess winter births, obstetric complications, left-handedness, soft neurological signs and cognitive impairment have all been replicated. Whilst some of the correlations may be weak, they appear to be robust[40]. There are also some

relatively new, though less robust[41], epidemiological data linking increased incidence of schizophrenia with maternal starvation[42] and influenza infection[43], both of which are recognised disruptors of intra-uterine development.

At the centre of the neurodevelopmental hypothesis is the notion that evidence of disrupted brain development will be found somewhere within the cytoarchitecture of the adult schizophrenic brain. Schizophrenia has long been regarded as "the graveyard of neuropathology"[44] on account of the historical failure to identify any consistent pathological findings in the disorder. However, in one sense the lack of readily identifiable neuropathology is supportive of Murray and Lewis' theory. If the macroscopic abnormalities of brain structure were due to neurodegeneration, one would expect to find evidence of gliosis. Absence of gliosis is perhaps the single most consistent neuropathological 'finding' in schizophrenia.

In recent years a small number of studies of the allocortex in and around the medial temporal lobe (hippocampus[45], parahippocampus[46], entorhinal cortex[47] and cingulate & prefrontal cortices[48]) have produced promising results consistent with disruption of early neurodevelopment. Each of these studies has identified abnormal cytoarchitecture associated with schizophrenia such that certain populations of cortical neurons are displaced inwards (located within a deeper than usual layer of the cortex). In the normal course of brain development these neurons migrate outwards, from deeper to more superficial layers of the cortex. This migration occurs during the second trimester of pregnancy. The discovery of these inwardly displaced neurons suggests disruption of the normal process of migration. The data

from these histopathological studies must be considered preliminary, however they are suggestive of neurodevelopmental insult during the second trimester. Indeed the findings are difficult to explain in any other way. Whilst this handful of small studies may not amount to indisputable evidence of Murray and Lewis' "persistence of immature patterns of cells and their projections"[29] (and are certainly not suggestive of "interference with neuronal fallout"[29]), they do at least illustrate the plausibility of the neurodevelopmental hypothesis. Critics argue that none of these neuropathological findings has been successfully replicated, that the phenotype of the affected neuronal populations is uncertain and that the relationship between neurochemical findings (see dopamine hypothesis above) and the neuropathology of schizophrenia remains unclear [Harrison 1999].

Murray and Lewis' hypothesis has survived fifteen years of scrutiny by brain imaging, epidemiology and neuropathology but unambiguous neuropathological evidence of abnormal neurodevelopment has not been found [Harrison, 1999]. The precise nature of the neurodevelopmental insult (or insults) remains the subject of open debate. Furthermore, the mechanism by which disrupted brain development gives rise to the signs and symptoms of schizophrenia also remains unclear, (although recent evidence is consistent with "dopamine sensitisation" [Murray et al., 2002]).

Whilst few schizophrenia researchers would now challenge the assertion that early developmental 'deviance' is one of several risk factors for schizophrenia, many would argue that there is also a neurodegenerative component to the disorder.

Proponents of the neurodegenerative hypotheseis cite evidence from treatment studies suggesting that treatment resistance is relatively uncommon in first-episode patients but becomes more likely wirth each successive psychotic relapse [Lieberman et al., 1993]. They also cite evidence from structural imaging studies suggesting progressive ventricular enlargement in association with poor outcome [Lieberman et al., 1996 & 1997, DeLisi et al., 1995, Gur et al., 1997 & Rapoport et al 1997] (this literature is discussed in greater detail in Chapter 1D). The questions raised by the neurodevelopmental/neurodegenerative debate continue to dictate the agenda of contemporary schizophrenia research, particularly in the field of structural imaging.

The Disconnection Hypothesis

Eugen Bleuler coined the term "schizo phrenia" ("split mind") to reflect what he saw as the disintegration, or 'disconnection', of psychic processes in certain patients[49]. Murray and Lewis suggested "an impairment of the organisation of axonal connections"[29] as a possible pathological mechanism for the disorder. The disconnection hypothesis represents a unification of these two ideas. It was first coherently formulated in detail in 1995 by the impressive pairing of eminent neuropsychologist Chris Frith and psychiatrist/ground-breaking functional brain imager Karl Friston[50]. The hypothesis states that schizophrenia can be understood in cognitive terms, *and* in terms of pathophysiology, as a failure of functional integration within the brain. The authors present an explanatory model for schizophrenia based upon functional disconnectivity between the prefrontal and temporal regions. The evidence cited in support of the theory reflects the two authors' areas of expertise – cognitive neuropsychology[51] and functional brain

imaging, particularly PET[52]. Only fleeting reference is made to structural imaging findings in the original paper.

The basis of Frith's argument is that the signs and symptoms of schizophrenia do not generally represent a single deficit, but can be seen as resulting from the abnormal integration of two or more processes (for example, auditory hallucinations result from "a failure to integrate 'inner speech' and 'attribution of agency'"[51]). Friston's argument is that 'functional segregation' models of the brain, which attribute certain functions to specific cortical regions are unlikely to be able to explain the signs and symptoms of schizophrenia. He believes that one must view the brain from the perspective of 'functional integration' if one is to understand the pathophysiology of schizophrenia. In terms of functional brain imaging he emphasises the distinction between studies of 'first order effects' (such as hypofrontality) and studies of 'functional connectivity' that look for evidence of temporal correlations between neuronal activity in different regions of the brain. He subjects PET data (and other neurophysiological measures) to a mathematical analysis to produce 'eigenimages', which illustrate the extent of functional connectivity between different regions of the brain. The main finding from this analysis is of *increased* prefronto-temporal functional connectivity. Friston attributes this somewhat counterintuitive result to a failure of normal inhibition of temporal activity by prefrontal or vice-versa.

Frith and Friston are at pains to point out that the disconnection hypothesis refers to *functional* and not to *anatomical* connectivity. However, the identification of structural correlates of prefronto-temporal dysconnectivity is unlikely to do their

hypothesis any harm. The most obvious support from structural imaging data is the fact that the medial temporal lobes and prefrontal cortex are arguably the two most macroscopically abnormal regions of the brain in schizophrenic subjects[53,54] (see chapter 1D). Since the publication of the disconnection hypothesis a number of groups have examined structural imaging data sets from the perspective of correlations between frontal and temporal lobe volumes. The majority of these analyses find reduced correlations between the volumes of these two brain regions in schizophrenics, a result which is interpreted as indicative of a lack of mutually trophic influences between the frontal and temporal regions during brain development[55,56,57]. Another potential strategy for examining connectivity using structural MRI is presented by the recently developed technique of Diffusion Tensor Imaging (DTI). This allows estimation of the directional 'purity' of white matter tracts. However, results from DTI studies of fronto-temporal tracts are, to date, inconclusive[58,59,60,61] (see chapter 2C).

The disconnection hypothesis is a relatively recent proposal. Frith and Friston would probably argue that, as a functional hypothesis, it makes no testable predictions about macroscopic brain structure. Its impact upon structural imaging research may therefore be limited. However, it will almost certainly play a central role in determining the direction of functional magnetic resonance imaging research in schizophrenia.

Towards a Unitary Theory: The Dysplastic Net Hypothesis

There is nothing inherently contradictory in the theories described above. Each of the 'biological' models is consistent with a range of 'environmental' modifying factors. Furthermore, it is perfectly possible to accept all three biological hypotheses simultaneously. This produces a model of schizophrenia in which disruptions to early neurodevelopment result in functional dysconnectivity between prefrontal and temporal regions of the adult brain, one consequence of which is increased sensitivity in mesolimbic dopaminergic pathways. This is the essence of the 'dysplastic net' hypothesis proposed by Ed Bullmore, Sophia Frangou and Robin Murray in 1997[62].

The 'net' in the title refers to the neuronal networks that are established during the second and third trimesters of pregnancy as axonal projections invade the cortical plate and establish early synaptic connections. As the authors point out, these neuronal networks are both anatomical and physiological (functional). Given that the development of cerebral cortex is dependent upon the trophic effects of afferent impulses, one would predict that the greater the functional connectivity between cortical regions during periods of neuronal proliferation and pruning, the greater the correlation between the volumes of those regions in the adult brain. The dysplastic net hypothesis therefore predicts that correlations between volumes of the temporal and pre-frontal regions will be reduced in schizophrenia. As described above, a number of studies have confirmed this prediction[55,56,57].

The dysplastic net hypothesis provides an elegant explanation for the complex, and at times ethereal phenomenon that is schizophrenia. Who knows, it might even be true! However intellectually appealing it may be, this proposal remains highly speculative.

As with any theoretical model, the ultimate determinant of its impact and longevity will be its credibility in the face of hard research data. With this in mind, an overview of the main findings to date from structural brain imaging studies of schizophrenia will now be presented.

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1C. Role of Structural Imaging in Schizophrenia Research

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104.

1D. Current State of Knowledge

Introduction

Kraepelin and his contemporaries were confident that the symptoms of schizophrenia reflected underlying brain pathology but they were unable to prove the association. Over the ensuing decades neuropathological explanations for symptoms were supplanted by psychodynamic theories to the detriment of biological research. As Ron and Harvey have pointed out "to have forgotten that schizophrenia is a brain disease will go down as one of the great aberrations of 20th century medicine."[1]

A number of factors probably contributed to the temporary decline in the biological view of schizophrenia. First, the flood of neuropathological discoveries that characterised late nineteenth and early twentieth century medicine (see Chapter 1A) slowed to a trickle. Second, a number of influential negative studies were published, for example in 1924 Dunlap published a paper in the American Journal of Psychiatry in which he stated "there are no abnormalities in the brains of schizophrenic patients that can not also be found in the brains of healthy subjects."[2] Third, Freud and the other psychoanalysts provided a fashionable and intellectually appealing alternative explanation for the peculiarities of thought and behaviour that characterise the disorder.

Whilst the Kraepelian argument (that schizophrenia is a disease of the brain) was undoubtedly drowned out by the clamour of psychoanalytic conjecture, it was never

completely silenced. For example, the highly successful and independent-thinking neuropathologists Oskar and Cecile Vogt (husband and wife) remained persistent and vocal champions of the biological theory of schizophrenia. With the arrival of safe and practical in-vivo brain imaging techniques, biological psychiatry finally had the means to regain lost intellectual ground.

The first task faced by brain imaging researchers was to demonstrate that the clinical syndrome of schizophrenia is indeed associated with abnormalities of brain structure. This was achieved with early studies, such as that by Johnstone and colleagues[3], which established the relationship between chronic schizophrenia and ventricular enlargement (thereby triggering a resurgence of interest in biological correlates of schizophrenia). However, these initial findings were challenged by critics who argued that ventricular enlargement was more likely to be a consequence of treatment than of illness per se. This debate continued until a large, well-conducted study by David Owens and colleagues demonstrated that "ventricular enlargement is unrelated to past physical treatment (neuroleptics, insulin coma and electroconvulsive therapy)."[4]

Subsequent advances in imaging technology, together with adroit study design, have enabled researchers to explore the relationship between schizophrenia and macroscopic brain structure in greater detail. Following the best scientific tradition, the field has advanced from question to question - each prompted by the last. The principal questions that schizophrenia poses to structural imaging researchers will now be addressed.

Is the brain abnormal in schizophrenia?

As described above, the first brain abnormality to be identified by in-vivo structural imaging in schizophrenia was ventricular enlargement[3]. This remains the most robust and consistently replicated finding in the field[5]. Even early X-ray CT machines (such as the original EMI 1010) provide good discrimination between ventricular spaces and brain substance, particularly at the level of the lateral ventricles (as demonstrated in figure 1Ciii). Although ventricular size can be reported in a variety of ways, the most widely used method in the CT literature is the Ventricle-Brain Ratio (VBR)[6]. The area of the ventricles is conventionally expressed as a percentage of the total area of the brain scan, at the level on the scan at which the ventricles appear largest (a method first described by Synek and Reuben in 1976[7]).

The most comprehensive review of this literature is the multivariate meta-analysis by Van Horn and McManus published in 1992. The analysis incorporates 39 studies of ventricular size in schizophrenia employing the VBR method. The authors conclude "there is a difference in VBR between schizophrenics and controls which would seem to be an indisputable characteristic of schizophrenia."[6]

Whilst increased VBR may be "an indisputable characteristic of schizophrenia", it falls short of being a pathognomonic biological marker for the disorder for a number of reasons:

(1) The difference between schizophrenics and controls is too small to be of practical significance in diagnosis[6]. In another quantitative review of the CT literature, Raz and Raz, estimate the effect size (*d*) to be between 0.57 and 0.70 standard deviation units[8] (and classified as 'medium' on the Cohen criteria[9]). This effect size corresponds to a 'non-overlap' of only 43% between schizophrenic and control groups of the same size.

(2) The finding is not specific to schizophrenia. Raz and Raz incorporate data from subjects with mood disorders into their analysis and conclude that the affective psychoses are also associated with increased VBR (effect size d=0.49 to 0.55, not significantly different from effect size in schizophrenia[8]). Siever and colleagues report increased VBR in patients with schizotypal personality disorder[10]. Other conditions such as hydrocephalus and various forms of dementia[11,12] are also known to be associated with increased VBR as is normal ageing[7] and closed head injury[13].

(3) The magnitude of the increase in VBR associated with a diagnosis of schizophrenia varies considerably from individual to individual and from study to study. There is evidence that the degree of ventricular enlargement may be related to certain clinical and demographic characteristics of the patient.

(4) The reliability and validity of VBR as a measure are open to question[14,15]. The model of scanner, the precise scanning protocol used to obtain the image and the method used to extract measurements from that image all influence the resultant VBR. It is therefore not possible to compute absolute values for VBR using in-vivo imaging techniques[14]. (Various methods have been

proposed for quantifying loss of brain tissue. Whilst VBR is the most widely used, alternatives may offer superior reliability and validity. As a ratio, VBR inevitably conflates any increase in lateral ventricular size with any reductions in whole brain volume. This problem can be avoided by adopting a statistical approach that either regresses lateral ventricular volume against volumes of brain structures or treats ventricular volume as a covariate[16].)

In their meta-analysis, Van Horn and McManus find that studies of VBR in schizophrenia report a wide range of effect sizes[5]. This is not at all surprising because many of the studies are small (n = 6 to 95) and therefore random sampling error would be expected to produce a spread of results. The rationale behind meta-analysis is that, by pooling data from many small studies, the true effect size can be determined with a greater degree of certainty. However, whilst much of the apparent discrepancy between the results of different studies may be accounted for by chance, a well-conducted systematic review will also examine the pooled data for evidence of 'moderator variables'[17].

Structural imaging studies of schizophrenia have identified correlations between ventricular enlargement and: cognitive impairment[2,18,19]; negative symptoms[20,21,22]; reduced frequency of positive symptoms[21,22]; unemployment[22]; poor premorbid social adjustment[20,23] ; poor prognosis[20,23]; and male sex[7,24]. These are all therefore potential moderator variables in a multivariate meta-analysis. Inevitably, most of the 39 studies examined by Van Horn and McManus did not collect data relating to all of these variables. In this sense the data set is incomplete. However, this is a universal

feature of meta-analyses in medical research. The solution is to examine those potential moderators for which data *are* available. In the case of the Van Horn and McManus analysis, these include: method of ascertainment of VBR; diagnostic criteria employed to determine caseness; type of control subjects; age of subjects; age of onset of schizophrenia; duration of illness; proportion of males among subjects; number of subjects in study; and year of publication of study. Of these nine 'candidate' variables, three (diagnostic criteria, duration of illness and year of publication) are found to have a moderating effect.

Van Horn and McManus' analysis reveals that studies employing DSM-III criteria for schizophrenia report a significantly larger increase in VBR than those employing RDC. Given that the DSM-III criteria are 'tighter' than the RDC (see chapter 1A) this finding suggests that ventricular enlargement may be related to severity of illness. However, Van Horn and McManus find that studies employing the *even tighter* inclusion criteria of simultaneous fulfilment of both DSM-III and RDC report the *smallest* increase in VBR. The authors offer an explanation based upon inclusion of patients with alcohol and other problems into the 'single criteria' studies. However, the 'both criteria' result is based upon a sample of only five small studies and may be misleading. What is clear from the meta-analysis is that the magnitude of increase in VBR is remarkably sensitive to small variations in the diagnostic criteria employed. The fact that even the most robust and consistent finding in structural imaging research is tarnished by unresolved issues relating to the definition of the disorder demonstrates the extent of the challenge faced by researchers in this field. (These issues are discussed in Chapter 1B.)

The second significant association identified by the meta-analysis is between magnitude of increase in VBR and duration of illness. Although this finding invites the conclusion that the pathological process underlying schizophrenia is progressive, there are a number of alternative explanations. First, duration of illness may be a proxy for severity. Second, and more controversially, the underlying pathological process may not be the same in all cases of schizophrenia. The evidence for and against these competing hypotheses is discussed later in this chapter.

The third moderator variable identified by Van Horn and McManus' meta-analysis is year of publication. The authors note a gradual reduction in effect size over time (recent studies reporting a smaller increase in VBR). Whilst there is no trend in the mean VBR reported for the schizophrenic subjects, VBR in controls increases with time (thereby narrowing the gap). The explanation offered is that this reflects an increasing awareness amongst clinical researchers of the importance of careful selection of controls for studies of this type. There is a small literature relating to the choice of control subjects in this type of research[25]. This may have informed a move away from the recruitment of 'super' controls such as medical patients previously identified as having normal brain scans.

In conclusion, the short answer to the question "is the brain abnormal in schizophrenia?" is "yes, it is indisputable that the ventricles are enlarged." However, this finding is not specific to schizophrenia and is not present in all cases. The

magnitude of the finding appears to correlate with indices of poor prognosis but is susceptible to variations in diagnostic criteria.

Ventricular enlargement is a somewhat 'general' finding, which presumably reflects a loss of surrounding brain tissue. The list of recognised causes incorporates a highly varied range of pathological processes. As a tool for differentiating between various proposed pathological mechanisms, any measurement of ventricular enlargement is therefore likely to be of limited value. Furthermore, whilst collapsing the information contained in a brain scan to a single number such as VBR may facilitate the demonstration of 'a difference' between schizophrenic subjects and controls, it does not allow the pathology to be localised. It is evident that a number of important questions relating to the brain pathology underlying schizophrenia can not be answered solely by measurements of ventricular size.

Is the whole brain affected or are the abnormalities localised?

The majority of CT studies of schizophrenia report lateral ventricular size (often expressed as VBR). However, in their comprehensive review of the literature, Raz and Raz include 23 studies that also report findings relating to the 3rd ventricle. They conclude: "increases in the volumes of the lateral and the third ventricles in schizophrenic subjects were linked [correlated]. . . When measurement method was taken into account, however, dilation of the third ventricle was found to be more pronounced than lateral ventricular enlargement."[7] This finding suggests that the pathological process underlying schizophrenia may not affect all regions of the brain equally. However, the poor soft-tissue discrimination of CT limits its utility as a tool for accurate measurement of specific regions within the brain, particularly regions as small as the 3rd ventricle where a slight *absolute* enlargement might produce a large *relative* enlargement.

Unlike CT, MRI provides excellent discrimination between white and grey matter. It is also capable spatial resolution comparable to the best X-ray CT scan[26]. With MRI it is possible to identify anatomically discrete regions within the brain. MRI therefore offers researchers the technological capability to examine grey and white matter in different parts of the brain for evidence of the pathological process responsible for the ventricular enlargement associated with schizophrenia. Two quantitative overviews of this literature have been published to date. The first, in 1998, by Lawrie and Abukmeil is described as a "systematic and quantitative

review" and incorporates 40 studies[27]. The second, in 2000, by Wright et al., is a more conventional meta-analysis of data from 53 studies[4].

Lawrie and Abukmeil argue that the limited amount of available data precludes the adoption of a conventional meta-analysis approach, (i.e. combining data from different studies into one large pool and then subjecting this to statistical analysis). Rather they calculate the *median* increase or decrease in volume of each particular brain region. They construct 'funnel plots' as a means of identifying publication bias and find little or none. By avoiding tests of statistical significance, their approach reduces the risk of reporting false negative findings where the available data are limited ('Type II' error). Lawrie and Abukmeil circumvent the potential problems associated with differences in diagnostic criteria by including only those studies that employ DSM IIIR criteria.

The principal finding of the review is that the volumetric MRI literature provides powerful support for the association between schizophrenia and ventricular enlargement. The average (median) increase in cerebral ventricular volume is 18%. Furthermore, this finding does not appear to be affected by differences in methodology and is not explained by publication bias. A few of the studies report data from sub-regions of the ventricles and, although the authors acknowledge potential inconsistencies in the definition of anatomical boundaries and possible publication bias, there is some evidence for differential enlargement of the body (median increase 50% left, 47% right) and occipital horns (31% left, 28% right) of the lateral ventricles. The suggestion within the CT literature of possible

disproportionate enlargement of the third ventricle[7] is not supported by this review of the MRI literature. However, the authors report a gender difference in this component of the ventricular system with a more prominent increase in male subjects (median 21%) than in females (median 5%).

One might expect ventricular enlargement to be associated with a corresponding reduction in brain substance. Lawrie and Abukmeil identify consistent reductions in whole brain volume of about 3%. These reductions are evident bilaterally and in both sexes. The data suggest that the temporal lobes may be affected to a greater extent than are other regions of the brain, (median reductions 6% left, 9.5% right). However, the authors note that this finding is not apparent in more recent studies that analyse data from male and female subjects separately. They argue that it may be a false positive attributable to examining only (severely affected) male subjects in early studies. The idea that results of structural imaging studies in schizophrenia may be affected by the gender of the subjects is supported by closer examination of findings relating to specific medial temporal lobe structures. It is in this region of the brain that Lawrie and Abukmeil find the largest reductions in volume. However, the literature is rather difficult to review because different studies sub-divide this part of the brain in slightly different ways. Ten studies report volumes for the 'amygdala-hippocampal complex' (median volume reduction 6.5%), although the two studies that report data from female subjects find a small *increase* in the volume of this region (0.5 & 3.5%). Seven studies report volumes for the hippocampus separately. The findings from male subjects suggest a differential volume reduction in this region (7% left, 8.5% right), whilst the two studies reporting data from female

subjects find volume reductions of 2.5 and 3%, comparable to overall reductions in brain volume. The five studies reporting volumes of the amygdala (10% bilaterally) and the three reporting volumes of the parahippocampal gyrus (14% left, 9% right) do not include any female subjects.

Of the 40 studies reviewed by Lawrie and Abukmeil, eight employ techniques that allow white and grey matter to be measured separately (so-called 'segmentation'). The results from these studies suggest that, whilst volumes of grey matter are reduced, white matter volume may actually be increased.

The meta-analysis conducted by Wright and colleagues incorporates 1,588 subjects with schizophrenia from 53 separate studies (including most of the 40 studies in the previous review). The methodology is more orthodox than that employed by Lawrie and Akumbeil. Data from individual studies are converted into a common form (ratio of volume in schizophrenics to corresponding volume in controls) before being combined into one large data pool for multi-variate analysis, using a random effects model. The findings are reassuringly similar to those of the previous review. Wright and colleagues report an increase in ventricular volume (mean 26%) with differential increases in the volume of the body of the lateral ventricle (mean 42%). They report no differential increase in the volume of the third ventricle (mean 26%). Whole brain volume is reduced by approximately 2-3% with no right-left or male-female differences. There is no clear evidence for a differential reduction in temporal lobe volume. However, medial temporal lobe structures including amygdala (9% bilaterally), hippocampus (7% left, 6% right) and para-hippocampal gyrus (11% left,

8% right) are subject to greater reductions in volume than would be expected were the whole brain affected equally. With respect to gender, Wright and colleagues replicate Lawrie and Abukmeil's finding that the magnitude of the schizophrenic-control differences is smaller in the five female only studies than in the eleven male only studies. However, when the pooled data from all 1,588 subjects are analysed, there is little evidence for a major gender effect. Wright et al. also examine data relating to white-grey segmentation. They conclude that reductions in grey matter volume are greater than reductions in white matter volume. They do not confirm Lawrie and Akumbeil's suggestion that white matter volume may be increased.

Given the current state of knowledge, the best available answer to the question "is the whole brain affected or are the abnormalities localised?" is a rather unsatisfactory "probably both!" Schizophrenia is associated with general reductions in brain volume (and with corresponding increases in ventricular volume) *and* localised reductions in volume of medial temporal lobe structures. It is intriguing to speculate that these may represent relatively distinct disease processes (a possibility that is explored later in this chapter).

Although the structural imaging literature relating to female schizophrenics is far from extensive, there is some evidence to suggest that certain localised macroscopic brain abnormalities identified in male subjects may not be present in female subjects. Whilst this apparent difference may be attributable to differential selection bias, the possibility remains that the underlying pathological process may be different in the two sexes (a notion supported by gender differences in age of onset[28]). As described

earlier, certain other findings from structural imaging studies (such as the association between chronicity of illness and ventricular size) can also be construed as providing evidence for more than one pathological process in schizophrenia. (The 'single' versus 'multiple' pathology debate in presented later in this chapter.) Of course the association between chronicity and ventricular size can also be interpreted as evidence for a progressive, neurodegenerative process in schizophrenia. Structural brain imaging has been employed to explore the temporal relationship between brain abnormalities and symptoms. The main findings from this literature will now be reviewed.

When do the abnormalities arise?

The definitive experiment to establish the temporal relationship between the development of macroscopic structural brain abnormalities and the clinical course of schizophrenia would be a large, longitudinal, cohort study. Subjects would be recruited before the emergence of any clinical symptoms. Detailed structural imaging examinations would be performed prior to the onset of illness, during the prodromal phase, the first psychotic episode, the first remission, and then at regular intervals for the duration of the illness and beyond. To borrow from the language of epidemiology, such an experiment would yield information relating to predisposing, precipitating and perpetuating factors in the aetiology of schizophrenia. It would address the following questions:

1. Do structural brain abnormalities pre-date the onset of symptoms?
2. Does the macroscopic appearance of the brain change in any way as the sufferer becomes unwell?
3. Do the structural brain abnormalities progress as the illness progresses?

Obviously such an experiment would be a massive undertaking, not least because of the difficulties inherent in the identification of pre-symptomatic schizophrenic subjects. This experiment has not and probably never will be conducted. However, the ambitious 'Edinburgh High-Risk Study' (EHRS) adopts a design that is as close to the ideal as is practicable. This is a cohort study of individuals who are at increased risk of developing schizophrenia by virtue of a strong family history of the

disorder (at least two affected first or second degree relatives). By selecting individuals who have 10-20 times the 'population-risk', the size of cohort required to generate adequate statistical power is reduced from over 2000 to around 150[29]. Unfortunately, the designs' greatest strength is also its greatest weakness. Inevitably the relative contribution of genetic factors to the genesis of schizophrenic symptoms in the EHRS cohort will be unusually high. The extent to which findings from this necessarily unrepresentative group can be generalised to the wider population of schizophrenic patients is unclear. The structural imaging literature contains some evidence for differences between familial and non-familial schizophrenia[30].

Recruitment for the EHRS began in 1994. To date, baseline assessments have been completed for over 160 high-risk subjects between the ages of 16 and 25. The assessment includes a comprehensive battery of psychometric tests and a volumetric MRI scan, which is analysed on a region of interest basis. Baseline assessments have also been completed for approximately 80 matched control subjects from two groups: first episode patients with schizophrenia; and 'normal' subjects with no known family history of psychotic disorder[31]. The baseline structural imaging findings suggest that high familial risk for schizophrenia is associated with reductions in the volume of the amygdalo-hippocampal complex and the thalamus. These regional reductions in volume resemble those observed in schizophrenic subjects but are of a lesser magnitude[32]. Although the study is in its early stages, preliminary findings from the first two-year follow-up assessments are starting to emerge. These appear to suggest that the development of psychotic symptoms is associated with a reduction in right temporal lobe volume[33].

As the study proceeds, comparisons of brain imaging data from those subjects who develop schizophrenia with data from those who do not will hopefully lead to the identification of features of macroscopic brain structure that pre-date (and may therefore predict) the onset of symptoms. It is also hoped that the study will reveal specific macroscopic changes in brain structure occurring during the early stages of the illness and thereby provide clues as to the nature of the pathological process responsible for the onset of symptoms. Finally, provided the cohort is followed-up over a sufficiently long period, it could provide information relating to the progression of structural brain changes over time.

The EHRS and other cohort studies may eventually provide definitive answers to the question: "When do the structural brain abnormalities associated with schizophrenia arise?" In the meantime, the only option available to researchers is to use the best evidence from cross-sectional studies to build up a longitudinal picture. With respect to the question "do structural brain abnormalities pre-date the onset of symptoms?", the best evidence comes from studies of patients during their first episode of schizophrenia. Shenton and colleagues review 124 MRI studies of first episode subjects. They conclude that the structural brain abnormalities consistently reported in chronic schizophrenia (enlarged ventricles and reduced whole brain volume) are present at the first episode of illness[34]. Furthermore, although the data relating to specific regions of the brain are less consistent, the authors conclude: "the brain regions involved [during the first episode of illness] are the same brain regions observed in more chronic patients."[34]

The question: "Does the macroscopic appearance of the brain change in any way as the sufferer becomes unwell?" is extremely difficult to answer without 'before', 'during' and 'after' data from individual cases. Even with unmedicated first episode cases it is impossible to exclude the possibility that structural brain changes occurring at the very earliest stages of the illness have been missed. The provisional finding from the EHRS of reductions in right temporal lobe volume[33] relates to 19 subjects with transient or isolated psychotic symptoms rather than schizophrenia per se and 12 of the subjects reported these symptoms at baseline.

Comparison of data from chronic patients with those from first episode subjects ought to provide information pertaining to the progression of structural brain abnormalities in schizophrenia. However, our ignorance of the effects of normal ageing upon brain morphology makes interpretation of findings from such comparisons rather difficult. Fortunately data are available from a small number of cohort studies. The largest of these is the Stony Brook First Episode Study conducted by Lynn DeLisi and colleagues[35]. 50 first episode subjects were reviewed at 4 year follow-up together with 20 matched controls. Both schizophrenics and controls showed general reductions in volumes of the right and left cerebral hemispheres and increases in the volumes of the lateral ventricles over time. However, the changes were more marked in the patient group. When results from specific brain regions were examined, the only areas showing statistically significant differential volume reductions in the patient group were the right cerebellum and the

isthmus of the corpus callosum. The authors interpret their findings as suggestive of a "subtle active brain process continuing through the first few years of the illness"[35].

A recently published ten year follow-up study from Japan reports significantly greater increases in lateral ventricular volume in schizophrenic subjects than in controls (23% v 5%)[36]. However, a number of other follow-up studies report no evidence of progression of structural brain abnormalities in schizophrenia[37,38]. Shenton and colleagues review 15 published studies relating to 5 cohorts of schizophrenic patients. They acknowledge the inconsistency of many of the findings and comment that further research is required. However, they conclude "there appear to be progressive changes in the frontal lobes and possibly in the parietal lobes, superior temporal gyrus, and lateral ventricles. Amygdala-hippocampal volume appears not to change over time."[34]

In conclusion, given the current state of knowledge, it is not possible confidently to answer the question "when do the structural brain abnormalities associated with schizophrenia arise?" A considerable body of evidence from studies of patients during their first psychotic episode suggests that most of the abnormalities are already evident at this stage. A small number of follow-up studies suggest that some of the brain changes may progress during the first few years of illness. However, the question of what happens to brain structure as someone becomes unwell remains a mystery. The Edinburgh High-Risk Study will hopefully provide more satisfactory answers to these questions within the next few years.

Is there more than one pathological process at work?

Our current concept of schizophrenia has its roots in the 'medical model' of the late nineteenth century (as outlined in Chapter 1A). This states that signs and symptoms reflect 'disease entities', each of which results from a single necessary and sufficient causative agent. However, the clinical picture in schizophrenia is characterised by a wide range of signs and symptoms, none of which is unique to the disorder. Furthermore, the course and outcome of the disorder are highly variable. The question of whether 'schizophrenia' reflects a single 'disease entity' has been the subject of open debate since the inception of the concept (see Chapters 1A and 1B). The identification of macroscopic structural brain abnormalities associated with the diagnosis provides powerful support for the presence of underlying brain pathology and supports the notion of 'schizophrenia' as a 'disease' of the brain. However, the picture is complicated by the fact that a substantial proportion of subjects with schizophrenia have no identifiable macroscopic brain abnormalities. This finding invites three rival explanations or theoretical models, each of which makes specific predictions that can be tested against research evidence.

1. Schizophrenia is a single disease entity of varying severity.

This model proposes a single, common pathological process underlying all cases of schizophrenia, reflected in changes in brain structure. However, the changes are only recognisable at a macroscopic level if they exceed a particular threshold of severity. This model predicts that the magnitude of structural brain changes will be proportional to indices of illness severity such as scores on symptoms rating scales,

poor treatment response and poor functional outcome. It also predicts that, (for a given severity of illness), the nature and extent of structural brain changes will be independent of risk factors and of gender. Finally, it predicts that structural brain changes evident in schizophrenic subjects will follow a unimodal distribution.

Nancy Andreasen and colleagues conducted one of the earliest CT studies examining the relationship between structural brain abnormalities and severity of clinical symptoms. They compared 16 patients with marked ventricular enlargement with 16 patients with normal ventricular volumes. The main finding was that increased VBR is associated with a preponderance of negative symptoms, whilst normal ventricular size is associated with an increased incidence of positive symptoms[21]. A more recent MRI study of 59 schizophrenic subjects identifies no clear relationship between brain imaging findings and symptom severity[39]. Marsh and colleagues in Stanford address the problem from the opposite direction. 56 'severely ill' patients are compared with 44 'moderately ill' patients and 52 healthy controls. Whilst increased clinical severity is associated with larger increases in CSF volume and greater reductions in fronto-parietal grey matter volume, reductions in temporal lobe grey matter volume are *smaller* in the more severely affected subjects than in the moderately affected group[40]. None of these results suggests a straightforward relationship between structural brain changes and a single dimension of 'severity'.

Poor symptomatic response to antipsychotic medication is an indirect indicator of illness severity in schizophrenia. Lee Friedman and colleagues review the VBR literature relating to treatment response and conclude that there is no statistically

significant association between treatment resistance and raised VBR[41], (although their analysis does identify a non-significant trend suggestive of a small positive correlation; effect size = +0.11). Lawrie and colleagues use both structural MRI and SPET to compare 20 treatment-responsive and 20 treatment-resistant patients matched for sex, age and duration of illness. They find no significant association between treatment resistance and any brain-imaging variable[42]. However, 'qualitative re-analysis' of the scans (by which the authors mean clinical ratings by an experienced neuro-radiologist blind to group) reveals a trend towards greater cerebral atrophy in the treatment-resistant subjects that is not identified by volumetric analysis[43]. On balance, the research evidence can probably be interpreted as indicating a weak association between treatment resistance and structural brain changes.

The literature relating structural brain changes to indicators of poor functional outcome (such as unemployment, loss of relationships, chronicity of in-patient care etc.) in schizophrenia is equally indeterminate. The first CT study to examine this relationship (Pearlson et al. 1984) identified a strong association between persistent unemployment and increased VBR in 46 psychotic patients[22]. A more recent review of the literature by Staal and colleagues (incorporating 15 CT and 6 MRI studies) highlights the wide range of outcome measures used by different groups and acknowledges that this hinders the review process. The authors tentatively conclude that ventricular enlargement is probably associated with global measures of poor outcome[44]. The same research group recently published their own MRI study comparing 20 'poor outcome' patients with 25 'favourable outcome' patients and 23

healthy controls. They conclude that poor outcome is associated with loss of frontal grey matter and increase in lateral and third ventricle volume[45]. These studies provide some support for the idea that patients with macroscopic structural brain abnormalities have a poorer functional outcome than patients with macroscopically normal brains. However, all of these studies share the intrinsic limitation of a cross-sectional design. The only longditudinal study published to date (Wassink, Andreasen et al. 1999) reports that the only structural brain abnormality to predict psychosocial impairment at seven year follow up is reduced cerebellar volume[46].

Another prediction of the 'single disease entity' model that can be tested against research evidence is that brain changes will be independent of specific risk factors. If macroscopic structural abnormalities reflect underlying brain pathology and the pathological process is the same in all cases of schizophrenia, then in-vivo structural imaging findings should be the same in all cases, irrespective of the aetiological risk factors that contributed to that pathological process. Most of the studies in this area examine the role of family history of psychosis as a risk factor for schizophrenia. The most recent meta-analysis of this literature concludes that patients with no known genetic predisposition for schizophrenia have lateral ventricles that are 20% larger than those with a recognised genetic predisposition[30]. However, the magnitude of this result is, at best, counter-intuitive. If correct, the degree of ventricular enlargement attributable to non-genetic determinants of schizophrenia is greater than the total ventricular enlargement associated with the disorder (estimated at 18%[27]). One has to wonder whether the literature relating to family history is subject to some form of selection bias (e.g. familial studies containing fewer chronic

or severe cases) although it seems improbable that the difference is *entirely* attributable to bias. One interesting aspect of this literature is that the familial/non-familial difference is not found in studies of female subjects. This suggests a significant sex by family history interaction. Finally, there is considerable evidence to suggest that a genetic predisposition for schizophrenia may be associated with particular structural brain abnormalities even in the absence of symptoms[32,47,48,49,50,51] (this literature is discussed in greater detail in Chapter 2A). This undermines the view that there is a simple, one-to-one, relationship between macroscopic structural brain changes associated with schizophrenia and the pathological process underlying the signs and symptoms of the disorder.

The argument relating to risk factors (outlined above) is equally applicable to gender – the single process theory predicts that the abnormalities should be the same in both sexes. As discussed in the previous section, the structural imaging literature relating to schizophrenia in women is limited. However, the available data indicate significant differences between male and female schizophrenics in terms of both the magnitude and the location of structural brain abnormalities.

The final prediction of the 'single process' model that lends itself to hypothesis testing relates to the statistical distribution of structural imaging data. If schizophrenia is a single disease entity of varying severity, one might reasonably expect indices of severity to follow a unimodal (probably Gaussian) distribution. If structural brain abnormalities reflect the underlying pathological process, quantitative measurements of brain changes ought to correspond to severity and

therefore ought to be distributed in a unimodal fashion within the schizophrenic population. David Daniel and colleagues test this hypothesis by examining the distribution of VBR amongst 691 schizophrenic subjects from 20 imaging studies. The results demonstrate a unimodal distribution of VBR. The authors conclude "the neuropathological process, which is obvious in patients with extreme VBR values, also exists but to a lesser degree in patients with 'normal'-sized ventricles."[52]

The concept of a single dimension of severity is also supported by imaging studies of subjects from either end of the severity spectrum. Studies of 'schizotypal' patients report structural brain changes resembling those found in schizophrenia but of a lesser magnitude[9,53]. Meanwhile a recent MRI study of patients with co-morbid schizophrenia and mild learning disabilities, which the authors consider to be a severe form of schizophrenia, reports structural brain abnormalities similar to those found in schizophrenia but of a greater magnitude[54]. However, as discussed in the previous chapter, the notion of a 'schizophreniform spectrum' does not sit comfortably with the 'single disease entity' view of schizophrenia.

2. Schizophrenia is a disease with a complex, multi-process pathology.

This model proposes a common pathological mechanism underlying all cases of schizophrenia. However, the mechanism is complex, incorporating more than one process. Not all of the processes produce the same macroscopic changes in brain structure. The appearance of the brain on in-vivo imaging reflects the relative impact of each of the different processes upon brain structure at the time the scan is conducted. Within this model, discrete structural brain changes are attributed to

particular neuro-pathological processes, each process making its own unique contribution to the clinical picture of schizophrenia. The model therefore predicts the presence of reliable correlations between specific structural imaging findings and identifiable clinical characteristics.

The theory that most clearly illustrates this view of schizophrenia is Crow's 'Two Process' proposal[55]. In this model, one pathological process ('Type I') involves biochemical imbalance and is reflected in positive symptoms, which tend to occur in acute episodes; meanwhile, a separate process ('Type II') involves loss of cerebral tissue and is reflected in negative symptoms, which tend to be persistent and progressive. The central prediction of Crow's theory is that ventricular enlargement will correlate strongly with negative symptoms. Data from early CT studies appeared to support this idea[21], however, subsequent CT and MRI studies have demonstrated that the association between ventricular enlargement and negative symptoms is less powerful than was first thought[7,56]. Furthermore, the experimental finding of a correlation between ventricular enlargement and measures related to brain dopamine activity (such as raised homovanillic acid and dopamine beta hydroxylase)[57] suggests that biochemical imbalance and loss of cerebral tissue are closely related phenomena and are therefore unlikely to reflect separate processes.

Although this early attempt to synthesise structural imaging findings into a complex, yet coherent pathological model for schizophrenia was unsuccessful, the primary aim of the majority of brain imaging studies in schizophrenia remains unchanged. Almost all studies are designed to identify correlations between specific

abnormalities of brain structure and particular clinical characteristics of the illness. The rationale behind this design is that with the aid of such correlations, one ought to be able to elucidate the nature of the individual processes that comprise the complex pathological mechanism underlying schizophrenia. In other words correlations will help to identify pieces of the schizophrenia jigsaw. Hundreds of such studies have been published. Numerous correlations have been identified, however, very few have been consistently replicated[27,34]. As previously discussed, the generalised findings of ventricular enlargement and reduced whole brain volume appear to be consistently correlated with cognitive impairment and also with negative symptoms, chronic hospitalisation, male sex and non-familiality (although these latter associations are relatively weak). Associations between regional brain changes and specific clinical characteristics are less reliable. The more frequently cited correlations include: increased basal ganglia volume with antipsychotic medication[58]; increased lateral ventricle (temporal horn) and third ventricle volume with severity of both positive and negative symptoms[59]; reduced volume of the left superior temporal gyrus (anterior portion) with auditory hallucinations[60]; reduced volume of the left superior temporal gyrus (posterior portion) with thought disorder[61,62]; reduced left hippocampal volume with severe obstetric complications[63]; and reduced right hippocampal volume with chronicity of illness[64].

Whilst these associations may be unreliable, there is no denying their attractiveness to schizophrenia researchers. A number of speculative aetiological theories have been advanced partly on the basis of preliminary structural imaging findings. A common theme amongst many of these proposals is the 'two hit hypothesis'[65,66].

This model, which stems primarily from epidemiological findings, states that both a pre-existing 'vulnerability' *and* exposure to a 'trigger' are necessary for the development of schizophrenia. Associations between structural imaging findings and known aetiological risk factors (e.g. left hippocampal volume reduction and obstetric complications) are interpreted as evidence for the neuro-pathology of vulnerability. Associations between brain abnormalities and symptoms (e.g. increased lateral ventricle volume and cognitive impairment) are viewed as expressions of the environmentally triggered neuro-pathological process. The appeal of the two hit model for structural imaging researchers is that it facilitates the construction of testable hypotheses (an example of a study designed to test a two hit hypothesis is given in chapter 2A). However, cynics might argue that the adaptability of the model encourages the over-interpretation of modest data.

3. Schizophrenia comprises a number of different disease entities.

Supporters of this model argue that the signs and symptoms that we call 'schizophrenia' can be produced by a number of unrelated pathological processes. For each patient, the nature and magnitude of macroscopic structural brain abnormalities is a reflection of the particular disease from which they are suffering. This model is the antithesis of the Kraepelian view of schizophrenia. It suggests that instead of searching for Kraepelin's "tangible morbid process", researchers ought to be looking for a range of different pathological mechanisms. Within this model, those patients with macroscopic brain abnormalities are viewed as suffering from a different disease (or group of diseases) from those with grossly normal brains. The model therefore predicts that these two (or more) groups of patients will differ on a

range of clinical indicators including treatment response, outcome, and symptom profile and that such disparities will not be accounted for simply by differences in illness severity. It also predicts that the two groups will differ in terms of aetiological risk factors and gender.

A number of early structural imaging studies were designed specifically to test this hypothesis. For example, in 1980, Weinberger and colleagues compared response to neuroleptic treatment in ten schizophrenic patients with enlarged cerebral ventricles with that in ten patients with normal ventricles matched for age, serum drug levels and various indices of severity. The principal finding was that patients with enlarged ventricles showed a poorer clinical response. This was interpreted, by the authors, as demonstrating that these patients were suffering from a "biologically different illness"[67]. Although this finding has not been consistently replicated[42], a recent review of outcome variables in schizophrenia, including treatment response, concludes that ventricular enlargement is a (weak) independent predictor of poor outcome[44]. Interestingly, attempted suicide (which is one of the more extreme outcomes in schizophrenia) has been found to be more common in patients with ventricular enlargement[68].

Andreasen and colleagues adopted an 'enlargement versus no enlargement' design in their 1982 study of clinical correlates of structural brain abnormalities. They reported poorer cognitive performance, greater incidence of negative symptoms and lower frequency of positive symptoms in the patients with ventricular enlargement[21]. These findings have subsequently been replicated on a number of occassions[19,69,70].

The association between ventricular enlargement and impaired cognitive performance was, of course, first identified by Johnstone and colleagues[2] and is arguably the single most robust finding in the field[18,34]. However, correlations between accepted clinical sub-types and specific structural brain changes have not been forthcoming. For example, an early CT study suggesting that schizophrenic patients with ventricular enlargement are more likely to show the paranoid subtype and less likely to show the hebephrenic subtype than are patients with normal ventricles has not been replicated[71].

If schizophrenic patients with significant ventricular enlargement are indeed suffering from a biologically distinct illness from those with normal ventricles, one would expect this to be reflected in differences in the risk factor and gender profiles of the two groups[72]. As discussed previously, there is considerable evidence to support the notion that ventricular enlargement is a more common finding amongst male schizophrenics than amongst females[7,24]. There is also evidence to suggest that schizophrenic patients with no family history of psychosis have larger cerebral ventricles than patients with a known genetic predisposition for the disorder[30]. If one accepts this finding, then one might expect ventricular enlargement to be positively associated with recognised non-genetic risk factors such as winter birth[73]. The available research evidence does indeed support the notion that ventricular enlargement is more common amongst schizophrenics born in winter[74].

Potentially the most powerful source of evidence in support of the 'different diseases' theory is molecular genetics. The ultimate aim of this type of research is

the identification of a single gene mutation that is responsible for several cases of schizophrenia within one extended family. This could, hypothetically, lead to the construction of a comprehensive explanatory model of the precise molecular mechanism responsible for the signs and symptoms of schizophrenia in those individuals. Strictly speaking any person with schizophrenia who did not share the mutation could be viewed as suffering from a different disease (in the sense that the pathological process is likely to be different at the molecular level). A variety of research strategies have been employed in the attempt to narrow down the search for a 'schizophrenia gene'. These include genetic linkage studies, association studies and 'candidate gene' approaches. Although this extensive field of research is beyond the scope of this thesis, it is probably not unreasonable to say that molecular genetic studies in psychosis have proved disappointing to date. Despite many promising preliminary findings, no positive linkage or association result has been satisfactorily replicated. This has not prevented researchers from exploring relationships between apparent linkage markers and structural imaging findings. For example Shihabuddin and colleagues in New York report that a linkage marker they identified on the short arm of chromosome 5 (5p14.1-13.1) is associated with increased VBR and increased fronto-parietal atrophy even in the absence of symptoms[75] (at least within the pedigree under study).

One 'fly in the ointment' for supporters of the 'different diseases' model is the finding that abnormalities of macroscopic brain structure in schizophrenia follow a unimodal distribution. The aforementioned analysis of VBR by David Daniel and colleagues[52] has recently been replicated in the form of a 'distribution analysis'

conducted by an Italian group[76]. These analyses demonstrate that any division of schizophrenic subjects into those with ventricular enlargement and those without is arbitrary. However, whilst a unimodal distribution may not be consistent with a 'two different diseases' model, it is consistent with a 'several different diseases' model provided one assumes considerable overlap between the different processes in terms of associated structural brain changes.

Conclusions

In this chapter the author has attempted to provide an overview of the current state of knowledge in the field of structural brain imaging in schizophrenia. He has also attempted to illustrate how findings from structural imaging research have informed the debate about the nature of the pathological process underlying schizophrenia.

The most important conclusion to be drawn from the structural imaging literature is that schizophrenia *is* associated with structural brain abnormalities. It is indisputable that the ventricles are enlarged and that brain volume is reduced. However, these finding are not specific to schizophrenia and are not present in all cases. As a group, schizophrenic women may show lesser structural brain abnormalities than do schizophrenic men. The magnitude of the abnormalities is, to some extent, dependent upon how one defines schizophrenia. The reductions in brain volume are probably attributable to reductions in grey more than white matter and certain regions of the brain (e.g. medial temporal lobe structures) appear to be disproportionately affected. Most of the structural abnormalities are apparent by the time the diagnosis is made (during the first psychotic episode) but some may then progress. Precisely what happens to brain structure as the patient is becoming unwell remains a mystery.

All of the findings outlined above relate to group differences between populations of patients with schizophrenia and 'normal' control populations. Just as there is considerable individual variation in the symptoms and course of schizophrenia, so

there is considerable individual variation in structural imaging findings. Structural imaging research has contributed to but has not resolved the debate surrounding the construct validity of Kraepelin's concept (see chapter 1B).

It seems increasingly unlikely that a "single necessary and sufficient causative agent"[77] will be found that will allow schizophrenia to sit comfortably within the nineteenth century 'medical model' concept of a 'disease entity'. However, the concept of 'disease' has evolved since Virkow's time. The nineteenth century view of 'disease entities' defined by specific pathological lesions has been superseded by the concept of diseases as "states incurring biological disadvantage"[78]. Pathognomonic disease markers are no longer required to justify a diagnostic category. Evidence of statistical difference from the norm is sufficient to lend validity to a diagnostic concept. The *clinical* validity of a concept is therefore not dependent upon the demonstration of a common underlying pathology shared by all patients with the diagnosis. Viewed from this perspective, the current diagnostic category of 'schizophrenia' has convincing clinical validity.

However, from the perspective of biological researchers working within the medical model, the limited *scientific* validity of the concept remains an important issue. Within the scientific community, the debate about whether all schizophrenic patients share a common underlying pathology continues. Lynn DeLisi refers to the "splitters" and the "lumpers"[79]. Although the arguments on either side of this debate have been explored in the previous section, in practice many of the differences boil down to semantics. For example, it is impossible to determine at what point "a

complex and varied pathological mechanism incorporating a number of different processes" becomes "a number of different pathologies". This debate, which has been running more or less continuously since at least 1920 when Kraepelin acknowledged "other illnesses may assume schizophrenic forms"[80], is unlikely to be resolved in the immediate future. For contemporary schizophrenia researchers, the most productive way forward is likely to involve acknowledging the limitations of schizophrenia as a scientific construct and working within those limitations.

Whilst the argument about whether schizophrenia is a single, heterogeneous disease or a collection of different diseases is intellectually stimulating, the practical issue of how best to advance our understanding of the condition should remain pre-eminent. Three strategies available to structural imaging researchers employed on this task are described in the next chapter. In section 2 of this thesis the author present three studies illustrating each of these strategies in turn.

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1D. Current State of Knowledge

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1E. Methodological Considerations in Structural Brain Imaging

Introduction

In the previous chapter the structural brain imaging literature relating to schizophrenia was reviewed and the current state of knowledge summarised. In this chapter various strategies available to structural brain imaging researchers hoping to advance our understanding of the disorder are discussed. The approaches presented by the author fall into three broad categories:

(1) Imaginative clinical study design employing carefully selected sub-groups from within the schizophrenic population
(2) Adoption of newly developed approaches to MRI data analysis
(3) Utilisation of novel structural imaging techniques

Some of the techniques discussed in this chapter represent the 'cutting edge' of structural imaging methodology. However, it is important to resist the temptation to employ novel techniques simply for the sake of it, (the so-called 'fallacy of facility'). Technological advances are only useful if they address a weakness in existing methods. As the previous chapter demonstrates, a number of fundamental questions relating to macroscopic brain structure in schizophrenia remain unanswered. Whilst there is probably no harm in schizophrenia researchers familiarising themselves with new imaging methods, this should not be at the expense of conventional imaging studies. We must not try to run before we can walk. The field is crying out for large, robustly designed and meticulously conducted studies aimed at answering

fundamental questions (such as the Edinburgh High Risk Study, described in chapter 1D). However, there is also room for small studies addressing specific research questions through imaginative design. This approach will be discussed first.

In chapter 1B, the clinical and aetiological heterogeneity that characterise schizophrenia are discussed. In chapter 1D, the apparent inconsistencies amongst structural brain imaging findings associated with the disorder are described. The high degree of disparity shown by individual patients presents a challenge to those wishing to develop an explanatory model applicable in all cases of schizophrenia. Indeed, it represents a challenge to anyone wishing to conduct research into the disorder. When adopting a straightforward 'schizophrenic cases versus controls' design, the within-group variability can reduce the between-group differences. However, individual differences may also provide clues about the complex pathology underlying schizophrenia. The key is to employ imaginative study design that may shed light upon the mechanisms underlying the clinical and aetiological heterogeneity. In chapter 2A the author illustrates this approach through the presentation of one of his own studies, which aims to identify those aspects of brain structure that represent endophenotypic markers for schizophrenia.

Exploring Clinical Heterogeneity

Symptoms

The most obvious form of clinical heterogeneity in schizophrenia occurs at the level of symptoms. Kraepelin combined a number of different clinical syndromes to produce 'dementia praecox'[1]. Bleuler talked not of 'schizophrenia' but of 'the group of schizophrenias'[2]. Modern classifications subdivide schizophrenia in accordance with the predominant symptoms[3,4]. However, attempts to identify reliable

associations between specific symptoms (or symptom clusters) and specific structural imaging findings have, to date, proved unsuccessful (see chapter 1D). It could be argued that this reflects the problem at the heart of schizophrenia research – that symptoms are too far removed from the underlying cause to allow pathological conclusions to be drawn from clinical observations.

Measures of brain function

One possible strategy for overcoming this problem would involve the identification of reliable phenotypic variants within the schizophrenic population, which are 'closer' to the underlying brain pathology than are symptoms. Electrophysiological research has been moderately successful in this regard. Reduced amplitude and increased latency of P300 auditory evoked potentials[5] and deficit of inhibition of involuntary saccadic eye movements[6] are neurophysiological markers with a reasonable degree of specificity for schizophrenia, although neither is present in all cases. To date, there is a small but fairly consistent body of evidence linking abnormalities of event-related potentials to structural abnormalities of the medial temporal lobe[7]. However, electrophysiology is likely to be superseded in this regard by functional magnetic resonance imaging (fMRI)[8] and magnetoencephalography (MEG)[9] both of which offer superior spatial and temporal resolution and greater flexibility.

Although functional imaging is beyond the scope of this thesis, it is inevitable that findings from fMRI and MEG will inform future structural imaging research. One possible route is through the sub-division of the schizophrenic population according

to phenotypes determined by neurophysiological measures. For example, the disconnection hypothesis (see chapter 1C) could be examined by comparing volumes of medial temporal lobe structures in schizophrenics with and without 'hypofrontality'. Daniel Weinberger and colleagues report a high degree of correlation between reduced hippocampal volume and reduced cerebral blood flow (measured using PET) in the dorsolateral prefrontal cortex in schizophrenic twins from discordant monozygotic twin pairs[10] (a result interpreted as being powerfully supportive of the disconnection hypothesis). This finding demonstrates a clear link between structural and functional brain abnormalities in schizophrenia. However, it also invites questions about the nature of the relationship. For example, do the volume reductions arise as a result of 'disuse atrophy' or is the abnormal function a consequence of pre-existing structural abnormality? The potential for combining structural imaging with measures of brain function is enormous and is likely to be facilitated by the development of voxel-based methods of analysis of structural imaging data (as described below). As with any productive area of research, studies of this design are likely to generate as many questions as answers.

Relapse and remission

As discussed above, the relationship between macroscopic changes in brain structure, abnormalities of brain function and symptoms is poorly understood. Given that in a substantial proportion of cases, schizophrenia is a relapsing and remitting illness, it would seem logical to design a study around the changes that presumably occur during the relapse-recovery cycle. For example, one might conduct a cohort study in which patients were scanned when they were well, during an episode of illness and

again once they had recovered. By using a patient's own scans as the comparator, many of the problems associated with individual variation would be circumvented. Such a design could be seen as taking full advantage of the safe repeatability that differentiates MRI from X-ray based imaging techniques.

Course

The modern operational definitions of schizophrenia[3,4], designed to maximise reliability of diagnosis, adopt an essentially Schneiderian approach with a relatively narrow focus upon psychotic phenomenology rather than taking into account the "total clinical picture"[1] as Kraepelin advocated (see Chapter 1B). Kraepelin believed that course was at least as important as symptoms. However, the clinical heterogeneity that characterises the symptoms of schizophrenia is echoed in the variability apparent in the course of the illness[11]. A number of structural imaging studies have (inadvertently) explored this heterogeneity by comparing chronic cases with first episode cases (see chapter 1D). However, the primary aim of these studies has been to look for evidence of progression through a cross-sectional study design. The fact that first episode cases differ significantly from chronic cases in terms of course (in that many will ultimately recover or follow a relapsing/remitting rather than a chronic course) is seen as a confounder. It would surely be more constructive to view variation in course as an interesting clinical feature worthy of study in its own right. The optimal study design would involve a large cohort of subjects recruited during the first episode and followed-up at regular intervals for several years (or even decades). The null hypothesis would be that differences in course are not reflected in differences in macroscopic brain structure at presentation. The Stony

Brook First Episode Study[12] (see chapter 1D) is arguably the closest to this design in the current literature. However, with only 50 subjects followed-up for only 4 years it lacks the statistical power required to draw meaningful conclusions about structural correlates of variations in clinical course.

One facet of the variable course of schizophrenia that has been examined by structural imaging is age of onset. Late onset schizophrenia ('paraphrenia') presents a challenge to neurodevelopmental theories of the disorder, which explain the latency between presumed neurodevelopmental insult and onset of symptoms in terms of brain maturation (see chapter 1C). To date, surprisingly few structural imaging studies of this interesting sub-group of patients have been published and findings are inconsistent[13,14]. Far more studies of early onset schizophrenia have been published and there is some evidence of progression of structural brain abnormalities in children and adolescents[15].

Treatment response

The final component of clinical heterogeneity that merits consideration is variability of response to treatment. As discussed in chapter 1D, the research examining the relationship between response to conventional antipsychotic drugs and abnormalities of brain structure is inconclusive. The only meta-analysis in the field finds a weak positive correlation between increased ventricular size and treatment resistance[16]. However, most of the studies use CT and it is possible that MRI would reveal regional effects not detected by CT (particularly if analysed using VBM, see below). There is currently only a small amount of (conflicting) evidence relating to

Clozapine[17,18]. It is possible that the differences between clozapine responders and non-responders may reveal information about the pathological process(es) that lead to symptoms. Also, changes in brain structure in response to medication (such as the increase in basal ganglia volume associated with conventional antipsychotics, see chapter 1D) may be informative. As new treatments are developed, so new research opportunities of this type will arise. In return, structural imaging findings may ultimately have a role to play in informing future prescribing decisions.

Exploring Heterogeneity of Risk Factors

One of the most striking features of schizophrenia is the variety of factors that have been identified as conveying an increased risk for the disorder (see chapters 1B and 1C). The strength of the 'neurodevelopmental hypothesis' (see chapter 1C) stems partly from its compatibility with these "curious epiphenomena"[19]. An appreciation of the mechanisms through which particular risk factors convey increased susceptibility for the condition would contribute directly to our understanding of the pathological process(es) within the schizophrenic brain.

Perhaps the most direct approach to this question is the comparison of patients with a particular risk factor against those without. Studies of this design, relating to family history of psychosis, winter birth and obstetric complications (including prematurity, intra-uterine growth retardation, prolonged labour etc.) have all been published (see chapter 1D). Whilst this is undoubtedly a hypothetically powerful design, in practice it can be difficult to establish the presence or absence of a given risk factor with the required degree of certainty (this is particularly true of family history where verbal

accounts are notoriously unreliable). It is also impossible to control perfectly for all other relevant variables. One way around this problem is to quantify each subject's exposure to every potential risk factor and then analyse the entire data-set with a statistical technique such as 'regression analysis'. This produces an estimate of the proportion of variance in a particular measurement (e.g. the volume of a particular brain structure) that is accounted for by differences in the level of exposure to a particular risk factor (e.g. family history of psychosis). Depending upon the statistical model employed, it may also be possible to identify interactions between different risk factors. This type of statistical approach relies upon large data sets (e.g. regression analysis requires a minimum of ten subjects per variable). For reliable conclusions to be drawn about interactions between risk factors, the numbers required can be enormous. Schizophrenia research, and imaging research in particular, is not renowned for large studies. There is a powerful argument for the introduction of large multi-centre studies to address fundamental aetiological questions relating to the disorder.

The role of risk factors can also be investigated from the opposite direction. Cases and controls matched precisely for the risk factor are compared, any differences are presumed *not* to be attributable to the risk factor. The archetype for this design is the study of discordant monozygotic twin pairs. However, monozygotic twins discordant for schizophrenia are rare. Recruiting sufficient numbers to allow meaningful interpretation of results is a monumental task. To date, only a handful of such cohorts have been assembled. The first structural brain imaging study of this type (Reveley et al. 1982) involved 7 twin pairs and revealed increased lateral

ventricular size in the affected twin[20]. In the 1980s, Fuller Torrey recruited 15 sets of twins from across North America. On structural MRI, the affected individuals were found to have larger ventricles and sulcii (12/15) and smaller hippocampii (14/15) than their unaffected twins[21]. These findings have recently been replicated by a Dutch group[22]. A further 7 twin pairs have subsequently been added to the Fuller Torrey cohort and analysis of the imaging and obstetric-history data suggests an association between structural brain abnormalities and birth complications (prolonged or traumatic labour)[23]. (This body of research is discussed in greater detail in Chapter 2A.)

Another research design that is particularly pertinent to conditions, such as schizophrenia, in which gene-environment interactions are implicated involves the study of 'obligate carriers'. This design (discussed and illustrated in chapter 2A) allows the effects attributable to high genetic risk to be separated from those attributable to illness per-se. As with studies involving monozygotic twins, the rarity of obligate carriers represents a significant obstacle to researchers wishing to adopt this design.

Exploring Co-Morbidity

A small group of individuals have the misfortune to suffer from both schizophrenia and some other brain disorder. A number of studies have focussed upon such patients in the hope that knowledge of the pathological processes responsible for the co-morbid condition can in some way be extrapolated to schizophrenia. Unfortunately there are a number of problems with this approach. First, the number

of subjects is often small (reflected by the preponderance of single case reports in this literature). Second, there is often as much ignorance surrounding the pathology of the co-morbid condition (e.g. 'socio-cultural' learning disability) as there is surrounding the pathology of schizophrenia. Third, the clinical diagnosis of schizophrenia can be very difficult in the presence of another disorder characterised by abnormalities of behaviour and cognition (e.g. pervasive developmental disorders such as autism).

However, these problems may not be insurmountable. For example, velo-cardio-facial syndrome (VCFS) is a complex congenital condition strongly associated with early onset schizophrenia[24]. The pathology is understood to result from a microdeletion in the q11 band of chromosome 22. Recent structural brain imaging studies of VCFS reveal reductions in whole brain volume with differential reductions in the hippocampus and superior temporal gyrus[25]. Work on VCFS has already informed genetic research into schizophrenia. It is not inconceivable that knowledge acquired from studies of this carefully selected (and in many ways unrepresentative) sub-group of patients will be of value to other schizophrenia researchers including those involved in structural imaging. On a similar theme, studies of schizophrenic subjects with gross structural brain abnormalities of developmental origin (thalamic adhesions, hippocampal fissures, lateral ventricular septae etc.) may be generally informative to structural imaging researchers.

New approaches to MRI data analysis

The development of structural brain imaging from pneumoencephalography through early X-ray CT scanning to modern MRI is described in Chapter 1C. As with any technology-based field, advances have been both evolutionary (the gradual refinement of existing methods) and revolutionary (the introduction of new, radically different techniques). In this section the author considers a number of recently developed approaches to MRI data analysis that may have an impact upon structural brain imaging research in the near future. In chapter 2B the author presents one of his own studies employing voxel-based morphometry (VBM).

Advances in the technical specification of MRI scanners appear with each successive generation. For example, whilst early scanners generated a magnetic field strength of around 0.5Tesla, the typical modern scanner is rated at 1.5T with strengths of 3T and above available (although very high field strengths introduce technical problems that need to be overcome e.g. high specific absorbtion rates due to induced fields). The quality of the raw imaging data available to researchers continues to improve in terms of tissue discrimination and spatial resolution. Meanwhile the time taken to acquire images is being steadily reduced. However, the main advances pertinent to schizophrenia research relate as much to how the data are analysed as to the quality of the data.

The methods of analysis traditionally used in structural MRI (such as VBR and ROI) are essentially evolutions of those used in CT and conventional X-ray radiography.

They rely upon information obtained from individual brain slices (or a series of slices) to calculate the length, area, or volume of particular brain structures. However, the ability of MRI systems to reassemble thin slices into a three-dimensional representation of the brain is now being exploited. For example, the notion of quantifying gyrification, traditionally associated with phylogenetics, has recently been introduced into in-vivo imaging. The 'gyrification index' (GI) is calculated by measuring the total length of the gyri within the cerebral cortex and dividing this by the total cortical surface area. The resultant number gives an indication of the extent of convolution of the gyri. Schizophrenia has been found to be associated with reductions in GI[26]. Given that gyrification is primarily an in-utero neurodevelopmental process, this finding is interpreted as supportive of the neurodevelopmental hypothesis. Another method that uses the three-dimensional information obtained by MRI is 'shape analysis'. This is a collective term embracing a number of techniques based upon comparisons of the curvature or asymmetry of various brain structures. For example, the shape of the corpus callosum has been demonstrated to be abnormal in schizophenia[27].

In contrast to structural MRI, the methods of analysis used in functional MRI are evolutions of those used in PET and nuclear medicine. They therefore tend to be computational and based upon comparisons between individual data points (as opposed to measurements obtained from two-dimensional representations). In recent years this type of data analysis has been introduced into structural imaging. Three different computational methods are currently available: voxel-based morphometry (VBM)[28]; deformation-based morphometry (DBM)[29]; and tensor-based morphometry

(TBM)[30]. VBM is the most widely used and is described and illustrated in chapter 2B. VBM uses statistical parametric mapping (SPM) (widely used in functional imaging research) to build up brain maps of statistical probabilities. In the case of VBM, the probabilities relate to differences in grey matter density either between two groups or between the study group and a 'normal' template. The process involves comparison of neuroanatomical differences on a voxel-by-voxel basis (this is discussed in greater detail in Chapter 2B). DBM provides information about global differences in brain shape, whilst TBM provides information about local shape differences. One of the main advantages of VBM over the other computational techniques is that it requires less computer-processing power. It can be performed reasonably quickly on conventional workstations.

Whilst computational methods may potentially revolutionise the way in which structural imaging data is analysed for research purposes, there remains considerable scope for evolutionary improvement of the conventional ROI approach. In the review of the literature presented in chapter 1D, the author argues that variations in the way in which ROI results are presented make it difficult to compare different studies directly. This is especially true of the medial temporal lobe structures that are of particular interest to schizophrenia researchers. These problems arise partly through the difficulties inherent in reliably identifying anatomical boundaries between relatively small, contiguous structures with complex three-dimensional shapes. There is a powerful argument for standardisation of ROI methodology in schizophrenia research and a number of groups have presented semi or fully automated parcellation methods designed to overcome these difficulties[31,32].

Novel structural imaging techniques

X-rays are X-rays. Whilst radiographers can alter the exposure time, the strength of the beam, the direction of the beam, the spread of the beam and even the means of detection, they can not alter the physical characteristics of the individual photons that constitute X-rays. By contrast, the production (and subsequent detection) of variations in the phase and frequency of radio waves that forms the basis of magnetic resonance imaging is an active and pliable process. In the hands of a skilled and ingenious operator, an MRI scanner can perform an impressively diverse array of imaging tasks. In chapter 2C the author illustrates the challenges inherent in adopting novel imaging techniques through the presentation of a proton magnetic resonance spectroscopy (^{1}H-MRS) and diffusion tensor imaging (DTI) study that he conducted with considerable help from colleagues at the Department of Medical Physics, University of Edinburgh.

As discussed in chapter 1C, magnetic resonance spectroscopy (MRS) predates magnetic resonance imaging (MRI) by some 30 years but it is only in the past decade that MRS has been commonly used as a tool in schizophrenia research. MRS allows the direct measurement of concentrations of particular chemicals in the brain in-vivo (see chapter 2C). Generally it is used in conjunction with other structural or functional imaging techniques (in the study described in chapter 2C it is used in conjunction with DTI). Early MRS studies used Phosphorus-31 (or occasionally Lithium-7 or Fluorine-19), however, the vast majority of recent studies relate to Hydrogen-1[33]. The most consistent finding is of a focal reduction in N-Acetyl

Aspartate (NAA) in the frontal and temporal lobes[34] and in the dorsolateral prefrontal cortex in particular[35].

Diffusion tensor imaging (DTI) is a relatively new MRI technique. It relies upon the measurement of the rate of diffusion of water molecules in different directions to produce a 'tensor' containing three-dimensional information. The deviation from isotropic diffusion ('anisotropy') described by this tensor reflects local cytoarchitechture (see chapter 2C). DTI is potentially valuable to schizophrenia researchers as it allows quantitative analysis of intra-cerebral white matter tracts and hence provides a means for examining structural correlates of functional connectivity within the brain. It therefore allows structural imaging researchers to contribute to the debate surrounding the 'disconnection hypothesis' (see chapter 1C). Only a handful of DTI studies in schizophrenia have been published to date and the findings are inconsistent[36]. However, there is some evidence for reduced anisotropy in the corpus callosum[37] and the arcuate fasiculus[38].

Arguably the most important new MRI techniques for schizophrenia research are those that examine brain function rather than brain structure. Although functional imaging is beyond the scope of this thesis, the author has alluded to it on a number of occasions. The primary goal of functional neuroimaging is to provide a clear map of neuronal activation or deactivation in the brain associated with a given mental operation. A number of techniques achieve this goal, however, the vast majority of fMRI studies in schizophrenia employ 'blood oxygen level dependent contrast' (BOLD). This technique relies upon the relative concentration of oxygenated and

de-oxygenated haemoglobin (although the signal is also influenced by changes in blood flow and blood volume). Neuronal activity within a particular brain region results in an increase in regional cerebral metabolism, which in turn produces an increase in perfusion. Over-compensation results in a decrease in the ratio of deoxyhaemoglobin to oxyhaemoglibin, which is reflected in an increase in the BOLD signal[8]. The author will not attempt to review the fMRI literature in schizophrenia. Suffice to say this technique has provided powerful evidence for abnormalities in neuronal activation in schizophrenia in response to a wide range of cognitive[39] and motor[40] tasks (usually in association with poor performance). It has also helped to identify biological correlates of specific psychotic symptoms[41,42] and demonstrated changes in patterns of neuronal activation attributable to antipsychotic medication[43]. It seems likely that results from functional imaging will inform future structural imaging research.

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Section 2: Studies

2A. Study 1

Structural Magnetic Resonance Imaging (MRI) in Obligate Carriers of Genes for Schizophrenia, their Affected and Unaffected Siblings

This study illustrates the use of imaginative study design employing a carefully selected sub-group from within the schizophrenic population.

2B. Study 2

Voxel Based Morphometry (VBM) and Region of Interest (ROI) Analysis of the Genotypic and Phenotypic Neuroanatomy of Schizophrenia

This study illustrates and explores the likely impact of a recenly developed computational approach to MRI data analysis.

2C. Study 3

Diffusion Tensor Imaging (DTI) and Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) in Schizophrenics and Normal Controls

This study illustrates the challenges inherent in the adoption of novel structural imaging techniques in schizophrenia research.

2A: Study 1

Structural Magnetic Resonance Imaging (MRI) of the Brain in Presumed Carriers of Gene(s) for Schizophrenia, their Affected and Unaffected Siblings

N.B. The study described in this chapter has been published. A reprint of the following paper is enclosed in a pocket attached to the inside of the back cover of this thesis.

Steel R.M., Whalley H.C., Miller P., Best J.J.K., Johnstone E.C. & Lawrie S.M. **2002.** "Structural MRI of the brain in presumed carriers of genes for schizophrenia, their affected and unaffected siblings." *Journal of Neurology, Neurosurgery and Psychiatry* **72(4):**455-458.

Background, Aim & Hypothesis

As discussed in chapter 1D, there is overwhelming evidence indicating that schizophrenia is associated with macroscopic structural brain abnormalities. The most consistently identified abnormalities include reduced whole brain volume and increased lateral ventricle volume. The balance of evidence supports the notion of differential volume reductions of the temporal lobes and, in particular, medial temporal lobe structures such as the amygdala, hippocampus and parahippocampus. It is not known for certain when these abnormalities arise: however, they have been consistently identified in patients during their first episode of illness. The pre-eminent contemporary theory, the 'neurodevelopmental hypothesis' proposes that they arise as a result of abnormal brain development (see chapter 1C).

A substantial minority of cases of schizophrenia are familial. First degree relatives of schizophrenic patients have a morbid risk of developing schizophrenia which is approximately ten times higher than in the general population[6,7]. The epidemiological evidence from adoption studies suggests that this familiality is primarily attributable to genetic rather than environmental factors[8,9]. The results of twin studies provide incontrovertible evidence for the role of both genetic and non-genetic ('environmental') factors in the aetiology of schizophrenia. The role of genetic factors is demonstrated by the substantially higher proband-wise concordance rates between monozygotic twins than between dizygotic twins (46% versus 14%)[13]. Meanwhile, the role of environmental factors is evident in the far from absolute concordance within monozygotic twin pairs - the monozygotic twin of a patient with

schizophrenia is more likely to be well (54%) than to suffer from the condition (46%)[13].

The aim of this study is to examine the relationship between genetic risk for schizophrenia and the abnormalities of brain structure associated with the condition. Central to the study design are individuals who have apparently transmitted schizophrenia from an affected parent to one or more affected children whilst remaining well themselves (see figure 2Ai). These individuals are considered to represent an unexpressed ('non-penetrant') genotype akin to unaffected monozygotic twins from discordant twin pairs. (Studies of the offspring of discordant monozygotic twin pairs reveal that children of both the affected *and* the unaffected twin share the same increased risk of developing schizophrenia[14] indicating that the genetic risk is present but not expressed in the unaffected twin). The authors have followed the example set by the Maudsley Family Study [Sharma et al. 1997] in using the term 'obligate carrier' to describe these individuals. The term 'obligate carrier' comes from Mendelian genetics where it is defined as "an individual who may be clinically unaffected but who must carry a gene mutation based on analysis of the family history" [NIH, 2003]. Although the term 'obligate' is borrowed from Mendelian genetics, the study design is not dependent upon a simple Mendelian single gene 'all-or-none' pattern of inheritance. Even if one assumes a complex, polygenic mode of inheritance (as the literature relating to genetics of schizophrenia suggests), the 'obligates' design remains valid because collectively 'obligates' carry a greater proportion of the genetic burden for the condition than do their unaffected

siblings with unaffected adult children (termed 'non-affected non-carriers' in this study).

The traditional methods of genetic research are 'linkage' and 'association'. Genetic linkage assesses the proximity between a given locus and a disease gene by estimating the frequency of separation between them at meiosis (termed 'independent segregation'). This method requires DNA samples from many affected and unaffected members of a multiply affected family. The precise polymorphisms in the linked locus can be different for each family (i.e. there may be linkage to a locus but no association with any particular allele – this is true of many linkage studies in schizophrenia [McGuffin et al., 2003]). For association, it is necessary to demonstrate that a specific allele at a polymorphic 'marker' locus segregates with the disease in many different families. This method requires DNA samples from a large number of affected individuals (from many different families) and from representatives of the general population from which the affected individuals are drawn. These methods have proved highly effective in identifying the genetic basis of rare inherited disorders caused by major single gene effects. However, the genes responsible for familial cases of schizophrenia have proved difficult to locate and identify[10,11,12]. Single genes conveying major risk for the disorder appear to be either rare or nonexistent [Tsuang et al., 1999]. Patterns of inheritance within multiply affected families are not easily explained by the single major gene model, the data have been shown to fit a multi-factorial, polygenic model far better [Gottesman 1991]. The consensus of opinion amongst contemporary genetic researchers is that genetic liability for schizophrenia almost certainly results from the combined effects

of multiple susceptibility loci interacting with environmental factors [Tsuang et al., 2001; McGuffin et al., 2003].

Despite relatively small effect sizes, many positive family based linkage studies have been published with reasonably consistent findings emerging on chromosomes 1, 6, 8, 11, 13, 15 and 22 [Seidman & Wencel, 2003]. A recent meta-analysis of the literature highlights statistically robust linkage with loci at 8p, 13q and 22q [Badner & Gershon, 2002].

Whilst the effect of each susceptibility gene may be too small to facilitate estimation of risk at either the population or individual level, an understanding of the biochemical pathways through which a gene conveys increased risk may provide insights into the molecular mechanisms underlying the illness. Experience from research into Alzheimer's disease provides an encouraging precedent: The identification of mutations in the β-amyloid precursor and presenilin genes in families at increased risk of Alzheimer's disease together with the discovery of a more general genetic susceptibility variant, Apo E4, has led to an understanding of the central role played by aberrant β-amyloid metabolism in the neuropathology of this relatively common disorder and serious attempts to link these susceptibility factors in common biochemical pathways [Sisodia & St George-Hyslop, 2002; Morishima-Kawashima & Ihara, 2001]. A number of recent studies have hinted at possible links between susceptibility loci and neuropathology in schizophrenia.

The Icelandic deCODE Genetics group refined the previously identified region of linkage within the short arm of chromosome 8 to a number of single nucleotide polymorphisms (SNPs) within the neuroregulin 1 (NRG1) gene, the core haplotype conveying a two fold risk for schizophrenia [Stefansson et al., 2002]. The same group has subsequently replicated this finding in a separate (Scottish) population [Stefansson et al., 2003]. The suggested association between variations in NRG1 and schizophrenia carries an attractive neurobiological plausibility – neuroregulin is understood to play an important role in neurodevelopment, promoting neuronal migration and cellular differentiation [Buonanno & Fischbach, 2001].

The susceptibility locus on the long arm of chromosome 22 has attracted the interest of a number of research groups not least because of the established association between microdeletions in this region and velocardiofacialsyndrome (see chapter 1E). Liu and colleagues reported associations between SMPs in the proline dehydrogenase (PRODH) gene and schizophrenia [Liu et al., 2002]. Shifman and colleagues found associations between SMPs in the gene coding for catechol-O-methyltransferase (COMT) and schizophrenia [Shifman et al., 2002]. This finding is particularly exciting as COMT is known to act directly upon monoaminergic neurotransmission and a polymorphism within the gene (Val 108/158 Met) has been found to have an effect upon prefrontal cognitive function [Egan et al., 2001]. Daniel Weinberger has described the COMT story as "describing the first plausible mechanism of genetic susceptibility for a mental illness" [Weinberger, 2002]. However, a recent meta-analysis of the COMT literature published by a group from Harvard suggests that the evidence for linkage is "modest" and that the evidence

from association studies is weak and may apply only to European populations [Glatt et al., 2003].

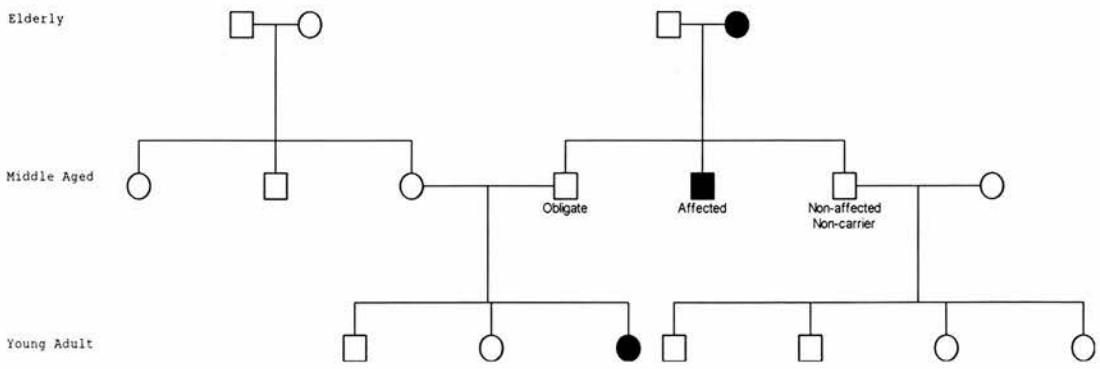
Whilst the role of polymorphisms in the COMT gene in the genesis of schizophrenia requires further clarification, the COMT literature provides an illustration of the way in which intermediate biological phenotypes may help to bridge the gap between genes and symptoms. As Daniel Weinberger observes, "biological effects at the level of cell and neural system function are far removed in biological space and time from the clinical psychopharmacology of schizophrenia and from phenotype at the level of clinical diagnosis" [Weinberger, 2002]. The reasonably reliable biological markers of impaired prefrontal cognition and reduced prefrontal dopaminergic transmission provide an endophenotype linking the COMT val allele to schizophrenia. The study in this chapter should be seen within this context. By exploring the link between macroscopic brain structure and genetic risk for schizophrenia, one ought to be able to identify those features of brain morphology which represent an endophenotypic marker for the genes conveying susceptibility for the disorder.

In this study volumetric data collected from obligate carriers are compared with data from both their affected and non-affected non-carrier siblings: The hypothesis being that structural brain abnormalities reflecting non-genetic factors will be apparent only in the schizophrenic subjects, whilst those resulting from genetically-mediated aetiological mechanisms (and therefore representing endophenotypic markers) will be found in both the schizophrenic subjects and in their obligate siblings.

Figure 2Ai. The study design can separate the effects of gene(s) from the effects of illness.

	INHERITED HIGH GENETIC RISK	*SHOWS SYMPTOMS OF SCHIZOPHRENIA*
AFFECTED SIBLING	Yes	Yes
OBLIGATE	Yes	No
UNAFFECTED SIBLING	No	No

Figure 2Aii. An ideal obligate family tree.



Methods

Subjects

As described above, the study was designed to separate out the genetic risk for schizophrenia from the illness itself. This involved recruiting specific sib-ships from multiply affected families. Each sib-ship consisted of an obligate carrier, an affected sibling, and a non-affected sibling with adult children without the disorder (and therefore presumed to be a 'non-carrier'). Volumetric data from these three groups was then compared (analysis described below). An anonymised example of an ideal obligate family tree is shown above (see figure 2Aii).

Exclusion criteria reflected known risk factors for altered brain volume: age>65 years; alcohol dependence (past or present); dementia or other "organic" brain disease; and any space-occupying intra-cranial lesion.

Families showing the ideal pattern of inheritance described in figure 2Aii are obviously rare. Family trees relating to 255 multiply affected Scottish families (originally identified for the Edinburgh High Risk Study[15]) were examined. Sixty obligates were identified, 14 of whom had both an affected sibling and a non-affected, non-carrier sibling. All fourteen families were contacted. Five families were unsuitable as one or more of the siblings met the exclusion criteria listed above (the majority of exclusions were due to age). Of the remaining nine families, three did not wish or were unable to participate. Data were therefore obtained from six sibling 'triples'. Five of the sib-ships were of the same sex (3 female, 2 male); the

remaining family included a female patient and obligate and male non-carrier (who was a twin with the patient).

Diagnosis

All subjects were interviewed using the Present State Examination (PSE)[16] and the Schedule for Affective Disorders and Schizophrenia – Life-Time version (SADS-L)[17]. This allowed diagnoses obtained through reference to psychiatric records or discussions with general practitioners to be confirmed or refuted. All six schizophrenic siblings fulfilled DSM-IV criteria for schizophrenia. Three of the obligates and three of the non-affected, non-carrier siblings fulfilled criteria for non-psychotic psychiatric disorders (all were either depressive disorder or brief depressive reaction, none was on psychotropic medication at the time of the scan). Psychiatric hospital case notes were examined to confirm duration of illness and current dose of antipsychotic medication. Diagnoses of schizophrenia in first degree relatives were confirmed through reference to psychiatric records.

Personal Data

The interview incorporated questions relating to relationships, education, employment, alcohol consumption, drug use, obstetric complications and childhood illnesses (see Table 2Ai for details). Pre-morbid IQ was estimated using the National Adult Reading Test (NART)[18]. There were no significant demographic differences between the groups although the obligates were slightly older than their siblings. As one might expect, the schizophrenic subjects were less likely to be in employment or a relationship than their well siblings. Two of the schizophrenic subjects reported

being told of complications surrounding their birth ("cord around my neck" and "coma after delivery") as did two of the obligates (both forceps deliveries) and one of the non-affected siblings ("unexpected twin, didn't breathe"). The only report of significant childhood illness was of scarlet fever in one of the obligates. Interestingly the obligates' IQs were on average 10-12 points higher than both their schizophrenic and non-affected, non-carrier siblings' (although the difference was not statistically significant: $F=0.84$, $p=0.45$).

Table 2Ai. Demographic and Clinical Details of Subjects by Group.

	Schizophrenic Siblings	*Obligates*	*Non-Affected, Non-Carrier Siblings*
Age in Years mean (sd)	46.2 (7.4)	49.0 (4.8)	45.2 (7.7)
NART IQ mean (sd)	95.6 (15.4)	107.4 (12.9)	97.3 (17.8)
Years in Education mean (sd)	11.0 (0.9)	11.2 (2.4)	10.8 (1.6)
Current Employment: no / part-time / full-time	3 / 2 / 1	0 / 1 / 5	1 / 1 / 4
Marital Status: single / divorced / married	4 / 1 / 1	0 / 2 / 4	0 / 0 / 6
Obstetric Complications: yes / no	2 / 4	2 / 4	1 / 5
Childhood Illness: yes / no	0 / 6	1 / 5	0 / 6
Psychiatric History: psychotic/neurotic/none	6 / 0 / 0	0 / 3 / 3	0 / 3 / 3
Duration of Illness in Years mean (sd)	20.8 (9.9)	N / A	N / A
Antipsychotic Dose (mg/day Chlorpromazine equivalent)	342 (256)	None	None
Current Symptoms: Psychotic / In Remission	3 / 3	N / A	N / A

Imaging Data

The MRI examinations included a dual spin echo sequence to exclude any significant brain lesions and a rapid volume acquisition sequence for volumetric data analysis. Any coil inhomogeneities were corrected for by scanning a flood phantom immediately after image acquisition and normalising to this prior to analysis. Unfortunately, it was not possible to examine all the subjects on the same machine. The first three sibling triples were scanned at the MRI Unit, City Hospital, Edinburgh (before the Unit closed) on a 1 tesla Magnetom scanner (Siemens, Erlanger, Germany) using a MPRAGE sequence , FOV 250mm, Flip angle 12°, TR=10 ms, TE= 4 ms, TI = 200 ms, and relaxation delay 500 ms, giving 128 partitions 1.88 mm thick. The remaining three triples were scanned at the Royal Infirmary, Edinburgh, using a 1 tesla Signa scanner (General Electric Company, Milwaukee, USA) using a SPGR sequence, FOV 250 mm, Flip angle 30°, TR = 16.4 ms, TE = 3.3 ms, RBW 8.93 KHz, giving 124 partitions 1.5 mm thick. Regions of interest were traced on a slice-by-slice basis according to well-established criteria using the semi-automated computer programme "ANALYZE" (Mayo Foundation, Rochester, MN). All data were analysed by the same experienced rater (Heather Whalley, Department of Psychiatry, University of Edinburgh) who was blind to group and has previously demonstrated high inter- and intra-rater reliability[15]. Boundaries between brain regions were determined by naturalistic boundaries in accordance with standard criteria[19,20,21]. Volumetric data for the following brain regions was collected: whole brain, third ventricle, fourth ventricle, right and left lateral ventricles, pre-frontal lobes, temporal lobes, caudate nuclei, lentiform nuclei, thalamic nuclei and

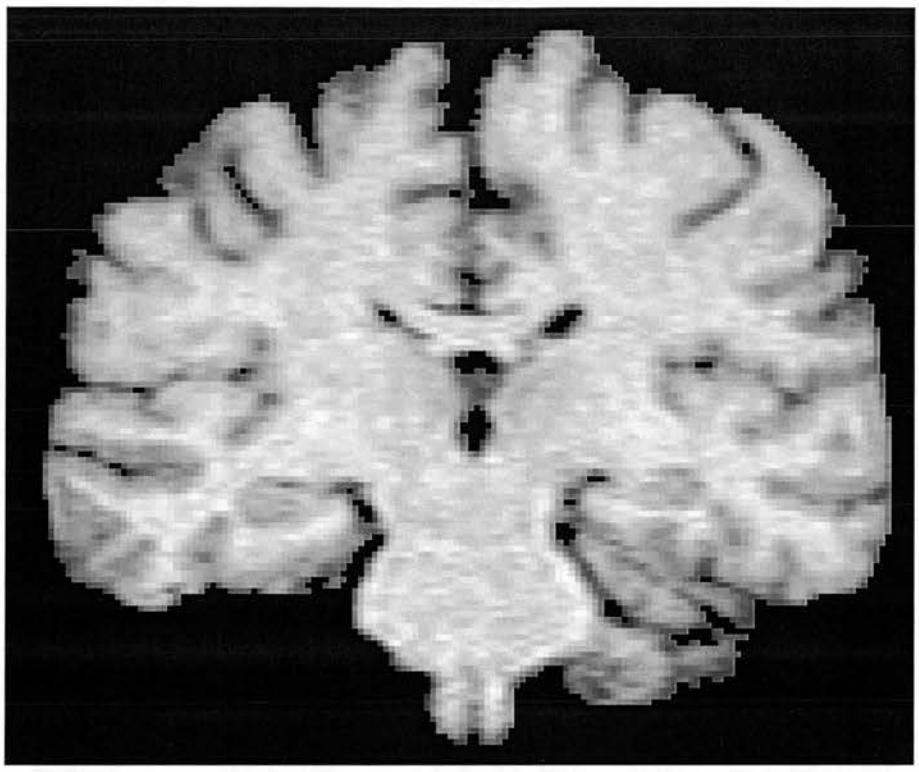
amygdalo-hippocampal complexes. Examples of the images used for volumetric analysis are shown in figure 2Aiii.

Figure 2Aiii. Coronal MRI scans as used for volumetric analysis.

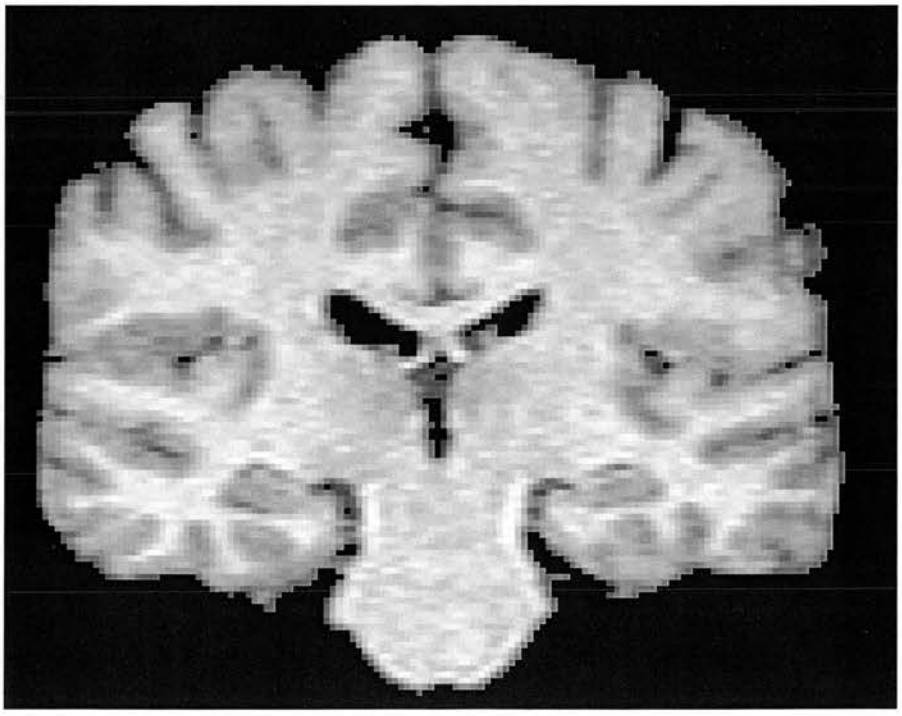
These images are from three siblings who participated in the study. They are approximately the same slice (at level of the third ventricle).

2Aiii-1 Schizophrenic Sibling

2Aiii-2 Obligate



2Aiii-3 Unaffected Sibling



Statistical Analyses

For the purposes of this thesis, the author felt it would be helpful to present two distinct statistical analyses. The first is a reproduction of the intentionally conservative and uncomplicated, 'original' analysis that was conducted for the purposes of publication. The second is a more ambitious, 'exploratory' analysis conducted primarily to facilitate comparison with the voxel-based morphometry (VBM) analysis described in chapter 2B (see Figure 2Bi) but also to satisfy the author's curiosity.

Original analysis

In order to reduce the number of statistical tests and thereby reduce the chance of false positive findings, brain regions and sides were collapsed into the following regions: cortical structures (incorporating pre-frontal and temporal lobes on both right and left); sub-cortical structures (caudate, lentiform and thalamic nuclei); amygdalo-hippocampal complexes; and intra-cerebral ventricular system (lateral, third and fourth ventricles). The null hypothesis was that there would be no difference between the three groups in terms of regional brain volumes. This was tested by straightforward repeated measures analysis of variance with the volumes of each collapsed region and whole brain volume entered as within-subjects factors. All tests were two-tailed with a threshold of $p < 0.05$ adopted for statistical significance.

Because each between-subjects factor within a repeated measures analysis of variance removes one degree of freedom (and therefore reduces statistical power), careful consideration was given to each potential confounder. It was felt that both

'family' and 'scanner' could potentially produce significant effects that would confound the main analysis. Both of these were therefore incorporated as between-subjects factors. Age and sex were also considered, however the groups were so well balanced for these variables that they were deemed unlikely to act as significant confounders. Finally, consideration was given to the issue of correction for whole brain volume. Because the central hypothesis was that reduced amygdalo-hippocampal volume *per se* may represent an endophenotypic marker for schizophrenia (rather than *relative* reduction of amygdalo-hippocampal volume), it was decided not to correct for whole brain volume. The authors acknowledge that this issue is open to debate – indeed both sides of the debate are evident in the structural imaging literature.

Exploratory analysis

The second analysis was designed to resemble as closely as possible the voxel-by-voxel comparisons that constitute VBM (see chapter 2B). Analysis of co-variance (ANCOVA) was conducted on data from the four 'collapsed' regions and all 16 regions of interest. All potential confounders - age, sex, family, scanner and whole brain volume were entered as co-variates. Three comparisons were carried out, testing for differences in regional brain volumes: schizophrenic siblings versus obligates (SZ v Obl); schizophrenic siblings versus non-affected non-carrier siblings (SZ v NA); and obligates versus non-affected non-carrier siblings (Obl v NA). In order to mimic the voxel-by-voxel probability map produced by VBM, the p-value of each between-group comparison for each individual region of interest was noted (see Table 2Aiii). A two-tailed threshold for statistical differences of $p<0.05$ was

adopted. Because the primary aim of the analysis was to ape VBM using ANCOVA, a number of statistical assumptions were made that would ordinarily be avoided. For example, correction for multiple-comparison (e.g. by Bonferroni) was considered over-conservative on the grounds that the confirmatory VBM analysis dealt with uncorrected *p* values (see Chapter 2B). The results from this exploratory analysis should therefore be treated with caution.

Power

The power of any study design is dependent upon sample size and the magnitude of any predicted effect. The unique design of this study renders accurate prediction of effect size problematic. There are no directly comparable data in the published literature. The only previously published study comparing obligate carriers with schizophrenic subjects and non-obligate relatives (the Maudsley Family Study [Sharma et al., 1998]) quotes effect sizes of 0.5-0.6 for differences in whole brain and cortical grey matter volume. However, these effect sizes relate to data collected from unmatched groups of relatives from sixteen different families rather than from sibling triples. A number of small MRI studies have been published suggesting that genetic factors account of over 70% of the individual variance in brain volumes [Bartley et al., 1997; Pennington et al., 2000]. A recently published volumetric MRI study of over 100 healthy twin pairs from the Netherlands found the heritability of whole brain volume to be comparable to that of height (90% cf. 89%) [Baaré et al., 2001]. One would therefore expect the sibling triples design to markedly reduce non-specific variation in regional brain volumes thereby increasing the predicted effect size. The Maudsley Family Study does not quote data relating to the

amygdalo-hippocampal complex (the region of greatest interest in this study). Evidence from the literature suggests differential volume reductions in this region associated with schizophrenia (approximately 6.5% cf. 3% for whole brain volume [Lawrie & Abukmeil, 1998]). One might therefore anticipate a larger effect size for comparisons relating to this region. Finally, in the original analysis (see above) data from sixteen discrete brain structures are collapsed into four larger composite regions. This is likely to further increase the predicted effect size. (In the event an effect size of 3.3 for the reductions in amygdalo-hippocampal volumes in schizophrenic subjects compared with non-affected non-carrier siblings was the largest found in this study.)

With so many factors potentially influencing the effect size, any power calculation would inevitably rest upon many assumptions. The authors felt that it would be impossible to reduce subjectivity to the level where an accurate estimation of power could be made. For this reason no formal power calculation was included in the design of this study.

Results of Original Analysis

The mean volumes of each region of interest (and of the collapsed regions) for each group are shown in Table 2Aii. The statistically significant differences identified by this conservative analysis are also shown in Table 2Aii (the right hand column). There were no statistically significant group by scanner interactions.

Differences between schizophrenic subjects and their non-affected, non-carrier siblings were largely consistent with the existing literature (see Chapter 1D). Whole brain volume was significantly smaller in the schizophrenic subjects (by approximately 5%), with a disproportionate reduction in the volume of the amygdalo-hippocampal complexes (12%). The expected increase in ventricular volume was, however, not found (see Table 2Aii).

In terms of whole brain volume and volumes of cortical structures, the obligates were indistinguishable from their non-carrier siblings (and significantly larger than their schizophrenic siblings). They also had significantly smaller ventricles than their schizophrenic siblings. However, with respect to the amygdalo-hippocampal complex, the obligates' brains did not differ significantly from their schizophrenic siblings' (both groups showing a significant reduction in volume compared with the non-affected, non-carriers).

Table 2Aii. Volumes of Brain Regions by Group (expressed in cm^3).

The collapsed regions used for the 'original' analysis

are in **bold** type.

	Schizophrenic Siblings (SZ)	Obligates (Obl)	Non-Affected, Non-Carrier Siblings (NA)	Significant between group differences by repeated measures ANOVA
	mean (sd)	mean (sd)	mean (sd)	(df = 2,3)
Whole Brain	**1193.2 (50.2)**	**1259.2 (85.6)**	**1262.6 (68.0)**	**SZ < Obl, NA** (F=7.8, p=0.01)
***Amygdalo-hippocampal* Complex**	**7.8 (0.8)**	**8.4 (0.5)**	**8.8 (0.3)**	***SZ, Obl < NA*** (F=16.5, p=0.001)
Left	3.6 (0.5)	3.9 (0.4)	4.1 (0.3)	
Right	4.1 (0.4)	4.5 (0.2)	4.7 (0.3)	
Sub-Cortical Structures	**28.1 (2.4)**	**27.6 (3.0)**	**28.8 (2.7)**	**No significant differences** (F=3.2, p=0.095)
Thalamus Left	5.2 (0.3)	5.3 (0.5)	5.7 (0.5)	
Thalamus Right	5.2 (0.5)	5.4 (0.6)	5.7 (0.6)	
Caudate Left	4.0 (0.5)	3.9 (0.5)	3.8 (0.4)	
Caudate Right	3.9 (0.5)	3.7 (0.7)	3.8 (0.3)	
Lentiform Nucleus Left	4.9 (0.8)	4.6 (0.7)	4.8 (0.8)	
Lentiform Nucleus Right	4.8 (0.6)	4.6 (0.8)	4.9 (0.7)	
Cortical Structures	**273.8 (9.5)**	**293.7 (16.0)**	**293.4 (13.4)**	***SZ < Obl, NA*** (F=4.7, p=0.04)
Pre-frontal Lobe Left	63.9 (6.3)	67.8 (6.1)	66.2 (3.4)	
Pre-frontal Lobe Right	65.9 (3.0)	71.1 (6.4)	73.5 (7.5)	
Temporal Lobe Left	70.9 (3.4)	75.6 (6.9)	74.2 (7.8)	
Temporal Lobe Right	73.0 (2.4)	79.3 (8.4)	79.5 (6.9)	
Intra-Cerebral Ventricular System	**16.6 (6.4)**	**9.6 (5.2)**	**18.9 (11.4)**	***Obl < SZ*** (F=5.4, p=0.03)
Lateral Ventricle Left	7.0 (2.9)	5.0 (2.9)	10.3 (7.6)	
Lateral Ventricle Right	7.9 (4.4)	3.3 (1.8)	7.0 (2.9)	
3^{rd} Ventricle	0.9 (0.3)	0.6 (0.2)	0.9 (1.0)	
4^{th} Ventricle	0.7 (0.3)	0.8 (0.5)	0.6 (0.1)	

Results of Exploratory Analysis

Comparing each region of interest individually whilst controlling for age, sex, family, scanner and perhaps most notably, whole brain volume, produces some interesting findings (see Table 2Aiii, which in essence equates to a VBM probability map).

Schizophrenic siblings have significantly smaller temporal lobes and thalami than their non-affected siblings but significantly larger lentiform nuclei (this last finding is probably attributable to medication[22]). Obligates have larger right temporal lobes but smaller thalami and smaller amygdalo-hippocampal complexes, particularly on the left than their non-affected siblings. Obligates also have larger cortical structures, especially the right temporal lobe, larger amygdalo-hippocampal complexes and smaller sub-cortical structures than their schizophrenic siblings.

These findings can be summarised as follows:

SZ < Obl < NA	**Amygdalo-Hippocampal Complexes**
Obl < SZ < NA	**Thalamus**
NA, Obl < SZ	**Lentiform Nucleii**
SZ < NA < Obl	**Right Temporal Lobe**

(Although it is perhaps worth noting that the SZ v NA comparison of amygdalo-hippocampal volumes actually has a *p*-value of $p = 0.08$ and therefore does not reach statistical significance when a threshold of $p < 0.05$ is adopted. This is partly explained by correction for whole brain volume (see below)).

Table 2Aiii. Results of 'exploratory' analysis.

Three between group analyses of covariance (as described in text) are shown. Results quoted as *p* values (probability of result arising if there is no between group difference). Results attaining statistical significance ($p < 0.05$) are shown in **bold** type.

	Schizophrenic Siblings V Obligates	**Schizophrenic Siblings V Non-Affected, Non-Carriers**	**Obligates V Non-Affected, Non-Carriers**
	p	*p*	*p*
Amygdalo-Hippocampal Cmpl.	**0.03**	0.08	**0.01**
Left	**0.04**	0.22	**0.01**
Right	**0.04**	0.14	0.22
Sub-Cortical Structures	**0.01**	**0.02**	0.10
Thalamus L	**0.05**	**0.01**	**0.01**
Thalamus R	0.09	**0.01**	**0.01**
Caudate L	0.07	0.33	0.93
Caudate R	**0.05**	0.49	0.86
Lentiform Nucleus L	**0.05**	**0.01**	0.23
Lentiform Nucleus R	**0.03**	**0.02**	0.18
Cortical Structures	**0.01**	0.11	0.33
Pre-frontal Region L	0.33	0.13	0.87
Pre-frontal Region R	0.30	0.07	0.71
Temporal Lobe L	0.18	**0.003**	0.11
Temporal Lobe R	**0.03**	**0.03**	**0.01**
Intra-Cerebral Ventr. System	0.23	0.08	0.20
Lateral Ventricle L	0.61	0.16	0.16
Lateral Ventricle R	0.15	0.06	0.25
3rd Ventricle	0.19	0.38	0.21
4th Ventricle	0.38	0.41	0.60

Influence of Covariates

Age, sex and whole brain volume were entered into the exploratory analysis as covariates despite not being corrected for in the original analysis (see above). It is possible that this may account for some of the differences between the two sets of results. Table 2Aiv (below) shows the strength of correlations between each of these covariates and volumes of each brain region. It is clear that neither age nor sex is likely to have had a major effect upon the results (the correlations are weak and the groups are well balanced for these variables). However, the powerful and statistically significant correlations between whole brain volume and regional brain volumes merit closer scrutiny.

The main finding of this study relate to volumes of the amygdalo-hippocampal complexes (AHC). The original analysis found reduced AHC volumes in schizophrenics and a statistically indistinguishable reduction in obligates (SZ, Obl < NA) whilst the exploratory analysis found obligates to be intermediate between their affected and unaffected siblings (SZ < Obl < NA). This difference can not be explained by the fact that whole brain volume was controlled for in the exploratory analysis because schizophrenics have smaller brains than the other two groups (hence when WBV is controlled for the relative AHC volume would increase rather than decrease).

The finding of reduced volumes of cortical structures in schizophrenics in the original analysis may simply reflect an overall reduction in brain volume. Similarly, the apparently larger lentiform nuclei of schizophrenics found on the exploratory

analysis may be a relative effect. However, the temporal lobe volume findings on the exploratory analysis (SZ < NA < Obl) are, if anything strengthened by the fact that they are robust in the face of whole brain volume correction. Finally, the somewhat counterintuitive thalamus finding on the exploratory analysis (Obl < SZ < NA) may be attributable to correction for WBV (the raw data suggests Obl, SZ < NA indicating that reduced thalamic volume may be an endophenotypic marker for schizophrenia).

Table 2Aiv. Correlations between covariates and regional brain volumes for all 18 subjects. Values quoted are partial correlation coefficients (controlling for other covariables) results attaining statistical significance ($p < 0.05$) are shown in **bold** type.

	Age	**Sex**	**WBV**	*p value*
Amygdalo-Hippocampal Cmpl.	-0.32	0.14	**0.80**	p=0.01
Sub-Cortical Structures	-0.36	0.32	**0.90**	p<0.01
Cortical Structures	0.04	0.20	**0.79**	p=0.01
Intra-Cerebral Ventr. System	0.16	0.16	-0.20	Not Sig.

Main Findings

In this study, the original analysis of the data reveals that presumed carriers of the gene(s) for schizophrenia ('obligates') share a significant reduction in amygdalo-hippocampal volume with their schizophrenic siblings (i.e. for amygdala-hippocampus: SZ & Obl < NA). However, in contrast to their affected siblings, they do not show reduced frontal or temporal lobe volumes (nor reduced whole brain volumes), and they do not have large ventricles (i.e. for cortical structures: SZ < Obl & NA). These results are most readily interpreted as suggesting that the reductions in amygdalo-hippocampal volume associated with schizophrenia reflect genetic risk for the disorder and may therefore represent an endophenotypic marker for schizophrenia. Meanwhile, ventricular enlargement and reductions in cortical volume reflect the schizophrenic phenotype and are presumably a consequence of environmental factors.

The second analysis puts a slightly different slant on the data. However, for the reasons explained above, it must be viewed as exploratory. The results are probably best regarded as interesting hypotheses worthy of further investigation rather than as definitive and robust research findings. On this analysis, whilst the obligates are found to have significant reductions in amygdalo-hippocampal volumes compared with their non-affected siblings, the schizophrenics' amygdalo-hippocampus are smaller again (i.e. for amygdala-hippocampus: SZ < Obl < NA). This suggests that reductions in amygdalo-hippocampal volumes (particularly on the left) reflect both genetic *and* environmental risk factors. Meanwhile the cortical region that reveals

the most significant between-group differences is the right temporal lobe. On this analysis the obligates' right temporal lobes are even larger than those of their non-affected siblings (i.e. for right temporal lobe: SZ < NA < Obl). One speculative interpretation of this finding is that larger temporal lobes may be in some way protective against schizophrenia. Interestingly the sub-cortical structures of thalamus and basal ganglia (which were collapsed into one large region for the first analysis) appear to show opposite effects. The schizophrenics have significantly larger lentiform nuclei than their siblings (i.e. for lentiform nuclei: NA & Obl < SZ). This presumably reflects the increase in basal ganglia volume associated with antipsychotic medication[22]. In contrast, the schizophrenics have smaller thalami than their non-affected siblings, however, the obligates have the smallest thalami of all (i.e. for thalami: Obl < SZ < NA). This suggests that reduced thalamic volume reflects genetic risk for schizophrenia even in the absence of disease. It would appear that on the original analysis this finding was masked by the increases in lentiform volume.

In chapter 2B, the two analyses described above are compared with a VBM analysis (see Figure 2Bi). The impact of different methods of statistical analysis upon the conclusions drawn from brain imaging data is an important issue for structural imaging research. This is discussed in chapter 2B.

One interesting and unexpected finding of this study was the relatively high IQ of the obligates (as shown in Table 2Ai their average NART score of 107 was 10 points higher than either their schizophrenic or non-affected siblings). This finding invites

a number of different explanations. One interesting possibility is that, amongst individuals at high genetic risk of schizophrenia, intelligence may be (or may reflect) a protective factor.

Limitations

The use of two separate MRI scanners in this study is an obvious and potentially serious limitation. However, the risk of bias was minimised by scanning all members of any given family on the same machine and by entering the scanner as a between-subjects factor in the first statistical analysis and as a co-variate in the second. As no significant scanner interactions were found, this study provides some evidence that regional volumetric analyses are reliable across scanners (although obviously this comparison has low power). The notion that results of brain imaging studies may be 'scanner-dependent' is a potentially serious problem for researchers in this field. The issue is discussed further in chapter 2B.

In any condition with apparently familial and non-familial cases, it can be argued that highly familial cases are unrepresentative of the condition as a whole[23]. This study involved subjects from families in which schizophrenia was not only unusually prevalent but also inherited in a pattern resembling that seen in autosomal dominant conditions. The genetic mechanisms involved in these families may therefore be different from those acting in families with a different pattern of inheritance and may be of little relevance to non-familial cases of schizophrenia. However, as discussed in chapter 1E, the heterogeneous nature of schizophrenia invites a research strategy

that employs carefully selected sub-groups from within the affected population to address specific aetiological questions. Hence this 'limitation' is also a strength.

The most obvious limitation of this study is its small size. For this reason the original analysis described above was designed to maximise power whilst minimising the risk of false positive findings. The study's relatively small size is a direct and perhaps inevitable consequence of the design, which relies upon the recruitment of carefully selected (and therefore rare) sibling groups. However, the use of siblings as controls carries the advantage of eliminating a number of potential sources of bias reducing in particular non-specific variation in regional brain volumes, thereby increasing the power of the study (see power calculations above).

Comparisons with Previous Research

Various research strategies have been employed to investigate the relationship between structural brain abnormalities and genetic risk for schizophrenia. These include twin studies, comparisons of familial versus sporadic cases and studies involving unaffected relatives of schizophrenic subjects. Some studies of unaffected relatives identify 'obligates' as a group of particular interest[24,25]. However, the author is not aware of any published study that shares the 'sibling triples' design employed in this study.

Twin studies

Because monozygotic twins share a genotype, differences between such twins can be assumed to be of environmental (non-genetic) origin. A small number of structural

brain imaging studies of monozygotic twin pairs discordant for schizophrenia have been published. The first such study, employing X-ray CT (Reveley et al. 1982)[26], reports increased ventricular size in affected twins when compared with their unaffected co-twins. However, both groups of twins had larger ventricles than healthy controls. Together, these findings suggest that ventricular enlargement may be related to both environmental and genetic factors. The first study to employ MRI (Suddath et al. 1990)[19] does not include a separate control group. The authors compare affected with non-affected twins and report increases in lateral and third ventricle volumes with small reductions in whole brain volume in the affected twins. They also report differential reductions in hippocampal volume, which they conclude are environmentally mediated. This conclusion is further supported by the subsequent finding of an association between reduced hippocampal volume and obstetric complications in the same (but extended) cohort[27]. A recent ambitious MRI study (Baare et al. 2001)[28] compares four groups: discordant monozygotic twin pairs; discordant same-sex dizygotic twin pairs; healthy monozygotic twin pairs; and healthy dizygotic twin pairs. The control ('healthy') groups are matched to the discordant groups for zygosity, sex, age and birth order. With respect to whole brain volume, subjects with schizophrenia show reductions. However, whilst the unaffected dizygotic twins from discordant pairs have normal sized brains, the unaffected monozygotic twins of schizophrenic subjects show significant reductions in brain volume (although their brains are not as small as their schizophrenic siblings'). These findings suggest that reductions in whole brain volume reflect both genetic and environmental factors. The results with respect to frontal lobe volume are very similar, inviting a similar conclusion. However, with respect to

hippocampal volume in discordant monozygotic twin pairs, there is almost no difference between affected and unaffected twins (both show considerable reductions in hippocampal volume when compared with healthy monozygotic twin pairs). This contrasts sharply with discordant dizygotic twins in which only the affected twin shows reduced hippocampal volume. These findings strongly suggest a predominantly genetic mechanism for the reductions in hippocampal volume associated with schizophrenia and support the main conclusion of this study - that reduced amygdalo-hippocampal volume may be an endophenotypic marker for schizophrenia.

Familial versus sporadic

A number of studies have compared schizophrenic patients with a known family history of schizophrenia with schizophrenic patients with no known family history. The most consistent finding in this literature (as discussed in chapter 1D) is of an inverse relationship between ventricular enlargement and familiality[29,30,31,32]. Jones and colleagues report a similar finding in relation to cortical sulcal widening[33]. This suggests that the ventricular enlargement associated with schizophrenia is of predominantly environmental origin. An interesting study from the Institute of Psychiatry adopted this design to explore the association between structural brain abnormalities and both genetic risk and obstetric complications (a recognised 'environmental' risk factor, see chapter 1D) in schizophrenia. 27 patients with no known family history but with a history of severe pregnancy and birth complications were compared with 21 patients with uncomplicated obstetric histories from multiply affected families[34]. The results suggest that reductions in right hippocampal volume

may be genetically mediated, whilst reductions in left hippocampal volume are related to birth trauma.

Studies of unaffected relatives

A number of previous studies report volumetric data from brain imaging in relatives of schizophrenics. A study of a Finnish cohort finds reductions in cerebral grey matter, particularly in the frontal and temporal regions (with corresponding sulcal widening) in both schizophrenic subjects and their unaffected siblings, however, the siblings do not show the ventricular enlargement associated with schizophrenia[35]. A Dutch study finds that, for a range of brain abnormalities, including whole brain volume, frontal grey matter, lateral and third ventricle size, siblings fall midway between schizophrenics and normal controls[36]. Data from the Edinburgh High Risk Study support the notion that, in terms of volumes of certain brain structures, asymptomatic individuals with a genetic risk for schizophrenia may lie somewhere between schizophrenics and 'population-risk' controls[37]. This appears to be particularly true of the prefrontal region, the thalamus and the amygdalo-hippocampal complex. Other studies support these region-specific findings. An American study comparing non-psychotic, non-schizotypal first degree relatives of schizophrenics with carefully matched controls reports reductions in thalamic and amygdalo-hippocampal volumes[38]. A Canadian study finds reduced amygdalo-hippocampal volumes in first degree relatives of schizophrenics in association with impaired delayed verbal memory[39]. Finally, a very recent study from Harvard finds that non-psychotic relatives from multiply affected families share left hippocampal volume reductions with their schizophrenic relatives, whereas non-psychotic

relatives of patients in whom the condition is sporadic do not show reduced left hippocampal volume reductions[40]. As with the Canadian study, left hippocampal volume reductions are found to correlate with impairment of verbal memory.

Obligate studies

Most of the studies of unaffected relatives described above adopt a straightforward 'schizophrenics versus relatives versus controls' design. By-and-large, relatives are not sub-divided according to the likelihood of their carrying the gene(s) for schizophrenia (although in the EHRS a computed estimate of genetic risk is treated as a continuous variable[37]). In the Maudsley Family Study, Tomnoy Sharma and colleagues separate first degree relatives into 'presumed obligate carriers' and 'presumed non-obligate carriers'. They find that, in terms of structural brain abnormalities, obligates more closely resemble their schizophrenic relatives than do non-obligates. This is particularly true of left lateral ventricle enlargement[24] and loss of normal prefrontal asymmetry (in normal, right-handed controls the right prefrontal region is typically larger than the left)[25]. Unfortunately, Sharma and colleagues do not report amygdalo-hippocampal volumes. The differences in brain structure between obligates and non-obligates highlight the potential value of research involving this particular group of individuals. However, the design adopted by the Maudsley Family Study does not allow such a clear separation of genetic and environmental factors as the 'sibling triples' design used in this study.

The data from this study suggest that reduced volume of the amygdalo-hippocampal complex (and also possibly the thalamus) in schizophrenia is predominantly

genetically determined. Meanwhile, increased ventricular volume and reductions in cortical volume (especially the right temporal lobe) are not. These findings are broadly compatible with previous research. There are, inevitably, a number of areas of disagreement. For example, the twin study by Suddath et al.[19] comes to the opposite conclusion (that hippocampal reductions are environmentally mediated, attributable to obstetric complications[27]). However, that study did not include a group without genetic risk for schizophrenia (equivalent to the 'unaffected, non-carrier sibling' group in this study). The genetic versus obstetric risk study by Stefanis and colleagues found that *right* hippocampal reductions were genetically mediated[34], whereas in this study (and in the recent study from Harvard[40]) the genetic effect was, if anything, greater on the left (although this was a speculative finding from the exploratory analysis). A number of studies including the EHRS found associations between genetic risk and reductions in prefrontal volumes[37] that were not replicated in this study. It is possible that this reflects a relative lack of power in this small study.

Implications

The data from this and other studies suggest that genes may play a central role in the development of certain structural brain changes associated with schizophrenia. Whilst the evidence is far from conclusive, a reasonable case can be made for predominantly genetic aetiology of volume reductions in the amygdalo-hippocampal complex (particularly on the left), thalamus and prefrontal cortex. Meanwhile other structural brain abnormalities associated with the disorder appear to be either partially genetically mediated (e.g. ventricular enlargement) or independent of genetic factors (e.g. right temporal lobe reduction).

It is highly probable that, even in families with an apparently dominant pattern of inheritance the development of psychosis is a consequence of highly complex gene-environment interactions. Studies that separate out the genetic risk for schizophrenia from the illness itself offer a valuable perspective upon the interplay between genes and environment in the aetiology of this multifaceted disorder. It is possible that the localised structural brain changes identified in this and other studies may prove to be a useful endophenotypic marker offering a bridge between genes and symptoms. More research will clearly be required before definitive conclusions can be drawn. The author would suggest that there is a role for studies of the 'obligate' design outlined in this chapter. This potentially valuable group has been under-utilised in schizophrenia research to date.

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2A. Study 1

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62.

2B: Study 2

A Voxel Based Morphometry (VBM) and Region of Interest (ROI) Analysis of the Genotypic and Phenotypic Neuroanatomy of Schizophrenia.

N.B. The study described in this chapter has been submitted for publication as follows.

Stamatakis E.A., Steel R.M., Whalley H.C., Job D.E., Johnstone E.C. & Lawrie S.M. 2002. "A Voxel Based Morphometry (VBM) and Region of Interest (ROI) Analysis of the Genotypic and Phenotypic Neuroanatomy of Schizophrenia." A*merican Journal of Psychiatry* (Submitted).

Statistical Parametric Mapping (SPM)[1] was originally developed as a tool for use in functional brain imaging research. It was first employed to detect patterns of regional cerebral activation in positron emission tomography (PET) imaging and has been adopted as the standard method of analysis in functional Magnetic Resonance Imaging (fMRI). It involves fitting scans to a standard anatomical atlas and then applying statistical techniques to detect clusters of activation and assess the significance of change. In recent years SPM has been adapted for use in structural imaging research. It now represents an alternative to conventional Region of Interest (ROI) analysis. One new procedure, designed to allow comparison of local concentration of grey matter between two groups of subjects, is referred to as Voxel Based Morphometry (VBM)[2]. As with other SPM techniques, the raw data are subjected to a series of mathematical transformations prior to analysis. In VBM, the pre-processing sequence consists of 'spatial normalisation' followed by 'segmentation' (into grey matter, white matter and CSF), and finally 'smoothing'. Although a number of different statistical techniques such as TBM and DBM (see chapter 1E) are now available for use by brain imaging researchers, VBM was chosen for this study because it is the 'front runner' in structural imaging and also because it is in a format that is easily available and firmly supported through an internet discussion list.

Computational techniques such as VBM offer a number of hypothetical benefits over conventional ROI analysis. First, the results ought to be reproducible because the

methodology does not depend on operator input (although there is a degree of subjectivity at the pre-processing stage). Second, because such techniques rely upon voxel-wise statistical tests performed on the whole brain, differences ought to be detected wherever they occur in the brain. This removes the focus from specific brain regions and avoids the need for (regional) hypothesis-driven design. However, results obtained by this type of analysis may prove rather difficult to interpret precisely because the technique need not test regional hypotheses. One approach designed to allow a specific regional hypothesis to be tested by VBM is 'small volume correction' (SVC). This entails performing detailed analysis on a small sub-region of the brain incorporating a particular region of interest. In this study an SVC centered upon the amygdala is described.

The extent to which the adoption of computational methods of data analysis such as VBM will influence future structural brain imaging research is not clear. The aim of this study is to identify inconsistencies and areas of commonality between the results obtained and conclusions drawn from a single, robust data set when analysed with a traditional method and with a computational approach. The structural MRI data set from the study described in chapter $2A^3$ is reanalysed using VBM. The results obtained by VBM are then compared with those obtained by ROI.

This particular data set was chosen for a number of reasons: First, the scanning sequences used were conventional and there were no technical problems with the collection of the imaging data. Second, rather than a simple 'case-control' design which would offer only one between-group comparison, there are three study groups

and hence three between-group contrasts. Third, the original ROI analysis of the data revealed a number of region-specific between group differences. This data set therefore ought to lend itself to a voxel-based, whole brain analysis such as VBM.

A total of three analyses are presented in this chapter (see Table 2Bi): the 'optimised' VBM analysis; the 'original', conservative ROI analysis (see Chapter 2A); and the 'exploratory' ROI analysis designed to mimic the voxel-by-voxel comparisons that constitute VBM (see Chapter 2A). The primary ROI versus VBM contrast is that comparing results *as they would appear in press* (i.e. the 'original' ROI versus the 'optimised' VBM).

Hypothesis

The main hypothesis of this study is that the new computational technique, VBM, will not substantially alter the results obtained or conclusions drawn from the data but that it will offer certain advantages over traditional ROI analysis (such as more precise anatomical localisation of between-group differences). The rationale behind the 'exploratory' ROI analysis is that it will help to elucidate any discrepancies between the reults obtained through VBM and those obtained through conventional ROI.

Table 2Bi. The different statistical analyses facilitate exploration of the origins of discrepancies between results obtained from the same data set when analysed using VBM rather than ROI.

'Original' ROI	**'Exploratory' ROI**	**'Optimised' VBM**
Conducted for purposes of publication.	Conducted for illustrative purposes.	Conducted for purposes of publication.
Designed to be methodologically and statistically robust.	Designed to mimic 'Optimised' VBM analysis.	Designed to be methodologically and statistically robust.
One repeated measures analysis of variance.	Three separate between-group analyses of covariance.	Three separate between-group analyses.
Compares volume of brain structures.	Compares volume of brain structures.	Compares local density of grey matter.
4 Collapsed Regions & Whole Brain Volume.	All 16 ROIs & 4 Collapsed Regions.	Hundreds of Thousands of Voxels.
'Family' & 'Scanner' as between-subjects factors.	'Family', 'Scanner', Age, Sex & Whole Brain Volume as covariates.	'Family', 'Scanner', Age, Sex & Total Grey Matter Voxels as covariates.

Methods

Subjects

The subjects comprised six sib-ships each consisting of an obligate carrier, an affected sibling and a non-affected sibling (as described in Chapter 2A).

Scanning Protocol

The scanning protocol is described in Chapter 2A.

ROI: methodology & statistical analyses

The regions of interest methodology is described in Chapter 2A. Two distinct statistical analyses were conducted: 'original' and 'exploratory' (as described in Chapter 2A and illustrated in Table 2Bi).

VBM: pre-processing methodology

Initially the images were converted to 'ANALYZE' format as for the ROI analysis (see Chapter 2A). The anterior commisure was then located manually in all the images within SPM (in order to assist spatial normalisation).

Spatial normalisation

This involves aligning the T1 images to a T1 template constructed by the Montreal Neurological Institute (MNI)[4]. The images need to be in the same stereotactic space to ensure that the comparisons that follow are carried out between anatomically corresponding voxels. The image alignment was carried out using a 12 point linear affine transformation. Nonlinear (or 'warping') transformations were avoided as

these have been shown to cause unwanted deformations where structural brain abnormalities are present[5]. The purpose of the alignment process is not to produce brains that are exactly matched to the template but to correct for global differences so that local changes across grey matter density are comparable for equivalent brain regions[2].

Segmentation

Following spatial normalisation, the grey matter probability maps were extracted with the segmentation procedure included in SPM[1]. This also involved correction for the non-uniformity of image intensity that is sometimes apparent in MR images (although this may not be strictly necessary - Ashburner and Friston[2] have shown that the non-uniformity correction makes little difference to the tissue classification of images without non-uniformity artifacts). The segmentation process works by comparing the images to probability images representing the likelihood that each voxel belongs to a tissue class (i.e. grey, white, CSF or other). The end result is a grey matter probability map.

Smoothing

The smoothing process ensures data are more normally distributed thereby increasing the validity of the statistical tests that follow. The process takes account of differences in local structure. Smoothing is equivalent to a convolution operator effectively converting voxel values into a measure of the local tissue composition[6]. The Gaussian kernel size adopted for smoothing determines the scale at which the subsequent statistical analysis will be most sensitive. In this study a Gaussian kernel

of 12mm FWHM was chosen as this is greater than the thickness of the cerebral cortex yet sufficiently small to allow gyrus-by-gyrus assignment of significant results within the Talairach[7] space[8].

VBM: statistical analyses

VBM comprises a voxel-by-voxel between-group comparison of the density (or concentration) of grey matter. Although it is a highly computational technique, it is not a 'one-size-fits-all' method. A degree of judgment is required at the pre-processing stage (as described above). Decisions also need to be made at the analysis stage. For example, which confounding variables should be entered as covariates in the analysis and what threshold will be adopted for statistical significance.

'Optimised' analysis

Potential sources of bias were addressed by entering *all* known confounders as co-variates (hence the 'optimised' appellation). These were: sex[9], age[10], total number of grey matter voxels[11] (GM) (this ensures that any positive results reflect regionally specific rather than generalised differences in brain morphology) and scanner (the data used in this study were obtained using two different machines, as described in Chapter 2A). The final covariate entered into the optimal analysis was family group (the rationale behind using siblings as controls is that this reduces non-specific variability in brain morphology).

Three one sided comparisons were carried out, non-affected non-carrier siblings > schizophrenic siblings (NA > SZ); non-affected non-carrier siblings > obligates (NA

> Obl); and obligates > schizophrenic siblings (Obl > SZ). A threshold of $p \geq 0.005$ uncorrected for multiple comparisons was considered significant (although probability peaks at the lower threshold of $p \geq 0.01$ were also examined). Correction for multiple comparisons was not carried out because it is considered to be overly strict when applied to the analysis of structural data with VBM in general (as Ashburner and Fritson observe "analyses based upon uncorrected peak height appear to be valid"[7]) and for confirmatory analyses in particular. Finally, because SPM produces results coordinates in MNI space[4], the results were converted to Talairach space[7] (with a nonlinear transform first described by Brett[12]).

SVC in the amygdala region

The rationale behind the small volume correction (SVC) technique is that it allows a specific regional hypothesis to be tested by facilitating a detailed analysis of the candidate region. The same procedure of pre-processing followed by voxel-by-voxel analysis is followed, however, the statistical parameters are optimised for the specific region under scrutiny. In this study an SVC analysis was conducted within the amygdalo-hippocampal complex (as there is more evidence implicating genetic factors in the genesis of morphological changes in this region than in any other, see Chapter 2A). The analysis incorporated voxels within a sphere of radius 10mm centered upon the amygdala (Talairach coordinates +/–24, -2, –18).

Results

ROI: results

The mean volumes of each ROI by group are shown in Table 2Aii.

Results of original ROI analysis

These are shown in Table 2Aii and discussed in Chapter 2A. A summary of the statistically significant findings is shown below:

SZ < Obl, NA	**Whole Brain Volume**
SZ, Obl < NA	**Amygdalo-Hippocampal Complexes**
SZ < Obl, NA	**Temporal & Prefrontal Lobes**
Obl < SZ	**Intra-Cerebral Ventricular System**

These results suggest that reduced AHC volume may be a genetic effect, whilst reduced temporal and prefrontal lobe volume may be phenotypic (see Chapter 2A).

Results of exploratory ROI analysis

These are shown in Table 2Aiii and discussed in Chapter 2A. A summary of the main statistically significant findings is shown below:

SZ < Obl < NA	**Amygdalo-Hippocampal Complexes**
Obl < SZ < NA	**Thalamus**
NA, Obl < SZ	**Lentiform Nucleii**
SZ < NA < Obl	**Right Temporal Lobe**

These results suggest that reduced AHC volume may be both a genetic and a phenotypic effect, whilst reduced thalamic volume may be a predominantly genetic effect and increased lentiform volume a phenotypic effect (possibly attributable to medication). Increased temporal lobe volume in obligates may reflect a protective factor (see Chapter 2A).

VBM: results

Results of 'optimised' VBM analyses

Results from the three VBM analyses: Obl > SZ; NA > SZ; and NA > Obl are presented in pictorial form in Figures 2Bi, 2Bii, and 2Biii respectively.

A summary of the main findings from all three analyses presented in the form of Talairich co-ordinates is shown in Table 2Bii.

The most striking feature of the Obl > SZ analysis is the large probability peak in the right medial temporal lobe region (centred upon Talairich co-ordinates 29, 2, -15 corresponding to the parahippocampal gyrus). This appears to correspond to similar medial temporal probability peaks *bilaterally* on the NA > SZ analysis (–12, -1, -18 and 29, 2, -15). The striking right-left asymmetry in the results from the medial temporal region (NA & Obl > SZ on the right, NA > Obl & SZ on the left) suggests lateralisation of the effects of risk factors. Genetic effects appear to predominate on the left. However on the right, reduced grey matter density in the medial temporal lobe appears to be an endophenotypic marker in that it is found in association with the schizophrenic phenotype but not with genetic risk for the disorder. (For the sake of brevity the term 'phenotypic' will be used to describe this type of effect.)

Examination of Figure 2Bii reveals a number of significant differences in cortical grey matter density between the schizophrenic subjects and their non-affected siblings. On visual inspection these appear to be scattered around the frontal, temporal, parietal and occipital cortices. The Talairach co-ordinates listed in Table

2Bii describe the precise location of these probability peaks (predominantly left middle frontal gyrus and right superior frontal gyrus). Comparison of the frontal and temporal regions of the brain in Figures 2Bi & 2Biii demonstrates far more differences in these regions between obligates and schizophrenics (Figure 2Bi) than between obligates and their non-affected siblings (Figure 2Biii). These findings suggest that the reduced grey matter density in these regions of the schizophrenic brain is unlikely to be attributable to differences in genetic predisposition for schizophrenia.

Interestingly, the VBM analysis also identifies a number of probability peaks in the occipital region (particularly the cuneus and pre-cuneus) and the cerebellum. Examination of these suggests largely mixed phenotypic/genotypic effects.

The VBM analysis identifies almost no localised differences in sub-cortical grey matter densities. It may be that pre-processing parameters designed to maximise the sensitivity of cortical comparisons render the data relatively insensitive to sub-cortical differences.

Figure 2Bi. Results of Optimised VBM analysis Obl > SZ at $p \leq 0.005$

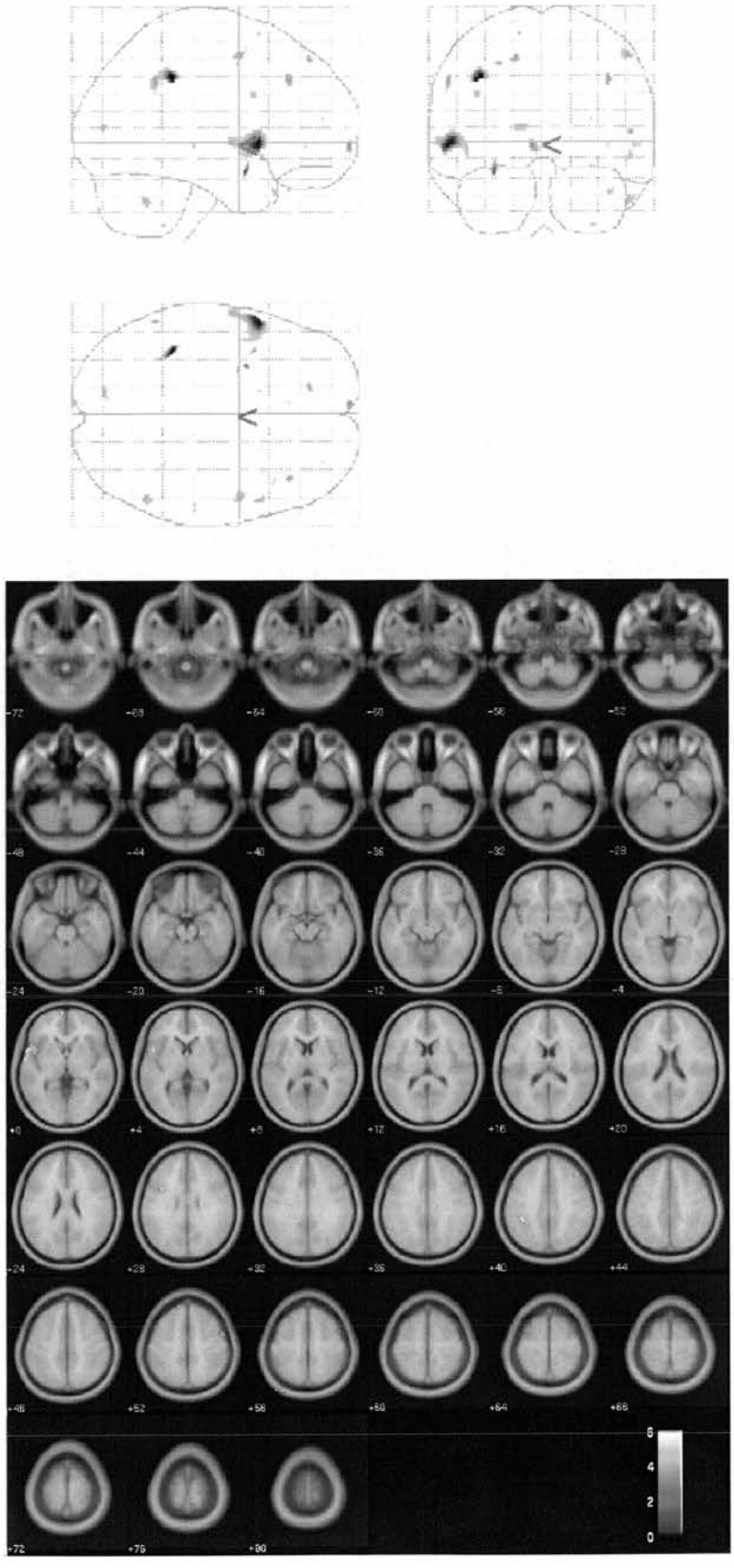

Figure 2Bii. Results of Optimised VBM analysis NA > SZ at $p \leq 0.005$

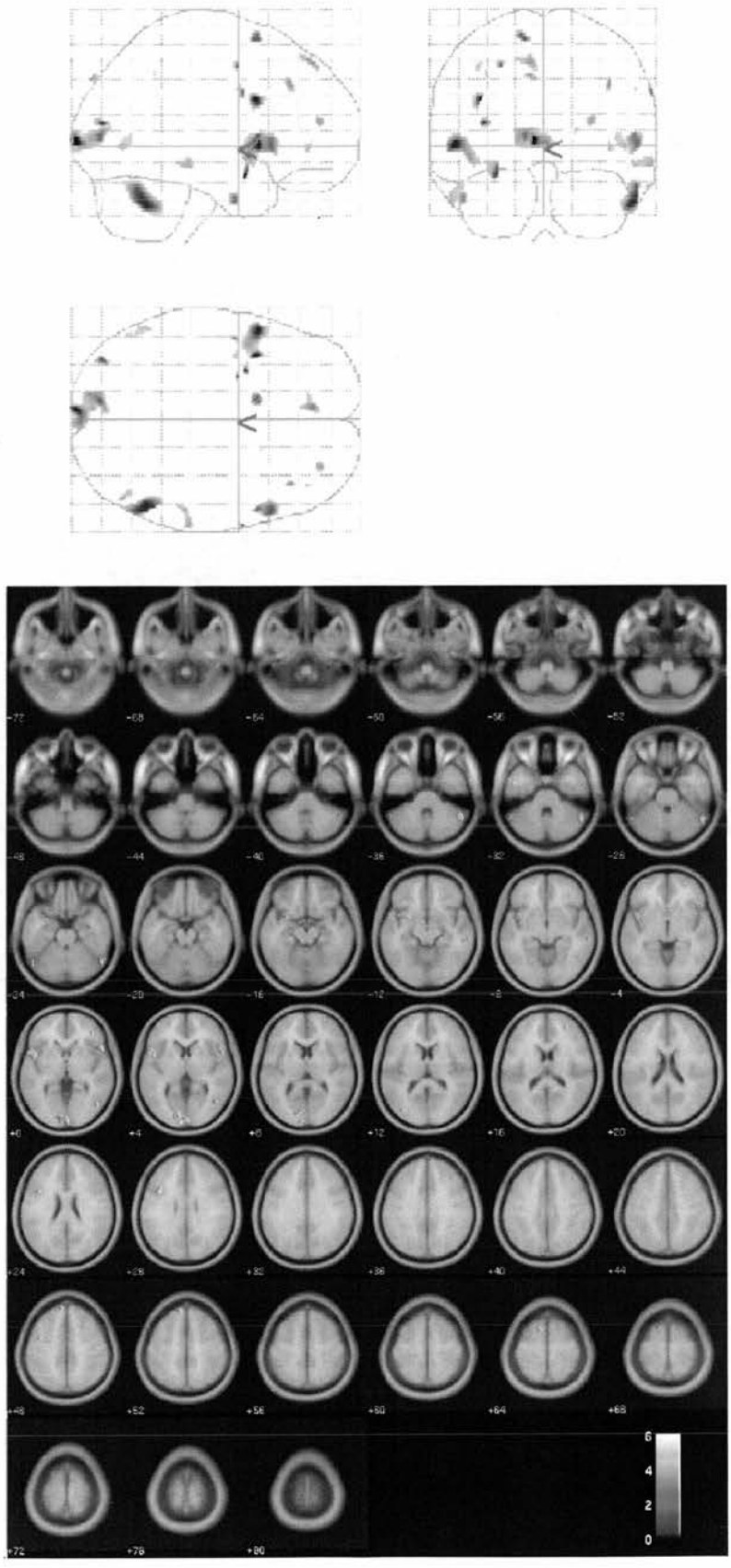

Figure 2Biii. Results of Optimised VBM analysis NA > Obl at $p \leq 0.005$

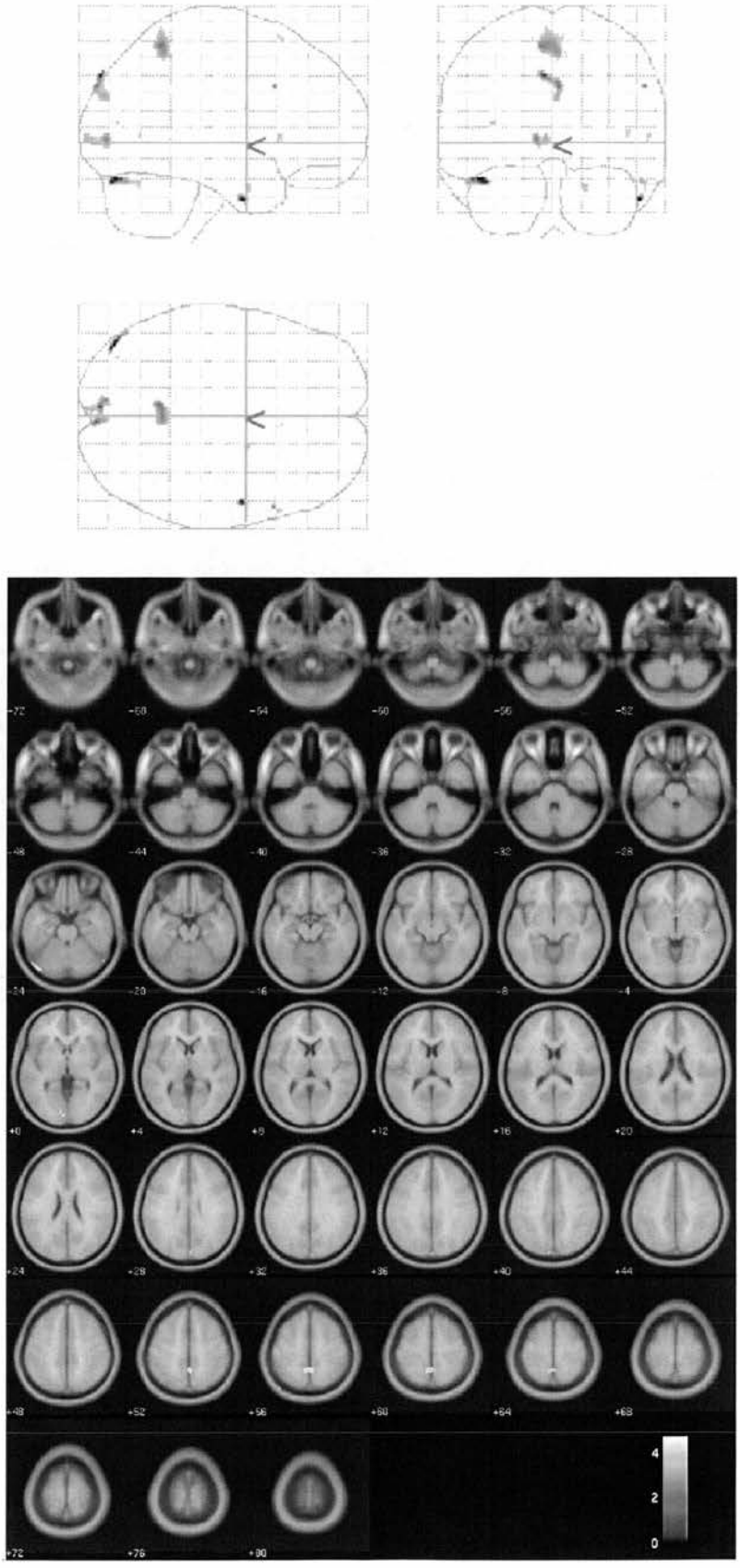

Table 2Bii. Results of Optimised VBM analyses (at $p \leq 0.005$ uncorrected)

Talairach co-ordinates & anatomical locations of voxel clusters showing significant between group differences.

Analysis	***Obl > SZ***					***NA > SZ***					***NA > Obl***				
Region	*Peak Hgt.* *T*	Talairach co-ordinates X	Y	Z	Location of Cluster	*Peak Hgt.* *T*	Talairach co-ordinates X	Y	Z	Location of Cluster	*Peak Hgt.* *T*	Talairach co-ordinates X	Y	Z	Location of Cluster
Limbic	*4.9*	29	2	-15	R Parahipp. gyrus	*3.0*	-12	-1	-18	L P.hipp. g. (p<0.01)	*3.6*	-19	-1	-22	L Uncus
	3.4	45	5	-5	R Insula	*5.6*	29	2	-15	R Parahipp. gyrus					
						4.1	46	7	-2	R Insula					
Sub-Cortical	*3.2*	-19	13	5	L Lentiform nucleus	*3.3*	-19	13	9	L Lentiform nucleus					
Frontal	*3.3*	-39	39	-4	L Middle Frontal gyr.	*4.4*	-39	32	32	L Middle Frontal gyr	*3.3*	-8	38	46	L Sup. Frontal gyrus
	3.3	-29	49	10	L Middle Frontal gyr.	*4.0*	-28	47	11	L Middle Frontal gyr	*3.2*	-6	21	58	L Sup. Frontal gyrus
	4.0	-40	31	33	L Middle Frontal gyr.	*3.2*	-54	18	30	L Middle Frontal gyr	*3.9*	-54	18	30	L Middle Frontal gyr.
	3.1	-49	18	3	L Inferior Frontal gyr.	*4.8*	-55	17	-1	L Inferior Frontal gyr	*3.4*	-57	20	3	L Inferior Frontal gyr.
	3.8	-51	2	48	L Pre-central gyrus	*4.9*	-63	3	29	L Pre-central gyrus	*3.5*	-60	-1	18	L Pre-central gyrus
	4.2	16	42	46	R Sup. Frontal gyrus	*4.3*	7	46	44	R Sup. Frontal gyrus					
	3.5	14	15	60	R Sup. Frontal gyrus	*4.9*	11	15	58	R Sup. Frontal gyrus					
	3.8	25	1	44	R Middle Frontal gyr.	*3.7*	15	43	46	R Sup. Frontal gyrus					
	4.2	6	64	-5	R Medial Frontal gry.	*3.6*	7	39	48	R Sup. Frontal gyrus					
	3.3	7	28	-25	R Rectal gyrus	*5.0*	25	1	44	R Middle Frontal gyr.					
						3.4	39	0	42	R Pre-central gyrus					
Temporal	*3.5*	-52	13	-7	L Sup. Temporal gyr	*4.0*	-64	-30	-9	L Mid. Temporal gyr.	*3.3*	-45	-62	9	L Mid. Temporal gyr.
	3.5	-57	27	0	L Sup. Temporal gyr	*3.9*	-53	-32	-9	L Mid. Temporal gyr.	*4.7*	-50	-4	-28	L Inf. Temporal gyr.
	3.3	-39	19	-26	L Sup. Temporal gyr	*4.7*	48	-3	-27	R Inf. Temporal gyr.					
	4.0	60	-4	0	R Sup. Temporal gyr										
	5.8	54	11	-1	R Sup. Temporal gyr										
Parietal / Occipital	*6.0*	38	-38	39	R Inf. Parietal Lobe	*4.0*	-48	-68	3	L Mid. Occipital gyr.	*3.8*	-2	-47	54	L Pre-cuneus
	3.3	7	-96	7	R Cuneus	*6.1*	6	-95	8	R Cuneus	*3.6*	-2	-81	28	L Cuneus
	3.7	13	-78	13	R Cuneus	*4.8*	12	-81	10	R Cuneus	*3.9*	7	-49	56	R Pre-cuneus
						5.3	36	-79	17	R Mid. Occipital gyr.	*3.6*	4	-92	6	R Cuneus
											3.9	10	-81	6	R Cuneus
											3.4	35	-74	15	R Mid. Occipital gyr.
Cerebellar	*3.5*	-30	-46	-40	L Post. Cbllr. tonsil	*5.5*	-51	-57	-26	L Post. Cbllr. tubcl.	*3.2*	-54	62	-20	L Post. Cbllr. declive
	3.7	-50	-56	-27	L Post. Cbllr. tubcl.	*3.9*	55	-55	-26	R Post. Cbllr. tubcl.					

Results of SVC in amygdala region

The greatest between-group differences on both the ROI and the VBM analyses relate to medial temporal lobe structures. It was therefore decided to perform an specific (and presumably confirmatory) SVC analysis in this region. The shape of the hippocampus renders it an unsuitable location for an SVC (the defined volume for an SVC in SPM must be spherical). It was therefore decided to centre the SVC in the amygdala.

The highest probability peaks identified on the SVC were as follows:

Result	*Anatomical Location*	*Talairach Co-ordinates* X	Y	Z	*Peak Height*	*Significance Level (corrected)*
SZ, Obl < NA	Left Amygdala	-19	0	-22	$T = 2.8$	$0.05 < p < 0.01$
SZ < Obl, NA	Right Amygdala / Uncus	29	2	–15	$T = 5.6$	$p < 0.01$

This suggests a phenotypic effect on the right and a genetic effect on the left, consistent with the main VBM analysis.

Comparison of results: VBM v ROI

The VBM analyses conducted for this study deal with grey matter voxels only (VBM was designed for use with grey matter and the validity of findings relating to white matter and CSF is open to question). The regions of interest data do not incorporate every possible brain region (only those regions that are widely believed to be implicated in schizophrenia are included). Therefore the only direct ROI v VBM comparisons that it is possible to make relate to grey matter in the prefrontal (corresponding to frontal lobe voxels with Talairach $Y > 35$) and temporal cortices, the thalamic, caudate and lentiform nuclei, and the allocortex of the amygdalo-hippocampal complexes. These comparisons are displayed in Tables 2Biv and 2Bv.

VBM versus original ROI

Direct comparison of results from the optimised VBM and original ROI analyses (for the regions listed above) reveals a remarkable degree of overlap. (This is illustrated in Table 2Biv, which shows the probability peaks on VBM that lie within the boundaries of the ROI data set – overlap is indicated by **bold** type.)

As discussed previously, the most striking features of the VBM analyses are the large probability peaks in the medial temporal lobe regions. These appear to correspond very well with the disproportionate reduction in the volumes of the amygdalo-hippocampal complexes identified on the original ROI analysis. However, the conclusion of the original ROI analysis was that, in terms of amygdalo-hippocampal volumes, the obligates resembled their schizophrenic siblings. The identification by VBM of differences between obligates and schizophrenics in the right

parahippocampal gyrus (29, 2, -15) and right insula (45, 5, -5) appears to contradict this conclusion. One of the acknowledged weaknesses of the original ROI analysis was that by collapsing the right and left amygdalo-hipocampii into one gross region, it became blinded to any right-left differences. This was one of the reasons for performing the 'exploratory' ROI analysis.

The original ROI analysis found no difference between obligates and non-affected, non-carriers in terms of prefrontal and temporal lobe volumes (both being significantly larger than schizophrenics). A glance at the almost empty right hand column of Table 2Biv shows that this is entirely in keeping with VBM.

Finally, neither analysis identified differences in sub-cortical structures (with the exception of an isolated voxel cluster in the left lentiform nucleus on VBM, -19, 13, 5/9). As proposed in Chapter 2A, it is possible that the original ROI failed to identify differences because reductions in thalamic volume were cancelled out by increases in lentiform volume when the regions were combined. Meanwhile, the VBM analysis would not be able to identify any *increases* in grey matter density in the lentiform nuclei as it tested only for *reductions* in density. Such an explanation does not, however, account for the absence of probability peaks in the thalamus.

Table 2Biv. Comparison of results of VBM versus 'original' ROI analysis

Voxel clusters showing significant between group differences on VBM that are in agreement with differences identified upon 'original' ROI analysis appear in **bold** type.

Analysis	***Obl > SZ***				***NA > SZ***				***NA > Obl***			
Region	Talairach co-ordinates X	Y	Z	Location of Cluster	Talairach co-ordinates X	Y	Z	Location of Cluster	Talairach co-ordinates X	Y	Z	Location of Cluster
Limbic	29	2	-15	R Parahippocampal gyr.	**-12**	**-1**	**-18**	**L Parahippocamp. gyr.**	**-19**	**-1**	**-22**	**L Uncus**
	45	5	-5	R Insula	**29**	**2**	**-15**	**R Parahippocamp. gyr.**				
					46	**7**	**-2**	**R Insula**				
Sub-Cortical	-19	13	5	L Lentiform nucleus	-19	13	9	L Lentiform nucleus				
Prefrontal	**-39**	**39**	**-4**	**L Middle Frontal gyrus**	**-28**	**47**	**11**	**L Middle Frontal gyrus**	-8	38	46	L Superior Frontal gyrus
	-29	**49**	**10**	**L Middle Frontal gyrus**	**7**	**46**	**44**	**R Superior Frontal gyr**				
	16	**42**	**46**	**R Superior Frontal gyr**	**15**	**43**	**46**	**R Superior Frontal gyr**				
	6	**64**	**-5**	**R Medial Frontal gryus**	**7**	**39**	**48**	**R Superior Frontal gyr**				
Temporal	**-52**	**13**	**-7**	**L Superior Temp. gyrus**	**-64**	**-30**	**-9**	**L Middle Temporal gyr**	-45	-62	9	L Middle Temporal gyrus
	-57	**-27**	**0**	**L Superior Temp. gyrus**	**-53**	**-32**	**-9**	**L Middle Temporal gyr**	-50	-4	-28	L Inferior Temporal gyrus
	-39	**19**	**-26**	**L Superior Temp. gyrus**	**48**	**-3**	**-27**	**R Inferior Temporal gyr**				
	60	**-4**	**0**	**R Superior Temp. gyrus**								
	54	**11**	**-1**	**R Superior Temp.gyrus**								

VBM versus exploratory ROI

The 'exploratory' ROI analysis was designed and conducted primarily to facilitate comparison with the optimised VBM analyses. Indeed the methodology adopted consciously mimics the VBM approach with probabilities of between-group differences being calculated for each individual region of interest. It is perhaps surprising therefore that the degree of overlap between the results of this analysis and VBM is considerably less than between the *original* ROI analysis and VBM. (This is illustrated visually by the relative sparsity of **bold** type in Table 2Bv compared with Table 2Biv.)

With respect to amygdalo-hippocampal volumes, the exploratory ROI findings are in broad agreement with the VBM findings – predominantly genetic effect on the left with predominantly phenotypic effect on the right.

The single sub-cortical probability peak identified by VBM (in the left lentiform nucleus: -19, 13, 5/9) is in agreement with the findings of the exploratory ROI analysis. However, it is notable that the VBM failed to detect the other basal ganglia differences and the thalamic differences identified by the exploratory ROI analysis. One possible explanation is that the VBM parameters (in particular the Gaussian kernal of 12mm employed for smoothing) were designed to maximise the chance of detecting differences in cortical grey matter. This may have rendered the analysis relatively insensitive to differences in sub-cortical grey matter.

With respect to cortical grey matter, the VBM analysis suggests a phenotypic effect (SZ < Obl, NA) in right and left prefrontal and right and left temporal lobes, whereas the exploratory ROI only identifies a statistically significant effect in the right temporal lobe.

Table 2Bv. Comparison of results of VBM versus 'exploratory' ROI analysis

Voxel clusters showing significant between group differences on VBM that are in agreement with differences identified upon the 'exploratory' ROI analysis appear in **bold** type.

Analysis	*Obl > SZ*				*NA > SZ*				*NA > Obl*			
Region	Talairach co-ordinates X	Y	Z	Location of Cluster	Talairach co-ordinates X	Y	Z	Location of Cluster	Talairach co-ordinates X	Y	Z	Location of Cluster
Limbic	**29**	**2**	**-15**	**R Parahippocamp. gyr.**	**-12**	**-1**	**-18**	**L Parahippocamp. gyr**	**-19**	**-1**	**-22**	**L Uncus**
	45	**5**	**-5**	**R Insula**	**29**	**2**	**-15**	**R Parahippocamp. gyr.**				
					46	**7**	**-2**	**R Insula**				
Sub-Cortical	**-19**	**13**	**5**	**L Lentiform nucleus**	**-19**	**13**	**9**	**L Lentiform nucleus**				
Prefrontal	-39	39	-4	L Middle Frontal gyrus	-28	47	11	L Middle Frontal gyrus	-8	38	46	L Superior Frontal gyrus
	-29	49	10	L Middle Frontal gyrus	7	46	44	R Superior Frontal gyrus				
	16	42	46	R Superior Frontal gyrus	15	43	46	R Superior Frontal gyrus				
	6	64	-5	R Medial Frontal gryus	7	39	48	R Superior Frontal gyrus				
Temporal	-52	13	-7	L Superior Temporal gyr	**-64**	**-30**	**-9**	**L Middle Temporal gyr**	-45	-62	9	L Middle Temporal gyrus
	-57	-27	0	L Superior Temporal gyr	**-53**	**-32**	**-9**	**L Middle Temporal gyr**	-50	-4	-28	L Inferior Temporal gyrus
	-39	19	-26	L Superior Temporal gyr	**48**	**-3**	**-27**	**R Inferior Temporal gyr**				
	60	**-4**	**0**	**R Superior Temp. gyr**								
	54	**11**	**-1**	**R Superior Temp. gyr**								

Discussion

Main findings

The aim of this study was to identify inconsistencies and areas of commonality between the results obtained and conclusions drawn from a single, robust data set when analysed with a traditional method and with a computational approach. The findings indicate considerable areas of commonality with relatively few inconsistencies allowing very similar conclusions to be drawn from the two analyses. Furthermore, the overlap is maximal when the two analyses are compared 'as they would appear in press'.

Both analyses identify abnormalities of limbic and cortical structures in association with a diagnosis of schizophrenia. The results from both analyses support the conclusion that there is an association between genetic risk for schizophrenia and limbic, but not cortical, abnormalities. The greatest difference between the two approaches is reflected in the extent to which the findings can be localised. The ROI analysis deals with relatively large anatomical structures (this is particularly true of the analysis described in this study which deals with conglomerated, 'gross' regions of up to $300cm^3$), whilst the VBM deals with voxels of the order of a millimetre across. The VBM analysis is therefore able to identify precisely where the between-group differences are at their greatest.

Limitations

The limitations of the original study are discussed in Chapter 2A. However, the limitations of the ROI versus VBM comparison and of VBM in general merit further discussion.

The most obvious limitation of this comparison is that it does not incorporate all regions of the brain. As the name suggests, the original 'Regions of Interest' data relate only to specific regions implicated in the pathophysiology of schizophrenia. In this respect it is typical of ROI studies, which are usually designed to test a specific regional hypothesis (in this case the amygdalo-hippocampal complex). Collecting ROI data from every brain region is not only time consuming but also leads to problems with multiple statistical tests. Meanwhile, the VBM analysis was restricted to grey matter. Although VBM has been used to identify differences in white matter and even CSF density, it was primarily designed to examine grey matter and the validity of VBM findings from other tissue types is questionable.

In the course of comparing two methods of data analysis, the original null hypothesis (that there would be no between group differences in amygdalo-hippocampal structure) is subjected to a total of four statistical tests: the original ROI; the 'exploratory' ROI; the optimised VBM; and the SVC. In the normal course of events this would be indefensible. However, the optimised VBM is essentially a confirmatory test, whilst the 'exploratory' ROI and SVC analyses are both exploratory tests. The subtle differences between the various results are informative. The original ROI collapses both sides into

one and finds SZ & Obl < NA (suggestive of a genetic effect). The exploratory ROI, optimised VBM and SVC all find SZ & Obl < NA on the left (suggestive of a genetic effect) and SZ < Obl & NA on the right (suggestive of a phenotypic effect). If one returns to the original volumetric data, one finds a similar trend (SZ < Obl < NA) on right and left, suggesting a mixed genetic and phenotypic effect on both sides (albeit non-significant). The apparent right-left differences may be an artefact of arbitrary statistical thresholds, which result in the intermediate (obligate) group being identified as statistically indistinguishable from one extreme or the other depending on how the data are treated. The arbitrary nature of statistical thresholds is an issue common to all forms of data analysis but appears to be particularly pertinent to structural brain imaging and perhaps especially VBM.

What statistical threshold to choose is not the only subjective judgement required in VBM. As mentioned previously, there is scope for adjusting the parameters at both the pre-processing and the analysis stages. The optimal protocol for detecting differences in cortical grey matter is not necessarily the best protocol for identifying differences elsewhere in the brain. Most of the major pre-processing parameters have now been standardised[2], however, the methods devised by Ashburner and Friston inevitably represent a compromise. In this study VBM appears to be relatively poor at detecting differences in the grey matter of the thalamic and caudate nuclei. Also the small volume correction facility, which offers the opportunity to test specific regional hypotheses, is limited by the fact that it can only examine spherical regions of interest rather than regions defined by anatomical boundaries. Other limitations of VBM include the

tendency for probability peaks to be located at anatomical boundaries (the so-called 'edge effect'[16]) and its inability to examine generalised, whole brain effects (an issue which may be pertinent to schizophrenia research, see Chapter 1D).

One issue of particular relevance to this study is the extent to which structural imaging findings, in particular those obtained through computational methods of analysis, may be 'scanner-dependent'. The apparent benefits of computational methods of analysis (reproducibility and removal of subjective judgements) would be undermined if systematic differences were identified between results obtained from different machines. The data analysed in this study were obtained using two different MRI scanners: a 1 tesla Siemens Magnetom model; and a 1 tesla General Eelectric Signa model (see Chapter 2A). The potential risk of this confounding the results was minimised by ensuring that all three members of a sibling 'triple' were scanned on the same machine and by entering 'scanner' as a between-subjects factor in the ROI analysis and as a covariate in the VBM analysis. No statistically significant scanner effect was identified in either analysis. However, one would not expect to identify subtle effects with such a limited number of subjects. Researchers conducting longditudinal studies, where scans are conducted years apart, often have no option other than to use different machines. The electron-magnets in MRI scanners have a limited life-span and, even if they lasted forever, technological advances would render an old model obsolete within years. Colleagues from within the author's department working on the Edinburgh High Risk Study (Hodges et al. 1999) have begun examining the very large data set (over 500 scans obtained from over 200 individuals) for evidence of between-scanner effects. They

believe that they have identified systematic yet unpredictable differences attributable to different machines. Their initial impression is that the effects are greatest when comparing machines of different field strength (in this case 1 tesla versus 1.5 tesla) and from different manufacturesrs. Furthermore they suspect that the scanning protocol chosen and the sequence of data collection may influence the results obtained. The author is not aware of any published studies supporting his colleagues' suspicions but it is likely that the issue will come to light in the near future.

Comparisons with previous research

The literature relating to the association between genetic risk of schizophrenia and structural brain abnormalities is discussed in Chapter 2A.

To date only a small number of structural brain imaging studies in schizophrenia have been published with a VBM analysis. The largest VBM study is from the Netherlands comparing 159 schizophrenic subjects with 158 controls[13]. The main findings are of reduced grey matter density in the left amygdala and hippocampus and the insula, posterior cingulate gyrus, thalamus, frontal and temporal lobes bilaterally. Grey matter density is increased in the basal ganglia. These results are entirely consistent with our findings. The frontal, temporal and insula findings are replicated in a German study of 48 patients, which also examined CSF and found increased ventricular volume[14]. A recent study from Queen's Square (20 patients, 20 matched controls) reports reductions of grey matter density in the mediodorsal thalamus and the ventral and medial prefrontal cortices, the latter correlating positively with a family history of schizophrenia[15] (a

finding that was not replicated in this study). It is important to remember that the VBM method has only been readily available for two years and it is therefore likely that the majority of studies are still in progress or in press. A study of 34 first episode subjects conducted in Edinburgh[16] reports grey matter density reductions in the right anterior cingulate, right medial frontal lobe, left middle temporal gyrus, left postcentral gyrus and left limbic lobe. SVC within the amygdalo-hippocampal complex identifies reductions in the right and left uncus and parahippocampal gyri and the right amygdala. Again such findings are in broad agreement with this study.

Implications

Computational methods of data analysis such as VBM offer a number of advantages over traditional ROI methods. As the comparison described in this chapter demonstrates, VBM allows differences to be localised with great precision. It also allows researchers to examine grey matter throughout the brain (rather than being restricted to pre-specified regions). Once the expertise has been acquired, VBM ought to be considerably less labour intensive than semi-automated ROI. However, the 'unique selling point' of computational analyses is that by producing results in the form of Talairach co-ordinates they have the potential to facilitate direct comparisons of functional and structural data. For these reasons, VBM is likely to become ever more popular in structural brain imaging research.

However, VBM is not perfect. As discussed above, its validity with respect to white matter and CSF is unclear. It is probably not as sensitive at detecting differences in sub-

cortical grey matter as in cortical. It is also important to recognise that, despite being highly computational, a degree of subjectivity remains in VBM and the conclusions drawn from a VBM analysis may be partially dependent upon the statistical threshold chosen. Finally the SVC facility is limited to spherical regions.

The outcome of this VBM versus ROI comparison is reassuring. VBM did not alter the main conclusions drawn from the data but it added detail. One might reasonably anticipate that the adoption of VBM will bring greater anatomical precision to the field of structural brain imaging in schizophrenia.

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2B. Study 2

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2C: Study 3

Diffusion Tensor Imaging (DTI) and Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) in Schizophrenic Subjects and Normal Controls.

N.B. The study described in this chapter has been published. A reprint of the following paper is enclosed in a pocket attached to the inside of the back cover of this thesis.

Steel R.M., Bastin M.E., McConnell S., Marshall I., Cunningham-Owens D.G., Lawrie S.M., Johnstone E.C. & Best J.J.K. **2001.** "Diffusion Tensor Imaging (DTI) and Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) in schizophrenic subjects and normal controls." *Psychiatry Research: Neuroimaging* **106:**161-170.

Background, Aim & Hypothesis

A number of explanatory models have been proposed as candidates for the morbid process underlying schizophrenia. However, no single theory has yet been developed which successfully explains the onset, clinical and pathological features and course of the disorder (see Chapter 1C). The 'Neurodevelopmental Hypothesis'[1,2] proposes that the disorder is due to developmental abnormalities arising before or around the time of birth, the psychopathology emerging in early adult life as the affected neuronal systems mature. It is suggested that the underlying pathology relates to disrupted neuronal migration during very early life[2,3]. The evidence in favour of this theory is by no means conclusive, but it finds support in the results of some post-mortem[4,5,6,7] and in-vivo structural brain imaging studies[8,9,10,11].

The findings of functional imaging studies (PET[12,13] and more recently fMRI[14]) suggest that schizophrenia may be associated with abnormal 'connectivity' between different brain regions. The 'Disconnection Hypothesis'[15] proposes that the signs and symptoms of schizophrenia can be understood in terms of a failure of integration of neurocognitive tasks reflecting a pathophysiological process characterised by disrupted functional connectivity between various brain regions. Precisely which neuronal connections (or circuits) are disrupted in the disorder is currently unclear. Cases have been argued for fronto-temporal pathways[16,17], fronto-cingulate connections[18] and trans-callosal fibres[19].

If one combines the neurodevelopmental and disconnection hypotheses, the result is a pathophysiological model in which the connections between various regions of the brain have developed abnormally and are therefore unable to function normally (the 'Dysplasic Net Hypothesis'[20]). If this model is accurate, one might expect to find (neurodevelopmental) abnormalities in the cyto-architecture of the white matter tracts (connections) within the brain. The study presented in this chapter uses proton magnetic resonance spectroscopy (^{1}H-MRS) and diffusion tensor imaging (DTI) to examine the anatomical basis of connectivity in the pre-frontal white matter.

^{1}H-MRS

Proton magnetic resonance spectroscopy measures the concentration of various biologically important metabolites in the brain. Although spatial resolution is relatively poor compared to conventional MR imaging, (15 mm cubic voxels in this study), the technique is useful because it allows brain chemistry to be studied in-vivo without the use of radioactive tracers. Most studies report N-acetyl aspartate (NAA) concentrations because the peak in the proton spectrum attributable to NAA is prominent, and can be measured reliably[21]. NAA is found predominantly within neurons though its intracellular function is unclear. It is thought to be a neuronal/axonal marker and its concentration may therefore provide a measure of both the number and integrity of neurons within a region of the brain. In connection with schizophrenia, many previous studies have reported reduced NAA concentrations in certain brain regions, particularly the hippocampus, frontal cortex and pre-frontal white matter[22,23,24]. In this study ^{1}H-MRS was used to measure NAA concentrations in pre-frontal white matter. Reductions

in NAA in this region may reflect a structural abnormality, (such as reduced axonal density or reduced viability of neurons), or it may reflect abnormal function of structurally normal neurons whose axons travel to and from the frontal lobes.

DTI

In the brain, the rate at which water molecules diffuse along a particular direction (the apparent diffusion coefficient, or ADC) can be measured in vivo from MR images by applying a diffusion sensitising gradient in the direction of interest. Structures such as myelin sheaths, axonal membranes and micro-filaments cause the diffusion to be slower perpendicular to axons than parallel to them, thereby leading to different rates of diffusion in different directions. This is reflected in differences between ADCs measured in different planes (called 'diffusion anisotropy'). From a set of diffusion-weighted images, a mathematical description of the rates of diffusion in three-dimensional space (the 'apparent diffusion tensor – **D**') can be derived. The extent to which diffusion is disrupted by cellular structures is then reflected in the degree of asymmetry of the tensor. Measures of diffusion anisotropy are indicators of the structural integrity of a neuronal tract. A commonly quoted diffusion anisotropy index is the 'fractional anisotropy' (FA), which measures the fraction of the total magnitude of the apparent diffusion tensor that is anisotropic[25,26].

DTI is a relatively new technique and the literature relating to schizophrenia is small and inconsistent (the study described in this chapter is only the fourth in the field). Buchsbaum and colleagues reported reduced diffusion anisotropy in the white matter of

the pre-frontal cortex in five schizophrenics compared with six controls[27]. Lim and colleagues studied ten US army veterans with schizophrenia and found widespread reductions in anisotropy throughout the brain[28], whilst Foong and colleagues studied the corpus callosum in twenty patients and reported reduced anisotropy in the splenium, but not the genu[29]. The primary aim of the present study was to measure diffusion anisotropy in the pre-frontal white matter. Diffusion anisotropy was also measured in a control region (occipital white matter), which is not generally implicated in the pathophysiology of schizophrenia.

Hypothesis

The study was designed to yield potentially complementary information from ^{1}H-MRS and DTI in the same patients and controls. The main hypothesis being tested was that NAA concentrations and diffusion anisotropy would be abnormal in the pre-frontal lobe white matter i.e. that aberrant connectivity in schizophrenia has structural and functional correlates.

Method

Subjects

Ten patients with established DSM-IV[30] schizophrenia were compared with ten healthy controls. Both male and female participants were studied. The groups were matched for age (see Table 2Ci).

Subjects were selected from patients with an unambiguous clinical diagnosis of schizophrenia. DSM-IV diagnosis was confirmed by SADS-L[31] semi-structured interview. Mean duration of illness was 15 years and all subjects were taking antipsychotic medication at the time of the study (see Table 2Ci). Because the study involved the piloting of a new technique (DTI) with a relatively long image acquisition time (45 minutes when all went well, longer when technical problems arose), it was decided to select patients who had participated in previous brain imaging studies. It was felt that subjects with prior experience of an MRI scanner would be more likely to tolerate the scanning procedure. (In the event, all subjects tolerated the procedure without difficulty). Clinical and volume acquisition scans were therefore available for every subject. Patients with gross structural brain abnormalities were not considered for inclusion in this study. However, patients known to have morphological brain changes consistent with schizophrenia (slightly reduced whole brain volume, enlarged lateral ventricles) were included (see table 2Ci).

Table 2Ci. Demographic, clinical and morphological details of subjects by group. Values presented are the group means (SD), and volumes (SD) in cm^3 with the corresponding *p*-value and effect size.

	Schizophrenic	*Control*	*p*	*Effect size*
Number of Subjects	10	10	-	-
Sex (Male/Female)	5/5	4/6	-	-
Age (years)	34 (14)	35 (7)	-	-
Duration of Illness (years)	15 (12)	N/A	-	-
Whole Brain Volume	1229 (123)	1259 (110)	0.62	-0.27
Left Lateral Ventricle Volume	5.9 (3.0)	3.7 (2.2)	0.12	+1.00
Right Lateral Ventricle Vol.	4.2 (2.7)	3.6 (1.8)	0.59	+0.50
Left Frontal Lobe Volume	69 (13)	70 (8)	0.94	-0.12
Right Frontal Lobe Volume	74 (13)	74 (9)	0.99	0.00
Left Amygdala-Hippocampus	4.1 (0.4)	4.5 (0.4)	0.18	-1.00
Rt. Amygdala-Hippocampus	4.7 (0.4)	4.7 (1.0)	0.98	0.00

effect size = $(mean_{sz.} - mean_{cont.})/SD_{cont.}$

Data Acquisition and Analysis

All imaging and spectroscopy data were collected on an Elscint 2T Prestige (Elscint, Haifa, Israel) clinical scanner (horizontal bore inner diameter 57cm), equipped with a 15mTm^{-1} actively shielded gradient set. The examination consisted of a T_2-weighted fast spin-echo sequence (TR/TE 4800/96; FOV 22 × 22cm; 10 axial slices beginning approximately 10mm above the base of the temporal lobes; slice thickness 6mm; slice gap 20%), a 3D gradient-echo volumetric sequence (TR/TE 28.5/9.254ms; FOV 18 × 18cm; slice thickness 1.5mm;) and then the following ^{1}H-MRS and DTI protocols. The complete examination took approximately 45 minutes.

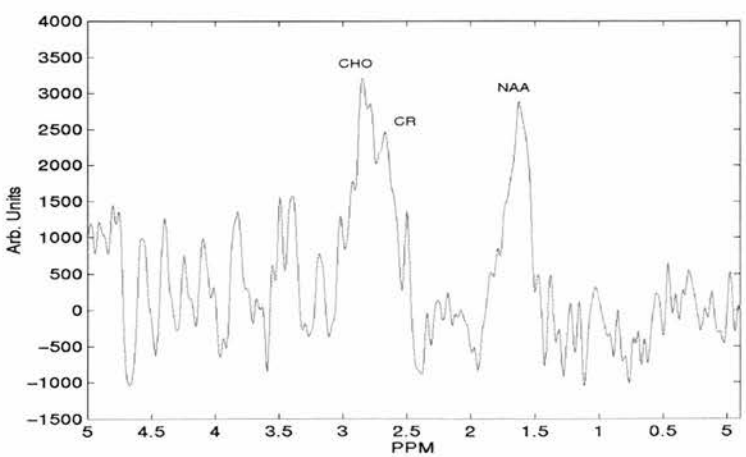
Proton Magnetic Resonance Spectroscopy

Using the set of axial fast spin-echo images (described above) for positioning, two PRESS-localised spectra (TR/TE 1500/145ms) were acquired from 15mm cubical volumes of interest (VOIs) in left and right hemisphere frontal white matter approximately 5mm from the most anterior part of the frontal horns in a line 45° from the midline (see Figure 2Ci). This voxel location ensured that proton spectra were acquired from homogenous white matter rather than from a heterogeneous mixture of tissue types. Following localised shimming and water suppression calibration for each VOI, 200 acquisitions with water suppression were collected. Eight acquisitions without water suppression were also collected to serve as a phase reference. The VOI positions within the head coil were noted, as was the scanner RF calibration figure to allow for the effects of coil loading.

Spectroscopy data were transferred to a Sun Ultra Sparc Station 10 (Sun Microsystems, Mountain View, CA, USA) workstation for analysis. Analysis consisted of phase correction using the water reference data[32], and removal of the residual water signal using Hankel-Lanczos singular value decomposition[33]. Spectral peak areas were quantified using the MRUI software package[34], which fitted Gaussian lineshapes to the peaks corresponding to choline (3.2ppm), creatine (3.0ppm) and NAA (2.0ppm). Finally, metabolite peak areas for each subject were corrected for coil loading and VOI position within the head coil. The resulting 'institutional' units enabled between-subject comparisons. An example of a ^{1}H-MRS spectrum is shown in figure 2Cii.

Figure 2Ci. A structural fast spin-echo image obtained at the level of the lateral ventricles from a 27-year-old female schizophrenic subject in this study. The box (□) indicates the position of the volume of interest from which the proton spectrum (see Figure 2Cii) was acquired.

Figure 2Cii. The proton magnetic resonance spectrum from the VOI indicated in Figure 2Ci. The N-Acetyl Aspartate (NAA), Choline (CHO) and Creatinine (CR) peaks are labelled.



Diffusion Tensor Imaging

Diffusion-weighted echo-planar (DW-EP) images were obtained using a non-cardiac gated, single-shot, spin-echo, half-Fourier sequence, in which two symmetric diffusion sensitizing trapezoidal gradient pulses of 35ms duration, and 43.9ms separation were applied sequentially along the six oblique gradient directions ($\mathbf{G}^1$ to $\mathbf{G}^6$) that constitute the uniform diffusion gradient sampling scheme of Basser and Pierpaoli[35]. In each of these six gradient directions 9 DW-EP images were acquired with a diffusion gradient strength of 11.0mT m^{-1}, giving an average value for Trace(**b**) of 686.55 ± 0.61(SD) s mm^{-2} for $\mathbf{G}^1$ to $\mathbf{G}^6$. A total of 54 DW-EP images per slice position were collected, plus 9 baseline images ($\mathbf{G}^0$) with no diffusion weighting. Values for the elements of the b-matrix were calculated numerically[36]. Other image acquisition parameters were as follows: 10 axial slices coincident with those prescribed in the fast spin-echo sequence; 6 mm slice thickness; 20% slice gap; 128 × 72 half-Fourier image matrix (zero filled to 256 × 128); 44 × 22cm FOV; TE of 103ms. The time from acquisition of one image slice to the next was 1.4s, so the effective TR was 14s. Patient head motion was minimized through use of a Velcro strip, which was comfortably tightened around the subject's forehead.

Magnitude Fourier transformed images in DICOM format were transferred from the scanner to a Sun workstation and converted into Analyze (Mayo Foundation, Rochester, MN, USA) readable form using software written in C. Within each slice the set of 9 images for each of the diffusion gradient directions $\mathbf{G}^0$ to $\mathbf{G}^6$ were separately realigned

to remove physiological motion using SPM95[37], and then averaged to give seven high signal-to-noise images[38]. Geometric image distortions in the six DW-EP images arising from the strong eddy currents present in the DTI experiment were corrected using a modified version of the iterative cross-correlation algorithm suggested by Haselgrove and Moore[39,40].

Within each voxel the six elements of the apparent diffusion tensor **D** (D_{xx}, D_{yy}, D_{zz}, D_{xy}, D_{xz} and D_{yz}) and the baseline T_2 signal intensity were estimated by multivariate linear regression[41] from the measured signal intensity using a program written in Matlab (The Mathworks, Natick, MA, USA). After diagonalization of **D** to yield the eigenvalues ($\lambda_{i=1,2,3}$), maps of the fractional anisotropy (FA)[26] were generated on a voxel-by-voxel basis and converted into Analyze format. An example of an FA map is shown in figure 2Ciii.

FA measures the fraction of the total magnitude of the apparent diffusion tensor that is anisotropic, and takes a value of 0 for an isotropic (cylindrically symmetric, $\lambda_1 >> \lambda_2 = \lambda_3$) medium (see expression below).

$$FA = \sqrt{\frac{3}{2}}\sqrt{\frac{\left(\lambda_1 - < D >\right)^2 + \left(\lambda_2 - < D >\right)^2 + \left(\lambda_3 - < D >\right)^2\right)}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

Values for FA in frontal white matter were calculated from regions-of-interest (ROIs) positioned by a radiologist to coincide with the centres of volumes of interest from

which the proton spectra were obtained (see Figure 2Ci). In the occipital lobes ROIs positioned manually (by the same radiologist) within the white matter adjacent to the optic radiation.

Figure 2Ciii. A Fractional Anisotropy (FA) map at the level of the lateral ventricles (obtained from the subject represented in Figure 2Ci).

^{1}H-MRS: results

Results from the proton magnetic resonance spectroscopy are presented in Table 2Cii.

NAA concentrations were reduced in male and female schizophrenic subjects compared with controls in both the right and left frontal white matter. The direction and magnitude (10-15%) of this difference is consistent with previously published data[22,23,24]. These findings failed to reach statistical significance (by one way ANOVA using a 2-tailed significance level of 5%), but this may be due to the small number of subjects in this pilot study.

Table 2Cii. ^{1}H MRS results by brain region, patient group and sex. Values presented are the mean (SD) N-Acetyl Aspartate peak areas in arbitrary units, *p*-value and the effect size.

Subjects	***Brain Region***	***Schizophrenic***	***Control***	***p***	***Effect Size (d)***
Males	Left Frontal	1072 (244)	1100 (218)	0.88	-0.13
Females	Left Frontal	992 (196)	1235 (164)	0.09	-1.48
All	**Left Frontal**	**1027 (208)**	**1178 (186)**	**0.16**	**-0.81**
Males	Right Frontal	1065 (218)	1122 (143)	0.71	-0.40
Females	Right Frontal	1019 (147)	1153 (134)	0.20	-1.00
All	**Right Frontal**	**1039 (171)**	**1140 (157)**	**0.22**	**-0.64**

effect size (d) = $(mean_{sz.} - mean_{cont.})/SD_{cont.}$

DTI: results

Results from the diffusion tensor imaging are presented in Table 2Ciii.

As illustrated in **bold** type in Table 2Ciii, there were no consistent differences between the FA values obtained from schizophrenics and controls. In schizophrenic subjects diffusion anisotropy was slightly reduced in left frontal white matter, but slightly increased in the other regions measured. All between group differences were small, and none was close to statistical significance. The absence of any discernable trend in these DTI data suggests that this was not simply a type-2 error caused by small numbers.

Examining the data from male and female subjects separately (as illustrated in non-bold type in Table 2Ciii) reveals that in male schizophrenics, anisotropy was slightly (but not significantly) reduced in all four regions of interest, whilst in females the only difference between the groups was a statistically significant ($p<0.01$) *increase* in anisotropy in the right occipital region in the schizophrenics.

Table 2Ciii. DTI results by brain region, group and sex.

Values presented are the mean (SD) Fractional Anisotropy (FA)

p-value and effect size.

Subjects	***Brain Region***	***Schizophrenic***	***Control***	***p***	***Effect Size (d)***
Males	Left Frontal	0.40 (0.08)	0.45 (0.06)	0.33	-0.86
Females	Left Frontal	0.44 (0.11)	0.42 (0.09)	0.83	+ 0.15
All	**Left Frontal**	**0.42 (0.09)**	**0.43 (0.08)**	**0.68**	**-0.22**
Males	Right Frontal	0.46 (0.15)	0.48 (0.50)	0.83	-0.31
Females	Right Frontal	0.41 (0.08)	0.39 (0.05)	0.66	+0.37
All	**Right Frontal**	**0.43 (0.11)**	**0.42 (0.07)**	**0.82**	**+0.15**
Males	Left Occipital	0.44 (0.06)	0.44 (0.06)	0.95	-0.05
Females	Left Occipital	0.46 (0.08)	0.45 (0.04)	0.77	+0.29
All	**Left Occipital**	**0.45 (0.07)**	**0.45 (0.05)**	**0.59**	**+0.06**
Males	Right Occipital	0.41 (0.02)	0.45 (0.08)	0.34	-0.46
Females	Right Occipital	0.53 (0.04)	0.46 (0.02)	>0.01	+3.29
All	**Right Occipital**	**0.47 (0.07)**	**0.45 (0.05)**	**0.90**	**+0.30**

effect size = $(mean_{sz.}–mean_{cont.})/SD_{cont.}$

Correlations

Correlations between ^{1}H-MRS and DTI measures were inconsistent. Within the schizophrenic group, NAA concentrations in the right frontal region showed a significant positive correlation with FA in both the right and left frontal regions (Pearson coefficients: Right 0.65, $p<0.05$; Left 0.69, $p<0.05$). However, no such correlation was found in the control group (Pearson coefficients: Right 0.09, $p>0.8$; Left 0.18, $p>0.7$). Furthermore, NAA in the left frontal region in schizophrenics showed a weak *negative* correlation with the FA (Pearson coefficients: Right –0.3, $p>0.4$; Left –0.2, $p>0.5$).

Correlations with volumetric data were also inconsistent. Within the schizophrenic, but not the control group, NAA concentrations in the left frontal region correlated strongly with the volume of the amygdalo-hippocampal complex on the left (Pearson coefficient 0.92, $p<0.05$), however, there was no correlation between the same measures on the right (Pearson coefficient 0.01, $p=0.98$). The FA showed no correlation with any volumetric measures in either group. Neither ^{1}H-MRS nor DTI measures correlated with duration of illness.

Main Findings

In this study schizophrenia appeared to be associated with reduced NAA concentrations in the pre-frontal white matter, but with normal diffusion anisotropy in the same region. These findings suggest that, whilst the neuronal tracts running to and from the frontal lobes may be *functionally* abnormal in schizophrenic subjects, they are *structurally* intact. This invites the conclusion that the aberrant functional connectivity associated with schizophrenia is not attributable to abnormalities of white matter cyto-architecture and that reduced NAA in the pre-frontal white matter reflects abnormal function of structurally intact neurones.

Limitations

Power

The most obvious limitation of this study is its relatively small size (and consequent limited statistical power). The consistent trend in the data suggesting reductions in NAA concentrations (of a magnitude consistent with the established literature) did not reach statistical significance (see Table 2Cii). The most likely explanation is that this reflects a Type II error. In contrast, the total absence of any discernible trend in the DTI data and the lack of any consistent correlations between the FA and any other measures (such as NAA concentrations and regional brain volumes) do not suggest a straightforward lack of power. Accurate estimation of power for the DTI part of this study is difficult as the few published studies differ markedly in terms of techniques employed and subjects recruited (most notably, none includes female subjects - our data suggest there may be a male/female difference). One of the aims

of this (pilot) study was to establish an estimate of effect size for future power calculations.

Volume of interest

The volume of interest approach has inherent limitations particularly when applied to white matter. It is difficult to ensure that the volume identified consistently reflects the same anatomical structures within the brains of different subjects. One might predict that as white matter tracts approach the cortex, diffusion anisotropy would fall as the fibres spread out. In this study, the volumes of interest were placed manually (always by the same experienced and experimentally blind radiologist). The reliability of this placement was not measured. Another limitation of this approach is that an apparently normal finding in the region studied does not rule out the possibility of significant structural abnormalities in important adjacent structures.

^{1}H-MRS

Single voxel proton MRS is slow and has a relatively poor spatial resolution. This increases the potential for contamination of the white matter spectrum by signal originating from grey matter and CSF. Such partial volume averaging is difficult to avoid, but since the MRS VOI is placed within a large region of white matter, such effects will be minimized. One new MRS technique, which could potentially circumvent this problem is called 'chemical shift imaging' (CSI). This involves the acquisition of a 2 (or 3) dimensional grid of spectra, the additional information allowing estimation of the tissue composition of the volume of interest.

DTI

DTI is a new technique and a number of issues relating to data acquisition and image analysis arose during the course of this pilot study. First, because single-shot diffusion-weighted echo-planar imaging suffers from artifacts caused by susceptibility changes at air/tissue boundaries (e.g. frontal sinus), accurate diffusion imaging data could not be obtained from all regions of the brain. Second, the gradient strength ($15mTm^{-1}$) and rise time ($30mTm^{-1}ms^{-1}$) of the scanner's gradient set were somewhat limited. This was reflected in the large field-of-view (44 × 22cm), relatively low image resolution (1.72 × 1.72mm voxels), low value of diffusion-weighting (Trace(**b**) ~ $700smm^{-2}$) and long echo train (200ms). Also the diffusion-weighted echo-planar imaging sequence could not be cardiac gated, which may result in artefactual increases in diffusion anisotropy close to the ventricles. The resulting FA maps therefore have relatively lower sensitivity and resolution than ideally required. Nevertheless, the absolute values for the FA of white matter reported in this study (~0.45) are in close agreement with those reported by Lim and colleagues[28]. Finally, the raw data must be processed to remove the effects of eddy currents and physiological movement before accurate diffusion anisotropy information may be obtained[42]. In this study DTI data acquisition was therefore limited to specific regions of interest and no attempt was made to acquire DTI data from other regions of the brain.

Comparisons with Similar Studies

Previous studies

The ^{1}H-MRS findings from this study were consistent with the existing literature[21,22], whilst the DTI findings were not[27,28]. However, DTI is a relatively new technique and the literature relating to schizophrenia is small. At the time this study was published, the literature relating to pre-frontal white matter in schizophrenia was limited to two small case control studies, both reporting reductions in diffusion anisotropy in association with schizophrenia.

Buchsbaum and colleagues[27] compared five male schizophrenic subjects with six male controls. They analysed their data using a statistical parametric mapping approach, which is not easily translated into an effect size for a volume of interest study (see Chapter 2B). However, the volume of interest examined in this study approximated to the region of the brain in which Buchsbaum and colleagues found the most highly statistically significant reduction in diffusion anisotropy.

The only previous study to employ similar techniques to this one was published by Lim and colleagues in 1999[28]. They report a statistically significant reduction in diffusion anisotropy in frontal white matter in ten schizophrenic US army veterans compared with ten male controls, (the largest effect size they report is 1.5 x SD which, for $n = 10$, translates to power > 0.99 at a two-tailed significance level of 5%). However there are important differences between their subjects and the subjects in this study. First, there is a large discrepancy in the age of subjects (mean of 48 years in Lim study versus 34 years in this study). One could speculate that the

differences they report are a consequence of decades of disease process or anti-psychotic exposure (although there was no correlation between fractional anisotropy and duration of illness in the present study). Second, a number of the schizophrenic subjects in the present study were known to have the characteristic macroscopic structural brain abnormalities associated with schizophrenia (reduced whole brain volume, increased ventricular size). It was anticipated, perhaps incorrectly, that pre-existing differences between the groups on established measures of brain structure, would increase prospects of detecting corresponding between group differences using DTI (i.e. increase the power of the study). Third, Lim et al. studied only male subjects whilst half of the subjects in the present study were female. It is possible that the different results may reflect male/female differences in diffusion anisotropy. Table 2Ciii shows that whilst fractional anisotropy was reduced in male schizophrenics (although the effect sizes –0.05 to –0.86 were smaller than those reported by Lim et al.), it was actually *increased* in female schizophrenics. This result, and in particular, the increased anisotropy in schizophrenic females in the occipital region was not anticipated, and is not understood. There is a relative paucity of imaging studies as a whole in female schizophrenic subjects, and this finding requires further exploration.

Subsequent studies

Since the publication of this study, three subsequent DTI studies have reported findings relating to diffusion anisotropy in the frontal white matter in schizophrenia. Two studies employed a computational (SPM) analysis to examine diffusion anisotropy of white matter throughout the brain. Both of these reported negative

findings in the pre-frontal region that was examined in this study. Foong and colleagues compared 14 patients (11 male, 3 female) with 19 controls and reported no significant differences anywhere in the brain[43]. Agartz and colleagues, adopting a similar approach, compared 20 schizophrenic subjects (11 male, 9 female) with 24 controls and found reductions in the splenium of the corpus callosum but nowhere else[44]. The only study to report a positive finding was by Kubicki and colleagues from Martha Shenton's group in Harvard. They compared 15 patients (male and female) with 18 normal controls. The method involved careful placement of a volume of interest within the uncinate fasciculus bilaterally. Although they found no overall differences in fractional anisotropy between patients and controls, they did report an asymmetry (FA: Left > Right) in normal subjects that was not present in schizophrenic subjects[45]. No such asymmetry was idenntified in either group in this study (see Table 2Ciii).

Although the three studies described above could be interpreted as validating of our negative DTI result, a subsequent study from within this department comparing 30 patients (15male, 15 female) with 30 matched controls employing a computational, voxel-based analysis technique has identified significant reductions in FA in the left uncinate fasciculus and left superior longditudinal fasciculus[46]. Not only is this the largest single study in the field, but the data was acquired on the latest generation of scanner (GE Signa LX 1.5T) and the data analysis protocol has been refined since the original study.

Implications for Future Research

^{1}H-MRS is a potentially useful adjunct to both structural and functional imaging research. Meanwhile DTI has the potential to develop into a powerful technique for the study of white matter in vivo. In the future it may usefully complement functional imaging techniques by providing information about the structural basis of abnormal functional connectivity. DTI certainly merits further development and ultimately may prove to be a useful tool in schizophrenia research. At present the research evidence is inconsistent. It is not possible to say for certain whether white matter cyto-architecture is normal or abnormal in schizophrenia. Clearly larger studies using proven techniques of data acquisition and analysis are required.

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Section 3: Closing Commentary

3. Closing Commentary

The search for biological correlates of schizophrenia using in-vivo structural brain imaging has undergone significant advances since Jacobi and Winkler's original pneumo-encephalographic study of 1927[1]. After a slow start, research took off following the publication of the first case-control CT study in 1976[2]. The field has benefited enormously from technological advances, which have delivered finer spatial resolution and superior soft-tissue discrimination, not to mention improved safety (see Chapter 1C). A large body of work has built up, comprising several hundred studies. Whilst many fundamental questions pertaining to brain structure in schizophrenia remain unanswered, scientific consensus has been achieved in a number of important areas (see Chapter 1D).

It is now accepted that, as a group, patients with schizophrenia have smaller brains and larger cerebral ventricles than healthy controls[3] and that particular brain regions are affected disproportionately[4]. It is also widely acknowledged that these morphological features can be identified during the first psychotic episode[5]. Griesinger's bold declaration in 1867 that "patients with so-called 'mental illnesses' are really individuals with illnesses of the nerves and brain"[6] would now be considered incontrovertible (at least in relation to certain cases of schizophrenia). Furthermore, the credibility of the pre-eminent contemporary aetiological theory of schizophrenia, the "neurodevelopmental hypothesis"[7], is largely attributable to its compatibility with structural imaging findings.

Each successive technological advance has brought both new insights and new challenges. Whilst the relationship between schizophrenia and ventricular enlargement was established by researchers employing pneumoencephalography and CT, the structural brain abnormalities could not have been localised without the superior white/grey matter discrimination offered by MRI. The results of MRI studies conducted to date suggest that the brain regions of greatest relevance to schizophrenia research may be the small and convoluted structures of the limbic/medial temporal lobes (amygdala, hippocampus, para-hippocampus, insula etc.)[4] Adequate imaging of these structures requires spatial resolution of the order of a millimetre combined with highly sensitive soft-tissue discrimination. MRI machines meeting the required specification have only become available in the last decade or so.

The study presented in Chapter 2C of this thesis demonstrates the types of difficulty encountered by schizophrenia researchers endeavouring to work at the limits of imaging technology. The early DTI literature described in that chapter is characterised by inconsistency. There is no established consensus in relation to methodology. Studies are small and technically flawed. It is perhaps not surprising therefore that results and conclusions vary from study to study. It is interesting to reflect that a little over 20 years ago the entire field of structural brain imaging in schizophrenia was in a comparable, embryonic state. One might anticipate that, as technology improves, techniques currently viewed as "cutting edge", (such as DTI) may become routine. Meanwhile, hitherto unimaginable techniques may emerge.

Whilst structural brain imaging research has undoubtedly benefited from technological developments, it could be argued that it has failed fully to exploit advances in data acquisition on account of limitations in computational methods. This is a problem shared by all technology-led fields. For example, molecular biology currently finds itself in a similar situation with vast amounts of data relating to 'proteomics' and 'cDNA micro-assays' (the molecular correlates of gene expression) lying dormant in anticipation of future advances in bio-informatics[8]. An MRI scan contains a vast amount of information relating to the three-dimensional structure of the brain. However, conventional approaches to image analysis (such as the Region of Interest analysis presented in Chapter 2A) are capable of dealing with only a fraction of this data. Recently developed computational methods such as Voxel-Based Morphometry (illustrated in Chapter 2B) and Shape Analysis (see Chapter 1E) are just beginning to unlock the full potential of MRI.

Brain imaging research will probably continue to ride the advancing wave of technology for years to come. However, sharper pictures and smarter computer programmes are unlikely to unlock the secrets of schizophrenia unless they bring about a conceptual shift. In the author's opinion, the failure of a whole generation of biological researchers to identify anything approaching a pathognomonic marker for this disorder is a reflection not of poor research but of a flawed concept.

Whilst Kraepelin's ambitious attempt to shoehorn his patients into a rigid 19th century medical model was in many respects admirable, there is now powerful evidence to suggest that it was wide of the mark (see Chapter 1B). The process by

which Kraepelin's original concept of 'dementia praecox' evolved into our current operational criteria for 'schizophrenia' is described in chapter 1A. It is clear that this process was driven as much by political expediency as by scientific rigor. The notion that Spitzer and his successors, through some unprecedented act of serendipity, may have chanced upon a "natural disease entity"[9] is risible. It is almost inconceivable that every patient who fulfils the ICD-10 or DSM-IV criteria for schizophrenia shares a common "necessary and sufficient"[10] "tangible morbid process"[11].

The creators of these diagnostic manuals do not claim to have found the holy grail of psychiatry. As Nancy Andreasen and William Carpenter Jr. concede "the construct must be recognised as provisional and based on a need to achieve consensus about definitions rather than on an understanding of pathophysiology and aetiology."[12] To the author's mind, this stance is more credible than that of critics such as Bentall who advocate the wholesale abolition of the concept whilst failing to present a viable alternative[13]. As argued in Chapter 1B, it is undoubtedly better to use the current concept as a starting point than to abandon it altogether. However, if advances in imaging technology are to lead to genuine breakthroughs in the understanding of the pathophysiological processes underlying the symptoms and behaviours that are currently called 'schizophrenia', researchers must be prepared to think 'out of the box'. Should researchers find themselves presented with data that are not easily interpreted within the boundaries of the current concept then they ought to feel able to question not only the data but also the concept. It would be an unfortunate irony if the very operational definitions created to facilitate research were to become a barrier

to scientific progress because researchers showed them too much respect. In the author's view the contemporary search for reliable endophenotypic markers (as illustrated in Chapter 2A) to act as intermediaries between pathophysiological theory and clinical observation is a welcome step in this direction.

The uncomfortable situation facing contemporary schizophrenia researchers is that, despite over 100 years of scientific endeavour, there are precious few certainties in the field that we have chosen to study. The traditional assertion that psychosis is categorically distinct from normality is by no means assured (it may be more accurate to view psychosis as one end of a continuum[14]). It is not clear that the Kraepelian distinction between schizophrenia and manic-depressive psychosis reflects clinical reality (see Chapter 1B). Finally, we can not be absolutely certain that the collection of individuals brought together by their compatability with the criteria listed under 'schizophrenia' in our diagnostic manuals have anything other than this in common. The author draws comfort from the Chinese proverb: "To be uncertain is to be uncomfortable, but to be certain is to be ridiculous."

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3. Closing Commentary

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PAPER

Structural MRI of the brain in presumed carriers of genes for schizophrenia, their affected and unaffected siblings

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Background: Schizophrenia is a highly heritable disorder associated with structural brain abnormalities. The aim of this study was to establish if the gene(s) for schizophrenia are associated with specific abnormalities of brain structure.
Subjects: Six sibships from multiple affected families were recruited. Each sibship consisted of one patient with schizophrenia, one "obligate carrier" without the disorder but with an affected child, and one "non-affected non-carrier". Such sibships are very rare, but present a powerful opportunity to separate the associations of genotype and phenotype. Obligates presumably have the gene(s) but not the disorder, affected siblings have both, whereas non-affected non-carrier siblings have neither.
Method: Brain MRI was conducted with a semiautomated region of interest analysis. The risk of false positive findings was reduced by collapsing brain regions and sides into five regions and comparing groups by repeated measures analysis of variance.
Results: In terms of whole brain volumes and volumes of cortical structures, obligates resembled their non-affected non-carrier siblings, both groups having significantly greater volumes than their schizophrenic siblings (p=0.01 and p=0.04). Obligates also had significantly smaller ventricles than their schizophrenic siblings (p=0.03). However, with respect to the amygdalohippocampal complex, the obligates' brains resembled those of their schizophrenic siblings, both groups showing a significant reduction in volume when compared with their non-affected non-carrier siblings (p=0.001).
Conclusions: In the families studied, reductions in volumes of cortical structures and reductions in whole brain volume seem to be associated with the phenotype of schizophrenia. By contrast, reduced volume of the amygdalohippocampal complex seems to be associated with genetic risk for the disorder even in the absence of disease.

Data from postmortem and in vivo brain imaging studies provide powerful evidence that schizophrenia is associated with abnormalities of brain structure [1–3] The most consistently identified abnormalities include reduced whole brain volume, increased lateral ventricle volume, and reduced volumes of the temporal lobes and medial temporal lobe structures. The origins and significance of these structural abnormalities are, however, not well understood. The preeminent contemporary theory proposes that they arise as a result of abnormal brain development.[4]

Many cases of schizophrenia are familial. The epidemiological evidence from twin and adoption studies strongly suggests that this familiality has a genetic origin.[5] However, to date, attempts to identify specific genes that might be associated with or even responsible for the disorder have proved inconclusive.[6 7] It is generally accepted that, even in highly familial cases, the development of schizophrenia is probably influenced by both genetic and environmental factors. The relative importance of genes versus environment in the development of the structural brain abnormalities associated with the illness remains unclear.

The aim of this study was to establish if the gene(s) for schizophrenia are associated with abnormalities of brain structure. We specifically hypothesised that amygdalohippocampal volumes are genetically mediated and tested this by taking a rare opportunity to compare specific sibships in multiply affected families.

METHODS

Subjects

The study was designed to separate out the genetic risk for schizophrenia from the illness itself (table 1). This was achieved by recruiting persons who had apparently transmitted schizophrenia from one affected parent to one or more affected children while remaining well themselves (so-called "obligate carriers"). Data from these obligates were compared with data from their schizophrenic siblings and their non-affected, presumed non-carrier siblings (as they had adult children without the disorder). An anonymised example of an ideal obligate family tree is shown in figure 1.

Table 1 The study design can separate the effects of gene(s) from the effects of illness

	Inherited high genetic risk	Shows symptoms of schizophrenia
Affected sibling	Yes	Yes
Obligate	Yes	No
Unaffected sibling	No	No

Exclusion criteria reflecting known risk factors for altered brain volume were age>65 years; alcohol dependence (past or present); dementia, or other "organic" brain disease; and any space occupying intracranial lesion.

Families showing the ideal pattern of inheritance described in figure 1 are obviously rare. Family trees relating to 255 multiply affected Scottish families (originally identified for the Edinburgh high risk study[8]) were examined. Sixty obligates were identified, 14 of whom had both an affected sibling and

Abbreviations: PSE, present state examination; SADS-L, schedule for affective disorders and schizophrenia—lifetime version; NART, national adult reading test

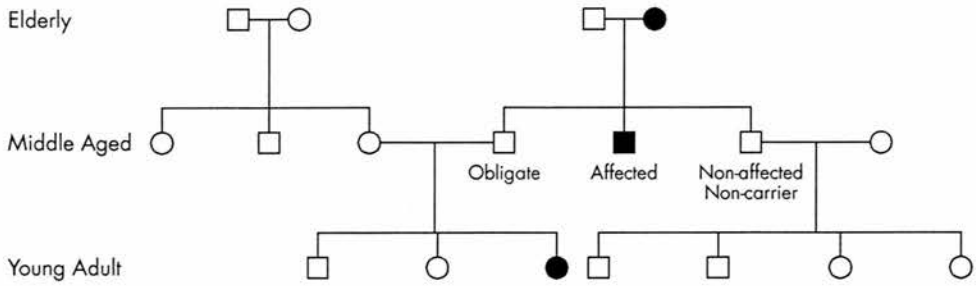

Figure 1 An ideal obligate family tree.

a non-affected, non-carrier sibling. All 14 families were contacted. Five families were unsuitable as one or more of the siblings met the exclusion criteria listed above (most exclusions were due to age). Of the remaining nine families, three did not wish or were unable to participate. Data were therefore obtained from six sibling "triples". Five of the sibships were of the same sex (three female, two male); the remaining family included a female patient and obligate and a male non-carrier (who was a twin with the patient).

Personal data

All subjects were interviewed (by RMS) with the present state examination (PSE)[9] and the schedule for affective disorders and schizophrenia—lifetime version (SADS-L).[10] Premorbid IQ was tested using the national adult reading test (NART).[11] Descriptive information was gathered from case notes and interviews (table 2 for details).

Imaging data

The MRI examinations included a dual spin echo sequence to exclude any significant brain lesions and a rapid volume acquisition sequence for volumetric data analysis. Any coil inhomogeneities were corrected for by scanning a flood phantom immediately after image acquisition and normalising to this before analysis. Unfortunately, it was not possible to examine all the subjects on the same machine. The first three sibling triplets were scanned at the MRI Unit, City Hospital, Edinburgh before the unit closed, on a 1 tesla Magnetom scanner (Siemens, Erlanger, Germany) using a MPRAGE sequence, FOV 250 mm, flip angle 12°, TR=10 ms, TE=4 ms, TI=200 ms, and relaxation delay 500 ms, giving 128 partitions 1.88 mm thick. The remaining three triplets were scanned at the Royal Infirmary, Edinburgh, with a 1 tesla Signa scanner (General Electric Company, Milwaukee, USA) using a SPGR sequence, FOV 250 mm, flip angle 30°, TR=16.4 ms, TE=3.3 ms, RBW 8.93 KHz, giving 124 partitions 1.5 mm thick. Regions of interest were traced on a slice by slice basis according to well established criteria using the semiautomated computer programme "ANALYZE" (Mayo Foundation, Rochester, MN, USA). All data were analysed by the same experienced rater (HCW) who was blind to group and has previously demonstrated high interrater and intrarater reliability.[8] Boundaries between brain regions were determined in accordance with criteria described in previously published studies from this department.[8] Volumetric data for the following brain regions were collected: whole brain, third ventricle, fourth ventricle, right and left lateral ventricles, prefrontal lobes, temporal lobes, caudate nuclei, lentiform nuclei, thalamic nuclei, and amygdalohippocampal complexes.

Statistical analysis

To reduce the chance of false positive findings, brain regions and sides were collapsed into the following regions: whole brain; cortical structures (prefrontal and temporal lobes); subcortical structures (caudate, lentiform, and thalamus nuclei); amygdalohippocampal complexes; and intracerebral ventricular system (lateral, third, and fourth ventricles). The null hypothesis was that there would be no difference between the three groups in terms of regional brain volumes. This was tested by repeated measures analysis of variance (ANOVA). The volumes of each collapsed region in each of the three subject groups was taken as a within subjects factor, with the scanner entered as a between subjects factor.

Table 2 Demographic and clinical details of subjects by group

	Schizophrenic siblings	Obligates	Non-affected, non-carrier siblings
Age (y; mean (SD))	46.2 (7.4)	49.0 (4.8)	45.2 (7.7)
NART IQ (mean (SD))	95.6 (15.4)	107.4 (12.9)	97.3 (17.8)
Years in education (mean (SD))	11.0 (0.9)	11.2 (2.4)	10.8 (1.6)
Current employment:			
No	3	0	1
Part time	2	1	1
Full time	1	5	4
Marital status:			
Single	4	0	0
Divorced	1	2	0
Married	1	4	6
Psychiatric history:			
Psychotic	6	0	0
Non-psychotic	0	3	3
None	0	3	3
Duration of illness (y; mean (SD))	20.8 (9.9)	NA	NA
Antipsychotic dose (mg/day chlorpromazine equivalent)	342 (256)	None	None
Current symptoms:			
Psychotic	3	NA	NA
In remission	3		

NA, not appropriate

Table 3 Volumes of brain regions by group

Brain region	Schizophrenic siblings mean (SD)	Obligates mean (SD)	Non-affected, non-carrier siblings mean (SD)	Significant between group differences by repeated measures ANOVA (df=2,3)
Whole				
Brain	1193.2 (50.2)	1259.2 (85.6)	1262.6 (68.0)	SCH<OBL, NANC (F=7.8, p=0.01)
Cortical				
Structures	273.8 (9.5)	293.7 (16.0)	293.4 (13.4)	SCH<OBL, NANC
Prefrontal lobe left	63.9 (6.3)	67.8 (6.1)	66.2 (3.4)	(F=4.7, p=0.04)
Prefrontal lobe right	65.9 (3.0)	71.1 (6.4)	73.5 (7.5)	
Temporal lobe left	70.9 (3.4)	75.6 (6.9)	74.2 (7.8)	
Temporal lobe right	73.0 (2.4)	79.3 (8.4)	79.5 (6.9)	
Subcortical				
Structures	28.1 (2.4)	27.6 (3.0)	28.8 (2.7)	No significant
Thalamus left	5.2 (0.3)	5.3 (0.5)	5.7 (0.5)	between group
Thalamus right	5.2 (0.5)	5.4 (0.6)	5.7 (0.6)	differences
Caudate left	4.0 (0.5)	3.9 (0.5)	3.8 (0.4)	(F=3.2, p=0.095)
Caudate right	3.9 (0.5)	3.7 (0.7)	3.8 (0.3)	
Lentiform nucleus left	4.9 (0.8)	4.6 (0.7)	4.8 (0.8)	
Lentiform nucleus right	4.8 (0.6)	4.6 (0.8)	4.9 (0.7)	
Amygdalohippocampal				
complexes	7.8 (0.8)	8.4 (0.5)	8.8 (0.3)	SCH, OBL<NANC
Left	3.6 (0.5)	3.9 (0.4)	4.1 (0.3)	(F=16.5, p=0.001)
Right	4.1 (0.4)	4.5 (0.2)	4.7 (0.3)	
Intracerebral				
Ventricular system	16.6 (6.4)	9.6 (5.2)	18.9 (11.4)	OBL<SCH
Lateral ventricle left	7.0 (2.9)	5.0 (2.9)	10.3 (7.6)	(F=5.4, p=0.03)
Lateral ventricle right	7.9 (4.4)	3.3 (1.8)	7.0 (2.9)	
3rd Ventricle	0.9 (0.3)	0.6 (0.2)	0.9 (1.0)	
4th Ventricle	0.7 (0.3)	0.8 (0.5)	0.6 (0.1)	

Volumes expressed in cm^3.

RESULTS

Results from volumetric analysis of the MRI are shown in table 3. There were no statistically significant group by scanner interactions.

Differences between schizophrenic subjects and their non-affected, non-carrier siblings were largely consistent with the existing literature. Whole brain volume was significantly smaller in the schizophrenic subjects (by about 5%), with a disproportionate reduction in the volume of the amygdalohippocampal complexes (12%). The expected increase in ventricular volume was, however, not found.

In terms of whole brain volume and volumes of cortical structures, the obligates were indistinguishable from their non-carrier siblings (and significantly larger than their schizophrenic siblings). They also had significantly smaller ventricles than their schizophrenic siblings. However, with respect to the amygdalohippocampal complex, the obligates' brains did not differ significantly from their schizophrenic siblings' brains (both groups showing a significant reduction in volume compared with the non-affected, non-carriers).

DISCUSSION

In this study, presumed carriers of the gene(s) for schizophrenia ("obligates") were found to share a significant reduction in amygdalohippocampal volume with their schizophrenic siblings. However, by contrast with their affected siblings, they were found not to have reduced frontal or temporal lobe or whole brain volumes; nor did they have large ventricles. This suggests genetic and phenotypic effects respectively.

The use of two separate MRI scanners in this study is an obvious and potentially serious limitation. However, the risk of bias was minimised by scanning all members of any given family on the same machine and by entering the scanner as a between subjects factor in the statistical analysis. Indeed, as no significant scanner interactions were found, our study provides some evidence that regional volumetric analyses are reliable across scanners (although obviously this comparison has low power).

One interesting and unexpected finding of this study was the relatively high IQ of the obligates (as shown in table 2 their average NART score of 107 was 10 points higher than either their schizophrenic or non-affected siblings). This raises the possibility that, amongst people at high genetic risk of schizophrenia, intelligence may be, or reflect, a protective factor, although various interpretations of this finding are clearly possible.

The design adopted for this study allows effects attributable to genetic risk to be separated from effects of disease without the need to identify the gene or genes responsible. The use of siblings as controls also carries the advantages of eliminating a number of potential sources of bias (reducing in particular non-specific variance in regional brain volumes), thereby increasing the power of the study. Sibling "triples" as described above are very rare and any study of this design will inevitably be small. Previous studies reporting volumetric data from brain imaging in relatives of schizophrenic patients suggest a range of brain abnormalities with relatives falling midway between schizophrenic patients and normal controls.[12] Most of these studies adopt a straightforward "schizophrenic patients versus relatives versus controls" design which does not allow persons with the gene(s) to be separated from those without. The only previously published study of obligates[13] did not report amygdalohippocampal volumes but found increased left lateral ventricle volume (which we did not find). That study adopted a slightly different design comparing normal unrelated controls with obligates, schizophrenic patients, and non-obligate relatives recruited from 16 multiply affected families. This design did not allow such a clear separation of genetic and environmental factors as the "sibling triples" design described above.

In any condition with apparently familial and non-familial cases, it can be argued that highly familial cases are unrepresentative of the condition as a whole. This study involved subjects from families in which schizophrenia was not only unusually prevalent but also inherited in an apparently dominant pattern. The genetic mechanisms involved in these

families may therefore be different from those acting in families with a non-dominant pattern of inheritance and may be of little relevance to non-familial cases of schizophrenia. The data from this study suggest that reduced volume of the amygdalohippocampal complex in schizophrenia is genetically determined whereas cortical changes and increased ventricular volume are not (and are presumably associated with the phenotype). However, data from the Edinburgh high risk study[8] does not support such a straightforward explanation. In that study genetic liability to schizophrenia in those from multiply affected families (with a range of patterns of inheritance) was not linearly associated with amygdalohippocampal volumes.[12] The genetic mechanisms involved in the development of schizophrenia are, however, not well understood and many different genes may be involved. We interpret our results as suggesting that obligates in this study have inherited some of the responsible genes (those causing amygdalohippocampal change) but not others (those responsible for cortical changes and symptoms). It seems likely that, even in families with an apparently dominant pattern of inheritance, highly complex gene-environment interactions are involved not only in the development of symptoms, but also in the genesis of structural brain abnormalities such as reduced brain volume and enlarged ventricles.

Genetic research into schizophrenia is currently a highly active area with many linkage and association studies in progress. The data from this study suggest that genes may play a part in the development of some, but not all, of the structural brain changes associated with the condition.

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Diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (^{1}H MRS) in schizophrenic subjects and normal controls

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Abstract

Several proton magnetic resonance spectroscopy (^{1}H MRS) studies in schizophrenia have found reduced *N*-acetyl aspartate (NAA) concentrations in pre-frontal and temporal regions of the brain. Reductions in NAA may reflect abnormalities of neuronal structure (e.g. reduced neuronal density or viability) or abnormalities of neuronal function. Diffusion tensor imaging (DTI) measures diffusion anisotropy, an indicator of the structural integrity of a neuronal tract. Both techniques were used to examine the anatomical basis of pre-frontal dysfunction in schizophrenia. Ten patients with DSM-IV schizophrenia were compared with 10 healthy controls. ^{1}H MRS and DTI were performed on a clinical MR system and analysed with a region of interest approach. NAA concentrations and diffusion anisotropy were measured in the same pre-frontal white matter region. Diffusion anisotropy was also measured in a control region (occipital white matter). ^{1}H MRS revealed non-significant but consistently reduced NAA concentrations (by 10–15%) in the pre-frontal white matter in schizophrenic subjects. Diffusion anisotropy measures revealed no such differences between schizophrenics and controls. It is concluded that the abnormalities of 'connectivity' reported in brain-imaging studies of schizophrenia may not be attributable to structural abnormalities of white matter and that reduced NAA in the pre-frontal white matter may reflect abnormal function of structurally intact neurons.

Keywords: Schizophrenia; Frontal lobe; Neurons; Magnetic resonance imaging

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1. Introduction

No single theory has yet been developed which successfully explains the onset, clinical and pathological features and course of schizophrenia. The 'Neurodevelopmental Hypothesis of Schizophrenia' (Murray, 1994) proposes that the disorder is due to developmental abnormalities arising before or around the time of birth, the psychopathology emerging in early adult life as the affected neuronal systems mature. It is suggested that the underlying pathology relates to disrupted neuronal migration during very early life (Raedler et al., 1998). The evidence in favour of this theory is by no means conclusive, but it finds support in the results of some post-mortem (Akbarian et al., 1993) and in-vivo imaging (Kotrla and Weinberger, 1995) studies.

The findings of functional imaging studies [PET (Soares and Innis, 1999; Fu and McGuire, 1999) and more recently fMRI (Kindermann et al., 1997)] suggest that schizophrenia may be associated with abnormal 'connectivity' between different brain regions (Andreasen et al., 1998). Precisely which aberrant connections are most important in the patho–physiology of the disorder is currently unclear. Cases have been argued for fronto–temporal pathways (Woodruff et al., 1997), fronto–cingulate connections (Fletcher et al., 1999) and trans-callosal fibres (Crow, 1998). If it is true that the connections between two regions of the brain have developed abnormally, one might expect to find abnormalities in the cyto-architecture of the white matter tracts carrying axons between those regions. This study uses proton magnetic resonance spectroscopy (^{1}H MRS) and diffusion tensor imaging (DTI) to examine the anatomical basis of connectivity in the pre-frontal white matter.

1.1. ^{1}H MRS

Proton magnetic resonance spectroscopy measures the concentration of various biologically important metabolites in the brain. Although spatial resolution is relatively poor compared to conventional MR imaging (15-mm cubic voxels in this study), the technique is useful because it allows brain chemistry to be studied in vivo without the use of radioactive tracers. Most studies report *N*-acetyl aspartate (NAA) concentrations because the peak in the proton spectrum attributable to NAA is prominent and can be measured reliably. NAA is found predominantly within neurons though its intracellular function is unclear. It is thought to be a neuronal/axonal marker, and its concentration may, therefore, provide a measure of both the number and integrity of neurons within a region of the brain. In connection with schizophrenia, many previous studies have reported reduced NAA concentrations in certain brain regions (Bertolino and Weinberger, 1999), particularly the hippocampus, frontal cortex and pre-frontal white matter. In this study ^{1}H MRS was used to measure NAA concentrations in pre-frontal white matter. Reductions in NAA in this region may reflect a structural abnormality (such as reduced axonal density or reduced viability of neurons), or it may reflect abnormal function of structurally normal neurons whose axons travel to and from the frontal lobes.

1.2. DTI

In the brain, the rate at which water molecules diffuse along a particular direction (the apparent diffusion coefficient, or ADC) can be measured in vivo from MR images by applying a diffusion sensitising gradient in the direction of interest. Structures such as myelin sheaths, axonal membranes and micro-filaments cause the diffusion to be slower perpendicular to axons than parallel to them, thereby leading to diffusion anisotropy in the measured ADCs. From a set of diffusion-weighted images, the apparent diffusion tensor, D, can be derived. The extent to which diffusion is disrupted by cellular structures is then reflected in the degree of asymmetry of the tensor. Measures of diffusion anisotropy are indicators of the structural integrity of a neuronal tract. A commonly quoted diffusion anisotropy index is the fractional anisotropy, FA, which measures the fraction of the total 'magnitude' of the apparent diffusion tensor that is anisotropic (Basser, 1995; Pierpaoli and Basser, 1996).

DTI is a relatively new technique and the liter-

ature relating to schizophrenia is small and inconsistent. Buchsbaum et al. (1998) reported reduced diffusion anisotropy in the white matter of the pre-frontal cortex in five schizophrenics compared with six controls. Lim and colleagues (Lim et al., 1999) studied 10 US army veterans with schizophrenia and found widespread reductions in anisotropy throughout the brain, whilst Foong et al. (2000) studied the corpus callosum in twenty patients and reported reduced anisotropy in the splenium, but not the genu. The primary aim of this study was to measure diffusion anisotropy in the pre-frontal white matter. Diffusion anisotropy was also measured in a control region (occipital white matter) which is not generally implicated in the pathophysiology of schizophrenia.

1.3. Hypothesis

The study was designed to yield potentially complementary information from ^{1}H MRS and DTI in the same patients and controls. The main hypothesis being tested was that NAA concentrations and diffusion anisotropy would be abnormal in the pre-frontal lobe white matter, i.e. that aberrant connectivity in schizophrenia has structural and functional correlates.

2. Method

2.1. Subjects

Ten patients with established DSM-IV (American Psychiatric Association, 1994) schizophrenia were compared with 10 healthy controls. Both male and female participants were considered, and the groups were matched for age (see Table 1).

Subjects were selected from patients with an unambiguous clinical diagnosis of schizophrenia (DSM-IV diagnosis was confirmed by SADS-L semi-structured interview). All had participated in previous brain-imaging studies and were known to be able to tolerate the scanning procedure. Mean duration of illness was 15 years and all subjects were taking antipsychotic medication at the time of the study. Clinical and volume acquisition scans were available for every subject. Patients with gross structural brain abnormalities were not considered for inclusion in this pilot study. However, patients known to have morphological brain changes consistent with schizophrenia (slightly reduced whole brain volume, enlarged lateral ventricles) were included (see Table 1).

Table 1
Demographic, clinical and morphological details of subjects by group*

	Schizophrenic	Control	*P*	Effect size
Number of subjects	10	10	–	–
Sex (male/female)	5/5	4/6	–	–
Age (years)	34 (14)	35 (7)	–	–
Duration of illness (years)	15 (12)	N/A	–	–
Whole brain volume	1229 (123)	1259 (110)	0.62	−0.27
Left lateral ventricle volume	5.9 (3.0)	3.7 (2.2)	0.12	+1.00
Right lateral ventricle volume	4.2 (2.7)	3.6 (1.8)	0.59	+0.50
Left frontal lobe volume	69 (13)	70 (8)	0.94	−0.12
Right frontal lobe volume	74 (13)	74 (9)	0.99	0.00
Left amygdala + hippocampus	4.1 (0.4)	4.5 (0.4)	0.18	−1.00
Right amygdala + hippocampus	4.7 (0.4)	4.7 (1.0)	0.98	0.00

*Values presented are the group means (S.D.) and volumes (S.D.) in ml, with the corresponding *P*-value and the effect size = $(\text{mean}_{sz.} - \text{mean}_{cont.})/\text{S.D.}_{cont.}$

2.2. Data acquisition and analysis

All imaging and spectroscopy data were collected on an Elscint 2T Prestige (Elscint, Haifa, Israel) clinical scanner (horizontal bore inner diameter 57 cm), equipped with a 15 mT m^{-1} actively shielded gradient set. The examination consisted of a T_2-weighted fast spin-echo sequence (TR/TE 4800/96; FOV 22×22 cm; 10 axial slices beginning approx. 10 mm above the base of the temporal lobes; slice thickness 6 mm; slice gap 20%), a 3D gradient-echo volumetric sequence (TR/TE 28.5/9.254 ms; FOV 18×18 cm; slice thickness 1.5 mm) and then the following ^{1}H MRS and DTI protocols. The complete examination took approximately 45 min.

2.3. Proton magnetic resonance spectroscopy

Using the set of axial fast spin-echo images for positioning, two PRESS-localised spectra (TR/TE 1500/145 ms) were acquired from 15-mm cubical volumes of interest (VOIs) in left and right hemisphere frontal white matter approximately 5 mm from the most anterior part of the frontal horns in a line 45° from the midline. This voxel location ensured that proton spectra were acquired from homogeneous white matter rather than from a heterogeneous mixture of tissue types. Following localised shimming and water suppression calibration for each VOI, 200 acquisitions with water suppression were collected. Eight acquisitions without water suppression were also collected to serve as a phase reference. The VOI positions within the head coil were noted, as was the scanner RF calibration figure to allow for the effects of coil loading.

Spectroscopy data were transferred to a Sun Ultra Sparc Station 10 (Sun Microsystems, Mountain View, CA, USA) workstation for analysis. Analysis consisted of phase correction using the water reference data (Ordidge and Cresshull, 1986) and the removal of the residual water signal using Hankel-Lanczos singular value decomposition (Van den Boogaart, 1994). Spectral peak areas were quantified using the MRUI software package ((http://carbon.uab.es/mrui)), which fitted Gaussian line shapes to the peaks corresponding to choline (3.2 ppm), creatine (3.0 ppm) and NAA (2.0 ppm). Finally, metabolite peak areas for each subject were corrected for coil loading and VOI position within the head coil. The resulting 'institutional' units enabled inter-subject comparisons.

2.4. Diffusion tensor imaging

Diffusion-weighted echo-planar (DW-EP) images were obtained using a non-cardiac gated, single-shot, spin-echo, half-Fourier sequence, in which two symmetric diffusion-sensitising trapezoidal gradient pulses of 35-ms duration and 43.9-ms separation were applied sequentially along the six oblique gradient directions (G^1 to G^6) that constitute the uniform diffusion gradient sampling scheme of Basser and Pierpaoli (Basser and Pierpaoli, 1998). In each of these six gradient directions, nine DW-EP images were acquired with a diffusion gradient strength of 11.0 mT m^{-1}, giving an average value for Trace (b) of 686.55 ± 0.61 (S.D.) s mm^{-2} for G^1 to G^6. A total of 54 DW-EP images per slice position were collected, plus nine baseline images (G^0) with no diffusion weighting. Values for the elements of the *b*-matrix were calculated numerically (Mattiello et al., 1997). Other image acquisition parameters were as follows: 10 axial slices coincident with those prescribed in the fast spin-echo sequence; 6-mm slice thickness; 20% slice gap; 128×72 half-Fourier image matrix (zero-filled to 256×128); 44×22 cm FOV; TE of 103 ms. The time from acquisition of one image slice to the next was 1.4 s, so the effective TR was 14 s. Patient head motion was minimized through use of a Velcro strip, which was comfortably tightened around the subject's forehead.

Magnitude Fourier transformed images in DICOM format were transferred from the scanner to a Sun workstation and converted into Analyse (Mayo Foundation, Rochester, MN, USA) readable form using software written in *C*. Within each slice the set of nine images for each of the diffusion gradient directions G^0 to G^6 were separately realigned to remove physiological motion using SPM95 ((http://www.fil.ion.ucl.ac.uk/spm)), and then averaged to give seven high

signal-to-noise images (Bastin et al., 1998). Geometric image distortions in the six DW-EP images arising from the strong eddy currents present in the DTI experiment were corrected using a modified version of the iterative cross-correlation algorithm suggested by Haselgrove and Moore (Haselgrove and Moore, 1996; Bastin, 1999).

Within each voxel the six elements of the apparent diffusion tensor D (D_{xx}, D_{yy}, D_{zz}, D_{xy}, D_{xz} and D_{yz}) and the baseline T_2 signal intensity were estimated by multivariate linear regression (Basser et al., 1994) from the measured signal intensity using a program written in Matlab (The Mathworks, Natick, MA, USA). After diagonalisation of D to yield the eigenvalues ($\lambda_{i=1,2,3}$), maps of the fractional anisotropy, FA (Pierpaoli and Basser, 1996)

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

were generated on a voxel-by-voxel basis and converted into Analyze format. The fractional anisotropy measures the fraction of the total 'magnitude' of the apparent diffusion tensor that is anisotropic, and takes a value of 0 (see formula above) for an isotropic (cylindrically symmetric, $\lambda_1 \gg \lambda_2 = \lambda_3$) medium. Values for FA in frontal white matter were calculated from regions-of-interest (ROIs) positioned by a radiologist (JJKB) to coincide with the centres of volumes of interest from which the proton spectra were obtained. In the occipital lobes ROIs were drawn by the radiologist to lie in the white matter adjacent to the optic radiation.

3. Results

Fig. 1 shows a structural fast spin-echo image, an FA map and a proton spectrum obtained from a 27-year-old female schizophrenic patient who participated in this study. The spectrum has been acquired from the predominantly white matter VOI indicated in the structural image. Note that although the baseline of this spectrum is relatively noisy, as is typical in clinical MRS studies, the NAA peak is still well resolved.

3.1. ^1H MRS

As illustrated in Table 2, NAA concentrations were reduced in male and female schizophrenic subjects compared with controls in both the right and left frontal white matter. The direction and magnitude (10–15%) of this difference is consistent with previously published data (Bertolino and Weinberger, 1999). These findings failed to reach statistical significance (by one way ANOVA using a 2-tailed significance level of 5%), but this may be due to the small number of subjects in this pilot study.

3.2. DTI

As illustrated in Table 3, when data from all subjects (male and female) were analysed there were no consistent differences between the FA values obtained from schizophrenics and controls. In schizophrenic subjects, diffusion anisotropy was slightly reduced in left frontal white matter, but slightly increased in the other regions measured. All between-group differences were small and none was close to statistical significance. The absence of any discernible trend in these DTI data suggests that this was not simply a type-2 error caused by small numbers.

Table 3 also shows the FA values for males and females, analysed separately. In male schizophrenics, anisotropy was slightly reduced in all four ROIs, but the findings were not statistically significant. In females the only difference between the groups was a statistically significant ($P < 0.01$) *increase* in anisotropy in the right occipital region in the schizophrenics.

3.3. Correlations

Correlations between ^{1}H MRS and DTI measures were inconsistent. Within the schizophrenic group, NAA concentrations in the right frontal region showed a significant positive correlation with FA in both the right and left frontal regions (Pearson coefficients: Right 0.65, $P < 0.05$; Left 0.69, $P < 0.05$). However, no such correlation was

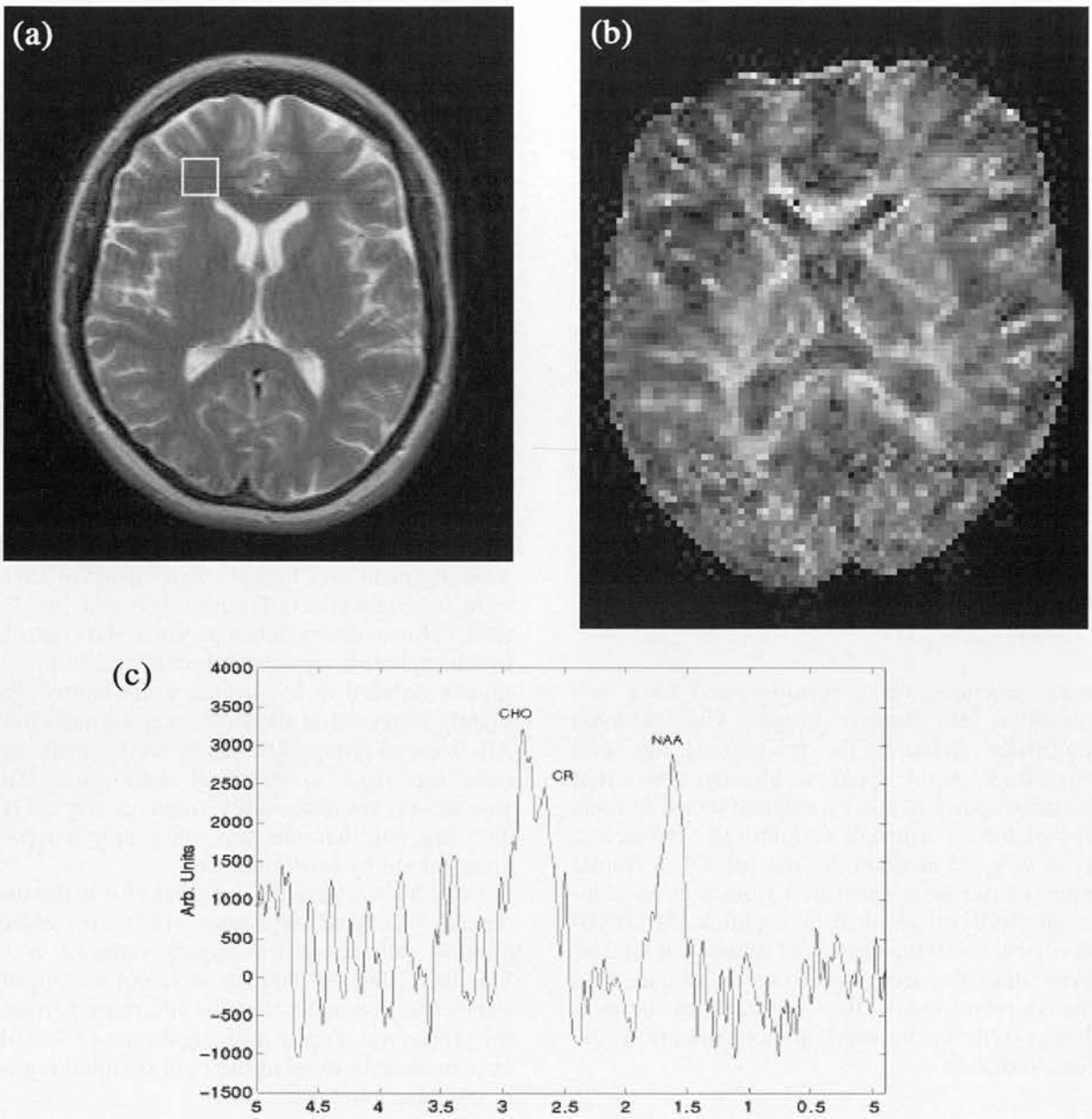

Fig. 1. A structural fast spin-echo image (a); a fractional anisotropy, *FA*, map (b); and a proton spectrum (c) obtained at the level of the lateral ventricles from a 27-year-old female schizophrenic subject in this study. The VOI from which the proton spectrum was acquired is indicated on the fast spin-echo image (□). The *N*-acetyl aspartate (NAA), choline (CHO) and creatine (CR) peaks are also indicated on the spectrum. In the *FA* map, high diffusion anisotropy is indicated by light pixels.

found in the control group (Pearson coefficients: Right 0.09, $P > 0.8$; Left 0.18, $P > 0.7$). Furthermore, NAA in the left frontal region in schizophrenics showed a weak *negative* correlation with the FA (Pearson coefficients: Right -0.3, $P > 0.4$; Left -0.2, $P > 0.5$).

Table 2
4k[1]H MRS results by brain region, patient group and sex*

Subjects	Brain region	Schizophrenic	Control	*P*	Effect size (*d*)
Males	Left frontal	1072 (244)	1100 (218)	0.88	−0.13
Females	Left frontal	992 (196)	1235 (164)	0.09	−1.48
All subjects	Left frontal	1027 (208)	1178 (186)	0.16	−0.81
Males	Right frontal	1065 (218)	1122 (143)	0.71	−0.40
Females	Right frontal	1019 (147)	1153 (134)	0.20	−1.00
All subjects	Right frontal	1039 (171)	1140 (157)	0.22	−0.64

*Values presented are the mean (S.D.) *N*-acetyl aspartate peak areas in arbitrary units, *P*-value and the effect size = $(\text{mean}_{sz.} - \text{mean}_{cont.})/\text{S.D.}_{cont.}$

Correlations with volumetric data were also inconsistent. Within the schizophrenic, but not the control group, NAA concentrations in the left frontal region correlated strongly with the volume of the amygdalo–hippocampal complex on the left (Pearson coefficient 0.92, $P < 0.05$); however, there was no correlation between the same measures on the right (Pearson coefficient 0.01, $P = 0.98$). The FA showed no correlation with any volumetric measures in either group. Neither ^{1}H MRS nor DTI measures correlated with the duration of illness.

4. Discussion

In this study schizophrenia was found to be associated with reduced NAA concentrations in the pre-frontal white matter, but with normal diffusion anisotropy in the same region. These findings suggest that, whilst the neuronal tracts running to and from the frontal lobes may be *functionally* abnormal in schizophrenic subjects, they are *structurally* intact. The authors conclude that the aberrant functional connectivity reported in schizophrenia may not be attributable to

Table 3
Fractional anisotropy by group, all subjects*

Brain region	Schizophrenic	Control	*P*	Effect size (*d*)
Left frontal	0.42 (0.09)	0.43 (0.08)	0.68	−0.22
Right frontal	0.43 (0.11)	0.42 (0.07)	0.82	+0.15
Left occipital	0.45 (0.07)	0.45 (0.05)	0.59	+0.06
Right occipital	0.47 (0.07)	0.45 (0.05)	0.90	+0.30
Fractional anisotropy by group, males only*				
Left frontal	0.40 (0.08)	0.45 (0.06)	0.33	−0.86
Right frontal	0.46 (0.15)	0.48 (0.50)	0.83	−0.31
Left occipital	0.44 (0.06)	0.44 (0.06)	0.95	−0.05
Right occipital	0.41 (0.02)	0.45 (0.08)	0.34	−0.46
Fractional anisotropy by group, females only*				
Left frontal	0.44 (0.11)	0.42 (0.09)	0.83	+0.15
Right frontal	0.41 (0.08)	0.39 (0.05)	0.66	+0.37
Left occipital	0.46 (0.08)	0.45 (0.04)	0.77	+0.29
Right occipital	0.53 (0.04)	0.46 (0.02)	> 0.01	+3.29

*Values presented are the mean (S.D.), *P*-value, and effect size = $(\text{mean}_{sz.} - \text{mean}_{cont.})/\text{S.D.}_{cont.}$

abnormalities of white matter cyto-architecture and that reduced NAA in the pre-frontal white matter may reflect abnormal function of structurally intact neurons.

The study successfully measured reductions in NAA concentrations of a magnitude consistent with the established literature. In contrast, the DTI results were characterised by the absence of any discernible trend and by the lack of any consistent correlation between the fractional anisotropy and NAA concentrations and volumetric data. The positive findings of previously published DTI studies have not been replicated. The authors acknowledge that the discrepancies between studies may be a consequence of limited statistical power. However, accurate estimation of power is difficult when the few published studies differ markedly in terms of techniques employed and subjects recruited. This is the first DTI study in schizophrenia to include female subjects and the data suggest a male/female difference.

DTI is a relatively new technique and the literature relating to pre-frontal white matter in schizophrenia is limited to two small case control studies. Buchsbaum et al. (1998) compared five male schizophrenic subjects with six male controls. They analysed their data using a statistical parametric mapping approach, which is not easily translated into a predicted effect size for a region of interest study. The region of interest examined in this study approximated to the region of the brain in which Buchsbaum and colleagues found the most highly statistically significant reduction in diffusion anisotropy.

The study by Lim et al. (1999) is the only study in the literature to employ similar techniques to this study. They report a statistically significant reduction in diffusion anisotropy in frontal white matter in 10 schizophrenic US army veterans compared with 10 male controls, (the largest effect size they report is $1.5 \times$ S.D. which, for $n = 10$, translates to power > 0.99 at a two-tailed significance level of 5%). However, there are important differences between their subjects and the subjects in this study. Firstly, there is a large discrepancy in the age of subjects (mean of 48 years in the Lim study vs. 34 years in this study). It is possible that the differences they report are a consequence of decades of disease process or neuroleptic exposure (although there was no correlation between fractional anisotropy and duration of illness in this study). Secondly, the schizophrenic subjects in this study were known to have the characteristic macroscopic structural brain abnormalities associated with schizophrenia. It was anticipated, perhaps incorrectly, that pre-existing differences between the groups on established measures of brain structure would increase prospects of detecting corresponding between-group differences using DTI (i.e. increase the power of the study). Finally, the different results may reflect male/female differences in diffusion anisotropy. Lim et al. (1999) studied only male subjects whilst half of the subjects in this study were female. Table 3 shows that whilst fractional anisotropy was reduced in male schizophrenics (although the effect sizes -0.05 to -0.86 were smaller than those reported by Lim et al., 1999), it was actually increased in female schizophrenics. This result and, in particular, the increased anisotropy in schizophrenic females in the occipital region was not anticipated and is not understood. There is a relative paucity of imaging studies as a whole in female schizophrenic subjects and this finding requires further exploration.

Single voxel proton MRS is slow and has a relatively poor spatial resolution. This increases the potential for contamination of the white matter spectrum by signals originating from the grey matter and CSF. Such partial volume averaging is difficult to avoid, but since the MRS VOI is placed within a large region of white matter, such effects will be minimized. Additionally, the chemical shift imaging (CSI) technique, which allows the acquisition of a two- (or three-)dimensional grid of spectra, may prove very useful in studies such as this where specific lesions are not evident.

DTI is a new technique and a number of issues relating to data acquisition and image analysis arose during the course of this pilot study. Firstly, because single-shot diffusion-weighted echo-planar imaging suffers from artifacts caused by susceptibility changes at air/tissue boundaries (e.g. frontal sinus), accurate diffusion imaging data could not be obtained from all regions of the

brain. Secondly, the gradient strength (15 mT m^{-1}) and rise time (30 mT m^{-1} ms^{-1}) of the scanner's gradient set were somewhat limited. This was reflected in the large field-of-view (44 × 22 cm), relatively low image resolution (1.72 × 1.72 mm voxels), low values of diffusion-weighting (Trace(b) ~ 700 s mm^{-2}) and long echo train (200 ms). Also the diffusion-weighted echo-planar imaging sequences could not be cardiac gated, which may result in artefactual increases in diffusion anisotropy close to the ventricles. The resulting *FA* maps, therefore, have relatively lower sensitivity and resolution than ideally required. Nevertheless, the absolute values for the *FA* of white matter reported in this study (~ 0.45) are in close agreement with those reported by Lim et al. (1999). Finally, the raw data must be processed, to remove the effects of eddy currents (Bastin and Armitage, 2000) and physiological movement before accurate diffusion anisotropy information may be obtained. In this study DTI data acquisition was, therefore, limited to specific regions of interest and no attempt was made to acquire DTI data from other regions of the brain.

The ROI approach has inherent limitations particularly when applied to white matter. It is difficult to ensure that the region identified consistently reflects the same anatomical structures within the brains of different subjects. One might predict that as white matter tracts approach the cortex, diffusion anisotropy would fall as the fibres spread out. Our regions of interest were placed manually (always by the same experienced and experimentally blind radiologist) and we did not measure the reliability of this placement. Another limitation of this approach is that an apparently normal finding in the region studied does not rule out the possibility of significant structural abnormalities in important adjacent structures.

DTI has the potential to develop into a powerful technique for the study of white matter in vivo. In the future it may usefully complement functional imaging techniques by providing information about the structural basis of abnormal functional connectivity. DTI certainly merits further development through pilot studies and ultimately may prove to be a useful tool in schizophrenia research. Clearly larger studies using proven techniques of data acquisition and analysis are required.

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