FUNCTIONAL NEUROIMAGING IN SUBJECTS AT HIGH GENETIC RISK OF SCHIZOPHRENIA

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The Edinburgh High Risk Study by necessity involves input from a large number of people, and I would like to acknowledge that the success of this study is attributable to great team effort. In particular I would like to offer my thanks to those involved in subject recruitment (Richard Cosway, Dr Lesley Harrison, Kirsten Russell and Caroline Brett), to Dr Dominic Job for technical support, and to the radiographers involved in scanning the study participants. The Edinburgh High Risk Study was funded by an MRC programme grant. Scanning was carried out at the Brain Imaging Research Centre (BIRC) for Scotland. Both BIRC and the Centre for Functional Imaging studies (CFIS) are funded by the Scottish Higher Education Funding Council (SHEFC).

Declaration

My primary role in the Edinburgh High Risk Study was to collect and document functional imaging data acquired for this study, and to analyse functional imaging data for the Hayling task.

Collection of data included in this thesis began in July 2000, and completion of the baseline data on the first 100 subjects (high risk and controls) ended in April 2002. During this period I was the primary operator of the experimental functional imaging equipment for the project (the IFIS system) and therefore collected imaging data (for the Hayling task, as well as the encoding and retrieval tasks) on approximately 90% of this sample. Second round data collection began in January 2002. Due to technical difficulties with the IFIS equipment, completion of second round data collection was delayed. The second round results presented here are therefore based on the number of subjects collected by the end of 2003. At the beginning of the second round data collection I trained another PhD student, Marie Claire Whyte (enrolled to analyse the encoding and retrieval data), to use the IFIS system in order to help with collection of the second round data. I therefore collected approximately half of the second round imaging data included in this thesis (Hayling, encoding and retrieval).

The analysis of the functional imaging data included in this thesis was conducted by myself under the supervision of Dr Enrico Simonotto and Dr Stephen Lawrie. The functional connectivity analysis methods were devised by Dr Enrico Simonotto, and for this reason Dr Simonotto was first author of the paper (in preparation) that constitutes the framework of chapter four. However, I was directly involved in testing and implementing these methods. I was also solely responsible for the second level (group) analyses, and I was primarily involved in reporting and interpreting the results, and in the writing of this paper.

Recruitment of subjects was co-ordinated by the research psychologists employed as part of the Edinburgh High Risk Study; Richard Cosway, Lesley Harrison, Kirsten Russell, and Caroline Brett. The clinical assessments were administered by Professor Eve Johnstone, Professor David C Owens, and Dr Stephen Lawrie.

This work as not been submitted for any other degree or professional qualification. I declare that this thesis is my own work, and the contribution to this thesis of others is clearly documented here and throughout the thesis where relevant.

Signed:

Dated: 22/12/04

Abbreviations

Abbreviation	Meaning	Definition
BA	Brodmann Area	Cytoarchitectonically defined anatomical areas of the brain (Brodmann 1925)
BOLD	Blood oxygen level dependent	Endogenous contrast used in fMRI based on paramagnetic properties of oxygenated and deoxygenated blood
CBF	Cerebral blood flow	Parameter related to cerebral haemodynamics
CBV	Cerebral blood volume	Parameter related to cerebral haemodynamics
$CMRO_2$	Cerebral metabolic rate of oxygen	Parameter related to cerebral haemodynamics
CT	Computerised tomography	X-ray based imaging technique
DSM IV	Diagnostic and Statistical Manual of the American Psychiatric Association (1994)	One of the two major classification manuals in psychiatry, see ICD-10
DTI	Diffusion tensor imaging	Imaging technique which examines the diffusion of water molecules, provides a measure of white matter integrity
EEG	Electroencepahalogram	Electrophysiological method for functional brain mapping based on recording transient electrical signals generated by neuronal depolarisation
EPI	Echo planar imaging	Fast imaging technique used in fMRI
FA	Fractional anisotropy	Measure used in DTI to describe the fraction of the magnitude of the tensor that can be ascribed to anisotropic (ellipsoid) diffusion
fMRI	Functional magnetic resonance imaging	Functional imaging technique measuring changes in blood oxygenation, based on differing magnetic properties of oxygenated and deoxygenated blood.
FOV	Field of view	Region of physical space to which the data corresponds
FWHM	Full width half maximum	Method for characterising the width of a peak on a graph. Commonly used to describe smoothing filter applied to neuroimaging data.
HbO_2	Oxygenated haemoglobin	Parameter related to cerebral haemodynamics
Hbr	Deoxygenated haemoglobin	Parameter related to cerebral haemodynamics
HRF	Haemodynamic response function	Parameter related to cerebral haemodynamics

Abbreviation	Meaning	Definition
ICD-10	International Classification of Diseases (World Health Organisation 1993)	One of the two major classification manuals in psychiatry, see DSM IV
MEG	Magnetoencepahalography	Electrophysiological method for functional brain mapping based on recording transient magnetic signals generated by neuronal depolarisation
MRI	Magnetic resonance imaging	Imaging technique based on observing changes in the behaviour of protons in the presence of a magnetic field
NART	National adult reading test	Pre-morbid measure of intelligence. This test requires people to pronounce words that do not follow the usual rules of pronunciation.
NIRS	Near infrared spectroscopy	Optical imaging method of functional brain mapping
OIS	Optical imaging based on intrinsic signal	Optical imaging method of functional brain mapping
PET	Positron emission tomography	Method of functional brain mapping based on measuring cerebral blood flow
PPI	Psychophysiological interaction	Regression based measure of the influence of that one brain region has on another
PSE	Present state examination	Clinical standardisation of diagnostic criteria based on structured interview (Wing *et al*, 1974)
SEM	Structural equation modelling	Technique used to describe effective connectivity, based on covariance matricies.
SOA	Stimulus onset asynchrony	Interval between successive onsets of the same trial type
SPECT	Single photon emission computerised tomography	Method of functional brain mapping based on measuring cerebral blood flow
SPM	Statistical Parametric Mapping	Software used to analyse structural and functional brain images
TMS	Transcranial magnetic stimulation	Method of functional brain mapping based on principles of electromagnetic induction
VBM	Voxel Based Morphometry	An automated method for analysing structural MR images using SPM software

Definitions

Term	Description / definition
Endophenotype	Or intermediate phenotype, a term used to describe measurable traits that represent internal or intermediate phenotypic expression of underlying genetic susceptibility to disease
Executive function	Set of cognitive processes involved in actively maintaining, and manipulating information in order to guide behaviour, e.g. volition, planning, purposive action and effective performance.
Effective connectivity	Type of connectivity analysis which infers the influence of one neuronal system on another
Functional connectivity	Correlation of activity between voxels of the brain across experimental conditions. Here there is no inference regarding the causal nature of the relationship.
Functional integration	Term used to describe connectionist models of brain functioning where the importance is on the interaction between distributed brain regions
Functional localisation	Term used to describe concepts of brain functioning focusing on the functions of discrete regions
Hypofrontality	Term used to describe reduced activation of the prefrontal cortex
'State' effects	Effects related to phenotypic expression of disease. In the current study, since no subjects met criteria for schizophrenia at baseline, this term is used to describe effects related to the manifestation of some of the characteristic features of the disorder rather than the disease itself . See also 'trait' effects.
Stimulus correlated movement	Movement during scanning session which periodically occurs in time with task onsets. This can result in artefactual signal change which is difficult to distinguish from true activations
Susceptibility artefact	Term used to describe distortions in EPI images occurring at air:tissue boundaries.
Time series	Series measurements that occur over time, for example a series of brain scans
'Trait' effects	Effects of presumed genetic origin

Summary of organisation of thesis

This thesis uses functional magnetic resonance imaging (fMRI) to examine brain activation patterns in subjects at high genetic risk of schizophrenia to address three issues. The primary aim was to identify the neural correlates of state and trait effects in these high risk individuals. There were two further subsidiary aims based on whether any subjects made the transition to illness over the course of the study. The second aim was to determine if it is possible to distinguish those who subsequently became ill from those who remained well and normal controls at baseline using functional imaging approaches. Finally, should there be sufficient numbers of subjects who became ill with two scans, the last aim was to determine if patterns of brain activity changed with the transition to illness, or varied with changes in symptomatic status of high risk individuals. Due to the rarity of data from individuals before illness develops, and since they were likely to be based on small subject numbers, the two subsidiary aims were considered exploratory analyses.

Overall this thesis therefore spans two main areas of study; neuroimaging, and schizophrenia research. For this reason two introductory chapters (chapters one and two respectively) cover these two topics. Historically, there are considered to be two distinct approaches to the understanding of brain function, functional localisation and functional integration, and the first two chapters are divided according to these main sub-headings. Subsequent chapters constitute the main experimental section of the thesis. Chapters three and four focus on functional neuroimaging in high risk subjects at baseline, addressing the first aim described above. A similar framework, this time dividing whole chapters into functional localisation (chapter three) and functional integration (chapter four) is used for consistency. Chapters five and six address the second aim, and examine functional localisation and functional integration at baseline in those who, over the period of study, developed schizophrenia. Chapter seven addresses the third aim, and examines changes in localisation of function over time. Change over time in terms of functional integration in these high risk subjects was considered beyond the scope of this thesis. Finally, chapter eight comprises an overall summary of the main findings and general discussion.

Publications

A list of relevant publications based on the functional imaging data contained within this thesis is presented below:-

Whalley HC, Simonotto E, Flett S, Marshall I, Ebmeier KP, Owens DGC, Goddard NH, Johnstone EC, Lawrie SM. 2004. fMRI correlates of state and trait effects in subjects at genetically enhanced risk of schizophrenia. *Brain*, 127: 478-490. *The data presented in chapter three formed the basis of this paper, which is included in the appendix.*

Simonotto E, Whalley HC, Meyer M, Whyte MC, Marshall I, Ebmeier KP, Owens GC, Goddard NH, Johnstone EC, Lawrie SM. Functional disconnectivity in subjects at high genetic risk of schizophrenia. *The data presented in chapter four forms the basis of this paper, which is currently in preparation.*

A list of published first author conference abstracts based on the functional imaging data contained within this thesis is detailed in the appendix. I was also involved in the analysis of structural imaging data (using both automated and semi-automated techniques) acquired as part of the Edinburgh High Risk Study, these publications are also listed in the appendix.

Abstract

Schizophrenia is an incapacitating psychiatric disorder characterized by hallucinations and delusions with a lifetime risk of around 1% worldwide. It is a highly heritable disorder which generally becomes manifest in early adult life. The established condition has been associated with structural and functional brain abnormalities, principally in prefrontal and temporal lobes, but it is unclear whether such abnormalities are related to inherited vulnerability, medication effects, or the presence of symptoms. Furthermore, the mechanisms by which the pre-morbid state switches into florid psychosis are unknown. The Edinburgh High Risk Study is designed to address these issues. The first phase (1994-1999) employed repeated clinical, neuropsychological assessments and structural imaging. In the current phase (1999-2004) functional magnetic resonance imaging (fMRI) has been added to the tests used previously.

As part of the Edinburgh High Risk Study, this study used a covert verbal initiation fMRI task (the Hayling Sentence Completion Test) known to elicit frontal and temporal activation, to examine a large number of young participants at high risk of developing schizophrenia for genetic reasons, in comparison with a matched group of healthy controls. Subjects were scanned at baseline, and after approximately one year. At the time of the baseline scan none of the participants met criteria for any psychiatric disorder, however, a number of subjects reported isolated psychotic symptoms on direct questioning. Over the course of the entire study (1994-2004), 21 individuals developed schizophrenia according to standard diagnostic criteria. Four of these subjects made the transition over the course of the current study (1999-2004), i.e. subsequent to the baseline functional scan.

There were three main aims of the current study (i) to use fMRI to identify the neural correlates of state and trait effects in high risk individuals, (ii) to determine if it is possible to distinguish those who subsequently become ill from those who remain well using functional imaging, and (iii) to determine if patterns of brain activity change with the transition to illness, or vary with changes in symptomatic status of these individuals.

Regarding the first aim, group differences of apparent genetic origin were found in prefrontal, thalamic, cerebellar regions, and differences in activation in those with symptoms were found in the parietal lobe. Functional connectivity analysis examining interactions between these regions also indicated similar abnormalities. These results may therefore reflect inherited deficits, and the earliest changes associated with the psychotic state, respectively. Although only a small number of subjects became ill over the course of the current study (n=4), initial findings suggested abnormalities in medial prefrontal and medial temporal regions (with an indication of parietal lobe dysfunction) were able to distinguish those who later became ill versus those that remained well. Finally, there were also indications of changes in activation patterns over time in a subgroup of subjects with varying symptomatic status.

To conclude, these results are consistent with previous findings in the Edinburgh High Risk Study – what is inherited by the high risk individuals is a state of heightened vulnerability manifesting, in the case of functional imaging, as abnormalities in activation and/or connectivity in prefrontal-thalamic-cerebellar and prefrontal-parietal regions. These finding also suggest that there are additional differences seen in those with psychotic symptoms, and to some extent in those who subsequently go on to develop the disorder. These results are not confounded by anti-psychotic medication since all subjects were anti-psychotic naïve at the time of assessment. The lack of findings traditionally associated with the established illness (dorsolateral prefrontal cortex and lateral temporal lobe) indicate these may be specifically associated with the established state, or when performance differences become manifest. Overall therefore these findings reveal information regarding the pathophysiology of the state of vulnerability to the disorder and about the mechanisms involved in the development of schizophrenia or schizophrenic symptomatology.

1 FUNCTIONAL NEUROIMAGING

1.1 INTRODUCTION

The desire to examine the inner workings of the human brain and to understand the relationships between brain and behaviour (in health and disease) have existed for millennia. The advent of revolutionary *in vivo* brain imaging techniques such as functional magnetic resonance imaging (fMRI) means that examination of these complex mental processes has now become a realistic prospect. The use of such techniques to identify areas of the brain associated with specific brain functions has led to the emergence of a new field of research, referred to as 'human brain mapping'. In addition to the exploration of normal brain function, these new techniques have also provided an invaluable means of examining the neural substrates of complex psychiatric disorders such as schizophrenia, which is the main focus of this thesis.

Historically, concepts of brain function were based on two theories, functional localisation (or specialisation), and functional integration (see Friston 2004a). As these are fundamental to the main functional imaging analysis techniques used within this thesis, they are discussed below. The first part of this chapter briefly outlines the historical background to concepts of localisation of function within the brain, followed by a summary of methods currently available for examining brain function, including a description of fMRI and its limitations. The second part of this chapter introduces ideas of functional integration and connectivity analysis techniques.

1.2 FUNCTIONAL LOCALISATION

1.2.1 Historical perspective

It has long been proposed that there was some degree of localisation of function within the human brain (for a comprehensive account see Young 1970). This concept is often identified with Gall's theorising, which suggested that particular psychological functions were associated with specific brain regions (Gall 1835). He

attributed these functions on the basis of external measurements of the skull, mistakenly assuming that the shape of the skull accurately reflected the shape of the brain, a field of study known as phrenology. This concept was of course later abandoned, but laid down important foundations regarding the idea that parts of the brain may have distinct functions. The opposing view at that time, based on cortical ablation studies in animals by Flourens, was that the loss of function depended on the *extent* of the damage to the cortex, rather than the *location* (Flourens 1824). This view was later developed by Lashley who termed it the 'law of mass action' or 'cortical equipotentiality' (Lashley 1929).

1.2.1.1 *Lesion deficit model*

Ideas of localisation, or functional segregation, were appealing since they made sense on practical and evolutionary levels; cells with a common purpose being grouped together. Support for this concept was based on methods of examining brain function available at that time, principally the effects of brain lesions (caused by disease, trauma or neurosurgery), and the resulting associated deficits of brain function. This 'lesion deficit model' was popular with nineteenth century neurologists, such as Broca and Wernicke who ascribed specific language deficits to particular brain regions (Broca 1869; Wernicke 1874). Localised lesions of the posterior region of the left frontal lobe (found later at post-mortem, and now referred to as Broca's area), were shown to result in a specific loss of language function. These subjects were unable to speak, but were still able to understand language (Broca 1869). Broca's area was therefore considered to be involved in speech production and was the first generally accepted evidence for localisation of function within the cerebral cortex. Damage to the posterior temporal cortex, now referred to as Wernicke's area, was shown to produce a different type of language deficit, this time concerning speech comprehension (Wernicke 1874).

By the end of the 19th century, following Broca's clinico-pathological localisation of the speech centre, most of the primary sensory and motor regions of the cortex had been defined (Young 1970). Subsequently detailed labelling systems based on architectonic subdivisions were also described (Brodmann 1925), see Figure 1.1.

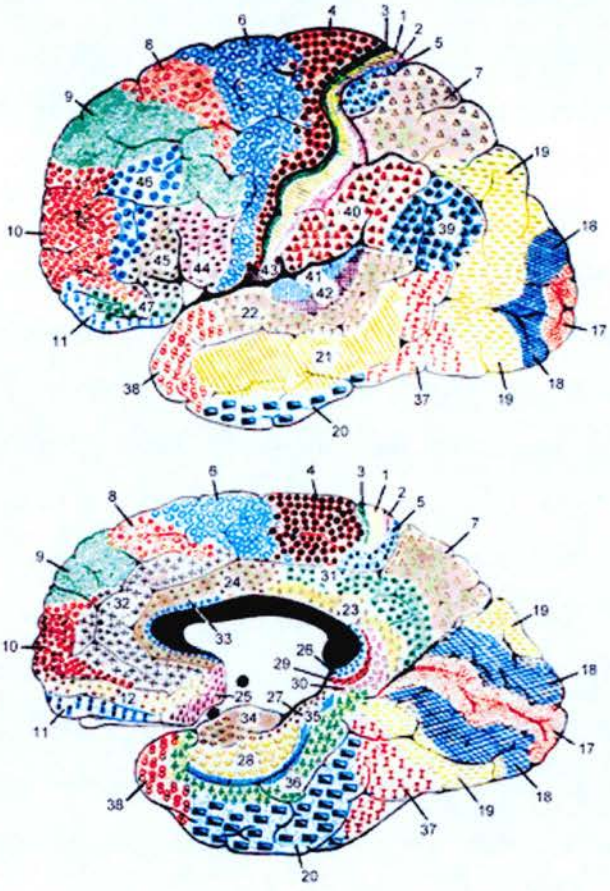

Figure 1.1 Brodmann areas of the brain (Brodmann 1925).

Brodmann segregated areas of the cortex based on detailed cellular structure and laminar arrangement of cells, and produced a map of the human cortex separated into 52 discrete cytoarchitectonic areas (Brodmann 1925). This nomenclature is still used in current brain imaging studies as a common standardised framework with which to locate areas of activation. Brodmann areas (BA) 44/45 correspond to Broca's area, and posterior portion of BA 22 corresponds to Wernicke's area.
From http://spot.colorado.edu/~dubin/talks/brodmann/brodmann.html

Around the mid-1900s associations between specific lesion sites and higher cognitive functions also began to be described. Most notable are descriptions of the subject 'H.M', who underwent surgical removal of bilateral medial temporal lobe structures in 1953 in an attempt to control intractable temporal lobe epilepsy. The resulting deficits, immediate and permanent global amnesia for new material, indicated the importance of the temporal lobe structures, particularly the hippocampus and surrounding cortex, in specific memory functions (Milner 1966). Damage to the frontal lobe has also been shown to result in deficits in higher cognitive functions. The classical example is that of the patient Phineas Gage. In this case a railroad construction accident caused damage to the frontal cortex of the patient resulting in dramatic changes in personality (Harlow 1869). Damage to the prefrontal cortex has also been shown to result in deficits in the planning and sequencing of complex behaviours, or 'executive dysfunction' (Mesulam 1986).

Neuropsychological studies of brain damaged patients have therefore provided important insights in terms of localising brain function, but have critical limitations. Pathological or surgical lesions are rarely confined to functionally discrete regions, and the neuropsychological profile of the subjects is often complex, involving overlapping cognitive domains and compensatory mechanisms. Furthermore, the lesions themselves may cause a degree of functional reorganisation in the brain, making it difficult to generalise findings to other populations.

1.2.1.2 *Direct cortical stimulation*

Another method available before the arrival of new functional imaging techniques was direct cortical stimulation, or electrical simulation mapping (ESM). This technique originated from Fritsch and Hitzig's discovery that electrical stimulation of specific parts of the motor cortex in dogs resulted in particular limb movements, depending on the site of stimulation, on the contralateral side of the body (Fritsch and Hitzig 1870). This finding was later confirmed by Ferrier (1873). These results added support to the notion that there was localisation of function in the brain, and furthermore that there was a degree of topographical organisation of the

cortex. A large body of work on cerebral localisation followed (see Young 1970). Fritsch and Hitzig's findings also established electrophysiological methods as important techniques for examining brain function. Much of our current understanding of the dynamics of brain function have been derived using similar electrophysiological stimulation and recording techniques in animals, such as single unit recording; from Hodgkin and Huxley's early model of action potential dynamics (Hodgkin and Huxley 1952), to more recent 'voltage clamp' or 'patch clamp' techniques examining complex models of learning (see Koester 1991; Kandel 1991).

The cortical stimulation technique was later performed in humans during neurosurgical procedures under local anaesthesia (Penfield 1954) in order to map regions involved in critical functions (such as language and motor control) prior to removal of diseased brain tissue to minimise functional loss. This also suggested that there was topographical organisation in the human sensory and motor cortex, where for example the leg is represented most medially, followed by the trunk, arm, face and the tongue most laterally (Penfield and Rasmussen 1950). These maps of the body surface represented in the brain are now referred to as the sensory (or motor) homunculus. These depict the extent and position of the cortex devoted to each part of the body. However, direct cortical stimulation as a technique for examining brain function is a highly invasive procedure with consequently limited usefulness in normal human subjects.

1.2.2 Current imaging methods

During the latter half of the 20th century methods of examining normal and diseased brain function which do not suffer such limitations have emerged. The advent of these new technologies has lead to a huge growth of research into human brain mapping. There are an abundance of functional neuroimaging approaches currently available, each with their own practical considerations, advantages, and limitations. The most extensively used methods fall into two major categories, those based on measuring the electromagnetic properties of neurones, and those based on measuring metabolic/vascular responses to neural activity.

1.2.2.1 *Electro- and magnetoencephalography*

Methods available for directly examining electrical activity in the human brain include electroencephalography (EEG), which measures electrical signals produced by the summation of neuronal electrical events, and magnetoencepalography (MEG) which records the magnetic fields induced by such activity. For a review of these methods see Gevins (1996) and Cheney (1996). These techniques can be used to measure the brain electrical activity, either spontaneous or synchronous with particular stimuli and events of interest (event related potentials, ERPs, or event related fields ERFs). These methods have excellent temporal resolution (in the order of milliseconds), and require only a modest amount of equipment, at least in the case of EEG. However, since they measure signals on the outer surface of the head they have limited spatial resolution. To overcome these limitations, combination of these imaging techniques with those capable of superior spatial resolution, such as magnetic resonance imaging, is becoming increasingly popular.

1.2.2.2 *Metabolic/vascular imaging techniques*

These methods include single photon emission computerised tomography (SPECT), positron emission tomography (PET), and functional magnetic resonance imaging (fMRI). SPECT and PET measure cerebral blood flow using radioactive tracers, and offer the ability to examine receptor functioning through the use of radioligand binding. fMRI indirectly measures changes in blood oxygenation due to the differing magnetic properties of oxygenated and deoxygenated blood, discussed in detail below.

The fundamental principles of the metabolic/vascular types of imaging technique are based on the empirical finding that changes in localised cerebral activity are associated with localised changes in blood flow and metabolism. The concept of this relationship can be traced back to 'The Principles of Psychology' by William James in 1890, where he put forward the idea that 'all parts of the cortex, when electrically excited, produce alterations both of respiration and circulation' (see Raichle 2000). The actual physiological relationship was further characterised in animals by Roy and

Sherrington (1890). They concluded that chemical products produced by neural metabolic processes caused changes in the diameter of the cerebral vessels, so that the vascular supply varied locally with local variations in functional activity. Numerous other studies of this relationship followed, but it was not until the mid 1960s that work began on humans. Ingvar and Risberg (1965) used injected radiotracers in the first study of functionally induced regional changes in blood flow in four human subjects during the performance of a task and at rest. In all four subjects they recorded an increase in blood flow during task performance, providing strong evidence that changes in regional cerebral blood flow occur in response to cognitive activity.

These metabolic/vascular techniques are a more indirect way of examining brain function than the electrophysiological methods, since they measure the vascular response to neuronal activation, but their main advantage is that they offer relatively good spatial resolution.

1.2.2.3 *Other methods*

Also becoming more widely used in the study of functional brain mapping are the techniques of transcranial magnetic stimulation (TMS), and optical imaging approaches.

1.2.2.3.1 TMS

TMS was first reported in humans by Barker and colleagues (1985); for a more comprehensive overview see Cheney (1996), and Haraldsson *et al* (2004). This method is based on principles of electromagnetic induction and has high temporal specificity. A pair of coils is placed near the subject's head and a strong, brief current is passed through the coil creating a strong transient magnetic field. The magnetic field passes through the skull and induces a weak electrical current in superficial cortex. This induced current can cause cortical neurones to discharge action potentials, and hence results in cortical stimulation (see Pascual-Leone *et al.*, 2000). TMS can be applied in a single pulse, or in rapidly repeated stimulations (rTMS) of

more than one pulse per second (>1Hz). Depending on where and how this technique is applied, it can provide information on various aspects of neurophysiology and cognitive processes (Haraldsson *et al.*, 2004). For example, single pulse TMS to the motor cortex results in muscle twitches in areas of the body dependent on the site of stimulation (according to the motor homunculus), and high frequency TMS applied to the prefrontal cortex can result in temporary deficits in short term memory. This technique has also been used to study patient populations, including those with schizophrenia, and has been suggested to play a role in the treatment of neuropsychiatric disorders, for example in reduction of auditory hallucinations in schizophrenia with slow TMS applied to temporoparietal cortex (Hoffman *et al.*, 2003). However, the use of this method is not an established form of therapy (see Haraldsson *et al.*, 2004), and it should be appreciated that TMS involves safety considerations which increase with higher stimulation frequencies (Wassermann 1998).

1.2.2.3.2 Optical imaging

There are also a number of optical imaging methods which are capable of examining the differences in reflectance and absorption spectra of haemoglobin and deoxyhaemoglobin, which can be used to detect changes in blood flow and oxygenation (Pouratian *et al.*, 2003). These techniques are more accurately referred to as 'optical imaging based on intrinsic signals' (OIS) and were developed from optical imaging methods using voltage sensitive dyes (see Bonhoeffer and Grinvald 1996). The latter was one of the first means to allow direct visualization of neuronal activity, but was prone to problems regarding 'phototoxicity' of the dyes, hence the current use of intrinsic sources of signal. Optical imaging techniques allow investigation of haemodynamic events at greater spatial (20-100 μm) and temporal (ms) resolution than, for example, with fMRI. Due to its exceptional spatial resolution this technique has been successfully used in animals to examine ocular dominance columns of the visual cortex, and to examine somatosensory and auditory cortices (Bonhoeffer and Grinvald 1996). In addition OIS techniques have also been

used to elucidate the coupling between electrophysiology and perfusion-related signals in fMRI (see Pouratian *et al.*, 2003). This technique does however require exposure of the cortex, since visible light would otherwise need to pass through many layers of material before reaching the brain. It is therefore difficult to perform in awake, behaving animals, and in terms of human study is restricted to neurosurgical patients (Haglund *et al.*, 1992).

Optical imaging can also be performed using longer wavelength near infrared light, which passes through skin and skull more easily than with visible light, hence may offer a non-invasive alternative (Strangman *et al.*, 2002). This method is termed near-infrared spectroscopy (NIRS). At present NIRS has more restricted spatial resolution than traditional optical imaging methods, and is confined to the surface of the cortex, although improvements in the technique are in progress (Tamura *et al.*, 2002), and such methods are beginning to be used to investigate psychiatric populations (Shinba *et al.*, 2004).

1.2.3 Summary

As can be seen there are a wide range of tools available for mapping brain function each with their own particular advantages, disadvantages and limitations. Issues such as spatial and temporal resolution, along with accessibility to specific brain sites, invasiveness, and cost, amongst others, define the most appropriate tool in each situation. A figure summarising the properties of the above techniques is presented below (Figure 1.2)

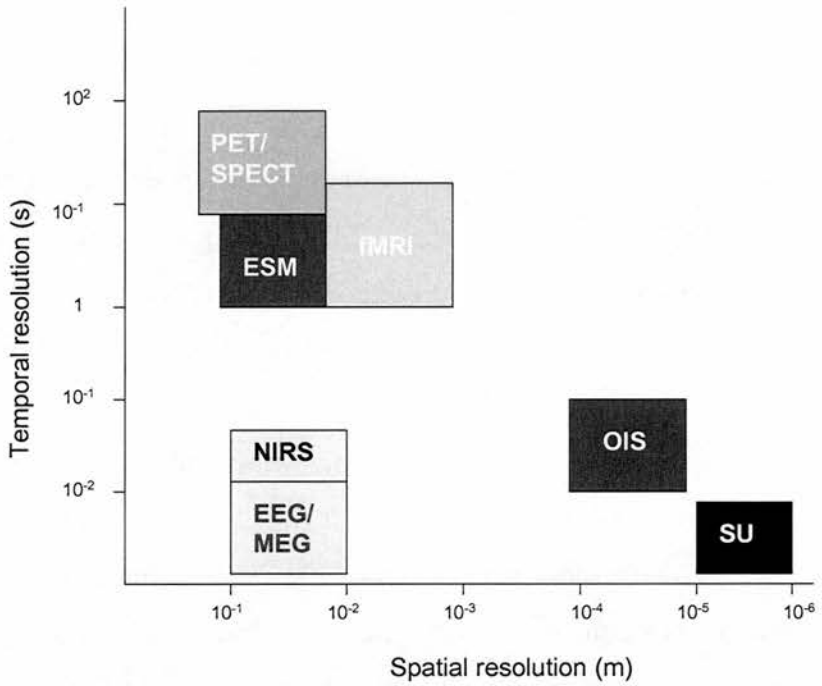

Figure 1.2 Comparison of functional imaging techniques

Figure illustrates spatial and temporal properties of different imaging modalities, along with the degree of invasiveness (represented as grey scale: darker indicated most invasive). Adapted from Pouratian *et al.*, 2003. Abbreviations: PET, positron emission tomogrpahy; SPECT, single photon emission computerised tomography; fMRI, functional magnetic resonance imaging; ESM, electrical-stimulation mapping; NIRS, near infrared spectroscopy; EEG, electroencaphalography; MEG, magnetoencephalography; SSEP, somatosensory evoked potentials; OIS, optical imaging of intrinsic signals; SU, single unit recording.

In the present study the technique of fMRI is used to examine human brain function in subjects at high genetic risk of schizophrenia and healthy controls. As indicated, fMRI provides a non-invasive method of functional imaging with a favourable balance between spatial and temporal resolution. Perhaps the greatest benefit of fMRI over techniques such as PET which require the use of radioactive tracers, are that measurements can be repeated on young healthy subjects for the purposes of research. As with any method, in order to interpret results, it is necessary to have an understanding of the basics of the technique and its limitations.

1.3 FUNCTIONAL MRI

Magnetic resonance imaging has been used for decades to examine brain structure. It is based on observing changes in the behaviour of protons within tissues in the presence of a magnetic field. This method was developed in the 1970s from principles previously described by analytical chemists in the 1940s, called nuclear magnetic resonance (Bloch 1946; Purcell *et al.*, 1946). The discovery that oxygenated and deoxygenated blood possess different magnetic properties (Pauling and Coryell 1936) in combination with the fast MRI technique known as echo-planar imaging (EPI, Mansfield 1977), opened up the possibilities for using this imaging tool to also look at brain function (Ogawa *et al.*, 1990a,b). Previously this had only been possible using techniques involving ionising radiation (PET and SPECT), requiring the injection of contrast agents. The term fMRI can refer to two different methods of studying brain function; techniques which examine blood flow directly, and techniques which examine changes in the oxygen concentration in the blood. In this thesis fMRI will be used as a term to refer to activation studies based on the latter, rather than techniques such as perfusion MR and arterial spin labelling which examine blood flow directly.

1.3.1 Basic principles

The principles behind fMRI are based on findings first demonstrated by Pauling and Coryell in 1936, where it was reported that the magnetic susceptibility of oxygenated arterial blood differed from that of fully deoxygenated venous blood (Pauling and Coryell 1936). The different magnetic properties of oxygenated (diamagnetic) and deoxygenated (paramagnetic) blood were further investigated many years later by Ogawa and colleagues who studied the effects of manipulating the concentration of deoxygenated blood using inhaled gas mixtures which altered metabolic demand or blood flow in anaesthetised rats in a 7 Tesla magnet (Ogawa *et al.*, 1990a,b). The result was the first *in vivo* demonstration that localised changes in blood oxygenation could be detected with fMRI, and this finding was termed 'blood oxygen level dependent' (BOLD) contrast (Ogawa et al., 1990a).

The first paper to demonstrate that MR techniques could be used to examine brain function in humans was published in 1991 by Belliveau and colleagues (Belliveau *et al.*, 1991). This study used an injected contrast agent to examine blood flow changes in response to visual stimulation, rather than the BOLD contrast methods. The potential for using the *non-invasive* BOLD technique for human studies of brain function was subsequently recognised, and the first successful studies were reported simultaneously by two independent groups in 1992 (Ogawa *et al.*, 1992; Kwong *et al.*, 1992).

1.3.2 fMRI and the BOLD signal

fMRI has come to dominate research into human brain function. The basis of the technique is that there are measurable changes in intravascular magnetic susceptibility due to localised activity of neurones.

As neurones become active they undergo membrane depolarisation, membrane repolarisation, and the release of neurotransmitters, which all require energy. It has been proposed that this increase in demand for energy causes vasodilation of local vessels resulting in an increased regional cerebral blood flow (rCBF) and volume (rCBV). Until relatively recently, the mid 1980s, it was assumed that increased blood flow was accompanied by localised increases in oxidative metabolism, and therefore oxygen consumption. However, PET data has demonstrated that functionally induced increases in cerebral blood flow were *not* coupled to similar changes in oxygen consumption (Fox and Raichle 1986a); so called 'uncoupling' of this relationship, discussed in more detail below. In other words, the increase in blood flow exceeds that of the local demand for oxygen, leading to an increased amount of oxyhaemoglobin in the blood nearby, and a relative localised reduction in the amount of deoxygenated blood (Figure 1.3).

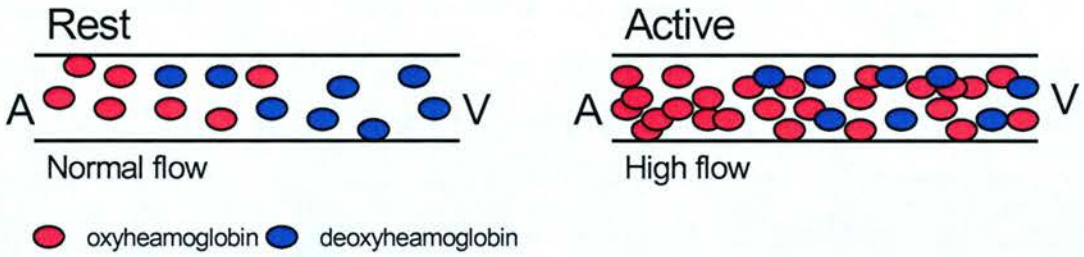

Figure 1.3 Illustrating proportions of oxy- and deoxygenated blood at normal and high flow.

Activated areas of the brain show increases in blood flow. The increased supply of oxygen exceeds the localised increase in oxygen utilisation. The concentration of oxygen in the venous blood increases, and the proportion of deoxyhaemoglobin decreases, resulting in the BOLD signal. A=arterial; V=venous.

It is this imbalance that is the primary physiological basis of the BOLD response (Figure 1.4 and 1.5). The changes in the levels of paramagnetic deoxyhaemoglobin (the presence of which distorts the magnetic field) causes changes in the MR decay parameter, T2*, leading to image intensity changes in T2* weighted images, producing the BOLD signal (Ogawa *et al.*, 1990a,b, 1992; Kwong *et al.*, 1992).

1.3.2.1 *Temporal dynamics of the BOLD response*

The temporal dynamics of the fMRI response have been well characterised and are often shown by averaging the response over several periods of activation, see Figure 1.4. This is also known as the haemodynamic response function (or 'hrf'). Due to the underlying physiological nature of the BOLD signal, it results in a delayed response function which is relatively slow (in seconds) compared to changes in neuronal activity (in milliseconds).

The response is considered to constitute three phases; after the initial onset of the stimulus the time for the BOLD response to increase from baseline is approximately 2 s, the response then plateaus at around 6-9 s (the positive BOLD response), and with the end of the stimulus the time to return to baseline is a further 3-4 s (Bandettini 2000).

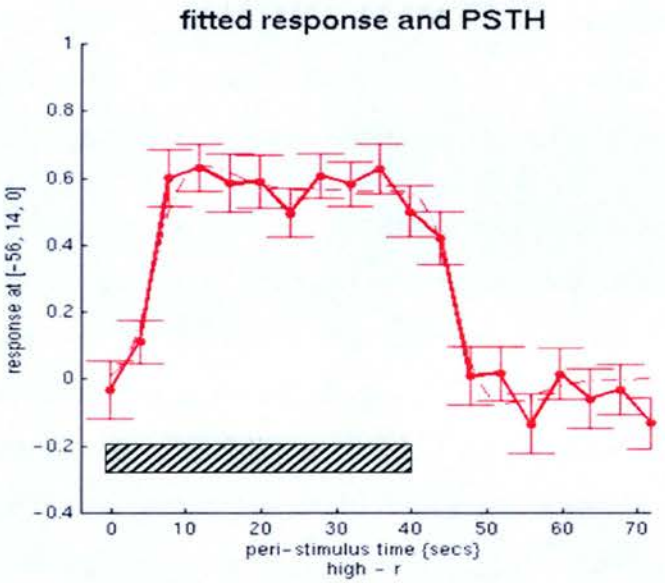

Figure 1.4 Example of an averaged BOLD response.

Fitted response and peri-stimulus time histogram: BOLD response using data from this thesis averaged over 42 subjects for a block of stimuli lasting 40 seconds (represented by the diagonally striped bar). Graph shows relatively slow rise in the BOLD response taking around 6-9s to plateau, followed by a delayed return to baseline after the stimulus has ended.

1.3.2.1.1 Pre- and post-stimulus undershoot

Some studies also report a 'pre-undershoot', or 'initial dip' during the first 500 ms-2 s of the signal, particularly at high field strengths (≥3 Tesla, Yacoub *et al.*, 2001). An illustration is represented below (Figure 1.5). The small amplitude of this initial dip means it is less consistently observed at low field strengths of around 1.5 Tesla (Grinvald *et al.*, 2000; Zarahn 2001). This pre-undershoot was first reported with optical imaging techniques, where differing light absorption spectra between oxy- and deoxyhaemoglobin were measured (Malonek and Grinvald 1996). These methods showed an early rise in the amount of deoxyhaemoglobin concentration in the cat visual cortex after visual stimulation, followed by a later more pronounced decrease in deoxyhaemoglobin (Malonek and Grinvald 1996). A proposed explanation of this phenomenon was that the initial activity of neurones uses the oxygen immediately available before the results of changes in flow occur, resulting in

an initial increase in deoxyhaemoglobin and hence the initial dip of the BOLD response (see Hu *et al.*, 2000). Indeed, a recent study has confirmed that there is a close relationship between neuronal activity and transient localised decreases in tissue oxygenation, shown by simultaneously measuring tissue oxygenation and single cell neuronal activity in the cat visual cortex (Thompson *et al.*, 2003).

In addition, some studies also report a post-stimulus undershoot which can take 20 s to up to a minute to return to baseline (noted in the early paper by Kwong *et al.*, 1992), although, like the initial dip, it is not always evident (Buxton *et al.*, 2000). The dynamics of this post-undershoot suggest that it is due to some physiological factor which is slow to return to baseline at the end of the stimulus. Proposed mechanisms are that cerebral blood flow returns to baseline faster than the cerebral metabolic rate of oxygen, or due to blood volume returning to baseline more slowly than the cerebral blood flow (Buxton *et al.*, 1998, 2000). There are, however, fewer studies examining the post-stimulus undershoot than those examining the initial dip, due to the interest in the pre-stimulus undershoot as a means of potentially improving spatial resolution of the fMRI technique (see later).

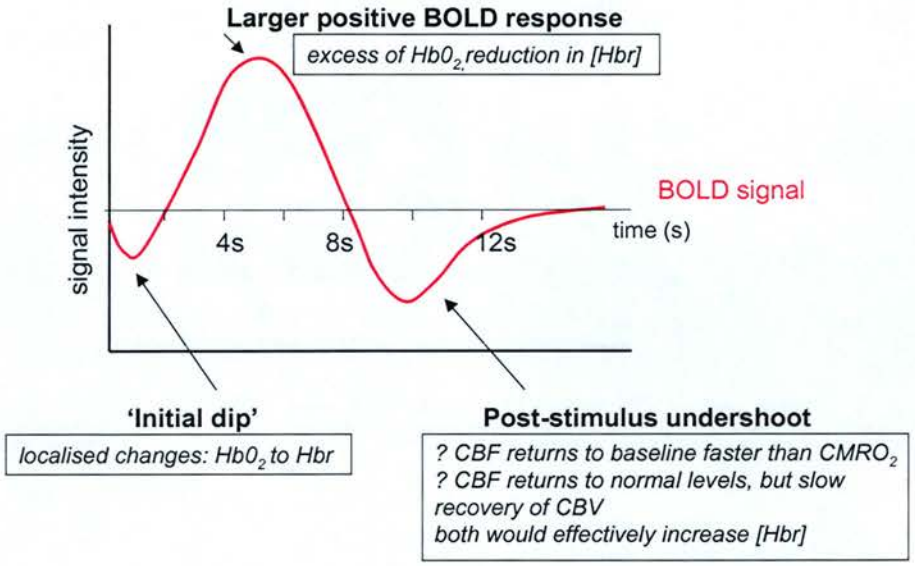

Figure 1.5 Example of BOLD signal to a brief stimulus

To illustrate the initial dip and post-stimulus undershoot of the BOLD signal.
HbO_2 = oxyhaemoglobin; Hbr = deoxyhaemoglobin

1.3.2.2 *Typical experimental design*

Illustrated below (Figure 1.6) is a schematic showing a typical blocked design fMRI experiment (based on the main experiment used in this thesis). The term blocked design refers to the way in which conditions are arranged. Here they are presented in groups or 'blocks' of the same condition, rather than 'event-related' paradigms where individual conditions are presented. In this example, scans are acquired throughout visual presentation of two conditions; the stimulus (a sentence completion task) and a baseline condition. More detail regarding the experimental design is presented in chapter three. The cognitive processes evoked by the conditions result in changes in neuronal activity which alter the proportions of oxy- and deoxygenated blood. As described above, these have different paramagnetic properties (the BOLD response) which result in intensity changes in the EPI images at, or near, the location of the active neurones. The resulting images are pre-processed and analysed using specifically designed software, resulting in maps indicating areas where neural activity is greater during the active conditions than during the baseline condition, and *vice versa*.

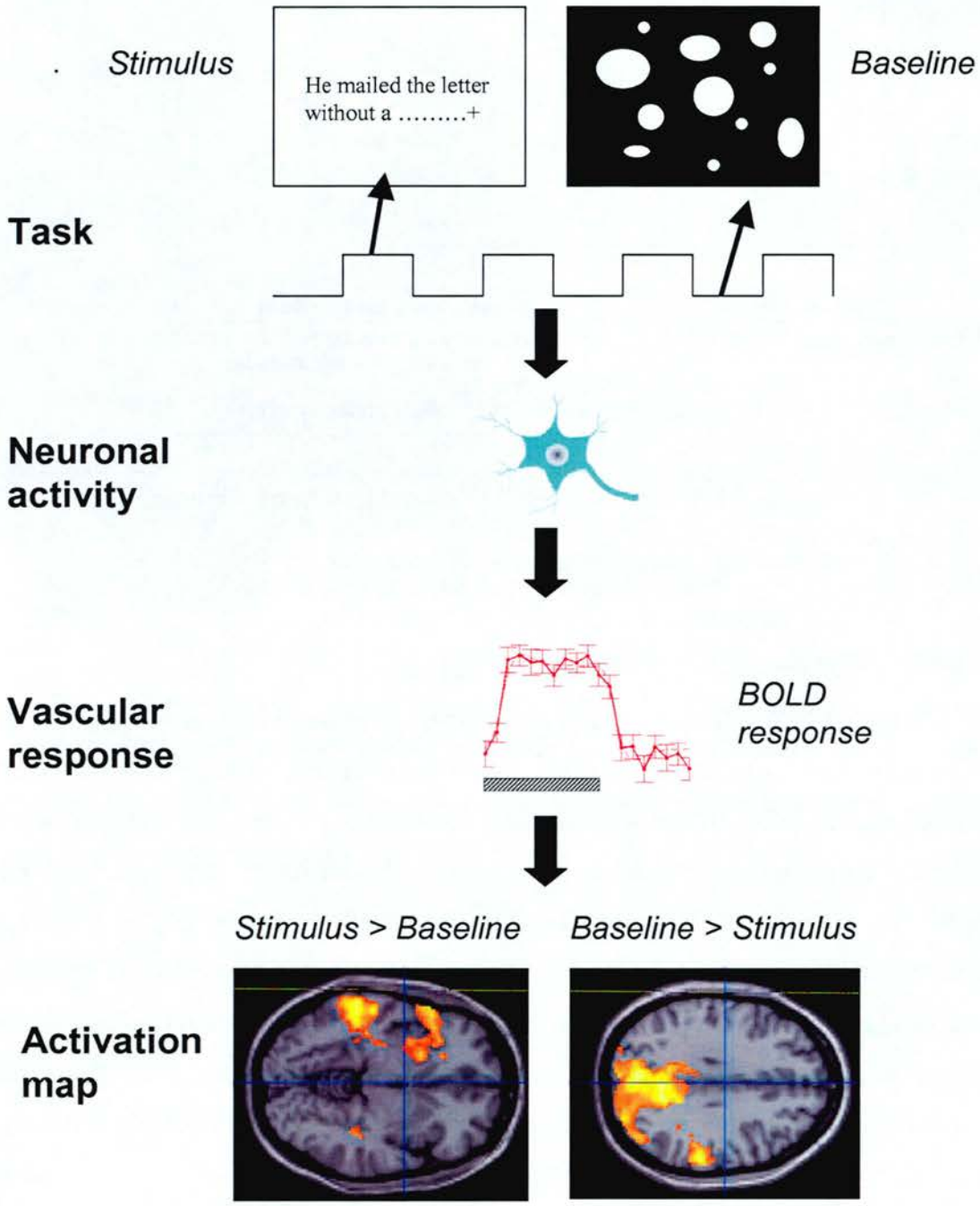

Figure 1.6 Schematic showing a blocked design fMRI experiment

Figure shows steps involved in an fMRI experiment based on the sentence completion paradigm used in this thesis. Activation map overlaid on a structural T1 image.

However, it should be appreciated that at the present time not all the steps in this sequence are completely understood. Although it is clear that the BOLD response is dependent on the imbalance between cerebral blood flow (CBF) and cerebral metabolic rates of oxygen ($CMRO_2$), the exact nature of the relationship between neuronal activity and the vascular response ('neurovascular coupling'), and the complex interplay between the many underlying physiological processes, remains uncertain (Arthurs and Boniface 2002).

1.3.2.3 *Uncoupling or coupling?*

One model proposed to account for the BOLD response includes an 'uncoupling' of the relationship between flow and oxygen metabolism, described earlier. This is based on PET studies, where CBF was found to increase by around 50% during neuronal stimulation, whereas $CMRO_2$ only increased by around 5% (Fox and Raichle 1986). When CBF increase is higher than $CMRO_2$ an excess of oxyhaemoglobin and reduction in deoxyhaemoglobin concentration would result. A localised decreased content of deoxyhaemoglobin produces an increase in the BOLD signal, seen as the main positive BOLD response. As $CMRO_2$ slowly increases to the new level, more deoxyhaemoglobin is created causing the response to plateau. At the end of the stimulus, CBF returns to baseline faster than the $CMRO_2$ producing the post-stimulus undershoot seen in the response.

The uncoupling model is however not a universally held view (Kim *et al.*, 1999). Buxton and Frank, amongst others, have put forward alternative models to describe the nature of the imbalance between CBF and $CMRO_2$. The 'balloon model' (Buxton and Frank 1997) is a biomechanical model based on a complex set of equations relating MR signal intensity changes to changes in blood volume and deoxyhaemoglobin concentration. Parameters involved are blood flow, the oxygen extraction fraction, the metabolic rate of oxygen consumption, and mean transit time. The model assumes that there is no capillary recruitment and treats the capillary bed as a fixed set of 'pipes', so that blood volume changes occur primarily in the venous compartment. The vascular bed is then modelled as an expanding venous

compartment, or balloon, that is fed by the output of the capillary bed. Essentially, the basics of this theory are that the elastic properties of the venous bed produce transient mismatches between cerebral blood volume and cerebral blood flow, which do not necessitate uncoupling between cerebral blood flow and the metabolic rate of oxygen. Buxton and Frank (1997) suggest that flow and oxygen metabolism are in fact 'coupled', but in a non-linear fashion, whereby a large flow increase is required in order to support a small increase in oxygen delivery, essentially due to the reduced time that the oxygenated blood spends in the capillaries ('decreased transit time'). This is also known as the 'oxygen limitation model'. Increases in flow inflate the venous balloon, diluting deoxygenated blood causing an increase in the BOLD signal. But, before the balloon is inflated sufficiently, the content of deoxygenated blood increases causing the initial dip in the response. After the flow has reached its peak, the balloon relaxes meaning there is reduced clearance of deoxygenated haemoglobin producing the post-stimulus undershoot.

1.3.2.4 *How does the BOLD signal relate to activity?*

A complete understanding of how well the fMRI BOLD response directly correlates with neuronal activity is yet to be achieved. In order to examine this relationship it is necessary to overcome difficulties associated with simultaneously measuring both the BOLD signal and the localised activity of neurones.

One breakthrough in the understanding of this relationship was described by Rees *et al* (2000) (and see Heeger *et al.*, 2000). Rees and colleagues measured the fMRI signal in area V5 in four human subjects and related it to the neuronal responses in the homologous area in the monkey visual cortex, which have previously been well characterised. Neurones in this area in the monkey are directionally selective and linearly increase firing rate with increased coherence of motion. The concept of motion coherence in such experimental terms relates to the proportion of dots in a stimulus moving in the same direction. If dots are moving in random directions this corresponds to 0% motion coherence, and if they are all moving in the same direction then this corresponds to 100% motion coherence. Rees

et al proposed that if the fMRI signal was indeed proportional to the localised average firing rate, then the fMRI signal would increase linearly with motion coherence. Their findings were in keeping with this hypothesis, lending important support to the previously assumed proportional relationship between neuronal firing and the BOLD response.

Another major breakthrough was reported by Logothetis and colleagues (2001). This was the first demonstration of simultaneous BOLD and multiunit electrical recording from the same animal. They recorded from the visual cortex in anaesthetised monkeys viewing rotating checkerboard patterns and directly examined correlations between the BOLD signal and neuronal activity (specifically action potentials, and local field potentials). This study reported that a spatially restricted increase in the fMRI signal in the visual cortex of the monkey directly reflected the increase in neuronal firing. The most direct relationship was between the fMRI response and the local field potential which is considered to reflect the synaptic input to a given neuronal population in the form of inhibitory and excitatory postsynaptic potentials. In other words this study suggested not only that the BOLD signal reflected increases in neuronal firing, but in addition suggested that this reflected the input to, and information processing within neurones.

1.3.3 fMRI limitations

fMRI has advantages over PET in terms of the non-invasiveness of the technique and the fact that repeated scans are not limited by maximal doses of radiation exposure. In addition it offers greater spatial and temporal resolution. This technique does however have limitations with respect to distortions due to movement, and inhomogeneity of the magnetic field, which can lead to signal loss in orbitofrontal and inferior temporal regions. In addition sequences that are routinely used can generate a lot of acoustic noise (restricting some auditory paradigms), and due to the strong magnetic fields inherent in the technique subjects with metallic implants can not be scanned with MR techniques.

1.3.3.1 *Movement*

Motion within the scanner during a single scanning session can cause different image artefacts. As a result of movement it is likely that a voxel within an image will not correspond to the same part of the brain throughout the scanning session. To some extent the effects of movement can be minimised using image processing techniques that are standard when preparing the data for statistical analysis. In the statistical parametric mapping software (SPM) (The Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, http://www.fil.ion.ucl.ac.uk/spm/) this stage is termed 'realignment'. Here all images within one session are realigned to a predefined scan, often the first scan in the series, or a mean of all the images. This registration is based on 'rigid body' transformations, where dimensions of size and position can be transformed, but not shape. While rigid body transformations can correct for movement between MRI volumes, subject movement can happen at any time during the acquisition of an MRI volume. Thus, a few brain slices can be acquired with the subject's head in one position, and the remaining brain slices are acquired with the head in a different position, and the image will be slightly distorted (for example see Figure 1.7). This distortion cannot be easily removed from the data and remains as an extra source of variance.

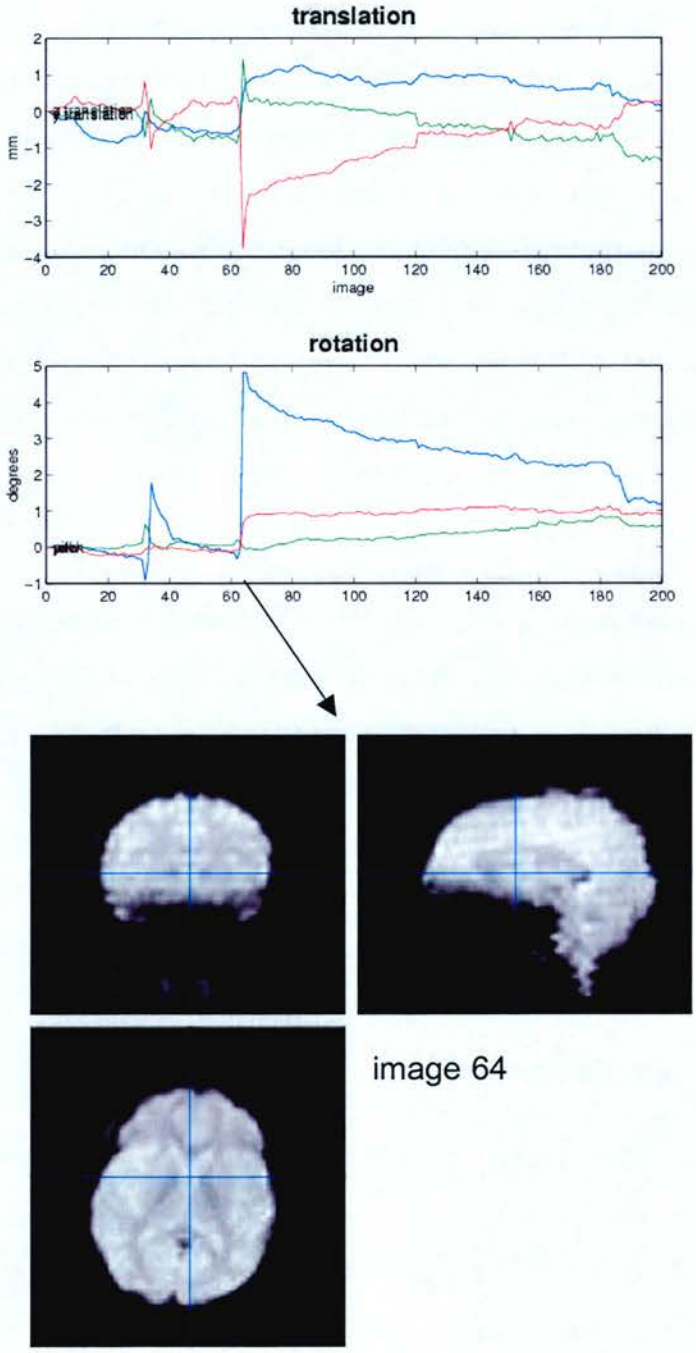

Figure 1.7 Movement effects

This figure demonstrates the effects of excessive movement. This subject was scanned as part of the Edinburgh High Risk data set and was subsequently excluded due to this artefact. The graphs represent the amount of movement (x,y,z) in translation and rotation (red=x, green=y, blue=z; red=pitch, green=roll, blue=yaw). The scan (prior to pre-processing) shows the effect of this movement peak. In this case scan acquisition was interleaved and the effects of movement here are seen as horizontal lines across the image. For example scans 1,3,5,7….are acquired with the subject's head in one position and scans 2,4,6….in another.

To reduce the effect of this noise, it is becoming customary to enter the movement parameters (translations and rotations in x, y, z) as 'nuisance covariates' into the model at the analysis stage. The techniques currently available are however unable to remove all effects of motion, particularly if a significant amount of motion occurs over a short period of time, hence the need to exclude subjects if movement is excessive. Also, the algorithms in general perform best if subject movement is kept to a minimum from the outset. This has lead to the introduction of various means of restricting subject movement, such as bite bars or head moulds, but these are not always well tolerated, and in most cases soft head pads are used.

There are further problems if the timing of the subject movement corresponds to the timing of stimuli of the task (termed 'stimulus correlated movement') (Hajnal *et al.*, 1994). Such correlated movement can result in artefactual signal changes of the order of 1-2%, which can lead to significant false positives (Ashburner and Friston 2000a). In this case it can be extremely difficult to distinguish between areas truly involved in the task, or areas that appear to be active, but whose signal changes over time are actually due to movement which is time-locked to genuine responses. Overall, it is therefore prudent when comparing groups of subjects to determine if there is any task correlated movement, and if there are systematic movement differences between groups (Bullmore *et al.*, 1999).

The distortions caused by movement also impose limitations with respect to the types of paradigms that can be successfully used in the scanner. Paradigms that involve overt speech in response to a stimulus become particularly problematical since movement of the musculature of the face and air in nasal cavities cause large image distortions (see Palmer *et al.*, 2001). New imaging techniques are beginning to be developed which permit verbal responses timed to occur in the gap between image acquisitions (see Henson *et al.*, 2002).

1.3.3.2 *Susceptibility artefacts*

Functional EPI data is also prone to signal reductions in regions with large differences in magnetic susceptibility, for example at air:tissue boundaries. These are termed 'susceptibility artefacts' (Ojemann *et al.*, 1997; Lipschutz *et al.*, 2001). It has been reported that the inferior part of the frontal lobes and the inferior temporal lobes bilaterally are particularly affected (Ojemann *et al.*, 1997; Lipschutz *et al.*, 2001). The reduced signal is thought to be due to the irregular shape of the head affecting the uniformity of the magnetic field which can create distortion. The frontal, sphenoidal, and ethmoidal sinuses affect the signal in the frontal lobes; and the ear canal, thick petrous bone, and mastoid air cells affect the signal in the inferior temporal lobes.

1.3.3.3 *Spatial and temporal resolution*

Other issues relating to fMRI involve spatial and temporal resolution. Spatial and temporal resolution are essentially dependent on scanner hardware and physiological limits. Since fMRI is based on examining secondary metabolic and haemodynamic events which follow neuronal activity, and not the electrical activity itself, the spatial resolution is ultimately dependent on the spatial extent of the vascular changes, in other words the smallest vascular unit involved in the response. It has been argued that theoretically this could involve regulation at the capillary level. The most commonly held view however is that the feeding arteriole is the smallest main functional unit involved in the main positive BOLD response, which has a vascular territory in the order of 1 mm^3 (Villringer 2000). In most human fMRI studies, at field strengths of 1.5 T, the spatial resolution is reported to be in the order of 3-5 mm^3 (Kim SG, *et al.*, 2000). In other words, the limits of spatial resolution are relatively large in relation to neuronal size, meaning populations of neurones are grouped together even at the level of a single voxel.

1.3.3.3.1 Spatial specificity

Furthermore, there are concerns regarding the spatial specificity of the BOLD response - in other words the co-localisation of the signal change and the actual site of neuronal activity. With the typically used fMRI acquisition parameters it has been suggested that activation signals may come from larger vessels downstream from the actual site of neuronal activation (Disbrow *et al.*, 2000; Ugurbil *et al.*, 2003). One study measured the BOLD signal and electrophysiological activity in anaesthetised monkeys at 1.5 T and indeed reported that the largest discrepancies between the two measurements were located close to large vessels (Disbrow *et al.*, 2000).

1.3.3.3.2 The 'initial dip' and spatial resolution

Most current fMRI studies use the larger positive BOLD response to map neuronal activity. However, it has been suggested that since the initial dip in the BOLD response, described earlier, represents localised increased oxygen consumption by active cells (Figure 1.5), this feature of the response may be a better indicator of the location of neuronal activity rather than the slower vascular features. Mapping this initial deoxyhaemoglobin increase may have implications for determining the ultimate spatial resolution of fMRI by minimising the contributions from large vessels (Malonek and Grinvald 1996). Indeed studies are beginning to be reported which support this suggestion. For example studies of the visual cortex which map the initial dip have been shown to produce sharper patterns of patches and stripes (representing the characteristic features of orientation and ocular dominance columns) than do studies using the traditional positive BOLD signals (Menon *et al.*, 1997, Grinvald *et al.*, 2000; Kim DS, *et al.*, 2000, Kim and Ogawa 2002, and see Ugurbil *et al.*, 2003), and have reported to be able to localise activation in the order of 0.5 mm (Duong *et al.*, 2000). However, it should be noted that the presence of the initial dip is still controversial (see Zarahn 2001). This inconsistency, together with its small amplitude, makes it unlikely at the present time to become the standard way of localising brain function in humans at conventional field strengths (Buxton 2001; Kim and Ogawa 2002).

1.3.3.3.3 Temporal resolution

As previously mentioned the time scale of vascular events is in the order of seconds, whereas the time scale of neuronal activation is in the order of milliseconds, resulting in a delay in the measured response. This therefore sets limits for the temporal resolution capabilities of current imaging techniques. There exist many different definitions of 'temporal resolution', as discussed in Bandettini (2000), including, amongst others, imaging acquisition rate, time for the response to rise and fall, and the smallest detectable activation duration.

The time of the response takes to rise and fall, or the temporal dynamics of the BOLD response, have been described above. It should be noted that the specific timings of the response, and to some extent the shape of the function, may vary depending on the region studied (see Bellgowan *et al.*, 2003), and across subjects (Aguirre *et al.*, 1998). Indeed newer versions of analysis software are beginning to permit more flexible models of response functions than those traditionally used.

With respect to the smallest detectable activation duration, early fMRI study paradigms were based on the standard minute-long epochs of averaged brain activity, or 'blocked' designs, as used in this current study. However, there have been a number of studies examining the shortest duration required to produce a measurable BOLD response. Signals have been measured in response to 2 s of visual stimulation (Blamire *et al.*, 1992), within 1.5 s of stimulus onset in a language task (Carpenter *et al.*, 1999), and even in response to 500 ms of finger tapping (see Bandettini 2000).

These studies have lead to one of the major advances in the development of fMRI paradigms: 'event-related' designs (see Rosen *et al.*, 1998). These allow measurement of the MR signal in response to single events (where typically responses to single events are averaged in order to generate time courses and activation maps). The discovery that the summation of individual responses was approximately linear (Boynton *et al.*, 1996), made the modelling of sequential and overlapping trials possible. Different types of events could now be mixed together, or

could be sorted with respect to the behavioural response. Initially event-related studies concentrated on primary sensory systems, but were later also shown to be possible in higher cognitive tasks (Buckner *et al.*, 1996). Although event related designs have advantages over blocked designs in terms of flexibility, this does not necessarily imply blocked designs are obsolete. Event-related studies can suffer from reduced detection power due to the decreased amplitude of the response as a result of shorter stimulus durations (see Aguirre and D'Esposio 2000, and Liu *et al.*, 2001).

1.3.3.4 *fMRI and repeatability*

fMRI is a relatively new imaging technique and it is becoming increasingly important to determine the reliability of the method in order to be able to draw firm conclusions about results, particularly with respect to longitudinal studies, and with regard to potential clinical applications (Maldjian *et al.*, 2002).

Test-retest reliability in terms of fMRI is generally measured as reproducibility of locations of activation ('centre of mass' differences), and/or some measure of the consistency of size, or extent, of activation. These are often reported as the reproducibility of the number of activated voxels, R_{size}, and the ratio of the common areas to the average, $R_{overlap}$; where values can range from 0 – 1 for both parameters (Rombouts *et al.*, 1998)

There have been a number of reports in the literature examining test-retest reliability for a variety of tasks including; visual stimulation (Moser *et al.*, 1996; Le and Hu 1997; Rombouts *et al.*, 1998; Miki et al., 2000), and simple motor tasks (Mattay *et al.*, 1996; Cohen and DuBois 1999). However it might be expected that such simple tasks may be more reproducible than higher cognitive tasks, due to the relatively higher signal change, the limited variety of strategies, and the decreased demands on attentional resources for the more basic tasks. Therefore tests of higher cognitive function such as spatial working memory (Casey *et al.*, 1998), visual encoding (Machielsen *et al.*, 2000), and language tasks (Veltman *et al.*, 2000; Chee *et al.*, 2003), or combinations of paradigms have been performed (McGonigle *et al.*,

1999; Waldvogel *et al.*, 2000; Marshall *et al.*, 2004). The study by Chee and colleagues also addressed the issue of reproducibility of relative differences in activation associated with differential levels of difficulty in a language task (Chee *et al.*, 2003).

1.3.3.4.1 Normal subjects

At the group level the overall conclusion from these studies is that the location or patterns of activation are generally reasonably reproducible in normal subjects (Mattay *et al.*, 1996; Casey *et al.*, 1998; Rombouts *et al.*, 1998; Machielsen *et al.*, 2000; Veltman *et al.*, 2000; Waldvogel *et al.*, 2000; Chee *et al.*, 2003); for example differences in the centres of mass were reported to be of the order of 2-4 mm for visual stimulation (Rombouts *et al.*, 1998). Results have also been shown to be consistent in a multi-centre study of spatial working memory at four different laboratories (Casey *et al.*, 1998). However, there does appear to be large variability between subjects; with some subjects showing good agreement between sessions, and others poor inter-session agreement (Miki *et al.*, 2000). Also, there appears to be considerably more variability in the size of the measured response, in terms of the amplitude and extent of activation, in comparison with the spatial variability (Machielsen *et al.*, 2000; Miki *et al.*, 2000; Waldvogel *et al.*, 2000; Marshall *et al.*, 2004). For example, in a visual stimulation paradigm Miki *et al* (2000) reported activated volumes ranging from 10-1220 voxels for thresholds of $Z > 4.5$. Furthermore, both Miki *et al.*, (2000) and Chee *et al.*, (2003) report at least one subject (out of a total 7 and 16 healthy volunteers respectively) with R_{size}, or $R_{overlap}$ of less than 0.1. These figures have negative implications regarding the possibility of achieving reproducible results particularly at the level of an individual subject (McGonigle *et al.*, 1999; Chee *et al.*, 2003).

1.3.3.4.2 Patient populations

There are also concerns regarding reproducibility in patient populations. The above studies were all conducted on normal subjects. However Manoach *et al.*,

(2001) examined reliability in normal controls and in a small group of schizophrenic subjects (n=7) performing a working memory task. In both groups the task performance was reliable across sessions, and the patients had a stable clinical status. In the group averaged data both groups activated all regions suggested *a priori* at both sessions. However, in individuals with schizophrenia the indicators of cognitive activation were not as reliable across sessions as they were for normal controls. Reliability was measured as the intra-class correlation coefficient, and these values were consistently lower for the patient group as compared to controls in all but one region. However, it should be noted that groups were not well matched for age or verbal IQ, and the normal subjects performed near ceiling levels during both sessions. Regardless, this highlights the need for further reproducibility studies in patient populations.

1.3.4 fMRI: Future perspectives

1.3.4.1 *Multi-modal approaches.*

fMRI is increasingly becoming the tool of choice for human functional brain mapping since it provides a non-invasive method of scanning which has excellent spatial and temporal resolution which does not require the use of radioactive tracers. That said, fMRI is still a relatively new imaging tool and there are still a number of outstanding issues yet to be resolved before we have a good understanding of the underlying mechanisms of the technique. There are an increasing number of reports in the literature regarding the integration of fMRI with other functional imaging methods (see Dale and Halgren 2001), both in humans and in animals, to elucidate some of these unresolved issues, and to offer methodological improvements. This multimodal approach to brain imaging exploits the individual advantages of different imaging tools and has the potential to lead to greater understanding of brain functioning and the underlying principles of fMRI.

1.3.4.2 *High-field fMRI*

As described above, the conventional BOLD approach has been used to investigate a variety of brain functions, including vision, motor, language and cognition, but there are an increasing number of reports regarding the improvements in spatial resolution and specificity offered by minimising large vessel contributions, i.e. by measuring the initial dip in the BOLD response. The magnitude of this portion of the response, and indeed the main positive BOLD response, is increased at higher field strengths (see Kim and Ogawa 2002), hence the popularity of imaging with machines at $\geq$3 T. The use of higher field strengths also offers the possibility of shorter scan sessions which may be of particular benefit when scanning patient populations who do not tolerate long scan times. Imaging at high field strengths is currently an active area of research. At the time of writing the highest field strength to be used in a published study examining human subjects is around 7T (Formisano *et al.*, 2003), and in animals is 9.4T (Duong *et al.*, 2000; Kim DS, *et al.*, 2000; Olman *et al.*, 2003). However there are associated difficulties with the use of higher field strengths (often the limitations described at conventional field strengths are magnified, such as acoustic noise, movement and most importantly susceptibility artefacts). Furthermore, the majority of studies using high field fMRI examine primary visual (e.g. Cheng *et al.*, 2001), or primary auditory cortices (e.g. Formisano *et al.*, 2003), areas which are not commonly associated with susceptibility artefacts. The main aim of such studies is to determine the practicalities and technicalities of imaging at such field strengths, and the ultimate resolution and functional specificity that can be obtained with fMRI (Kim DS *et al.*, 2000).

1.4 FUNCTIONAL INTEGRATION

1.4.1 Historical perspective

Historically, the prevailing view of brain organisation was based on concepts of functional specialisation, or localisation. However, at an anatomical level the brain is a highly interconnected structure; few regions are separated by more than three synaptic junctions, and connections between regions are often reciprocal (Dolan and Friston 1997). It should be recognised that, although functional specialisation and functional interaction have often been considered opposing models of brain function, they are complimentary rather than mutually exclusive concepts (Friston 2004a). In functional terms the brain relies on efferent and afferent connections between remote, locally specialised regions, particularly when performing complex cognitive tasks. The approach to examining these interactions between regions is termed functional integration.

This type of integrative approach in brain imaging is a re-emergence of the connectionist views of brain function originally described by Wernicke (1874, 1906) and Goltz (1881). Wernicke proposed that only the most simple, and basic mental functions of the brain were localised, and that interconnections between these sites made the more complex functions possible. Wernicke formed a model of language perception based on the connections between Broca's and Wernicke's areas. He proposed that initial sensory perceptions of language occurred in their respective primary and secondary cortical areas, this information was then passed via areas of association cortex to Wernicke's area where it was associated with meaning. He then used this model to predict a new type of aphasia which was later described clinically. This involved damage to the tract linking these two language areas; the arcuate fasciculus. The resulting 'disconnection syndrome', a term coined later by neurologist Geschwind (1965a,b), was characterised by the inability to repeat sentences presented to the patient, yet there is relative preservation of speech production and comprehension (which are lost with damage to Broca's and Wernicke's areas respectively). Other evidence followed, in 1892 Dejerine examined

two patients with reading disorders, alexia (an inability to read or recognise written words of letters) without agraphia (an inability to write), and postulated that a 'disconnection' prevented information passing from the intact right visual cortex to the language centres in the left hemisphere (Dejerine 1892, and see Absher and Benson 1993). Despite these early examples however, connectionism had less of an impact on scientific research in the late 19th and early 20th century than concepts of functional localisation described above.

By the mid-20th century however significant observations regarding the functional consequence of disconnection began to be described: the split-brain patients described by Gazzaniga (see Gazzaniga 2000), and a group of clinical syndromes described by Geschwind (see Absher and Benson 1993).

The split-brain patients described by Gazzaniga had undergone surgical section of the corpus callosum (the tract linking the hemispheres) as a treatment for intractable epilepsy in the 1950's. Symptoms of this disconnection often only appeared when specific tests were administered to these individuals. Stimuli presented to the left visual field or to the left hand (therefore perceived by the right hemisphere) could not be named by the patient since there was no cross hemisphere communication with the left sided language centres of the brain.

Geschwind also studied disconnection disorders and published a comprehensive and influential article entitled 'Disconnection disorders in animal and man' (Geschwind 1965a,b) in which he described callosal disconnection, conduction aphasia, alexia without agraphia, and agnosia (inability to verbalise description or knowledge of an object) (see Absher and Benson 1993). Although disconnection disorders had been described previously by Wernicke and Dejerine, this article did a great deal to promote connectionist principles as a useful model with which to examine brain function in both humans (for example see Mesulam 1990) and in animals (for examples see Gaffan and Harrison 1988; Gaffan and Eacott 1997).

1.4.2 Connectivity in functional brain imaging

In line with connectionist theories, functional imaging analysis techniques have developed methods to examine networks of connecting brain regions. These originated from the analysis of spike trains in electrophysiological data acquired from multiple recording sites (Aertsen *et al.*, 1989; Aertsen and Preissl 1991; Gerstien and Perkel 1969), and are based on the principles of collecting data from multiple sites simultaneously, and then performing correlation or covariance analysis. Such studies are the origin of some of the current definitions of connectivity in functional imaging (Aertsen and Preissl 1991). This functionally integrative approach to brain mapping is termed 'connectivity analysis', and has opened up the possibility of using functional imaging data to examine interactions in the normal human brain, and how these interactions may be altered in disease states. Other techniques have also been used to examine inter-regional brain connectivity, for example by assessing the anatomical substrates of these interactions (see Catani *et al.*, 2000).

1.4.2.1 *Definitions and terminology*

The concept of connectivity in functional imaging terms was devised using PET data where it was suggested that when activity of two regions displays a high degree of covariance, or correlation, these regions may be considered operationally as functionally connected (or functionally coupled) (Friston *et al.*, 1993a,b). The generally accepted terminology makes a distinction between functional connectivity and effective connectivity, as described by Friston *et al.*, 1993a,b (and Aertsen and Preissl 1991). Functional connectivity refers to the observed correlations over time between different brain areas, or 'correlations between remote neurophysiological events'. So for example, brain regions A and B may be considered to be functionally connected if an increase (or decrease) of activity in brain region A is associated with an increase (or decrease) of activity in brain region B. However, this association does not imply any causal relationship between these two areas, since there is no indication of the direction of the relationship. Areas A and B may be considered as being effectively connected if it can be shown that there is a *causal* relationship

between these regions. The term 'effective connectivity' refers explicitly to the influence that one brain region has over another (see Friston and Price 2001).

There are a variety of mathematical approaches to examining functional interactions in the brain, ranging from data-led functional connectivity techniques to complex model-based effective connectivity methods, discussed below.

1.4.2.2 *Functional connectivity studies*

The main approach to examining functional connectivity is based on examining the correlation of activity between regions. At its simplest, the analysis of functional connections in fMRI data is based on detecting inter-regional temporal correlations of the BOLD signal. This may be performed at rest, or during task-activation studies.

1.4.2.2.1 'Resting' state studies

During the resting state, spontaneous firing of neurones occurs. This can affect firing in remotely located brain regions through efferent connections, hence it is possible to examine areas which are functionally connected at rest. Early functional connectivity studies examined correlations in brain activity in the resting state, first with PET data (e.g. Horwitz *et al.*, 1984; Metter *et al.*, 1984; and see Horwitz 2003) and then with fMRI (e.g Biswal *et al.*, 1995; Lowe *et al.*, 1998). Here it was demonstrated in single slice fMRI time series data that regions which were simultaneously activated in a standard fMRI motor paradigm, also showed a high correlation in spontaneous low frequency fluctuations at rest (Biswal *et al.*, 1995). Lowe and colleagues (1998) also used a similar paradigm to detect correlations between motor cortex, visual cortex and amydalae at rest, this time using both single- and multi-slice data.

1.4.2.2.2 Task-activation studies

Later studies began to examine functional connectivity in subjects with the conventional task-activation methods; beginning with PET data (e.g. Friston *et al.*,

1993a; Horwitz *et al.*, 1995), followed by fMRI (e.g Bokde *et al.*, 2001; Lawrie *et al.*, 2002a). One approach is based on selecting a reference voxel, or reference region, and then studying the correlations between the time course of this voxel, or region, with all the other voxels in the brain: the 'seed voxel' approach (Horwitz *et al.*, 1995).

In an alternative approach, two or more regions of interest are selected *a priori*, and summary measures of the voxel time courses within each of the regions are then extracted. The correlation coefficients between these values are determined, and if activity in each region shows a high degree of correlation, then these regions are considered to be functionally connected. An example of this method is described in Lawrie *et al.*, (2002a) where regions of interest in the left temporal cortex and left dorsolateral prefrontal cortex were selected *a priori,* and the correlations between these regions compared between schizophrenic subjects and normal controls.

1.4.2.2.3 Confounds of task performance

To minimise the possibility of falsely assuming functional connectivity between regions, the effect of the task activation should be considered. At first this seems counter-intuitive and is best explained using an example (taken from Xiong *et al.*, 1999). Consider a particular psychological task in which both visual and auditory components are involved. Since both components are involved in the task, visual and auditory areas will be seen to be co-active. Task-related co-activation could therefore be considered a rather unsatisfactory index of inter-regional connectivity.

Resting state covariance analyses obviously do not suffer from confounds related to task activation, or indeed subject performance. It has therefore been suggested that resting state correlations represent genuine task-independent connections between brain regions. However, unconstrained mental processing during resting conditions, particularly at a single subject level, can be highly unpredictable. An alternative is to attempt to remove task effects from the analysis. An example of such an approach is detailed in Arfakanis *et al.*, (2000), where independent component analysis (ICA)

was used to remove effects of task activation for three separate tasks (bilateral finger tapping, passive listening, and viewing alternating checkerboard). This approach reduces confounds related to task activation, but also has the advantage over resting state studies in that there is more control over the subject's cognitive activity.

1.4.2.3 *Psycho-physiological interactions*

Another approach is to look at differences in connectivity under different psychological contexts (the psycho-physiological interaction or 'PPI' approach). PPI analysis refers to the observation of task or context dependent inter-regional covariance, or 'explaining the regionally specific neuronal responses in terms of the interaction between influences from a remote brain area, and from sensory or task related parameters' (see Friston *et al.*, 1997). The method is essentially based on simple regression analysis, where activity in one region is regressed against activity in a second brain region. The slope of the regression is considered to reflect the influence that the second brain region has on the first. If this regression between regions is then performed under different contexts, for example different attentional conditions, then the change in the regression slope in response to the change in context of the task, reflects the PPI. An example of this approach is described in Friston *et al.*, (1997), where information about the modulatory activity of the posterior parietal cortex (mediating attention to a particular stimulus), and information about the stimulus itself, was used to identify regions that respond to the stimulus when activity in the posterior parietal cortex is high. This approach has also been used to examine the influence of the amygdala on other brain regions during the processing of emotional facial expressions (Morris *et al.*, 1998), to examine cerebellar involvement in altering responses to self generated tactile stimulation (Blakemore *et al.*, 1999), and to examine anterior cingulate modulation of prefronto-temporal connectivity in schizophrenia (Fletcher *et al.*, 1999).

1.4.2.4 *Effective connectivity methods*

Since effective connectivity can be used to infer a causal relationship between regions, it may be considered to be a more meaningful approach to examining connectivity. However, methods for examining effective connectivity tend to be more complex than those used for the analysis of functional connectivity. Development of such analysis methods is a rapidly expanding field of research, and there are an increasing variety of mathematical techniques available allowing a flexible range of models; including linear, non-linear, static and dynamic approaches. For a comprehensive account see Harrison and Friston (2004).

The most commonly used approach to examine effective connectivity is structural equation modelling (SEM, see McIntosh and Gonzalez-Lima 1994). Here optimisation methods are used to determine connection strengths between a hypothesised set of regions (based on an anatomical model) in order to account for the observed pattern of covariances in the neuroimaging data. As with all methods of examining effective connectivity, a network of connected regions, or model, is proposed based on knowledge about anatomical connections and the regions involved in the task. The imaging data is then fitted to this model based on determining the covariance structure between the regions. In this method the principles differ from the usual statistical approach where parameters of the model are based on minimising the sum of the squared differences between predicted and observed variables. SEM does not consider variables individually; the emphasis is on the covariance structure. Parameters are estimated by minimising the differences between the observed covariances and those implied by the model, and a statistical estimate of goodness of fit can be determined. The parameters of the model are the connection strengths, which give an estimate of effective connectivity. The goal of this approach is not to account for all the variance in the imaging data, but to determine which regional interactions are central to the model. Examples of this method of connectivity analysis are described in McIntosh *et al.*, (1994), where PET data was used to determine pathways involved in object and spatial visual processing. Another example, this time using fMRI data, involved examining modulation due to

attention on the effective connectivity between visual motion area V5 and the posterior parietal cortex (Friston and Büchel 2000).

More recently, methods have been devised which allow non-linear components to be modelled. Inherent to this approach is the factorial experimental design, where inputs are organised as to whether they modulate the system, or whether they have a direct driving effect on the system. Examples of such methods include Volterra kernels, and dynamical causal models (see Friston 2004b,c).

1.4.2.5 *Validity*

In general functional connectivity is an exploratory tool that provides a way of examining coherent brain activity. Effective connectivity is a more complex approach which allows for additional inference. It should be appreciated that these are relatively new analysis methods, and the validity of these techniques is often difficult to establish. The statistical estimate of goodness of fit in effective connectivity can provide a measure of the model's validity; however true construct validity relies more heavily on the accuracy of the underlying neuroanatomical model. It is therefore important that the model tested is based on prior anatomical knowledge of connections within the brain. These are commonly derived from neuroanatomical tracer studies in non-human primates. It should however be appreciated that models are always an over-simplification of reality. Truly correct models would be too difficult to understand, so there is a need to balance the complexity of the model and interpretability.

In relation to validity of the technique, one interesting study examined low frequency fluctuations between the two cerebral hemispheres in fMRI data from three normal subjects and in one subject with almost complete callosal agenesis (Lowe *et al.*, 1997). The study focused on functional connectivity between the left and right auditory cortex, with the region of interest placed on the left side. The normal subjects showed many supra-threshold voxels in the contralateral right auditory cortex. The subject with callosal agenesis however had strikingly fewer significant

voxels in the homologous right sided region. It is reasonable to suppose that the subject with callosal agenesis would have *normal* bilateral blood flow, but *abnormal* connectivity between the brain hemispheres. This study therefore supported the hypothesis that the observed correlations in fMRI time series data are indeed suggestive of neuronal connectivity, rather than artefacts associated with blood flow. Although this is only a case study, it is in agreement with EEG studies in similar clinical groups where interhemispheric EEG coherence is reduced in subjects with callosal agenesis (see Lowe *et al.*, 1998).

1.5 CONCLUSION

In summary, fMRI is an exciting new functional imaging technique that provides good spatial and temporal resolution along with the opportunity to study functional localisation and functional integration. This, together with the low invasiveness of the technique, and the widespread availability of MRI scanners capable of performing functional imaging, offers the potential to address many unresolved issues in numerous areas of research, including the study of neuropsychiatric disorders such as schizophrenia.

2 NEUROIMAGING IN SCHIZOPHRENIA

2.1 INTRODUCTION

Schizophrenia is a serious psychiatric disorder of largely unknown aetiology with a lifetime risk of 1% worldwide (Torrey 1987; Jablensky *et al.*, 1992). It is a highly heritable disorder, where typically sufferers are well as children but become psychotic in early adulthood. The most characteristic symptoms of early psychosis are delusions (abnormal beliefs) and hallucinations (abnormal perceptions). The associated disabilities are considerable and are frequently long lasting. In this chapter the disorder of schizophrenia will be briefly outlined. Since this thesis is primarily focused on brain imaging in schizophrenia, the chapter will include a review of localised regions previously implicated in schizophrenia by both structural and functional imaging techniques, followed by an overview of the evidence for disordered functional integration in the disorder. The potential contribution of medication effects will not be discussed since the cohort studied in the experimental section of this thesis were all anti-psychotic naïve at the time of assessment. Studies concerning high risk individuals will be discussed in following chapters.

2.2 SCHIZOPHRENIA: GENERAL OVERVIEW

2.2.1 Historical perspective

Schizophrenia was first described as a clinical syndrome over a hundred years ago by Kraepelin and given the name 'Dementia Praecox' (Kraepelin 1896). This term was coined to describe a perceived deterioration in function over time, and the relatively young age of onset of the disorder. Bleuler further developed the concept, and used the term 'schizophrenia' in his book; 'Dementia Praecox, or the group of Schizophrenias' (Bleuler 1911). Bleuler used this term to describe a split or disintegration of associations between different cognitive processes, which he considered to be a defining feature of schizophrenia; he did not consider hallucinations and delusions as primary in the disorder. In the mid-1950s, Schneider

shifted the focus of schizophrenic symptomatology to such symptoms, referred to as 'first-rank' symptoms (Schneider 1959).

2.2.2 Current terminology and diagnosis

Modern descriptions of the disorder refer to positive and negative symptoms, from notation originally described by Reynolds (1861) (see Berrios 1985), and later by Hughlings Jackson (1869). The term positive symptoms are used to symbolise behavioural excesses, while negative symptoms represent loss of normal function (Strauss *et al.*, 1974). The four classic positive symptom types are described as hallucinations, delusions, disorganised speech, and abnormalities in behavioural monitoring and control. The negative symptoms consist of abnormalities of the flow of ideas and language (alogia), in expressing emotion (affective blunting), in the ability to initiate goal directed activity (avolition), and in the ability to initiate, seek out and experience pleasurable experiences (anhedonia) (Fuller *et al.*, 2003). Studies have generally shown that negative symptoms are more stable than positive symptoms and are less likely to improve over the course of the illness (Fuller *et al.*, 2003).

At present there is no objective diagnostic test for schizophrenia. The diagnosis of the disorder is made depending upon the description and observation of the patient's mental state using standardised criteria outlined in the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM IV) (American Psychiatric Association 1993), or the International Classification of Diseases (ICD – 10, World Health Organisation 1993) summarised in Figure 2.1. It should be appreciated however, that symptoms can vary widely between patients, creating diverse symptom profiles (Fuller *et al.*, 2003).

In addition to the clinical symptoms described above, associated cognitive deficits have been reported. Findings indicate that schizophrenia is characterised by compromised intellectual functioning, consisting of a general reduction in IQ (Johnstone 1991), and on detailed testing, a general decline in many cognitive

domains; including deficits in language, attention, memory, and executive functions (Heinrichs and Zakzanis 1998; Goldberg *et al.*, 2003).

ICD-10 criteria

Characteristic symptoms
- *At least one of:*
  - Thought echo, thought insertion/withdrawal/broadcast
  - Passivity, delusional perception
  - Third person auditory hallucinations, running commentary
  - Persistent bizarre delusions
- *Or two or more of:*
  - Persistent hallucinations
  - Thought disorder
  - Catatonic behaviour
  - Negative symptoms
  - Significant behaviour change

Duration
- More than 1 month

Exclusion criteria
- Mood disorders, schizoaffective disorder
- Overt brain disease
- Drug intoxication or withdrawal

DSM_IV criteria

Characteristic symptoms
- *At least one of:*
  - Bizarre delusion
  - Third person auditory hallucinations
  - Running commentary
- *Or two or more of:*
  - Delusions
  - Hallucination
  - Disorganised speech
  - Grossly disorganised behaviour
  - Negative symptoms

Duration
- One month characteristic symptoms
- With 6 months of social/occupational dysfunction

Exclusion criteria
- Schizoaffective or mood disorders
- Direct consequence of substance abuse or general medical condition
- Pervasive developmental disorders

Figure 2.1 Summary of diagnostic criteria for schizophrenia

2.2.3 Genetic component

Although the nature of genetic transmission in schizophrenia is not yet known, many studies have demonstrated that there is a genetic component to the disorder. The world-wide lifetime risk is reported to be 1%, but this is greatly increased among first degree relatives: 9% for a sibling of an affected individual and 13% for a child of an affected parent (Gottesman 1991). Twin studies examining concordance rates reveal the risk to the second twin developing schizophrenia as being 28% (range 18-65%) for a monozygotic pair, for a dizygotic pair the rates are the same as for ordinary siblings (Torrey 1992). Adoption studies also support the genetic theory of transmission, with 11-19% of offspring of affected parents adopted at birth later developing the disease (McGuffin *et al.*, 1995). Studies of affected family members

(described more fully in the following chapter) have also shown that close relatives often share a number of subtle neurobiological abnormalities that are typically seen in patients, suggesting that some deficits are inherited. Such abnormalities are typically referred to as the 'endophenotype' or 'intermediate phenotype'. Studies of the intermediate phenotype have been suggested to provide great promise in terms of elucidating the molecular genetics of the disorder, since these measures may be closer to the actual biological effect of the genes (see Egan *et al.*, 2003).

Although all these studies implicate a genetic component, it can not be the only aetiological mechanism since these figures do not indicate simple Mendelian inheritance. To illustrate, in Mendelian dominant diseases such as Huntington's disease the concordance rate for monozygotic twins is 100% as the twins are genetically identical. Since this is not the case in schizophrenia, this suggests that people can apparently carry the genotype for schizophrenia without ever developing the disease itself (i.e. without expressing the full phenotype). However, in terms of satisfying the epidemiological criteria for risk factors, the only class reported to satisfy the major risk factor category for schizophrenia are the genetic influences (Jablensky 1997). Other risk factors such as urbanicity, seasonality of birth, and obstetric complications, findings which have been more difficult to replicate, are reported to have only a minor risk-increasing effect (Jablensky 1997).

2.2.4 Age of onset and the neurodevelopmental model

The typical age of onset of the disorder is in adolescence or early adulthood, and the condition occurs slightly earlier in males than in females (Häfner *et al.*, 1993, 1998). The onset in men typically peaks at around 20-24 years. In women there is a less prominent peak around this age, followed by a second smaller peak over 35 years (Jablensky 2003).

A convergence of findings from the mid-1980s onwards began to suggest abnormalities of developmental origin which were hard to reconcile with the age of illness onset. These findings included the presence of neurobiological deficits at the

time of disease onset (Weinberger *et al.*, 1982), the lack of the normal pathological response of the central nervous system to brain insults, namely gliosis (see Harrison 1999), and the findings that individuals who later go on to develop the disorder show behavioural abnormalities in childhood (Done *et al.*, 1994; Jones *et al.*, 1994). To account for these discrepancies one of the primary hypotheses regarding the pathophysiology of schizophrenia suggests an early neurodevelopmental disruption occurs (of genetic, pre- or peri-natal origin) which predisposes individuals to schizophrenia. This would be consistent with the lack of gliosis, as brain insults early on in life are not associated with this response (see Harrison 1999). This disruption later becomes unmasked by subsequent developmental events such as synaptic pruning which occurs during adolescence or early adulthood, or when compromised regions are placed under high functional demand (Weinberger 1986; Murray and Lewis 1987; Marenco and Weinberger 2000). In other words, neural systems are primed from early in life to develop abnormalities which account for the illness, but these are sub-clinical or compensated for until early adult life. There is continued debate on the timing of these abnormalities, whether these can be identified prior to illness onset, and whether these progress throughout the duration of the disorder; hence the importance of prospective studies examining subjects before illness occurs.

2.3 LOCALISATION: BRAIN REGIONS IMPLICATED

Over the past 100 years technical advances have contributed greatly to the understanding of the neural basis of the disease, however, it is only in the past few decades that *in vivo* neuroimaging tools have become widely available for the study of the neural correlates of the disorder. Before describing the findings from structural imaging studies, a brief description of such techniques is outlined below.

2.3.1 Structural imaging studies of schizophrenia

2.3.1.1 *Structural imaging techniques*

Early in vivo structural imaging techniques available included pneumoencephalography and computerised tomography (CT). Pneumoencephalography was an invasive imaging technique which involved using air as a contrast agent in order to visualise the lateral ventricles. Air, unlike calcified structures such as the skull, absorbs very little radiation and appears dark on the X-ray film. The air-filled ventricles could therefore be relatively easily distinguished from the surrounding tissues. This invasive method was associated with risks and was therefore considered unethical to use except in the investigation of disease, which imposed limitations on its use in research. This was superseded by safer techniques such as computerised tomography (or CT) and MRI.

CT is a non-invasive X-ray based technique introduced in the 1970's which has the ability to distinguish grey matter, white matter and cerebrospinal fluid. It does however involve the use of X-rays, and due to methodological problems such as beam hardening effects around bony structures, gives a restricted view of the posterior fossa. Magnetic resonance imaging (MRI) does not involve radiation, permits visualisation of all areas of the brain clearly, and has better resolution than CT images. For research purposes MRI has therefore superseded CT.

Principles of structural MRI are based on observing changes in the behaviour of protons in the presence of a magnetic field. When a subject is placed in the MR scanner, the protons of the tissue align longitudinally with the static magnetic field, either in the lower energy state (parallel), or the higher energy state (anti-parallel). Magnetic effects of protons in parallel and anti-parallel alignment cancel each other out, however there are more protons aligned in the lower energy state than the higher, resulting in a longitudinal magnetisation. A radiofrequency pulse is then introduced, producing synchronous precession ('wobble') of the protons, which results in transverse magnetisation. The radio frequency pulse also provides sufficient energy

to some of the protons to move to the higher (anti-parallel) state, which has the effect of cancelling out some of the longitudinal magnetisation. As the radiofrequency pulse is turned off, the protons move back to their original energy state, returning energy to the surrounding structural lattice, hence increasing longitudinal magnetisation, this being termed 'T1 relaxation time'. Simultaneously, the transverse magnetisation decreases as the precession of the protons becomes asynchronous once more, this being termed the 'T2 relaxation time'. Different tissues have different relaxation times depending on the amount of hydrogen atoms they contain. This technique has excellent discrimination between tissue types, and produces images of high spatial resolution.

2.3.1.2 *Early findings in schizophrenia*

Research into structural abnormalities associated with schizophrenia initially concentrated on ventricular enlargement after the landmark finding that schizophrenic subjects showed dilation of the lateral ventricles on CT images (Johnstone *et al.*, 1976). Since this time there has been extensive study of the structure of the brain in people with schizophrenia using both CT and MRI.

Due to technical and practical limitations, early methods were based on simple linear, area, or ratio measurements. One of the most common area measurements was the ventricle to brain ratio, or VBR (Synek and Reuben 1976, and see van Horn and McManus 1992). The VBR provided a relative measure of the area of the ventricles as a proportion of the size of the brain, most commonly derived from axial slices. Reviews of studies using such measures indeed confirmed the presence of ventricular enlargement in schizophrenia (Raz and Raz 1990). However these techniques suffered from fundamental methodological limitations, including issues associated with the variation in slice selection and position, of particular importance in these early studies involving large inter-slice gaps (Woods *et al.*, 1991).

2.3.1.3 *Semi-automated structural analyses: techniques*

After advances in image acquisition and analysis techniques, such as improved spatial resolution of MR images and smaller slice thickness, later studies focused on volumetric measurements of brain structures. Although considerably more labour intensive than earlier methods, they provided more detailed information. The advent of semi-automated volumetric measurement of brain structures greatly aided standardisation, and therefore interpretability of results, and highlighted regions other than the ventricular system as being abnormal in schizophrenia. These methods used a combination of computer-aided edge detection techniques along with manual delineation of anatomical boundaries to determine volumetric measurements, see Figure 2.2.

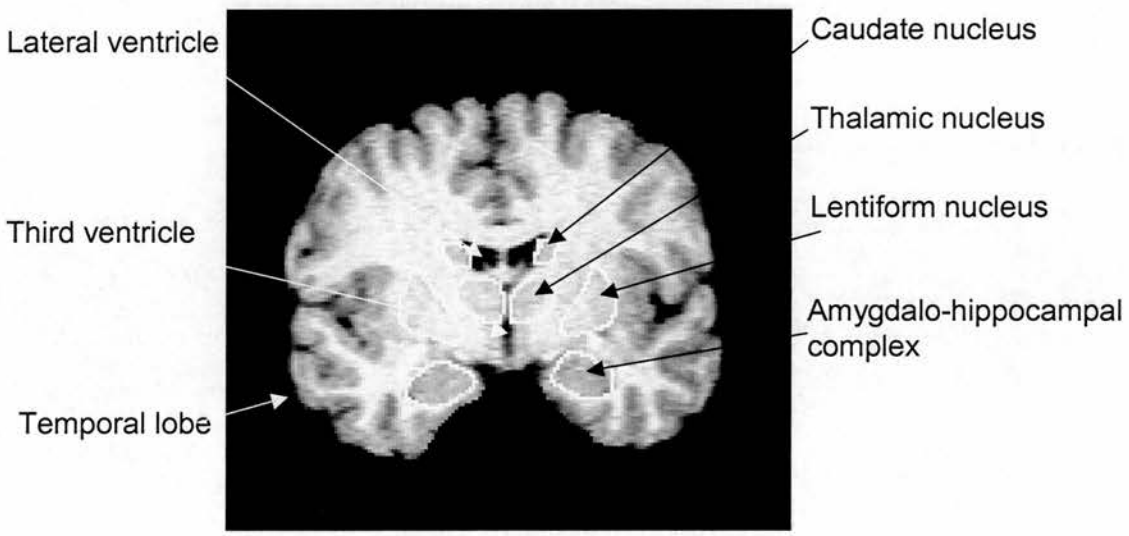

Figure 2.2 Semi-automated region of interest delineation of structural MR image.

Regions are defined by a combination of automated edge-detection and manual outlining of structures. Volumetric measurements are obtained by multiplying the area of the structure on each slice by the slice thickness, or slice thickness plus inter-slice gap if slices are not contiguous.

2.3.1.4 *Semi-automated structural analyses: findings*

There have been many studies using semi-automated assessment of structural MRI scans of schizophrenic subjects versus controls, and many different brain regions have been examined. Interpreting this breadth of literature has been a formidable task for researchers, particularly since there are often differences in methodologies, including heterogeneity of subject groups (chronicity of disease, sex, age, and handedness), differences in scanner parameters (slice thickness, scanner sequence, plane of acquisition), and subtle differences in the measurement processes, including anatomical definition criteria. Meta-analytical and systematic review techniques have been applied to the literature making the interpretation of the results considerably more practicable. Meta-analysis refers to the statistical analysis of a large collection of results from individual studies for the purpose of integrating findings (Glass 1976). It offers a standardised way of interpreting results from a large number of studies, providing measures of the magnitude and consistency of the evidence.

2.3.1.4.1 Whole brain size and ventricular system

Overall, the findings from these reviews and meta-analyses indicate that there is a small global reduction in brain size in schizophrenia (Ward *et al.*, 1996; Lawrie and Abukmeil 1998; McCarley *et al.*, 1999; Wright *et al.*, 2000; Shenton *et al.*, 2001), but by far the most replicated finding is of ventricular enlargement (Lawrie and Abukmeil 1998; McCarley *et al.*, 1999; Pearlson and Marsh 1999; Wright *et al.*, 2000; Shenton *et al.*, 2001).

2.3.1.4.2 Frontal lobes

In terms of more localised deficits, it had long been recognised that many of the symptoms of schizophrenia such as flattening of affect, and thought disorder, resemble some of the symptoms exhibited by patients with frontal lobe lesions. Anatomically the frontal lobes are divided into four strips, the motor cortex, the premotor cortex, the prefrontal cortex and the anterior cingulate cortex. The first

three run vertically and wrap around the medial surface of the frontal lobes, and the later runs horizontally along the medial surface.

The main focus of interest in schizophrenia is the prefrontal cortex (the region anterior to the precentral sulcus, see Figure 2.3). In semi-automated volumetric techniques the prefrontal cortex is generally defined on coronal section as the region anterior to the first slice showing the genu of the corpus callosum. However this is a large region which is further anatomically subdivided based on sulcal/gyral patterns and by cytoarchitecture, see Figures 2.3 and 1.1.

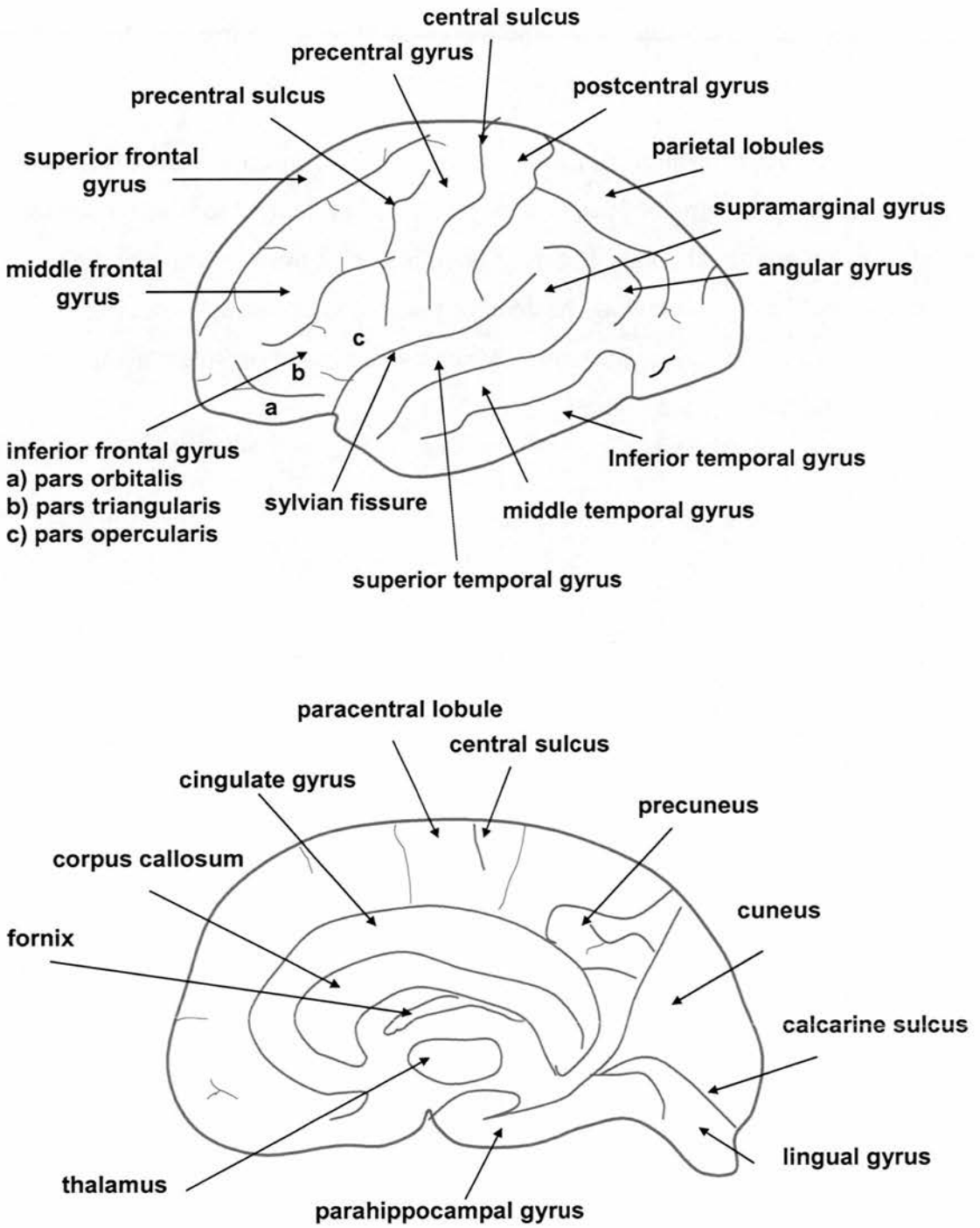

Figure 2.3 Stylised diagram of main sulci/gyri

Lateral view above, medial view below, to illustrate main regions of interest in schizophrenia referred to in text.

Reviews of the semi-automated volumetric analyses indicated a modest volume reduction in the prefrontal lobes in schizophrenia, however these are less consistently found than differences in whole brain and ventricular volumes (Chua and McKenna 1995; Lawrie and Abukmeil 1998; McCarley *et al.*, 1999; Wright *et al.*, 2000, Shenton *et al.*, 2001). The lack of convincing evidence for the involvement of the prefrontal lobe from these semi-automated methods may in part be due to differences being localised to subdivisions of this large area, which may be diluted in measurements of the entire lobe. Improvements in analysis techniques including detailed parcellation methods (Crespo-Facorro *et al.*, 1999a), and automated methods (discussed below), may prove more useful.

2.3.1.4.3 Temporal lobe

With regards the temporal lobe, the superior temporal gyrus includes several functional zones of potential interest in schizophrenia, including primary auditory cortex, the auditory association cortex, and Wernicke's speech area (Figure 1.1). This gyrus is located on the lateral surface of the temporal lobe and runs parallel to the sylvian fissure (Figure 2.3). Reductions in this region have indeed been reported in schizophrenia (Lawrie and Abukmeil 1998; Pearlson and Marsh 1999; Wright *et al.*, 2000), and there are some suggestions that volumetric findings are more consistent in those studies which examined grey matter only (McCarley *et al.*, 1999; Shenton *et al.*, 2001).

Small volume reductions in medial temporal lobe structures, in particular the amygdalo-hippocampal complex are also consistently reported, see Figure 2.2 (Chua and McKenna 1995; Lawrie and Abukmeil 1998; Nelson *et al.*, 1998; McCarley *et al.*, 1999; Pearlson and Marsh 1999; Wright *et al.*, 2000; Shenton *et al.*, 2001). The hippocampus, part of the limbic cortex, has long been known to play an important role in memory (Milner 1966). Since patients with schizophrenia have been shown to be impaired on particular memory tests, this has been an obvious site for study. However, due to difficulties in separating the amygdala and hippocampus, particularly on early MRI scans, the two regions have often been measured together.

This is not ideal as they are functionally distinct structures which may be differentially affected (Hopkins and Lewis 2000). Indeed there is increasing evidence that that there are greater decreases in volume of the amygdala compared to the hippocampus (Lawrie and Abukmeil 1998; Wright *et al.*, 2000), and inclusion of the amygdala along with volume measures of the hippocampus has been shown to increase effect sizes across studies (Nelson *et al.*, 1998). Evidence also suggests a relatively large regional decrease in volume, of the order of 10%, in the parahippocampal gyrus in schizophrenia (Lawrie and Abukmeil 1998). However there are fewer studies examining this region than those described above.

2.3.1.4.4 Sub-lobar: thalamus

The thalamus is connected to regions including the prefrontal, cingulate and temporal cortices, all of which have been implicated in the schizophrenia and hence has been a focus of interest in studies of the disorder. However, thalamic volume reductions are reported to be small and inconsistent in schizophrenia (McCarley *et al.*, 1999; Pearlson and Marsh 1999; Wright *et al.*, 2000; Shenton *et al.*, 2001), and one meta-analysis cautioned that there was marked variability in effect sizes across studies (Konick and Friedman 2001).

2.3.1.5 *Automated structural analyses: techniques*

Although semi-automated volumetric analysis techniques are, as the name suggests, partly automated, they do still involve a considerable amount of operator input (often in the order of 4-5 hours per brain), which seriously restricts the number of brains, and regions, which can be measured. There are also issues concerning the reliability of these measurements, particularly for smaller brain structures (Whalley *et al.*, 1999). Hence, more automated, data-lead techniques like brain averaging (Andreasen *et al.*, 1994) and voxel based morphometry (VBM) (Wright *et al.*, 1995, 1999a; Ashburner and Friston 2000b) are beginning to replace semi-automated volumetric analysis methods. Automated methods have the advantage that they allow

analysis of the brain as a whole and may therefore implicate different regions, or potentially sub-regions of larger structures which have not previously been studied.

VBM is an automated technique which uses software originally designed for use on functional images called statistical parametric mapping, or 'SPM'. Studies employing these techniques broadly follow similar procedures, including spatial normalisation of individual subject's scans to a standardised space, segmentation of the image into the three tissue compartments (grey matter, white matter and cerebrospinal fluid) and then spatially smoothing the images prior to statistical analysis. However it should be considered that methodological refinements to these methods are frequent at present and may vary between studies (see Good *et al.*, 2001; Ashburner and Friston 2000b). The majority of studies focus on the analysis of grey matter segments, which are discussed below. Reports of differences in studies that have examined white matter are reported in later sections regarding anatomical substrates of altered interregional connectivity in schizophrenia.

2.3.1.6 *Automated structural analyses: findings*

Results of VBM studies in schizophrenia are in general consistent with the volumetric studies described above, indicating localised reductions of grey matter in prefrontal and temporal regions.

2.3.1.6.1 Frontal lobe

In the prefrontal cortex, there are reports of grey matter reductions, in lateral (Wright *et al.*, 1999a; Gaser *et al.*, 1999; Job *et al.*, 2002; Suzuki *et al.*, 2002), and orbital regions (Hulschoff Pol *et al.*, 2001; Wilke *et al.*, 2001; Ananth *et al.*, 2002). The most consistently reported region of localised reduction is in the anterior cingulate/medial prefrontal region (Wright *et al.*, 1999; Wilke *et al.*, 2001; Paillière-Martinot *et al.*, 2001; Sigmundsson *et al.*, 2001; Ananth *et al.*, 2002; Job *et al.*, 2002; Kubicki *et al.*, 2002a; Suzuki *et al.*, 2002).

2.3.1.6.2 Temporal lobe

In the temporal lobe there are reported reductions in the lateral temporal neocortex (Gaser *et al.,* 1999; Sigmundsson *et al.*, 2001; Wilke *et al.,* 2001; Ananth *et al.*, 2002; Job *et al.*, 2002; Kubicki *et al.*, 2002a; Suzuki *et al.*, 2002), the parahippocampal gyrus and amygdala/hippocampus (Wright *et al.,* 1999a; Hulschoff Pol *et al.*, 2001; Pailliere-Martinot *et al.,* 2001; Sigmundsson *et al.*, 2001; Job *et al.*, 2002; Suzuki *et al.*, 2002a), and in the insular cortex (Wright *et al.,* 1999a,b; Hulschoff Pol *et al.*, 2001; Pailliere-Martinot *et al.,* 2001; Sigmundsson *et al.*, 2001; Wilke *et al.,* 2001; Ananth *et al.*, 2002; Kubicki *et al.*, 2002).

2.3.1.6.3 Sub-lobar and other regions

There are fewer studies reporting localised increases in grey matter; in the putamen (Gaser *et al.,* 1999, Wilke *et al.,* 2001), in the caudate and globus pallidus (Hulschoff Pol *et al.*, 2001) and one study reported grey matter increases in the parietal lobe and cerebellum (Suzuki *et al.*, 2002a).

2.3.1.6.4 Summary

In general therefore these studies support findings from semi-automated volumetric methods, but in addition appear to indicate localised regional reductions of grey matter in the insula and anterior cingulate cortex. The insula is a region of buried cortex overlaid by cerebral operculum. It occupies the medial wall of the lateral sulcus and is considered, due to its extensive connections with limbic regions, to be part of the paralimbic system. The anterior cingulate is situated on the medial surface of the hemisphere, bordering the corpus callosum (Figures 1.1, 2.3). It forms the largest part of the limbic system, and is considered to be involved in processes such as attention, inhibition, and the evaluation of strategy in normal subjects (Smith and Jonides 1999). These automated studies, in addition to confirming previous structural studies, therefore also reveal involvement of limbic and paralimbic regions in the pathology of schizophrenia.

2.3.2 Functional imaging in schizophrenia

For much of the last century attempts have been made to define specific lesions underlying psychiatric illnesses such as schizophrenia. As described above, structural imaging studies have greatly advanced knowledge regarding structural deficits of the disorder, more recently however the use of functional imaging methods has opened up the possibility of examining the functional underpinnings of the disease.

2.3.2.1 *Hypofrontality*

Early functional imaging studies suggested that a core deficit of schizophrenia was a failure to activate the prefrontal cortex ('hypofrontality'), supported by neuropsychological findings of impaired executive performance in schizophrenia, and by findings that patients with frontal lobe damage are also impaired on similar types of tests. The first study to report hypofrontality in schizophrenia was by Ingvar and Franzen in 1974. In this study, positron emission tomography (PET) was used to compare brain activity in schizophrenic subjects and a matched normal control group at rest. In the schizophrenic group there was reduction in blood flow to the prefrontal cortex relative to posterior regions of the brain (Ingvar and Franzen 1974). Since this original report, hypofrontality has become one of the most prominent findings reported in the functional imaging literature, particularly in response to tasks in which patients perform worse than controls, such as working memory (see Manoach 2003), and verbal initiation tasks (Liddle *et al.*, 1992; Yurgelun-Todd *et al.*, 1996; Curtis *et al.*, 1998). Indeed early review articles indicated that hypofrontality was a robust finding; one review article published in 1996 stated that there were no reported PET or SPECT activation studies that failed to find hypofrontality during a frontal lobe activation task (Velakoulis and Pantelis 1996). More recently however it has been suggested that performance differences between patients with schizophrenia and controls could potentially confound these results (Ebmeier *et al.*, 1995; Ramsey *et al.*, 2002). In studies which have controlled for task performance, findings of hypofrontality are less consistently reported (Frith *et al.*, 1995a; Fletcher *et al.*, 1998; Ramsey *et al.*, 2002). There are also suggestions and that there may be a complex

relationship between performance and either decreased (or even increased) prefrontal activation, particularly in relation to working memory load (see Manoach 2003).

2.3.2.2 *Language processing*

More recent functional imaging studies have also focussed on neuropsychological deficits which have been repeatedly implicated in schizophrenia, broadly including deficits in language, attention, memory, and executive function (Gourovitch and Goldberg 1996), on the basis that these impairments will be reflected by activation differences in the relevant brain areas. Since the main paradigm used in the experimental section of this thesis involves a task which requires self-generated word production, descriptions of functional imaging studies in schizophrenia will be restricted to similar aspects of language processing, specifically verbal fluency, and sentence completion tasks.

2.3.2.2.1 Verbal fluency: background

Both Bleuler (1911) and Kraepelin (1896) considered disturbances of speech and language to be core symptoms of schizophrenia. This is reflected in the literature where self-generated word production deficits are among the most consistently reported functional impairments. Deficits on such tasks are considered to reflect the poverty, incoherence, and disorganisation of speech observed in those with the disorder. However, the underlying mechanisms of the deficit remain unclear.

Functional imaging studies of self-generated word production have centred on verbal fluency tasks. These classically fall into two main types; letter fluency, and categorical, or semantic, fluency. These tasks require the subject to name as many words beginning with a specified letter, or as many examples from a predefined category as possible in a limited period of time. Performance is judged based on the number of words produced, and on the number of errors (including repetitions, or 'perseverations', and inappropriate responses). Successful performance involves multiple cognitive processes with high demands on executive resources, including

processing of the stimulus, self initiated searches of the subject's own word-store (or lexicon) in order to retrieve appropriate words, followed by covert or overt articulation of the word, and finally sustained attention throughout the task.

2.3.2.2.2 *Verbal fluency: imaging in normal subjects*

The neural correlates of word production have been examined by numerous imaging studies. Using PET, verbal fluency tasks in normal subjects have repeatedly shown activation of the left dorsolateral prefrontal cortex, and deactivation of the superior temporal regions (Frith *et al.*, 1991, 1995). A recent meta-analysis of both PET and fMRI studies also identified these main regions as important areas in the core processes of word production determined from a variety of tasks; the left posterior inferior frontal gyrus (Broca's area), mid segments of the left superior and middle temporal gyri, posterior segments of the left superior and middle temporal gyri (Wernicke's area), and the left thalamus (Indefrey and Levelt 2000).

2.3.2.2.3 *Letter and semantic fluency in schizophrenia*

It has been suggested that there are subtle differences in the cognitive processes involved in the two types of verbal fluency tasks. Performance of the letter fluency involves a search of the lexicon based on phonology/orthography. The categorical task involves a search of semantic networks which require that the subject understands the features that the correct response must posses in order to meet criteria for inclusion in the category. Production of the letter fluency relative to semantic fluency is reported to involve the left prefrontal cortex and left inferior parietal cortex (Mummery *et al.*, 1996; Gourotovich *et al.*, 2000); consistent with regions considered to be involved in the phonological loop of working memory. Semantic fluency relative to letter fluency is reported to involve prefrontal and temporal regions (Mummery *et al.*, 1996; Gourovitch *et al.*, 2000); consistent with regions considered to be involved in gaining access to the semantic lexicon (Gourovitch *et al.*, 2000).

Reports in the literature indicate deficits in both letter and semantic fluency in schizophrenia; however historically there are conflicting reports on whether there is a disproportionate deficit in one or the other. Consequently there are differing views on where the largest functional impairment arises. Reduction in fluency have been suggested to arise from disturbances in the executive processes involved in internal retrieval strategies or in accessing lexical information (e.g. Allen *et al.*, 1993), due to abnormalities in the organisation of semantic memory (e.g. Paulsen *et al.*, 1996; Elvevåg *et al.*, 2002), or due to a combination of the above (Vinogradov *et al.*, 2002). A recent meta-analysis examining 13 studies of both letter and semantic fluency concluded that subjects with schizophrenia are considerably more impaired on semantic fluency (Bokat and Goldberg 2003). The authors of this meta-analysis stress that the results do not contradict previous findings of retrieval impairment, but suggest it may be that semantic organisation is more markedly affected in schizophrenia (Bokat and Goldberg 2003).

2.3.2.2.4 Verbal fluency; imaging in schizophrenia

One of the first imaging studies in schizophrenia to examine patterns of brain activity during a verbal fluency task was undertaken by Frith *et al.*, (1995a) using PET. In this study subjects were categorised into three groups, selected according to their performance on a letter verbal fluency test, and were compared to normal controls. One group of schizophrenic subjects showed normal performance, the second showed impaired performance, and the last group were quantitatively normal but produced more than five erroneous words. In the fluency condition compared to the baseline repetition condition, the normal control subjects showed increased activation in the dorsolateral prefrontal cortex, the anterior cingulate, and in the thalamus, and decreases in the posterior cingulate, and right superior temporal cortex. Taken together, the schizophrenic patients did not show hypofrontality in relation to the control subjects, as may have been predicted from earlier studies. They did however differ from the controls in that they showed a lack of temporal cortex deactivation. In this study therefore activity in the superior temporal cortex was

negatively correlated with activity in the prefrontal cortex in normal subjects. This relationship was *not* seen in the schizophrenic subjects, where activity in the superior temporal cortex positively correlated with prefrontal activity (Friston and Frith 1995a). This pattern of fronto-temporal activity has also been observed in un-medicated patients, where it was reported that there was a failure to deactivate the left superior temporal gyrus (and left inferior parietal lobule, Fletcher *et al.*, 1996). These results therefore hint at a reversal of the normal prefronto-temporal interaction in schizophrenia. Other PET studies examining asymptomatic schizophrenic subjects (Dye *et al.*, 1999), and patients with mild symptoms (Spence *et al.*, 2000) however failed to find temporal lobe abnormalities, indicating that the possibility that active symptomatology of the subjects may be an important factor in temporal lobe dysfunction.

Studies of verbal fluency have also been performed with fMRI, the most consistent finding being abnormal frontal activation in schizophrenic subjects (Yurgelunn-Todd *et al.*, 1996; Curtis *et al.*, 1998, 1999). However, there are important methodological issues regarding the comparison of these two imaging techniques, the main difference being the requirement for covert responses in fMRI designs due to the problems associated with movement during articulation of overt verbal responses. This may account for differences in temporal lobe activation seen with PET and fMRI studies, since regions within the temporal lobe are considered to be involved in internal perception of overt speech, and are reported to show reduced activation in response to self-generated vocalisations in normal subjects (see Curtis *et al.*, 1998).

2.3.2.2.5 *The Hayling sentence completion test*

The Hayling sentence completion test is a relatively new test of self-initiated word production designed to assess aspects of frontal lobe functioning (The Hayling and Brixton tests, Thames Valley Test Company). In the standard 'pen and paper' format, this test consists of two sections each comprising 15 sentences in which the final word is missing. Sentences are adapted from Bloom and Fischler norms (1980).

In the first section, referred to as 'response initiation', sentences are read aloud to the subjects and they are requested to complete the sentence sensibly in as little time as possible. This condition could be considered an extension of the verbal fluency tasks, in that both tests recruit the executive processes involved in generating intrinsic responses in the form of word retrieval. However, this test uses the sentence context to constrain the word response choices. In the second section ('response inhibition' or 'suppression') the subject is asked to produce a word which is unconnected to the sentence in every way. For section one, performance is assessed in terms of response latencies. This is considered to reflect a measure of response initiation which has shown to be impaired in some patients with frontal lobe lesions (Burgess and Shallice 1996). In section two, performance is assessed by error scores, and the time taken for the subject to respond. Patients with frontal lobe lesions are also impaired in this condition (Burgess and Shallice 1996). In section two where the subject has to inhibit sensible responses, errors are categorised into two groups. Responses which are entirely plausible in the sentence context are termed 'category A' errors. Responses which are somewhat connected to the sentence meaning are referred to as 'category B' errors (The Hayling and Brixton tests, Thames Valley Test Company).

The Hayling test has been used in a variety of clinical groups including Alzheimer's disease (Collette *et al.*, 1999), attention deficit hyperactivity disorder (Clark *et al.*, 2000), post-traumatic stress disorder (Kimble *et al.*, 2002), amyotrophic lateral sclerosis (Abrahams *et al.*, 1999), and of course in schizophrenia (Kircher *et al.*, 2001; Lawrie *et al.*, 2002a; Waters *et al.*, 2003). It has also been used in the previous phase of the Edinburgh high risk project (Byrne *et al.*, 1999, 2003), discussed in chapter three.

2.3.2.2.6 Hayling test: imaging in normal subjects

The Hayling test has also been adapted for use in imaging studies. Imaging in normal subjects performing the Hayling test have generally implicated areas consistent with those reported to be involved in verbal fluency tasks, i.e. the left lateral and medial prefrontal cortex, and lateral temporal cortex. However, there is

some inconsistency regarding differential activations of the two conditions, response initiation and response suppression.

In an early PET study, the left frontal operculum (BA 45) and left anterior cingulate cortex (BA 32) were reported to be involved in both the initiation and suppression conditions when compared to a baseline reading condition (Nathaniel-James *et al.*, 1997). This study reported additional activation of the left inferior frontal region and left middle temporal gyrus in the initiation condition versus suppression, but surprisingly, no additional activation differences when the harder suppression condition was compared to the initiation condition. A following PET study confirmed increased activation occurred in the initiation condition versus reading in the left frontal operculum (BA 45). However this study found differential activation in the suppression condition versus initiation in the left middle frontal gyrus (BA 9/10) and left inferior frontal gyrus (BA45) (Collette *et al.*, 2001).

Another PET study by Nathaniel-James and Frith (2002) investigated the effects of varying sentence constraint. Previously, studies have used sentences with high cloze frequency, or constraint, where there is a high probability that one word will be produced to complete the sentence (derived from the set of sentence completion norms as detailed in Bloom and Fischler, 1980). In the study by Nathaniel-James and Frith (2002) sentences were selected in which there was a systematic variation in the number of possible words available to complete the sentences (varying from high to low constraint). Initially taking all levels of constraint together, this study reported activation of the left dorsolateral prefrontal cortex (BA46/9) in the suppression condition relative to the initiation condition, and activation in the medial orbitofrontal cortex (BA 11) in the initiation condition relative to the suppression condition (Nathaniel-James and Frith 2002). With regards the differing levels of contextual constraint for both tasks combined, significant right middle temporal lobe activity was reported in the high constraint condition (easiest level of the task), and the left dorsolateral prefrontal cortex was activated in the low constraint condition (hardest difficulty level). Interactions between task and constraint level indicated that

the left dorsolateral prefrontal cortex was activated at all levels of constraint in the suppression task, but only in the low (most difficult) constraint condition for the initiation condition.

2.3.2.2.7 Hayling test: imaging in schizophrenia

There are a small number of imaging studies which have employed the Hayling task to examine patterns of neural activity in schizophrenic subjects, but methodological differences preclude generalisations. The study by Kircher *et al.*, (2001) used fMRI to examine patients with and without formal thought disorder and normal controls using an overt version of the Hayling task, comprising an initiation condition, a decision condition (subjects given a choice of two words), and baseline reading condition. The patients with formal thought disorder showed a decreased response of the right superior temporal gyrus for both initiation versus baseline, and initiation versus decision compared to the other groups, indicating the differential activation of the temporal cortex was dependent on symptomatology (i.e. the presence of formal thought disorder).

The fMRI study by Lawrie *et al.*, (2002) used the response initiation condition, but did not find localised differences between schizophrenic and control subjects, nor differential effects of varied sentence constraint, potentially due to small group numbers. Decreased fronto-temporal connectivity was however reported in schizophrenic subjects compared to controls, and in addition, fronto-temporal connectivity was found to be negatively correlated with the severity of auditory hallucinations. These results are consistent with findings from earlier verbal fluency tasks hinting at a disruption in fronto-temporal integration in schizophrenia.

2.4 FUNCTIONAL INTEGRATION

2.4.1 The disconnection hypothesis and schizophrenia

Schizophrenia is typically characterised by a variety of symptoms but attempts to explain and account for the symptoms and complex cognitive deficits of schizophrenia by the presence of specific focal structural or functional abnormalities have not proved definitive. Evidence that regions such as the prefrontal cortex may be under-, or over-activated in certain circumstances also suggests that the functional abnormalities seen in schizophrenia may be dynamic in character (Liddle and Pantelis 2003). As indicated above, this has led to the suggestion that deficits of the disorder could arise from abnormal interactions, or 'disconnectivity' between a distributed network of brain regions (Friston and Frith 1995a). Indeed, Bleuler's suggestion of the fragmentation or disintegration of cognitive functions in schizophrenia could be interpreted as one of the earliest accounts of altered functional integration in the disorder (Bleuler 1911).

2.4.1.1 *Corollary discharge*

One of the neurophysiological theories behind disconnectivity and the symptoms of schizophrenia is based on deficits in self-monitoring and corollary discharge (Frith 1992, 1995b). Self-monitoring forms the basis of normal cognitive function, in planning, controlling, and anticipating the consequences of our actions. Corollary discharge is a term used to describe the copy of the neural command after it has been modulated by the components attributed to self-generated actions, and is considered to inform or prepare relevant parts of the brain for the consequences of internally generated actions. Hence, it allows us to distinguish between events due to our own actions, and externally generated events. It is the balance of these signals which forms the basis of our sensori/motor world, and disruption to this system may result in abnormal perceptual experiences.

The concept that motor signals could be used by the brain to interpret sensory consequences of movement is based on work described by Helmholz in the study of oculomotor proprioception (Helmholtz 1879). During self-generated eye movements our view of the world to remains stable. However when the eye is moved passively there is no internal motor command to modify sensory effects and the visual world appears to move. The modern concepts of corollary discharge, or efference copy, are attributed to Sperry (1950), and von Holst and Mittelstaedt (1950). Similar examples of the effects of corollary discharge have been reported in the auditory system (see Frith 1995b and Friston 1999). In the monkey, there are cells in the auditory system which respond to externally generated sounds, but not to self-generated vocalisations (Muller-Preuss and Ploog 1981). It has been proposed that during self-generated vocalisations inhibition of these cells by the corollary discharge associated with generating vocalisation prevents misattribution of these internally generated sounds as being externally generated. Similar neuronal responses during self-generated speech from recordings performed pre-operatively in the auditory cortex in humans have also been reported (Creutzfeldt *et al.*, 1989). Here, neurones in the middle temporal gyrus showed a dampened response, starting 100 ms before and continuing during the subject's own speech, which did not occur when another person spoke to the subject (Creutzfeldt *et al.*, 1989). It is conceivable that dysfunction in this system could result in an inability to recognise internally generated vocalisations as originating from the self (Frith 1995b). In humans the prefrontal cortex and its interactions is thought to play a major role in modulating activity in different sensory systems (see Friston *et al.*, 1995b), hence it is plausible that a disruption of the normal influence between frontal and other brain regions could result in perceptual disturbances. In particular it has been suggested that a disruption in the interactions between the areas where speech is generated (the frontal lobes), and where speech is perceived (the temporal cortex), through corollary discharge may cause a failure in recognising inner speech, giving rise to auditory hallucinations (Frith 1995b). This model of disruption of corollary discharge has also been applied to explain other characteristic features of schizophrenia, including disruptions in the sense of self and

will resulting in delusional symptomatology (Feinberg 1978, Feinberg and Guazzelli 1999).

2.4.1.2 *Anatomical substrates of altered connectivity.*

One of the central characteristics of functional integration is that for there to be interaction between two regions, there must also be anatomical connectivity between them. Therefore before describing aspects of functional integration it is useful to consider briefly the anatomical substrates of these connections (the white matter fibre tracts of the brain), along with the developmental processes involved in forming these connections. As a proviso however, it should be noted that although functional interactions depend on these substrates of anatomical connectivity, the disconnection hypothesis referred to regarding schizophrenia does not necessarily imply there is anatomically localised pathology, as the disconnection in schizophrenia is proposed to be primarily a *functional* abnormality (see Friston *et al.*, 1999).

2.4.1.2.1 White matter fibre tracts

A variety of techniques have been used to examine the anatomy and integrity of white matter fibre tracts, beginning with early work by Dejerine (1895, 1901) who used histopathological staining methods to produce a detailed atlas in which the course of the white matter tracts were depicted, through the use of axonal transport of exogenous tracers such as horseradish peroxidase in non-human primates (for example see Seltzer and Pandya 1989), to modern day *in vivo* imaging techniques such as DTI tractography which use algorithms to portray colour coded orientation maps based on fibre trajectories (see Catani *et al.*, 2002; Mori *et al.*, 2002).

The white matter of the human brain consists of glial cells and myelinated axons with different orientations which connect regions of the cerebral cortex to other brain regions. The axons form white matter tracts which fall into three categories; commissural, association, and projection fibres.

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White matter pathway	Approx size, cross section (mm)	Orientation
Superior longitudinal fasciculus	6-10	AP
Cingulum bundle	5-8	AP
Inferior longitudinal fasciculus	5-7	AP
Occipitofrontal fasciculus	4-6	AP
Uncinate fasciculus	3-5	AP
External capsule	2-6	AP
Fornix	2-8	AP
Corpus callosum	5-15	ML
Anterior commisure	2-5	ML
Internal capsule	5-12	SI

Figure 2.5 Features of the main white matter tracts

AP anterior-posterior; ML medio-lateral; SI superior-inferior, from Dejerine (1895, 1901).

2.4.1.2.2 Arcuate fasciculus

One of the most important connections in terms of language is the connection in the left hemisphere between Wernicke's area in the superior temporal association cortex and Broca's area in the premotor (ventrolateral) cortex, known as the arcuate fasciculus. The arcuate fasciculus is a large tract of fibres which sweeps around the insula and extends from the superior temporal region to the inferior frontal gyrus. The arcuate fasciculus is part of a more extensive tract, the superior longitudinal fasciculus, which in addition to connecting regions of the frontal and temporal lobes, also makes connections with the parietal lobe.

2.4.1.2.3 Uncinate fasciculus

Another important fronto-temporal connection is the uncinate fasciculus which is a hook shaped bundle of long association fibres which pass across the bottom of the lateral fissure uniting the gyri of the frontal lobes with anterior portion of the temporal lobe. This tract is considered to connect the medial temporal lobe with the orbital frontal lobe, and the inferior and lateral parts of the temporal lobe with the

lateral frontal lobe. In the detailed description of the uncinate fascicle in 10 human post-mortem subjects by Ebeling and von Cramon (1992), the fibres were found to originate from the anterior three temporal convolutions of the tip of the temporal lobe (BA 20 and 38) and the nuclei of the amygdala. The fibres then united in the anterior temporal stem and formed a tract of dimensions 3-7 mm in width and 2-5 mm in height which ran in the extreme and external capsule through the limen insulae (the floor of the basal part of the lateral fissure). The fibres then terminated in the gyrus rectus (BA11), medial frontal cortex (BA12), and sub-callosal area 25. The ventro-medial fibre bundle was found to connect the uncus, amygdala, and inferior temporal gyrus with the gyrus rectus and sub-callosal area. The dorsolateral bundle was found to connect the tip of the superior and medial temporal gyri with the inferior frontal gyrus, lateral and medial orbital gyri.

2.4.1.2.4 Cingulate fasciculus

The cingulum bundle, or cingulate fasciculus, is a 'C' shaped longitudinal bundle which runs beneath the cingulate gyrus connecting the frontal, parietal and temporal lobes. Its fibres extend from below the rostrum of the corpus callosum in the frontal lobe, they then arch around the corpus callosum proceeding to the parahippocampus, uncus, and surrounding regions of the temporal lobe. The anterior portion is strongly connected to the amygdala, nucleus accumbens, thalamus, and dorsolateral prefrontal cortex, and the posterior portion is connected with lateral and medial temporal lobe, parietal and orbitofrontal cortex (see Kubicki *et al.*, 2003).

2.4.1.2.5 Fornix

The fornix is the major pathway connecting the hippocampal formation with sub-cortical brain regions, and provides an indirect connection between the hippocampal formation and the prefrontal cortex. There is one fornix in each cerebral hemisphere which unite in the midline and are often described as one structure. Fibres enter the fornix via the fimbria of the hippocampus (the narrow strip of white matter on the medial aspect of the hippocampus). These fibers enter at the crus of the

fornix which arches upwards under the splenium of the corpus callosum, and then become continuous with the body of the fornix. Anteriorly the fornix splits into two columns which terminate in the mamillary bodies in each hemisphere.

2.4.1.2.6 Developmental processes

Abnormal connectivity could arise from mechanisms which determine neuronal connectivity early in life and/or those that continue throughout brain maturation (see Penn 2001). Early in development neurones from one brain region, after they have undergone cell specialisation, extend axons along specific pathways to target brain regions in order to form functional links. These are considered to be controlled by genetic and molecular factors and are characterised by an over-elaboration of synapse formation. The next stage is a refinement, or pruning, of these initially imprecise connections, a process which is thought to be 'activity-dependent'. In other words synapses are strengthened by the synchronous firing of pre- and post-synaptic neurones (Hebb 1949): or as in the popular *aide-mémoire*, "cells that fire together – wire together". Hence, *in utero* the overriding processes are genetic and epigenetic mechanisms, later there is greater emphasis on activity-dependent plasticity. In the human prefrontal and association cortex these activity-dependent processes are not thought to be complete until mid-adolescence (Huttenlocher 1997, and see McGlashan and Hoffman 2000), which coincides with the time when symptoms of schizophrenia generally become manifest. Although the foundations for neural connections that are laid down early in development are of importance, the consequences of altering the later activity-dependent refinement of these connections can also be considerable. Disruptions in these types of connections in animals during critical developmental periods have been shown to produce permanent behavioural and neurological deficits; depriving monkeys of normal sensory input, in terms of social interaction during infancy can result in irreversible pathological behaviours (Harlow 1958, and see Penn 2001), and temporary monocular visual deprivation in kittens has also been shown to permanently alter the pattern of connections in the visual cortex (Hubel and Wiesel 1965, and see Penn 2001).

2.4.2 Evidence for disrupted white matter.

As described above, the disruption of neural interactions thought to underlie the symptoms of schizophrenia is primarily considered to be a functional deficit which may, or may not, co-exist with localised structural white matter abnormalities. A number of studies have therefore aimed to examine whether anatomical substrates of altered connectivity are in fact present in those with the disorder. An overview of studies reporting evidence for localised disrupted structural white matter or neuronal connections from a range of imaging techniques and post mortem studies are described below. This summary will focus on those studies reporting specific, localised abnormalities rather than differences generalised to whole lobar regions. Functional imaging studies reporting evidence for abnormalities in functional integration in schizophrenia are described in the following section.

2.4.2.1 *Post-mortem studies*

Anatomical correlates of functional disconnectivity in schizophrenia could be a consequence of an alteration in the structural integrity of the white matter tracts (discussed below), or a physical change in the number, density, or morphology of synaptic connections (see Blanpied and Ehlers 2004). Post-mortem studies have in general reported inconsistent findings, and a comprehensive account of the post mortem literature in schizophrenia is beyond the scope of this thesis. For a detailed critical review see Harrison (1999). Post-mortem studies have however provided growing evidence for abnormal structural connectivity in schizophrenia.

Prefrontal: One of the most prominent post-mortem findings was reported by Selemon and colleagues. Increased neuronal density was found in Brodmann areas 9 and 46 (17% and 21% respectively) in patients compared to normal controls, with a non-significant reduction in cortical thickness (Selemon *et al.*, 1995, 1998). Since other studies indicated no difference in cell number in the frontal lobes (Pakkenberg 1993; Akbarian *et al.*, 1995), the authors proposed that this finding of increased neuronal density could result from a reduction in surrounding neuropil, and

consequent shrinkage of interneuronal spaces (Selemon *et al.,* 1995; Selemon and Goldman-Rakic 1999). Neuropil, the substance surrounding neurones, constitutes the principle components of cortical synapses. It comprises (a) dendrites and their spines, (b) synaptic terminals, (c) axons and collateral branches, (d) glia and their processes, and (e) vasculature. The 'reduced neuropil' hypothesis therefore suggests that the disturbances seen in schizophrenia may result from atrophy of neuronal processes in the prefrontal cortex, i.e. a deficit in neuronal *connections* (Selemon and Goldman-Rakic 1999). Indeed this theory of altered neuronal connectivity is further strengthened by neurocytochemical reports of decreased levels of synaptic protein expression, such as synaptophysin (a protein component of synaptic vesicles and a marker of synapse density) in the prefrontal cortex of patients with schizophrenia (Glantz and Lewis 1998), by studies reporting decreased density of dendritic spines (a measure of the amount of synaptic contacts between neurones, Garey *et al.,* 1998), and abnormalities of layer III pyramidal neurones in the dorsolateral prefrontal cortex (Rajkowska *et al.*, 1998), which are the origin of cortico-cortical projections (for more detail see Harrison 1999), all suggesting dysfunction of pathways to and from prefrontal regions. However other studies have shown that the size distribution of the actual nerve fibres in schizophrenic subjects is within the normal control range (Marner and Pakkenberg 2003), and other studies rather than finding increased neuronal density, report decreased (actually in area 10: Colon 1972, Benes *et al* 1986), or unchanged neuronal density (in areas 9, 46: Akbarian *et al.,* 1995). Furthermore, the majority of studies of the prefrontal cortex have concentrated on dorsolateral areas, so it remains unclear as to whether there are abnormalities in other subregions of the prefrontal cortex which have yet to be examined.

Thalamus: An early study reported a significant loss of neurones, astrocytes and oligodendrocytes in the mediodorsal nucleus of the thalamus in schizophrenic patients compared to controls (Pakkenberg 1990). This has since been confirmed by other studies also reporting decreased neuronal number in this region (Popken *et al.*, 2000, Young *et al.*, 2000). The study by Popken *et al* (1998) additionally discovered that the neuronal loss was restricted to the parvo and densocellular nuclei of the

mediodorsal nucleus, which is the source of the main subcortical input to the dorsolateral prefrontal cortex (Goldman-Rakic and Porrino 1985). Along with replicating this finding Young *et al* (2000) also found a lesser, but significant reduction in the number of neurones in the anteroventral-anteromedial nucleus of the thalamus in the schizophrenic patients, which projects to the limbic cortex (the cingulate and entorhinal cortices, Vogt *et al.*, 1987).

2.4.2.2 *Quantitative semi-automated structural studies*

There have been a number of studies employing quantitative measurements of white matter tracts from MR scans. However, due to difficulties in visualising and accurately measuring such regions, these studies have tended to focus on the most prominent fibre bundles, in particular the corpus callosum and fornix. Regarding the corpus callosum, this is often measured as a total area on mid-sagittal section, and then subdivided into regions based on anatomical criteria. In a meta-analysis of 11 studies published in 1995 it was reported that significant reductions were only found in a minority of cases, potentially due to methodological differences and small sample sizes (Woodruff *et al.*, 1995). Fornix measures have also not proved to be conclusive, with some studies reporting no case control differences (Zahajsky *et al.*, 2001), while others report quantitative increases in patient groups (Davies *et al.*, 2001). These inconsistencies again may be due to methodological differences, small sample sizes, and the difficulties in accurately measuring such structures by hand tracing.

2.4.2.3 *Gyrification studies*

Cortical folding begins *in utero* at around 16 weeks starting with the primary convolutions, and then proceeds through ordered developmental stages which are largely complete by birth (see Harris *et al.*, 2004; Narr *et al.*, 2004). It has been suggested that if abnormal patterns of gyrification exist in schizophrenia, then this would be of potential importance in establishing the timing of such disturbances. This measure is also considered to reflect cortico-cortical connectivity since it has

been shown that lesions to axonal tracts with inputs to specific regions results in abnormal sulcal-gyral patterns in the target areas (Rakic 1988, and see Harris *et al.*, 2004). Hence abnormalities in gyral patterning may reflect underlying disturbances in anatomical connectivity.

The level of cortical folding is often measured as the gyrification index. This is a ratio of the inner and outer contours of the convolutions; where the inner contour follows the pial surface into the depths of the sulci, and the outer contour refers to the line connecting maximal points of the gyral curvatures (see Vogeley *et al.,* 2000). Increased gyrification values therefore reflect increased gyral complexity. In terms of studies that have reported regionally defined measures of the gyrification index, the most consistently reported findings have included hypergyria of the right prefrontal region in male schizophrenic subjects (Vogeley *et al.*, 2000; Narr *et al.*, 2004), and increases in right temporal lobe gyrification (Kikinis *et al.*, 1994; Harris *et al.*, 2003). However, other studies have failed to report abnormalities in such areas (e.g. Highley *et al.*, 2003). Inconsistencies in the results perhaps reflect gender effects and methodological differences such as variability in the criteria for regional delineations (see Highley et al., 2003).

2.4.2.4 *Automated structural studies*

Automated analysis techniques (as described in the previous section, for example voxel based morphometry, VBM) have shown reductions in white matter in a number of brain regions including; anterior prefrontal regions (Paillière-Martinot *et al.,* 2001), in the anterior limb of the internal capsule (which connects the medial and anterior thalamic nuclei to the prefrontal cortex and cingulate gyrus, Sigmundsson *et al.*, 2001; Suzuki et al., 2002; Hulshoff Pol *et al.*, 2003; Zhou *et al.*, 2003) in the region of the superior occipitofrontal fasciculus (Suzuki *et al.*, 2002), in the region of the uncinate (Sigmundsson *et al.*, 2001; Spalletta *et al.*, 2003), and arcuate fasciculus (Spalletta *et al.*, 2003), the corpus callosum (Hulshoff Pol *et al.*, 2003), anterior commisure (Hulshoff Pol *et al.*, 2003) and in the regions of cortico-striatal and thalamo-cortical fibres (Spalletta *et al.*, 2003).

2.4.2.5 *Diffusion tensor imaging studies*

Diffusion tensor imaging (DTI) permits the examination of the coherence or orientation of diffusion of water molecules along white matter tracts, termed fractional anisotropy (FA). Reduced anisotropy implies more random diffusion of water molecules and hence reduced white matter integrity. Decreased anisotropy may therefore represent structural damage, or a disruption in the organisation or integrity of the white matter tracts. Decreased anisotropy has been associated with demyelinating diseases such as the leukodystrophies and multiple sclerosis, and comparisons have been made between the symptoms of schizophrenia and some of the clinical presentations of these demyelinating diseases, particularly if the prefrontal cortex is affected (see Davis *et al.*, 2003).

Buchsbaum *et al.*, (1998) were first to use this method to examine white matter within the frontal lobe and striatum in a small number of schizophrenic subjects and normal controls. Reduced anisotropy was found in the schizophrenic subjects compared to the controls in frontal regions adjacent to the putamen, and in temporal regions. Reductions have also been reported in the splenium, the thickest posterior part of the corpus callosum (Foong *et al.*, 2000a). Abnormalities have also been reported in the uncinate fasciculus (asymmetry: Kubicki *et al.*, 2002b, and reduced anisotropy: Burns *et al.*, 2003), in the arcuate fasciculus (Burns *et al.*, 2003), cingulate fasciculus (Kubicki *et al.*, 2003), and anterior cingulum (Wang *et al.*, 2004). Other studies have found no differences in anisotropy values for pathways to and from the cerebellum, (the superior and middle cerebellar peduncles, Wang *et al.*, 2003), or in frontal regions (Steel *et al.*, 2001). In two studies, decreased anisotropy was found to be less localised (Lim *et al.*, 1999; Minami *et al.*, 2003), and in two uncontrolled studies reduced anisotropy in inferior frontal regions was found to be associated with severity of negative symptoms (Wolkin *et al.*, 2003), and impulsivity (Hoptman *et al.*, 2002).

2.4.2.6 *Magnetisation transfer imaging studies*

Magnetisation transfer imaging, originally described by Wolff and Balaban (1989), generates contrast based on the exchange of proton magnetisation between water molecules and macromolecules in tissue (myelin and cell membranes). The magnetisation transfer ratio (MTR) provides a measure of myelin or axonal membrane integrity. MTR reduction has been shown to correlate with myelin and axonal loss in conditions such as multiple sclerosis (Gass *et al.*, 1994). Foong *et al.*, (2000b) used this method in schizophrenic subjects and healthy controls using a region of interest method and found reductions in MTR in the patient group predominantly in temporal regions. The same group reported reductions in left inferior frontal gyrus and right inferior temporal regions in the patient group using a global voxel-based method of analysis (Foong *et al.*, 2001). Bagary *et al.*, (2003) also used this imaging technique and reported reduced MTR in patients in their first episode of schizophrenia in the region of the uncinate fasciculus.

To summarise, although not entirely consistent, structural studies of white matter fibre tracts do begin to provide some evidence for disrupted connectivity in schizophrenia. Tracts reported to be abnormal include the uncinate fasciculus (connecting frontal and temporal regions), the arcuate fasciculus (connecting frontal, temporal and parietal regions), frontal-thalamic tracts, the cingulate fasciculus and fornix (connecting limbic regions), and the corpus callosum (inter-hemispheric connectivity).

2.4.3 Functional disconnectivity in schizophrenia

The view that there are disruptions in functional integration in schizophrenia is now increasingly commonly held. There are however surprisingly few functional imaging studies reporting direct empirical evidence, perhaps in part due to the fact that functional and effective connectivity analysis methods are still relatively new techniques with continuing methodological refinement. In the following section an

overview of the evidence for disrupted connectivity in schizophrenia from functional imaging studies will be reviewed (see appendix for summary table).

2.4.3.1 *Prefrontal-temporal*

One of the primary candidates thought to underlie the deficits of schizophrenia is a dysfunction of connectivity between the frontal and temporal lobes. The current re-emergence of the disconnection hypothesis in schizophrenia has, in part, been prompted by evidence of a reversal in normal prefronto-temporal interactions from PET studies of chronic schizophrenic subjects during word generation tasks, described above (Frith *et al.*, 1995a). Other indirect evidence to support this hypothesis, before the more widespread use of functional connectivity analysis techniques, came from the analysis of correlations between volume measurements of localised brain regions. These were proposed to be of interest with respect to functional integration since it has been suggested that positive correlations between regional brain volumes further strengthen the notion of a functional relationship between them. When connections between regions are forming *in utero*, reciprocal connections between regions may confer a mutually protective effect, and therefore subsequent growth of the anatomically connected regions is likely to be positively correlated. Reports of correlations between volumes of prefrontal and temporal lobe structures have however been conflicting, with some showing increased correlations (Wible *et al.*, 1995), and some showing reduced correlations in schizophrenic subjects compared to normal control subjects (Woodruff *et al.*, 1997a, Bullmore *et al.*, 1998).

There have been very few studies reporting direct evidence for disrupted fronto-temporal interaction in schizophrenia. The study by Friston *et al.*, (1996) used a multidimensional scaling approach to examine functional connectivity in patients with schizophrenia and normal controls performing a verbal fluency task in a PET scanner. This study involved three groups of 6 schizophrenic subjects, categorised according to task performance, and one group of 6 normal controls performing a word generation task. The multidimensional scaling technique transforms and

presents the data in such a way that the proximity of the relationship between regions reflects the degree functional connectivity between them (Friston *et al.*, 1996). In the normal subjects there appeared to be negative fronto-temporal connectivity, however in all three schizophrenic groups there was a markedly different organization. The superior temporal region moved across to areas spanning high positive correlations to independence with prefrontal regions, and in particular the poor performance group suggested an absence of connectivity between these regions. These results support the hypothesis of a disintegration of fronto-temporal connectivity, specifically between the left superior temporal gyrus and left dorsolateral prefrontal cortex.

The study by Lawrie *et al.*, (2002) used fMRI to examine connectivity in eight patients with schizophrenia and ten controls whilst they performed the Hayling sentence completion task. Connectivity was determined between two pre-specified regions of interest; the dorsolateral prefrontal cortex and the left middle/superior temporal gyrus. Here reduced connectivity was reported between these regions in the patient group, and in addition, this correlation was lowest in patients with auditory hallucinations than those without, although there were only three hallucinating subjects.

Shergill *et al.*, (2003) also examined connectivity patterns in patients with auditory hallucinations. This study performed fMRI scans of eight patients with a history of hallucinations and eight control subjects whilst varying the rate of inner speech. The time series data for the maximal response for fast versus slow covert articulation in the left inferior frontal gyrus was selected and entered as a 'covariate of interest' in order to examine functional connectivity with the rest of the brain. In the patient group there were reduced fronto-temporal interactions (between the left inferior frontal gyrus and the right superior/middle temporal gyri) along with reduced connectivity with other regions; right insula, right parahippocampal/fusiform gyrus, right precentral gyrus, and left medial parietal lobe.

There is also evidence of disturbed fronto-temporal interaction from EEG coherence data. It should be considered that although EEG provides excellent

temporal resolution, spatial resolution of this technique is inferior to that provided by both PET and fMRI methods (as described in chapter one), therefore regions are categorised more generally in these studies. One study of task-activation EEG data (the auditory oddball task) indicated disturbed fronto-temporal connectivity, both in schizophrenia patients, and in unaffected siblings (Winterer *et al.*, 2003). In another task-activation EEG study, twelve patients with schizophrenia and ten controls performed two tasks: talking aloud and listening to their own speech played back to them (Ford *et al.*, 2002, 2004). In certain EEG frequency bands patients failed to show the normal increase in coherence during the talking aloud condition, indicating a disruption in fronto-temporal integration in schizophrenia. Furthermore, this effect was reported to be stronger in those patients who hallucinated than those who did not.

A recent study examined task-uncorrelated measures of functional connectivity within the auditory cortex, using the approach described previously by Arfanakis *et al.*, (2000) to remove task effects (Calhoun *et al.*, 2004). Seventeen schizophrenic patients and seventeen control subjects were examined. The patient group demonstrated more dorsolateral synchrony within the auditory cortex than the controls, and the controls demonstrated more ventromedial synchrony than the patient group. Connectivity between auditory areas and prefrontal regions were not described, however these patterns of connectivity within the auditory cortex were found to reliably distinguish between patients and controls.

There are some studies however which suggest that alterations in fronto-temporal connectivity involve medial temporal lobe structures rather than lateral temporal lobe. Meyer-Lindenberg *et al.*, (2001) examined functional connectivity from PET data of 13 patients and 13 control subjects performing a working memory paradigm. Canonical variates analysis was used to characterise the differences in patterns of connectivity between patients and controls. This method involves computing a correlation between the imaging data and the set of regressors contained in the design matrix (here modelling group by time interactions). These are then

decomposed into a set of 'eigenimages' that represent the variance in this correlation. It was reported that 69% of the variance was attributed to two patterns of connectivity. The pattern in the patient group was characterised by loadings in the temporal lobe, particularly the inferior temporal region and the hippocampus, and cerebellum. In the comparison subjects the pattern was characterised by loadings in the dorsolateral prefrontal cortex and cingulate cortex bilaterally. Overall these results therefore suggest that disturbed prefrontal-hippocampal interactions may underlie schizophrenia. This is consistent with an earlier study by Weinberger *et al.*, (1992) who studied prefrontal function (assessed in terms of performance on frontal lobe tests) and hippocampal structure using neuroimaging in identical twins discordant for schizophrenia. This study reported a correlation between abnormalities in these areas only in the affected twins.

2.4.3.2 *Prefrontal-subcortical-cerebellar*

Other studies have implicated disruptions in subcortical and cerebellar connections to the prefrontal cortex in schizophrenia. Based on empirical findings of frontal, thalamic and cerebellar abnormalities in schizophrenia from post-mortem, structural, and functional imaging studies, Andreasen *et al.,* (1996) proposed a model suggesting that the core symptoms of schizophrenia could arise from abnormal connectivity between these regions. The term 'cognitive dysmetria' has been ascribed to this model and is used to infer the difficulties in prioritising, processing, co-ordinating, and responding to information which has been suggested to account for the diverse symptoms and deficits seen in schizophrenia. Studies that have examined nodes in this network or reported deficits in connectivity in these regions are described below.

One study, published in 1996 by Katz and colleagues, reported regional correlation patterns of metabolic rates of glucose in 18 never-medicated schizophrenic patients and 22 normal subjects during performance of the continuous performance test (CPT). Structures comprising two circuits were selected, and the correlation for each structure of the circuit with connected structures was examined.

The first circuit comprised of signals averaged from the main cortical regions (frontal, parietal, temporal and occipital), the basal ganglia and thalamus. The second more complex circuit comprised of the following areas: the left inferior frontal gyrus, whole temporal, parietal areas, the primary visual cortex, thalamus, caudate, putamen, globus pallidus, nucleus accumbens, ventral tegmentum, midbrain, anterior cingulate, hippocampus and amygdala. The main finding from this study, consistent across both circuits, was a more negative correlation between frontal regions and the thalamus in patients with schizophrenia versus controls. The authors suggest that this indicated these structures were operating differently between the groups. Additional differences were seen in the more extensive circuit including more positive correlations in the patient groups between inferior frontal and parietal areas, and the reverse between inferior frontal regions and temporal regions.

Stephan *et al.*, (2001) examined functional connectivity of the cerebellum using a seed voxel approach to investigate the effects of the atypical drug anti-psychotic olanzapine on 6 schizophrenic subjects (scanned in the drug free condition and treated) and 6 healthy controls performing a simple finger tapping task. The analysis suggested that treatment with this atypical antipsychotic drug caused widespread changes in functional connectivity of the cerebellum, including increases in connectivity with lateral and medial prefrontal regions, and decreased connectivity with the thalamus.

Schlösser *et al.*, (2003) examined effective connectivity from fMRI data from twelve patients with schizophrenia and six controls performing a 2-back working memory task using SEM. The schizophrenia group were further subdivided in to those on typical and atypical anti-psychotics. Compared to the normal controls, both patient groups showed reduced connectivity between left dorsolateral prefrontal regions and the right cerebellum, and between the cerebellum and thalamus, however connections between the left dorsal and ventral prefrontal cortex and thalamus were found to be enhanced. The authors suggested that the increased thalamo-cortical connections could represent a compensatory increased thalamic input in the presence

of the disrupted prefrontal-cerebellar connections. It should be noted however that there was a significant difference in performance between the study groups.

Another study examined the effects of nicotine on patterns of functional connectivity in 13 smokers with schizophrenia and 13 smokers with no history of mental illness (Jacobsen *et al.,* 2004). The subjects underwent two fMRI sessions performing an auditory *n*-back working memory task, once with a placebo patch and once with a nicotine patch. Functional connectivity was assessed using the seed voxel approach with the region of interest placed in the thalamus. Nicotine was found to enhance performance, and to enhance connectivity between medial prefrontal cortex and thalamic nuclei. This was modulated to a greater degree in the schizophrenic subjects at the most difficult task condition, leading the authors of the study to conclude that nicotine may enhance performance in the schizophrenic subjects by enhancing connectivity in the fronto-thalamic network.

Menon *et al.*, (2001a) examined functional connectivity within basal ganglia structures in 8 subjects with schizophrenia and 12 control subjects performing a motor sequencing task. Regions of interest included the caudate, lentiform nucleus (anterior and posterior regions) and thalamus. Analysis of covariance was used to examine connectivity, i.e. whether activation differences in one region of interest was accounted for by activation differences observed in another region of interest. The findings indicated that thalamic deficits in subjects with schizophrenia were accounted for by deficits in the posterior lentiform nuclei (posterior putamen plus globus pallidus).

2.4.3.3 *Prefrontal-parietal*

Disruptions in prefrontal-parietal networks have also been investigated in schizophrenia. These regions, along with other cortical areas such as the cingulate and temporal cortex, are considered to subserve cognitive functions such as working memory. Working memory deficits, or deficits in the process of holding and manipulating information on-line, are consistently reported in schizophrenia, and

abnormal activation in both prefrontal (Manoach *et al.*, 1999) and parietal (Quintana *et al.*, 2003) regions have been reported during tasks requiring such functions.

Kim *et al.*, (2003) investigated deficits in the working memory network in twelve patients with schizophrenia and twelve control subjects performing the *n*-back working memory task in the PET scanner. Correlations between activity in lateral prefrontal activation and other regional activations were computed. Marginal differences in performance between the groups were reported. In the control subjects the right lateral prefrontal cortex activity was found to be significantly correlated with activation in bilateral inferior parietal regions. In the task activation maps the patient group showed an absence of bilateral inferior parietal activation, and hence the pattern of normal prefrontal-parietal connectivity was not seen in this group, suggesting prefrontal-parietal functional disconnection in working memory processing in schizophrenia.

2.4.3.4 *Lateral-medial prefrontal*

As previously described, performance of the verbal fluency test in normal subjects involves activation of the dorsolateral prefrontal cortex and deactivation of superior temporal regions, and this relationship was not seen in schizophrenic subjects (Frith *et al.,* 1995a). The study by Spence *et al.*, (2000) sought to investigate functional connectivity relationships in a similar experimental design in schizophrenic patients, normal controls and in obligate carriers (unaffected relatives of schizophrenic probands who have both a parent and a child with schizophrenia, who are therefore assumed to have transmitted the genotype from one generation to the next without expressing the phenotype) during performance of a verbal fluency task. In this study the common maximally activated voxel within the left dorsolateral prefrontal cortex (determined by the combination of groups involved in each separate contrast) was used as a covariate of interest to derive maps of functional connectivity. Compared to the control subjects the obligate carriers had reduced connectivity between the dorsolateral prefrontal cortex and precuneus, but no differences in fronto-temporal connectivity. Comparing controls and schizophrenic subjects

suggested the patients had decreased connectivity between left dorsolateral prefrontal cortex and left anterior cingulate cortex, but not temporal regions. Comparisons between the obligate carriers and schizophrenic subjects also suggested reduced connectivity between left dorsolateral prefrontal cortex and anterior cingulate regions.

Medial prefrontal regions, specifically the anterior cingulate cortex, have also been implicated in the study by Fletcher *et al.*, (1999) using the psychophysiological (PPI) method, or regression analysis, whereby an assessment of the measured activity in a selected brain region is made in terms of the extent to which it predicts activity in other regions. This study examined twelve patients with schizophrenia and seven control subjects performing learning and recall of word lists in a PET scanner. In the control group the product of the activity in the prefrontal cortex and anterior cingulate was found to significantly predict the decrease in activity in the superior temporal gyrus, this relationship was not observed in the schizophrenic group. The authors suggested that this reflects a disruption of the cingulate modulation of the interactions between the prefrontal and temporal lobes.

2.4.3.5 *Widespread network deficits.*

Other studies examining functional connectivity have reported more extensive disruptions of integrative networks in schizophrenia. These are discussed below.

The study by Mallet *et al.*, (1998) examined patterns of connectivity from PET data in three groups of psychiatric patients (depressive, obsessive-compulsive and patients with schizophrenia) versus controls at rest. Six correlation coefficients were found to differ between controls and patients with schizophrenia: between contralateral frontal regions, between contralateral medial frontal regions, between right frontal and right posterior associative regions, between right frontal and medial occipital regions, between right temporal and medial occipital regions, and between left posterior associative regions and the right thalamus.

The study by Jennings *et al.*, (1998) examined networks involved in semantic processing in eight patients with schizophrenia and eight control subjects using structural equation modelling (SEM) of PET data. A set of regions shown to be involved in the semantic task were selected, these consisted of left inferior frontal gyrus (BA 45), left anterior cingulate (BA 32), bilateral middle frontal gyrus (BA 10), and left superior temporal gyrus (BA 22). The functional networks were found to be significantly different between the groups. The patient group showed more positive correlations between: the left inferior frontal gyrus and left middle frontal gyrus, between right middle frontal gyrus and left anterior cingulate, between left anterior cingulate and left middle frontal gyrus, and between left superior temporal gyrus and left anterior cingulate. In addition the patient group showed more negative correlations between: left inferior frontal gyrus and right middle frontal gyrus, between left anterior cingulate and right middle frontal gyrus, between left anterior cingulate gyrus and left inferior frontal gyrus, between left inferior frontal gyrus and left superior temporal gyrus, and finally between left inferior frontal gyrus and left anterior cingulate.

Josin and Liddle (2001) used a neural network to examine functional connectivity in PET data from 16 schizophrenic and 6 control subjects performing a word generation task. The neural network was trained on 2 controls and 7 schizophrenic subjects, and subsequently correctly classified the remaining 4 controls and 9 schizophrenic subjects. Connectivity patterns which differed between groups involved less positive correlations between left lateral prefrontal cortex and midbrain/thalamus in the schizophrenic group, greater positive correlations between left lateral prefrontal cortex and anterior cingulate cortex, and more negative correlations between left lateral prefrontal cortex and left lingual gyrus. Regions where there were negative correlations in controls, but positive correlations in schizophrenic patients were found between left lateral prefrontal cortex and left lateral temporal cortex and left inferior parietal lobe (Josin and Liddle 2001).

Welchew *et al.*, (2002) used multidimensional scaling techniques on fMRI data of 19 patients with schizophrenic and 20 controls subjects performing a semantic categorisation task with sub-vocal articulation of response. This report did not find global or local differences in the patterns of interregional correlations between groups, but did find in general greater variability in the interactions in the schizophrenic group.

2.4.3.6 *Discussion*

The conclusion from the above studies is that there is evidence emerging for widespread abnormal connectivity between the prefrontal cortex and other cerebral sites in schizophrenia. In particular deficits fall into the general categories of prefrontal-temporal, prefrontal-thalamic-cerebellar, prefrontal-parietal, and lateral-medial prefrontal connectivity. It is also clear from these studies that a wide variety of methods for examining connectivity have been used. This lack of standardisation causes problems for the generalisation of results. Differences not only include statistical analysis techniques but other methodological differences, including the types of tasks used. It may be the case that functional disconnectivity is task dependent, which could account for the range of connectivity deficits reported. Indeed studies are beginning to be reported which examine task uncorrelated measures of connectivity (see Arfanakis *et al.*, 2000; Calhoun *et al.*, 2004). At present most of the evidence for altered connectivity in schizophrenia suggests disruption of fronto-temporal connectivity; but this may in part be due to the fact that the majority of the studies examining connectivity have used tasks which involve these particular regions, for example word generation tasks, or tasks relating to inner speech. Evidence from the studies above also suggests dysfunctional fronto-temporal interaction may be related to the current symptomatology of the patient groups. Fronto-temporal disconnectivity was seen chronically ill patients (Frith *et al.*, 1995a, and in those with hallucinations (Lawrie *et al.*, 2002; Ford *et al.*, 2002; Shergill *et al.*, 2003) but was not reported in obligate carriers (Spence *et al.*, 2000). Indeed, this is consistent with previous activation studies of self-generated word production tasks

reporting abnormal temporal lobe activation in chronically ill or symptomatic patients (Frith *et al.*, 1995a; Fletcher *et al.,* 1996; Shergill *et al.*, 2000; Kircher *et al.*, 2001), but not in asymptomatic patients (Dye *et al.*, 1999).

There is also inconsistency in terms of connectivity seed selection criteria. Furthermore, detailed descriptions are not always explicitly outlined in the methods sections of these papers. A variety of options are possible. Seed regions may be selected *a priori*, or they may be selected based on areas of maximal activation, which in turn could originate from an 'all subjects' analysis, analysis of control, or patient groups separately, or even based on group differences. Also, connectivity can be examined based on prior hypotheses between specific regions, or alternatively at the 'whole-brain level' where the seed region is used as a reference with which to compare the time courses of all other cerebral voxels. It should be noted that the majority of studies have performed connectivity analysis on task activation data, which can artificially inflate interregional correlations, as described in the previous chapter. There are also issues relating to task performance differences between groups, described earlier in relation to findings of hypofrontality, which are not always considered. The extent of movement differences between groups are also not always described fully. In addition to these differences it is noted that the majority of studies have used a relatively small number of subjects. All of these factors could fundamentally impact on the connectivity results seen.

2.5 SUMMARY AND CONCLUSIONS

Schizophrenia is a highly heritable and disabling disorder. The use of new imaging techniques and advances in analysis methods have contributed greatly to the understanding of the illness. It is now well established that schizophrenia is associated with both structural and functional abnormalities, particularly but not exclusively, involving frontal and temporal brain regions. Along the lines of connectionist models of brain function, current theories suggest that the principal symptoms could arise from disconnections between brain regions. It is difficult to draw firm conclusions from these studies due to methodological variation. However

evidence is beginning to suggest underlying deficits in connectivity with prefrontal regions. It is also unclear whether abnormalities seen in the established state relate to the presence of symptoms, exposure to anti-psychotic medication, or to genetic vulnerability. The timing of these abnormalities is also uncertain. In order to address such issues, crucial to the understanding of the pathophysiology of the disorder, investigators have turned towards prospective studies of individuals before they become ill.

3 FUNCTIONAL LOCALISATION IN HIGH RISK SUBJECTS

3.1 INTRODUCTION

As described in the previous chapter, schizophrenia is a highly heritable disorder which generally becomes manifest in early adult life. The established condition has been shown to be associated with structural and functional brain abnormalities, most notably in prefrontal and temporal lobes, but previous studies have not been able to clarify the extent to which the abnormal findings relate to the presence of a schizophrenic predisposition (or genotype), or to the presence of symptoms (expression of the phenotype) and/or medication effects. This, in combination with evidence suggesting neurodevelopmental origins of the disorder, has lead to the prospective study of relatives of affected individuals, which allows the investigation of whether abnormalities predate development of the illness and reflect genetic vulnerability or 'trait' effects, or whether there are changes specifically associated with the manifestation of symptoms or 'state' effects. There is also increasing interest in this area due to the lack of knowledge regarding the nature of the prodromal phase of the disorder, and the possibilities of predicting those who may become unwell.

3.2 OVERALL AIMS OF THE STUDY

This thesis examines brain activation patterns in young subjects at high genetic risk of schizophrenia using functional magnetic resonance imaging (fMRI) techniques. Due to the design of the study it is possible to address the following issues in a situation un-confounded by the effects of anti-psychotic medication and prolonged illness. It has three main aims:

1) The primary aim was to identify the neural correlates of state and trait effects in these individuals in terms of functional localisation and functional integration. Based on findings in the established condition it was hypothesised that if abnormalities in prefrontal and temporal brain regions are related to inherited vulnerability ('trait') then they would be observed in high risk individuals. Conversely, if they were related to phenotypic expression

('state') then they may be observable in those at high risk displaying some of the characteristic symptoms of the disorder.

There were two further subsidiary aims should any subjects go on to develop the disorder over the course of the study:

2) To determine if it is possible to distinguish those who become ill using functional imaging approaches (fMRI). It was hypothesised that state and/or trait abnormalities identified above may be seen to a greater extent in those who, although well at the time of assessment, subsequently become ill.

3) To determine if changes in patterns of brain activity occur with the transition to illness, or the development or improvement of psychotic symptoms.

These two secondary aims were considered exploratory given the rarity of the data and the small number of subjects likely to make the transition.

The analysis presented throughout this thesis pertains to the first 100 subjects seen in the current phase of the Edinburgh High Risk project. This chapter will describe a functional localisation study of high risk individuals from the Edinburgh High Risk Study performing the verbal initiation section of the Hayling Sentence Completion Test to address the first aim of the study. The following chapter also addresses this aim in terms of functional integration. The baseline analyses presented in these chapters reflects the clinical status of the subjects at the time of the first fMRI assessment. The analyses presented in the remaining chapters (regarding those who have developed schizophrenia), addressing the second and third aim of this thesis, are based on clinical data collected at the end of April 2004. It should be appreciated however that this work forms part of a large longitudinal project. Consequently, the status of the individuals under study may still change, which could affect these findings.

This chapter will begin with a brief introduction to the high risk research strategy. A full description of the background of high risk studies has been presented elsewhere, Byrne (2000a).

3.2.1 High risk studies: background

In the past, prospective longitudinal national birth cohort studies of large numbers of individuals from the general population have been undertaken (Done *et al.*, 1994, Jones *et* al., 1994). These studies provided evidence that those who go on to develop schizophrenia show abnormalities of language and behaviour compared to those who do not (Done *et al.*, 1994, Jones *et a*l., 1994). However, the difficulties involved in following such a large number of subjects (required since the lifetime risk within the general population is estimated to be around 1%) until the typical age of onset, can be prohibitive. For this reason cohorts selected with a higher chance of developing schizophrenia than the general population have been undertaken; the high risk research strategy.

Since the most important risk factors for developing schizophrenia are genetic, this has meant recruiting participants who have relatives affected with the disorder. This strategy was originally developed in the 1950s and 60s with the aim of following children who were at higher risk of developing schizophrenia than the normal population due the presence of a positive family history. A number of high risk studies have been undertaken throughout the world, including the New York High Risk project (Erlenmeyer-Kimling *et al.*, 1993), the Copenhagen High Risk project (Cannon and Mednick 1993), and the Israeli High Risk study (Mirsky *et al.*, 1995). For a review of these and other high risk studies see Niemi *et al* (2003). However, these studies suffered from the long delay between subject recruitment during childhood and the individuals reaching the period of maximum risk of onset of schizophrenia. This has lead to the diversification of the high risk strategy, for example, by recruiting adolescents or young adults of affected relatives with the aim of examining them over the time period of maximum risk of developing the disorder (Johnstone *et al.*, 2000, 2002a), by selectively recruiting from populations who are

already displaying some of the characteristic symptoms of the disorder (the 'ultra high risk' approach; see Yung *et al.*, 2003), or by studying those with the highest genetic risk, i.e. monozygotic twins discordant for schizophrenia (Weinberger *et al.*, 1992).

3.2.2 High risk studies: neuroimaging

The opportunity to prospectively examine such high risk individuals with neuroimaging techniques is now being addressed by several research groups with a view to determining the timings of when such abnormalities arise, and characterising the structural brain abnormalities seen in the established state and their relation to genetic liability. The latter is often referred to as endophenotype, a term used to describe measurable traits that represent internal or intermediate phenotypic expression of underlying genetic susceptibility to disease (Gottesman and Gould 2003). It is proposed that if abnormalities in unaffected high risk relatives are similar to abnormalities seen in patient groups then this would suggest a presumed genetic origin (whereas, if differences lie closer to controls, this would suggest that the deficits seen in the patient group were disease specific effects).

The broad term 'high risk' will be used to refer to individuals with a higher genetic risk than the general population due to the presence of a positive family history, however it should be considered that the subject's age is also an important factor, due to the typically restricted age of onset of schizophrenia. Older high risk subjects may be past the age of maximum risk of developing the disorder and therefore in a different risk strata than younger high risk relatives. The individuals in the current project are part of the Edinburgh High Risk Study, and are all young high risk subjects (recruited between the ages of 16 and 25 years). It should also be noted that, for simplicity, the origins of abnormalities are categorised according to the nomenclature of 'trait' (presumed genetic origin), or 'state' (related to phenotypic expression) effects. However, it should be appreciated that in reality this distinction is likely to be more complex; presumed genetic factors could be related to early environmental factors, or to interactions between genetic vulnerability and

environmental influences. It should also be noted that at baseline none of the study participants met criteria for schizophrenia, therefore the term 'state' effects is used to describe early phenotypic expression of some of the characteristic symptoms of the disorder.

3.2.2.1 *Structural imaging*

3.2.2.1.1 Semi-automated analysis

On region of interest tracing of MR images, abnormalities observed in high risk subjects generally fall in between those seen in normal controls and those seen in the established illness, suggesting an interaction between genetic and disease specific effects. By far the most replicated finding is a reduction in volume of the hippocampus, or amygdala-hippocampal complex, compared to controls, and increases in comparison to patient groups. This is seen both in older high risk relatives (Seidman *et al.*, 1999, 2002; O'Driscoll *et al.*, 2001; Steel *et al.*, 2002; van Erp *et al.*, 2002; Tepest *et al.*, 2003), and in young subjects before the typical age of illness onset (Schreiber *et al.*, 1999; Keshavan *et al.*, 2002a), and in our own young high risk subjects (Lawrie *et al.*, 1999, 2001). Twin studies also report decreased hippocampal volume in monozygotic twins discordant for schizophrenia compared to healthy monozygotic twins (Baaré *et al.*, 2001). However, a few studies report no differences between relatives and controls (Staal *et al.*, 2000; Schulze *et al.*, 2003), or even increases (in well parents of patients with schizophrenia, Harris *et al.*, 2002).

Thalamus reductions are also reported by a number of studies versus control subjects (Lawrie *et al.*, 1999, 2001; Seidman *et al.*, 1999; Staal *et al.*, 1998). In our own study of young high risk relatives increased genetic liability to the disorder was found to be associated with decreased volumes of bilateral thalamic nuclei (and prefrontal lobes, Lawrie *et al.*, 2001). Studies of other brain regions, such as the ventricular system have been less consistent (Lawrie *et al.*, 2001).

3.2.2.1.2 Automated analysis

Automated analysis has permitted the examination at the whole brain level rather than focussing on pre-determined regions of interest. In our own study of young high risk relatives, reductions in the grey matter of the bilateral anterior cingulate gyrus were reported compared to control subjects (Job *et al.*, 2003). Reductions in medial temporal regions were also confirmed by such techniques. In general these regions are consistent with the manual and automated methods both in high risk subject and in those with the established illness.

3.2.2.2 Functional imaging

3.2.2.2.1 PET and SPECT

Functional imaging studies in high risk relatives have also been conducted but are fewer in number than structural imaging studies (see summary table in appendix). Berman *et al.*, (1992) used PET scanning to examine monozygotic twins discordant for schizophrenia performing the Wisconsin card sorting test. In all of the 10 discordant twin pairs, the individuals with schizophrenia demonstrated hypofrontality compared with the unaffected co-twin indicating hypofrontality may be related to non-genetic factors. However, Blackwood *et al.*, (1999) performed SPECT scanning in 19 schizophrenic patients, 36 first degree relatives and 34 healthy controls and reported decreased perfusion of the left inferior prefrontal cortex and anterior cingulate region in both patients and relatives compared to the controls, suggesting a genetic contribution to prefrontal functional deficits.

Spence *et al.*, (2000) performed PET scans on 10 obligate carriers (phenotypically normal individuals lying between two affected generations, typically parent and child), 10 patients with schizophrenia, and 10 healthy controls performing a verbal fluency test. No differences in activation were reported between the obligate group and controls, however the patient group exhibited over-activation of the precuneus compared to the high risk relatives. The main differences reported were prefrontal functional connectivity differences between the groups. The obligate group

showed decreased connectivity between the left dorsolateral prefrontal cortex and precuneus compared to the controls, and the patients demonstrated decreased connectivity between the left dorsolateral prefrontal cortex and anterior cingulate.

3.2.2.2.2 fMRI

Studies of high risk relatives have also been conducted with fMRI. In a small preliminary study using high field fMRI (3T), Keshavan *et al.*, (2002b) examined activation patterns in four high risk subjects and four controls performing a memory guided saccade task. The high risk subjects demonstrated hypofunction of bilateral dorsolateral prefrontal cortex, right middle frontal gyrus and right inferior parietal cortex.

In a larger fMRI study of 23 high risk subjects and 18 healthy controls (replicated in 25 relatives and 15 controls) performing the *n*-back working memory task, unaffected relatives showed an increased response in the right dorsolateral prefrontal cortex (Callicott *et al.*, 2003). Many other widespread differences were reported in this study, which may be attributed to the low statistical threshold applied ($p<0.01$ uncorrected), however the main prefrontal results were replicated in the additional group of subjects.

In another fMRI study, this time examining 12 relatives and 12 healthy controls performing an auditory working memory task, the relatives showed increased activation of the prefrontal cortex, and thalamus. After controlling for task performance, greater activation of the anterior cingulate region was reported in the high risk relatives (Thermenos *et al.*, 2004).

Overall these studies demonstrate altered prefrontal functioning in high risk relatives, both in lateral and medial prefrontal regions. However the direction of these differences is less consistent.

3.2.3 The Edinburgh High Risk Study

3.2.3.1 *Background*

The Edinburgh High Risk Study began in 1994 and is funded by the Medical Research Council. The original idea for this study was conceived by Professor Johnstone. The study serially examines, in comparison with matched healthy controls, young people (aged between 16-25 at ascertainment) who are at increased risk of schizophrenia for genetic reasons (Johnstone *et al.*, 2000). The design of the study differs from other high risk research since subjects are recruited in early adult life, i.e. over the time period of greatest risk of developing the disorder, to address the difficulties seen in long follow-up studies such as high attrition rates and the superseding of data collection and analysis techniques (Johnstone *et al.*, 2000). Subjects were recruited based purely on having two or more first or second degree relatives with the disorder, and were not selected based on emerging symptomatology, distinguishing this study design from the ultra-high risk approach described above.

3.2.3.2 *First phase (1994-1999)*

This first phase of the Edinburgh High Risk study (1994-1999) employed repeated assessments every 18-24 months, including clinical and neuropsychological assessments, and structural brain imaging. On completion of recruitment, at the end of 1998, 229 high risk individuals had been identified. At baseline assessment 162 high risk participants from 110 families provided some data; clinical, neuropsychological, or had a structural MRI scan. Due to rolling recruitment, smaller numbers reached the 18-24 month follow-up assessments, but a substantial proportion of those due for assessment (n=80) were assessed on a second occasion. Finally, 29 had a third assessment (Johnstone *et al.*, 2000). Ten to fifteen percent of the high risk group were predicted to develop schizophrenia by the age of 30 years on the basis of the known frequency of schizophrenia in individuals with this degree of heredity, and the actual occurrence of schizophrenia by this age (Johnstone *et al.*,

2002a; Craddock and Owen 1996). These numbers were used to determine the sample size of the control groups of which there were two; a matched normal healthy control subjects with no known psychotic relatives (n=36), and a matched group of individuals in their first episode of the disorder (n=37; the latter group were not followed longitudinally). For further details see Johnstone *et al*, (2000).

As background, a summary of the clinical and neuropsychological findings from this first phase are presented below. Findings from the structural imaging analyses have been described above (Lawrie *et al.*, 1999, 2000; Job *et al.*, 2002), and findings relating to changes over time in high risk subjects will be described in a following chapter.

3.2.3.3 *Main findings from the first phase*

3.2.3.3.1 Demographic and clinical measures

In terms of demographic details, at baseline there were no significant differences between high risk and controls subjects in age, gender, or social class at birth (paternal) (Johnstone *et al.*, 2000). There were also no significant differences in terms of alcohol, cannabis or other drug use (Johnstone *et al.*, 2000). The high risk subjects did however present poorer educational and employment attainment, and had more social work contact than the normal controls (Johnstone *et al.*, 2000).

In terms of psychopathology, high risk subjects presented more symptomatology than controls, including partial and definite psychotic, and non-psychotic symptoms. Isolated psychotic symptoms occurred in 2-3 times as many subjects as were expected to develop schizophrenia, but was not found to be associated with genetic liability. However, in those subjects that have gone on to develop the disorder, the florid condition has been preceded by isolated psychotic symptoms in approximately 70% of cases (Johnstone *et al.,* 2002a).

3.2.3.3.2 Neuropsychological findings

In terms of neuropsychology, the main functional domains of current and premorbid intellectual function, executive function, mental control/encoding, perceptual motor speed, language, learning and memory, and attention were tested (see Byrne *et al.*, 1999, 2003; Cosway *et al.*, 2002). At baseline the high risk subjects were found to perform worse than controls and better than first episode subjects in a number of tests. Many of these differences did not survive controlling for IQ, which was significantly lower in the high risk group; however significant differences in the domain of executive function remained (Byrne *et al.*, 1999, 2003). Comparing neuropsychological functioning in those with psychotic symptoms versus those without revealed no significant differences, suggesting that deficits in performance seen against the controls could not be accounted for by the presence of psychotic symptoms. Also, many more high risk subjects than those expected to develop schizophrenia presented impaired neuropsychological performance with respect to controls. For example, 41% of the high risk group were reported to have scores on the Rivermead Behavioural Memory Test one standard deviation below the mean of the control group. A similar pattern was seen for other tests of learning and memory, and executive function (Byrne *et al.*, 2003). In addition, significant associations were seen between genetic liability and performance on a number of neuropsychological tests. Increased genetic vulnerability was found to be related to decreased performance on the Hayling test (Section B, Type A errors), the Rivermead story (delayed recall), and semantic verbal fluency (Byrne *et al.*, 2003).

Overall, these findings indicate that high risk individuals inherit a state of vulnerability to the disorder manifested by the presence of neuropsychological impairments (Byrne *et al.*, 1999, 2003), psychopathology (Johnstone *et al.*, 2000, 2002a) and structural brain abnormalities (Lawrie *et al.*, 2001; Job *et al.*, 2003). Whether similar patterns of findings would be seen with functional imaging, and whether extremes of these abnormalities can predict those who subsequently become unwell, are amongst the aims of the current phase of the Edinburgh High Risk study.

3.2.3.3.3 Second phase (1999-2004)

In the current phase of the study (1999-2004), a covert verbal initiation fMRI activation paradigm, the Hayling sentence completion test, has been added to the tests used in the first phase to examine these issues. This test had previously been shown to elicit activity in areas which have been repeatedly implicated in the disorder, i.e. frontal and temporal lobes (Burgess and Shallice 1997), and had been shown to distinguish between schizophrenic subjects and controls (Lawrie *et al.*, 2002). This test was also selected with the aim of examining connectivity in these individuals, which is the focus of the following chapter. This chapter will describe the baseline functional localisation analysis of these young high risk subjects performing the Hayling Sentence Completion Task (section A).

A number of high risk participants in the current study reported, on direct questioning at standardised interview, isolated and/or transient psychotic symptoms in the presence of unimpaired functioning, although none of the participants in this current study met diagnostic criteria for any psychiatric disorder at the time of this investigation. This study therefore allowed consideration, not only of the way in which regional brain function differs between normal controls and those at enhanced risk (referred to in this thesis as 'trait' effects), but also how that function is altered in those with isolated psychotic symptoms (referred to as 'state' effects; although it should be remembered that these subjects did not meet criteria for schizophrenia), all in a situation uncontaminated by the effects of prolonged illness and /or anti-psychotic medication. It was hypothesised that if abnormalities in frontal and temporal regions were related to inherited vulnerability, or if they were symptom-related effects, then they would be observed as trait or state effects respectively in these high risk individuals.

3.3 METHODS

3.3.1 Study populations

Subjects were recruited in the first phase of the Edinburgh High Risk study between 1994 and 1998 along with an age matched normal control group. Recruitment details have been presented previously (Hodges *et al.*, 1999; Byrne 2000b; Johnstone *et al.*, 2000; Johnstone *et al.*, 2002). Recruitment originally centred in Edinburgh and Southeast Scotland, but later extended to include Argyll and Clyde, Borders, Galloway, Forth Valley, Highlands and Islands, and the towns of Dumfries and Perth. Potential high risk participants were identified from families with at least two family members with a confirmed diagnosis of schizophrenia using the OPCRIT (Operational Criteria Checklist) computer program (McGuffin *et al.,* 1991). Individuals were identified by examining case-notes of patients with schizophrenia known to individual hospitals. Where there appeared to be two related cases, consent was sought to speak to a healthy family member to obtain a detailed family history to determine if there were was a possibility of there being a close relative between the ages of 16-24 years. As described above, a total of 229 people were identified, and 162 provided some data in the first phase of the study. The control group were recruited from youth groups in Edinburgh or from the social network of the subjects themselves, and had no known family history of the illness.

This chapter presents the results from the first 100 subjects reviewed in the second phase of the study. At the time of the current study, all participants regarded themselves as being in good health and functioning well. Basic demographic variables are presented in Table 3.1. Out of the first 100 subjects, six did not participate in scanning. A further four scans were subsequently excluded (two due to minor vascular malformations, and two due to movement artefact, see below), leaving 90 fMRI scans: from 21 normal controls and 69 high risk subjects. Of these remaining subjects the high risk sample originated from 50 families with between one and three family members involved in the study. The control group originated from 16 families with between one and two family members involved in the study.

Around the time of the scan all subjects underwent a structured psychiatric interview to identify any psychotic symptoms (the Present State Examination, PSE, Wing *et al.,* 1974). A simple scoring system (Johnstone *et al.*, 2002) was administered by three experienced clinicians (Professor E Johnstone, Professor D Owens and Dr S Lawrie). A score of 4 was assigned for definite schizophrenia based on the PSE, and a clinical diagnosis of schizophrenia in terms of the ICD-10 (World Health Organisation, 1993). A score of 3 was assigned for any fully rated psychotic feature from PSE items 55-92 (including thought reading, echo broadcast; auditory, visual or other hallucinations; delusions of control, misinterpretation, reference, persecution, grandiosity, influence or other) and from PSE items 128-9 and 135-7 (blunted affect, incongruous affect, neologisms or idiosyncratic use of words, incoherence of speech, flight of ideas). A score of 2 was assigned if any of the features of 3 were partially held or present to a mild degree, plus items 49-54 (perceptual disorders other than hallucinations) and behavioural items 108-9, 118, and 125-6 (self neglect, bizarre appearance, behaves as if hallucinated, suspicious, perplexed) fully rated, and items 133 (muteness) partially or fully rated. In essence:

4 = schizophrenia
3 = fully rated psychotic symptoms
2 = partially rated psychotic symptoms
1= fully or partially rated non-psychotic symptoms
0 = no symptoms.

For the purposes of this study subjects were classified according to the presence of psychotic symptoms (fully or partially held: scores 2/3) or absence of psychotic symptoms (scores 0/1), or schizophrenic (score 4).

Twenty seven high risk subjects reported the presence of psychotic symptoms (usually isolated delusions or hallucinations); the remainder of the high risk group and all of the controls had no such symptoms (Table 3.1). At the time of the baseline fMRI scan none of the subjects were on anti-psychotic medication, seeking treatment, or indeed saw themselves as unwell; they therefore could not be said to fulfil operational diagnostic criteria for any psychiatric disorder. It was subsequently

discovered that one of the high risk subjects had been referred by his GP with positive symptoms. However this referral had not been reported at the time of the PSE assessment and it has not been possible to access hospital notes for further clarification. However, since this subject did not meet criteria for any psychiatric disorder at the time of assessment, was not functionally impaired, and was not on medication, he was therefore included as a high risk participant in the current and subsequent analyses. In addition, a further high risk subject reported having had very fleeting psychotic symptoms whilst abroad, and as described by her at the time of the PSE this did not appear to be anything more than transient/partial psychotic symptomatology. At the time of assessment this subject did not fulfil criteria for any psychiatric disorder, had returned to work, was not receiving medication, and was not suffering any functional impairment. Based on the data that was available at the time, this subject was included as a high risk participant in this and subsequent analyses. Throughout this thesis 'high risk without symptoms' and 'high risk with symptoms' will be used to refer to those without and with *psychotic* symptoms.

Where possible the PSE was conducted on the same day as the scan, where this was not possible (for example due to work commitments of the subjects, or difficulties co-ordinating scanner slots) they were conducted as near in time as possible. Since the PSE assessment covers symptoms over the previous month, these small delays were not considered a major issue, and in fact in the majority of cases the PSE assessment and scan were conducted within 3 days of each other (n=60, 67%). Overall the mean time in days between the time of the PSE and the scan was 9 (sd 15.2), 14 (sd 33.6), and 5 (sd 9.2) for the controls, high risk without symptoms and high risk with symptoms respectively. Five subjects (one control, three high risk without symptoms, and one high risk with symptoms) had to be re-scanned due to scanner malfunction at the time of their original visit. The time intervals between the scan and clinical assessments for these subjects was therefore longer, >100days. There was no significant difference between groups for the time intervals between the scan and PSE assessment. All subjects provided written informed consent, and the study was approved by the relevant ethics committees.

Table 3.1 Demographic details.

	Controls ($n = 21$)	High risk without symptoms ($n = 42$)	High risk with symptoms ($n = 27$)
Mean age (std dev)	26.8 (2.7)	26.8 (3.4)	25.1 (3.1)
Gender (male:female)	13:8	17:25	13:14
Mean NART IQ (std dev)	97.95 (24.02)	99.56 (18.12)	97.86 (10.60)
Handedness (R:L:mixed)	19:2:0	39:2:1	21:4:2
Categorical genetic liability ($1^{st°}$:$2^{nd°}$)*	N/A	32:10	16:11
Continuous genetic liability	N/A	0.3005 (0.1848)	0.2355 (0.1742)

*any 1^{st}, or only 2^{nd} degree relatives with schizophrenia

3.3.2 Scanning procedure

Imaging was carried out at the Brain Imaging Research Centre for Scotland (Edinburgh, UK) on a GE 1.5 T Signa scanner (GE Medical, Milwaukee, USA) equipped with 23 mT/m "Echospeed" gradients having a rise time of 200 μs. After a localiser scan, subjects were imaged with a multislice T2-weighted fast spin-echo sequence (TR/TE = 6300/102 ms). Twenty slices (5 mm thickness, 1.5 mm gap), aligned parallel to the anterior commisure-posterior commisure (AC-PC) line, covered the brain. A structural scan with 1 mm pixel size was next acquired using a 3D inversion recovery-prepared T1-weighted sequence (inversion time, TI = 600 ms). 124 slices (thickness 1.7 mm) were aligned perpendicular to the AC-PC line. Finally an axial gradient-echo planar images (EPI) (TR/TE = 4000/40 ms; matrix 64×128; FOV 220×440 mm) were acquired continually during the experimental paradigm. Thirty eight contiguous 5 mm slices were acquired within each TR period. Each EPI acquisition was run for 204 volumes of which the first 4 (baseline) volumes were

discarded. Imaging data were transferred to a multiprocessor computer in the Centre for Functional Imaging Studies.

3.3.3 Experiment

The participants in the study performed the verbal initiation section of the Hayling sentence completion test (Burgess and Shallice 1997; Lawrie *et al.*, 2002a) in the scanner. This paradigm was originally adapted for use in a functional imaging environment by Professor C Frith at the Functional Imaging Laboratory, Institute of Neurology in London. Paradigms were programmed in E-Prime (Psychology Software Tools (PST), Pittsburgh, PA) by Dr H Lakany. Instructions and stimuli were presented visually on an LCD display mounted on the head coil (IFIS; PST, Pittsburgh, PA) and corrective lenses were used where necessary. Subjects were provided with left- or right-hand pushbutton units (IFIS; PST, Pittsburgh, PA) depending on their own preference, to allow their reaction times to be logged by the software. Subjects were shown sentences with the last word missing and were asked to silently think of an appropriate word to complete the sentence (i.e., without speaking the word), and press a button when they had done so. Verbal instructions were given prior to scanning and were standardised across subjects. Sentences were selected from Bloom and Fischler's set of sentence completion norms (Bloom and Fischler 1980). The task was adapted for fMRI to have four levels of difficulty, according to the range of suitable completion words suggested by the sentence context (cloze probabilities for the most frequent response of 0.95-1.00 were used for the high constraint condition; 0.74-0.80 for medium high; 0.53-0.59 for medium low; 0.09-0.26 for low constraint). Constraint levels high to low therefore represent increasing task difficulty. Examples of each difficulty level are presented in Table 3.2.

Table 3.2 Examples of sentences from each constraint level and corresponding cloze frequencies.

Constraint	Example sentence	Frequencies of responses*
High	"He mailed the letter without a ....."	STAMP (0.99)
Medium high	"The train was still on ....."	TIME (0.79); SCHEDULE (0.09); TRACK (0.03); COURSE (0.02); HOLD (0.02); ROUTE (0.02)
Medium low	"Not even the cast liked the ....."	PLAY (0.58); SCRIPT (0.17); SHOW (0.11); PERFORMANCE (0.04); PRODUCTION (0.03); DIRECTOR (0.02); STORY (0.02)
Low	"Rushing out he forgot to take his ....."	COAT (0.24); WALLET (0.15); LUNCH (0.13); BOOKS (0.11); KEYS (0.10); HAT (0.06); VITAMIN (0.05); PILLS (0.04); UMBRELLA (0.04)

*from Bloom and Fischler 1980

A schematic showing the basics of the experimental design are shown in Figure 3.1. Sentences were presented in blocks of fixed difficulty. Each block lasted 40 seconds and included eight sentences. Sentences were presented for a period of 3 seconds followed by a fixation cross for 2 seconds. Subjects were instructed to respond at any time (by button press) until the next sentence appeared. The rest condition consisted of viewing a screen of white circles on a black background for 40 seconds. The order of the blocks was pseudo-random, and each block was repeated four times (different sentences were used for each sentence block). This design allowed both a standard subtraction (sentence completion versus rest) and parametric analysis (examining areas of increasing activation with increasing task difficulty). Four sequences of presentation were used, which were cycled between subjects. During the same scanning session a further functional imaging study was performed (a verbal encoding and retrieval task), the results of which are not discussed here.

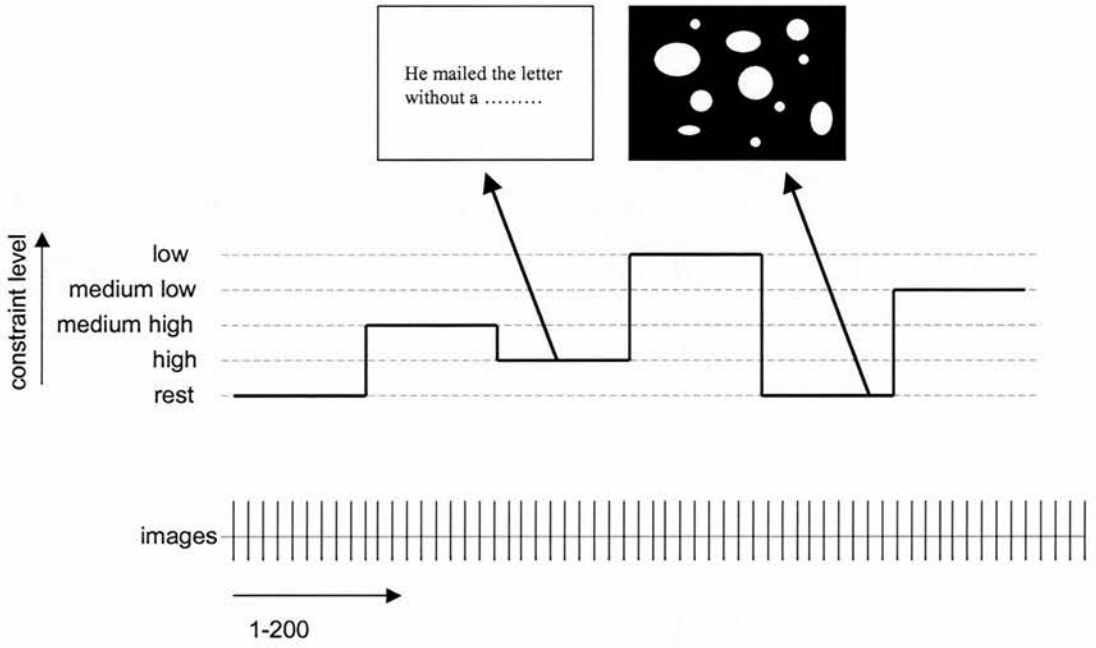

Figure 3.1 Experimental design

Eight sentences presented per block, each sentence presented for 3 s, fixation cross for 2 s (block length 40 s). Ten volumes collected per block, time to repeat (TR) was 4 s. Four levels of task difficulty (increasing difficulty; high to low sentence constraint)

3.3.4 Behavioural data

Immediately after scanning, subjects were given the same sequence of sentences on paper and requested to complete each sentence with the word they first thought of in the scanner. If they couldn't remember the word they first thought of in the scanner they were requested to fill in the first word that came to them at that time and add an asterisk next to this sentence. 'Word appropriateness' scores were determined from the word frequency list of sentence completion norms (Bloom and Fischler, 1980) which provides respective probabilities of possible responses. A score of one was given to the most frequently produced word in the word frequency list, a score of two for the next most frequently produced word etc. Mean scores for both word appropriateness and reaction time were determined for each constraint level. The

mean number of words, the mean number of reaction times recorded, and the mean number of asterisks reported for each constraint level were also determined. For a number of subjects, (6 controls, 8 high risk without, and 5 high risk with symptoms), no reaction time measures were recorded in the scanner. Since these subjects presented activation patterns consistent with performance of the task, and since they indicated at debriefing that they had indeed performed the task in the scanner, they were included in the analysis.

3.3.5 Scan processing

The EPI images were reconstructed offline into ANALYZE format (Mayo Foundation, Rochester, MN, USA). Scan analysis was performed using SPM99 (The Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, http://www.fil.ion.ucl.ac.uk/spm/) running in Matlab, version 5.3 (The MathWorks, Natick, MA, USA), and was performed blind to the group status of the individual.

3.3.5.1 *Discarded acquisitions and setting the origin*

Initially the first four volumes were discarded to ensure that brain magnetisation had reached steady state before the paradigm began (to allow for these discarded acquisitions there was a 16 s delay before the initial onset of the stimuli). The next step was to manually set the origin of the images. This was performed using the 'display' function in SPM. The anterior commisure was visualised in axial, sagittal and coronal section, the co-ordinates were noted, and then the 'header edit' function in SPM was used to define these co-ordinates as the origin for all the images in the acquisition. This was to ensure optimal starting estimates for the pre-processing steps to follow. A figure detailing the remaining steps involved in the pre-processing of the EPI volumes (n=200) and first level statistical analysis is shown in Figure 3.2.

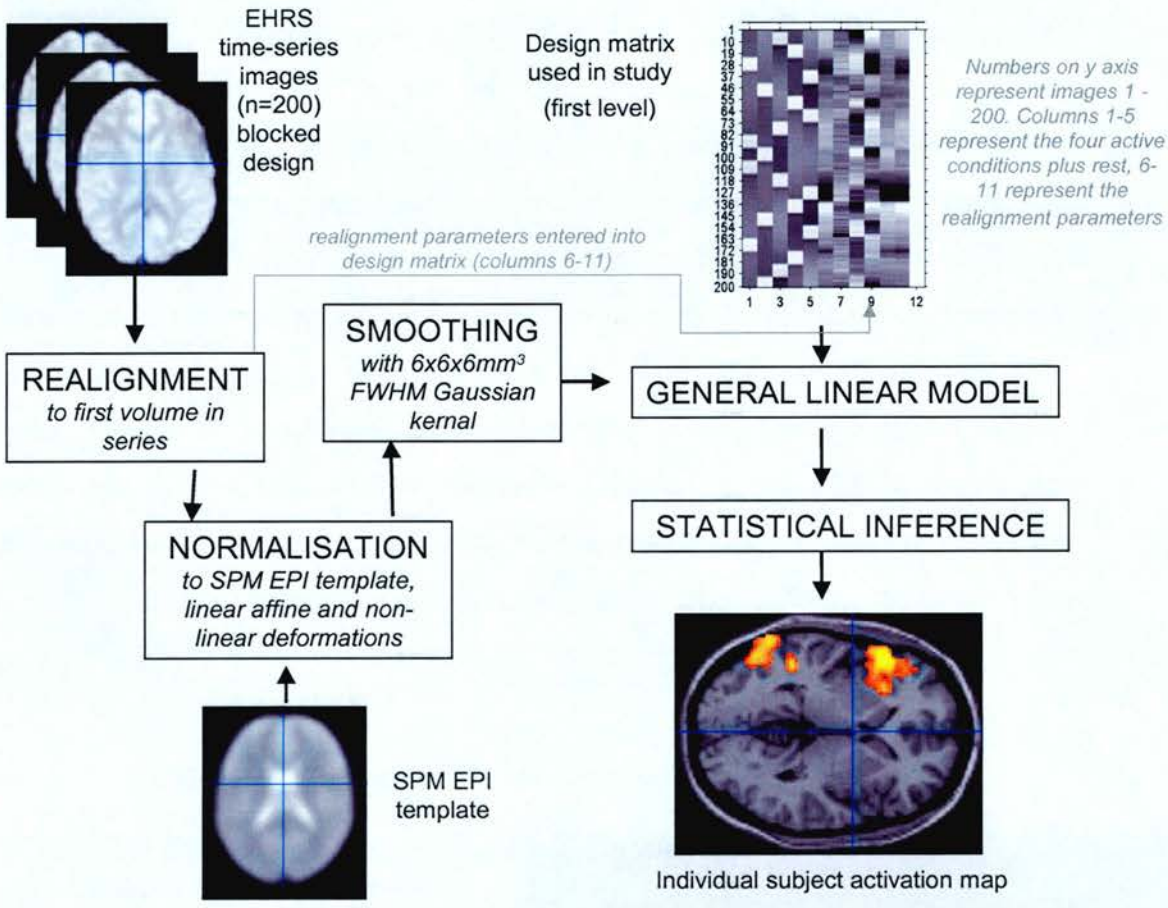

Figure 3.2 Steps involved in pre-processing and first level analysis

See text for further details.

3.3.5.2 *Realignment*

For each subject the EPI volumes were realigned to the first volume in the series and the mean image was created. Realignment involves first estimating the 6 parameters of the rigid body affine transformations that minimise the sum of squared differences between each scan and the reference image, and then applying these transformations to the images. This step attempts to correct for movement throughout the period of image acquisition or 'run', and provides information in graphical and

text format regarding the extent of subject movement that is corrected (translation and rotation in x, y, and z). For each subject the graphical output in SPM from this stage of pre-processing was checked for drifts, periodic events, and spikes. Two matlab scripts, 'rd_motion.m' (written by Dr K Christoff available online http://www-psych.stanford.edu/~kalina/), and 'cc.m' (written by Dr E Simonotto, see appendix) were used to summarise the absolute maximum extent of movement and to identify if there was significant correlation between movement parameters and task regressors, respectively. Two subjects were found to present significant motion artefacts and were excluded from further analysis; one subject was excluded on the basis of having moved > 3 mm, peak to peak over less than 20 images (see chapter one Figure 1.7), and one subject presenting large correlations (>0.5) between movement parameters and task regressors was also excluded. Details of within scanner movement for the remaining subjects are presented in Table 3.3.

Table 3.3 Estimates of within scanner movement

	Controls (n=21)	High risk without symptoms (n=42)	High risk with symptoms (n=27)
Max movement in x (mm), (std dev)	0.80 (0.51)	1.10 (0.83)	1.45 (0.93)
Max movement in y (mm), (std dev)	0.80 (0.47)	0.82 (0.38)	0.99 (0.46)
Max movement in z (mm), (std dev)	1.11 (0.74)	1.06 (0.65)	1.25 (0.83)

The estimate of movement parameters determined from realignment stage of pre-processing

3.3.5.3 *Normalisation*

The mean image created during the realignment step was then used in the normalisation stage of pre-processing. Normalisation of images from individual subjects into a standardised space is a prerequisite for performing group comparisons and permits the use of standard brain atlases for the reporting of results. The mean image was selected as the 'image to determine parameters', and then all images in the run were selected as the 'images to write normalised'. The images were normalized to the SPM99 EPI template (created from an average of 13 subject's mean fMRI images acquired at 2T) using linear affine transformations followed by non-linear deformations, and resampled using sinc interpolation to cubic voxels of size 8 mm^3.

3.3.5.4 *Spatial smoothing*

Normalized images were spatially smoothed with a 6x6x6 mm^3 full width half maximum (FWHM) Gaussian filter to minimize residual inter-subject differences, and in order to meet assumptions for parametric statistical analysis regarding the distribution of residuals.

3.3.5.5 *Visual inspection*

Visual inspection of the images prior to the realignment stage and after pre-processing was used to verify image quality and to ensure that the image processing has been successfully completed. This inspection identified images from five subjects that contained significant image artefact (thought to be due to excessive mechanical vibration of scanner hardware), as described in the 'subject populations' section above. All five subjects were subsequently re-scanned. This inspection also identified the two subjects with minor vascular malformations (shown in appendix), confirmed by a consultant neuroradiologist, who were also excluded from further analysis.

3.3.6 Statistical analysis

3.3.6.1 *First level analysis*

Statistical analysis was performed using the general linear model approach as implemented in SPM, generally simplified as:

$$Y = \beta X + \epsilon$$

Where Y is the observed response, i.e. the fMRI time series data; X is the linear combination of explanatory variables (also called 'covariates' or 'regressors') comprising the design matrix (see Figure 3.2); β are the regression weights or parameters to be estimated for each of the explanatory variables in the design matrix; and ϵ is the residual error term.

In SPM each column in the design matrix corresponds to effects built into the model. In the current analysis, at the individual subject level, the data was modelled with 5 conditions (the four sentence completion difficulty levels and the rest condition), each modelled by a boxcar convolved with the synthetic haemodynamic response function (hrf) provided in SPM99. This convolution is performed since if the boxcar function was not convolved it would remain a sharp transition between conditions. With convolution applied, this is smoothed and delayed as seen in the true hrf. The resulting conditions are represented by the first 5 columns in the design matrix. The estimates of the subject's movement from the realignment stage of pre-processing (translations and rotations in x, y and z) were entered as 'covariates of no interest' in the model, represented by columns 6-11 in the design matrix.

Before fitting the model, the subject's data was filtered in the time domain using both a low pass (Gaussian kernel, 4 s (FWHM) and a high pass filter (400 s cutoff). High and low pass filtering in the analysis of fMRI time series are used to remove low frequency drifts (such as physiological noise) and to address issues relating to serial autocorrelations seen in fMRI data, respectively. Temporal smoothing or low pass filtering, is a standard technique to correct for correlated residuals in fMRI analysis (see Henson 2004). After temporal smoothing the autocorrelation is

dominated by the smoothing filter applied and can be estimated from the filter parameters.

Contrasts were then constructed to examine all four sentence completion conditions versus rest ([1 1 1 1 -4] and *vice versa* (-1 -1 -1 -1 4]), and areas of increasing activation with increasing task difficulty (the 'parametric' contrast) ([-2 -1 1 2] and *vice versa* [2 1 -1 -2]).

3.3.6.2 *Batch scripting and changes to default settings*

As this was a large study, the image reconstruction, pre-processing, and first level statistical analysis was performed using batch scripts devised and tested by Dr E Simonotto and the author, based on examples provided with SPM. These scripts are detailed in the appendix. In general the default settings in SPM99 were selected for the first level analysis. The two exceptions were the specification of a new study specific mask image in the first level analysis, and the inclusion of realignment parameters. The latter has been discussed previously.

3.3.6.2.1 Mask image

With regards to the first level analysis the mask image refers to the image that determines which voxels are to be included in the analysis. The mask image created for each individual subject in SPM, by default, only includes those voxels with intensities higher than 80% of the global brain mean. Regions of signal drop-out (susceptibility artefacts often seen in orbitofrontal and inferior temporal regions with EPI), often have intensities lower than 80%, hence voxels within this region would be excluded from the first level analysis. This has further implications for random effects analysis, since the group mask would only include voxels that are common to all individual subjects. To try and ensure that as many brain voxels as possible were entered into the analysis a new, more generous, mask image was created and the processing scripts were edited to explicitly select this new mask image. This mask image was created by smoothing (at 6mm) and averaging the normalised mean images from the first 75 subjects scanned in this study. This image was then

dominated by the smoothing filter applied and can be estimated from the filter parameters.

Contrasts were then constructed to examine all four sentence completion conditions versus rest ([1 1 1 1 -4] and *vice versa* (-1 -1 -1 -1 4]), and areas of increasing activation with increasing task difficulty (the 'parametric' contrast) ([-2 -1 1 2] and *vice versa* [2 1 -1 -2]).

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3.3.6.3 *Second level analysis*

Contrast images for each subject representing a subject-specific summary of brain responses to the different conditions (sentence completion versus rest, and the parametric contrast) were entered into a second level random effects analysis to make inferences about activations within and between groups. A one sample t test was used to determine areas of activation within each of the three groups, and an ANOVA model was used to examine differences between groups.

3.3.6.3.1 Main trait and state effects

Differences in activation due to 'trait' effects were initially examined by comparing controls versus all high risk subjects (and *vice versa*). Symptomatic 'state' effects were initially examined by comparing the non-symptomatic groups (controls plus high risk without symptoms) versus high risk subjects with symptoms (and *vice versa*). This analysis structure was chosen to simplify the reporting of results and to minimize the number of group comparisons. As the groups were matched on demographic variables, and there were no significant differences in movement parameters, these factors were not included as potential confounds in the model. However to be confident that these factors were not having a significant effect, further examination of the influence of these variables was performed. Potential confounders (detailed in Tables 3.1 and 3.2: age, gender, handedness and within scanner movement in x (mm)) were examined by entering them as covariates in the second-level random effects analysis. Even at lenient thresholds (regions considered significant at voxel-level p corrected <0.10, for maps thresholded at 0.01, F test) none were found to have a significant effect.

3.3.6.3.2 Post-hoc trait and state effects

In order to further clarify any trait/state related findings, *post-hoc* group comparisons were conducted. The criteria followed for differences to be identified as potential trait-specific effects were that similar differences should be found between controls versus high risk with symptoms, and between controls versus high risk

without symptoms, but *not* between high risk with, versus high risk without symptoms. Criteria for any potential state-specific effects were that similar differences should be found between high risk with symptoms versus controls, and between high risk with, versus high risk without symptoms, but *not* between high risk without symptoms versus controls.

In addition to these more detailed group comparisons, a masking procedure in SPM was performed to examine regions fulfilling the above criteria for trait and state effects. In order to derive regions signifying potential trait effects, an exclusive mask was generated between controls versus high risk without symptoms and high risk with, versus high risk without symptoms, at an uncorrected mask threshold of 0.05. This mask was then converted into binary format to define an image to be used to examine areas of overlap between this exclusive mask and the contrast controls versus high risk with symptoms. Along similar lines, in order to derive regions signifying potential state effects an exclusive mask was generated between high risk with, versus high risk without symptoms and high risk without symptoms versus controls which was then used to examine areas of overlap in the contrast high risk with symptoms versus controls.

3.3.6.3.3 Interactions

Finally, interactions between trait and state effects were examined by looking at areas where there were increasing or decreasing effects across the groups, i.e. controls greater than high risk without symptoms, greater than high risk with symptoms, and vice versa. This was performed using the contrast weightings of [3, -1, -2] for decreases across groups, and [-3, 1, 2] for increases across the groups.

3.3.6.3.4 Genetic Liability

Categorical and continuous measures were also used to examine associations between measures of brain activity and the degree of inherited vulnerability in the high risk group. These measures of genetic liability were determined during the first

phase of the Edinburgh High Risk Study. These analyses were performed primarily to clarify whether trait effects found using the above contrasts were indeed influenced by the degree of genetic risk.

For the categorical measure, high risk individuals were classified as having any first degree relatives with the disorder, or only second degree relatives (details in Table 3.1). Group comparisons were made by examining differences between those with first versus those with second degree relatives.

The technique for generating the continuous measure of genetic liability in high risk subjects is based on matrix algebra and was developed by Professor Sham, and has been described previously (Lawrie et al., 2001). This measure takes into account the total number of ill and well relatives of each subject and their degree of relationship to the high risk individual. Briefly, this measure assumes a multifactorial polygenic liability-threshold model of schizophrenia with a heritability of 0.7 for the liability to schizophrenia. Expected liabilities are 2.86 for patients with schizophrenia, and -0.014 for all other individuals in the families, based on the mean values of the liability above and below a threshold assuming a prevalence of 0.5% of schizophrenia. An index of the genetic loading for each individual was computed, based on the expected liabilities for all family members, by multivariate regression. Matrices were constructed to describe the genetic relationships of all individuals in each high risk family to each other (i.e. relationship to self is 1, to spouse is 0, to first degree relative is 0.5, second degree relative is 0.25 etc). Assuming genes are the only source of familial resemblance (as shown in twin studies of schizophrenia), the correlation matrix of liabilities within a family is given by a second matrix, where the off diagonals in the above matrix were multiplied by the heritability estimate. A matrix of regression coefficients for a family is then obtained by multiplying the correlation matrix of genetic loadings by the inverse of the correlation matrix of liabilities. Finally, vectors of expected genetic loadings are given by multiplying the matrix of the regression coefficients by the vector of expected liabilities, given the affected states of the family members. These computations were conducted in Matlab

and generated a continuous but bimodal distribution of liabilities. Details of the continuous genetic liability measure are shown in Table 3.1 and distributions are shown in Figure 3.4. The lowest peak essentially corresponds to those with second degree relatives, and the highest peak to those with first degree relatives (see Byrne *et al.*, 2003). To determine if were any associations with the degree of inherited risk a regression analysis was performed using a single covariate of interest representing the continuous measure of genetic liability within the high risk group.

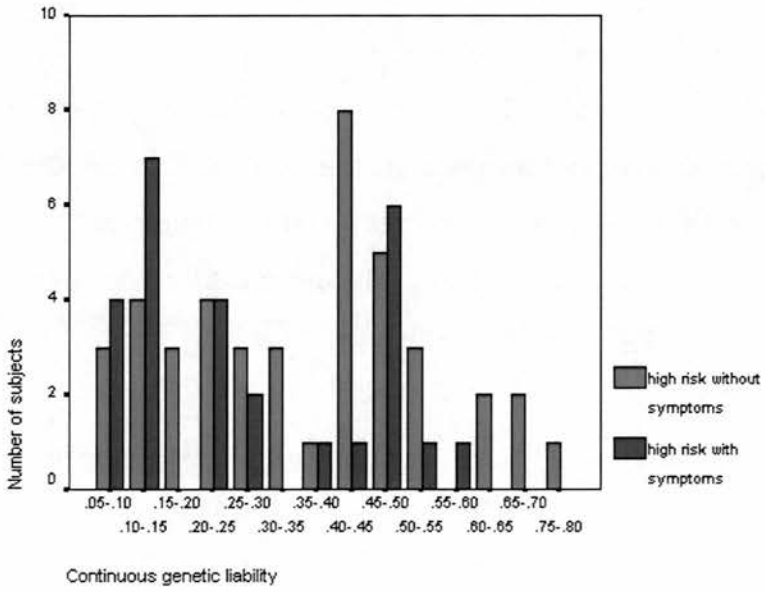

Figure 3.4 Distributions of the continuous genetic liability measure.

Continuous measure of genetic liability developed by Professor Sham, described previously (Lawrie et al., 2001).

Analyses for both categorical and continuous measures of genetic liability were initially performed at the 'whole brain' level, and then using restricted search volumes spatially determined by the clusters reported in the trait contrasts as an inclusive mask in order to unambiguously determine if there were regions of similarity between the analyses.

For all analyses, statistical maps were thresholded at a level of $p=0.001$ uncorrected, and regions were considered significant at $p<0.05$ cluster level corrected for multiple comparisons. For the analyses reported here, and throughout this thesis, all results are from random effects analyses. All p values quoted in the text are at the corrected cluster level, and co-ordinates were converted from MNI (Montreal Neurological Institute) to Talairach co-ordinates using a non-linear transformation, as described in (http://www.mrc-cbu.cam.ac.uk/Imaging).

Identification of regions was performed using a combination of electronic resources listed in order below (the Talairach Deamon, the Talairach Space Utility and MNI Space Utility), together with two brain atlases, Talairach and Tournoux (1988), and Duvernoy (1991):

http://ric.uthscsa.edu/projects/talairachdaemon.html
http://www.ihb.spb.ru/~pet_lab/TSU/TSUMain.html
http://www.ihb.spb.ru/~pet_lab/MSU/MSUIntro.html

3.4 RESULTS

3.4.1 Demographic details

There were no statistically significant differences in mean age, gender, handedness or mean NART IQ between the subject groups. There were also no significant differences between the high risk groups (with and without psychotic symptoms) on either measure of genetic liability (Table 3.1).

3.4.2 Behaviour

All three groups showed the expected pattern of quicker reaction time and higher word appropriateness scores with greater contextual constraint (Table 3.4). This pattern of reaction time confirms that the subjects were performing the task appropriately during scanning. There were no significant differences between the groups in terms of their performances on word appropriateness scores, reaction time measures, the numbers of words, or reaction time measures recorded, or the number of asterisks reported (Table 3.4).

Table 3.4 Behavioural results: word appropriateness scores and reaction times.

Group	Mean word appropriateness (std dev)				Mean reaction time (ms) (std dev)			
Constraint levels	Low	Med low	Med high	High	Low	Med low	Med high	High
Controls	6.40 (1.25)	3.25 (0.53)	1.93 (0.32)	1.10 (0.08)	2643 (528)	2543 (605)	2413 (615)	2375 (630)
High risk without symptoms	6.30 (1.00)	3.17 (0.59)	1.97 (0.35)	1.12 (0.09)	2501 (547)	2366 (591)	2242 (587)	2237 (618)
High risk with symptoms	6.38 (0.79)	3.35 (0.59)	2.01 (0.40)	1.12 (0.08)	2530 (671)	2441 (671)	2246 (699)	2337 (703)
	Mean number of words recorded (std dev) *(Mean number of asterisk reported)*				**Mean number of reaction time measures recorded (std dev)**			
Controls	7.80 (0.40) *0.80*	7.88 (0.19) *0.46*	7.98 (0.08) *0.17*	7.95 (0.10) *0.17*	7.93 (0.46)	7.83 (0.26)	7.87 (0.28)	7.83 (0.24)
High risk without symptoms	7.50 (0.99) *1.37*	7.74 (0.64) *0.93*	7.78 (0.62) *0.67*	7.87 (0.35) *0.39*	7.40 (0.68)	7.41 (0.63)	7.68 (0.51)	7.73 (0.39)
High risk with symptoms	7.39 (0.72) *0.96*	7.74 (0.41) *0.55*	7.90 (0.19) *0.50*	7.95 (0.12) *0.29*	7.39 (0.69)	7.46 (0.78)	7.66 (0.61)	7.72 (0.51)

3.4.3 Within groups results

3.4.3.1 *Sentence completion versus rest*

Figure 3.5 a-c displays the results for the sentence completion versus rest contrast (all levels of difficulty versus baseline) within each of the groups (further details are presented in Tables 3.5-7). This demonstrated activation in regions commonly activated with this task, including the left precentral gyrus, inferior frontal gyrus, medial/superior frontal gyrus, middle/superior temporal gyrus, right posterior lobe of the cerebellum, and the occipital lobes bilaterally. Visual inspection of these maps indicated an additional region of activation in the high risk subjects with psychotic symptoms in the left parietal lobe, not seen in either of the other two groups.

An example of the BOLD response in a region activated during the sentence completion versus rest contrast, the left posterior middle temporal gyrus, is shown in Figure 3.6.

Table 3.5. Sentence completion versus rest: controls (*n*=21)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	746	5.94	-50, -2, 44	L frontal: precentral gyrus, BA 4
			-44, 6, 49	L frontal: middle frontal gyrus, BA 6
			-46, 9, 31	L frontal: inferior frontal gyrus, BA 44
<0.001	1106	5.55	-59, -35, -2	L temporal : middle temporal gyrus, BA 21
			-51, -22, -9	L temporal: middle temporal gyrus, BA 20
			-44,-37, -12	L temporal: fusiform gyrus, BA 20
<0.001	762	5.51	-4, 6, 51	L frontal: superior frontal gyrus, BA 6
			-2, -1, 65	L frontal: medial frontal gyrus, BA 6/32
			-2, 14, 49	L frontal: superior frontal gyrus, BA 6
<0.001	163	5.36	18, 16, 17	R sub-lobar: border of caudate
			22, 3, 22	R sub-lobar: border of lentiform
<0.001	1622	5.09	-22, -5, 18	L sub-lobar: thalamus
			-6, -19, 18	L sub-lobar: thalamus
			-16, -11, 15	L sub-lobar: thalamus

For Tables 3.5-10 analyses thresholded at 0.0001 uncorrected cluster level, extent threshold = 50. Co-ordinates represent the three maxima within one cluster in order to give an indication of the spatial extent.

Table 3.6 Sentence completion versus rest: high risk without symptoms (*n*=42)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	311	Inf	25, -99, 0	R occipital : cuneus, BA 17/18
<0.001	2111	7.75	-2, 7, 53	L frontal: superior frontal gyrus, BA 6/32
			-6, 3, 59	L frontal: medial frontal gyrus, BA 6
			-8, 14, 40	L limbic: cingulate gyrus, BA32
<0.001	3156	7.71	-28, -95, -5	L occipital: lingual gyrus, BA 18
			-51, -37, -2	L temporal: middle temporal gyrus, BA 21
			-44, -55,-16	L temporal: fusiform gyrus BA 37
<0.001	7768	7.12	-51, 14, 18	L frontal: inferior frontal gyrus, BA 44
			-46, 17, 23	L frontal: inferior frontal gyrus, BA 9
			-36, 3, 20	L frontal: frontal operculum
<0.001	1607	5.88	-2, -72, -10	L cerebellum
			24, -66, -39	R cerebellum: posterior lobe
			2, -50, -28	R cerebellum: anterior lobe
<0.001	437	5.51	50, -39, 4	R temporal
			50, -29, 0	R temporal: superior temporal gyrus, BA 22
			48, -18, -9	R temporal
<0.001	259	5.30	46, -61, -22	R cerebellum: posterior lobe, declive
			32, -59, -22	R cerebellum: posterior lobe, declive

Table 3.7 Sentence completion versus rest: high risk with symptoms (*n*=27)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	6448	6.68	-42, 4, 35	L frontal: precentral gyrus, BA6
			-48, 4, 42	L frontal: middle frontal gyrus, BA 6
			-18, 0, 9	L sub-lobar: lentiform nucleus
<0.001	922	6.50	30, -64, -39	R cerebellum: posterior lobe
			32, -59, -24	R cerebellum: anterior lobe
			18, -79, -30	R cerebellum: posterior lobe
<0.001	1954	6.12	0, 10, 49	L frontal: superior frontal gyrus, BA 6
			-2, 1, 55	L frontal: medial frontal gyrus, BA 6/32
			-6, -11, 59	L frontal: medial frontal gyrus BA 6
<0.001	1623	5.81	-51, -37, -3	L temporal: middle temporal gyrus, BA 21
			-55, -22, -9	L temporal: middle temporal gyrus, BA 21
			-59, -44, 6	L temporal: middle temporal gyrus, BA 21
<0.001	257	5.69	-46, -44, 46	L parietal: inferior parietal lobule BA 40
			-40, -45, 26	L parietal: inferior parietal lobule,
<0.001	255	5.67	-28, -64, 47	L parietal: superior parietal lobule, BA 7
			-30, -60, 40	L parietal: inf parietal lobule/supramarginal g
<0.001	187	5.51	-22, -97, -5	L occipital: lingual gyrus BA 18
			-34, -93, -2	L occipital: inferior occipital gyrus, BA 18
<0.001	126	5.35	28, -95, -5	R occipital: lingual gyrus, BA 18
<0.001	300	4.93	10, 8, -2	R sub-lobar: border caudate/lentiform
			16, 1, 11	R sub-lobar: lentiform
			18, 1, 18	R sub-lobar: border of caudate
<0.001	139	4.22	50, -37, 0	R temporal: middle temporal gyrus, BA 21
			46, -28, -7	R temporal: superior/middle temporal gyrus
			48, -33, 7	R temporal: superior temporal gyrus,
<0.001	119	4.20	34, 23, 3	R cerebrum: insula
			40, 16, 1	R cerebrum: insula

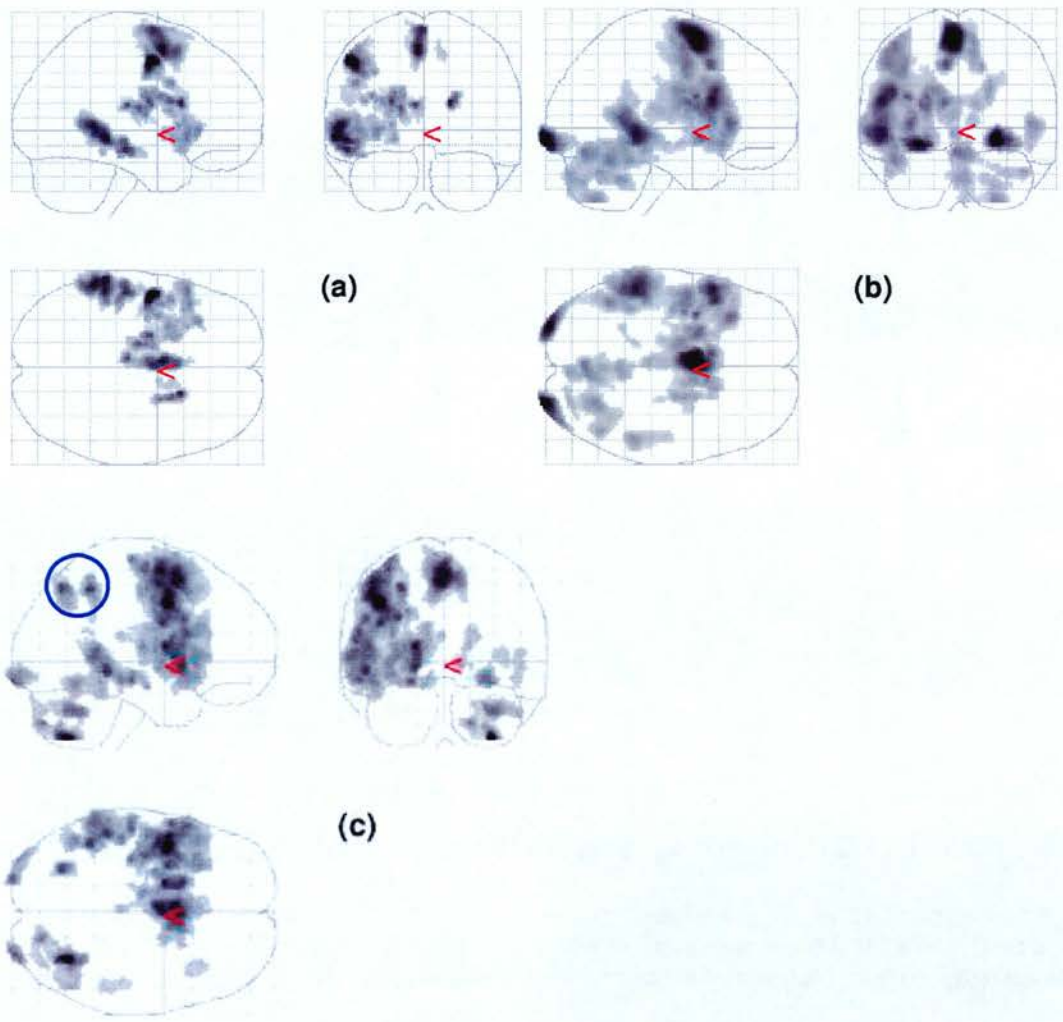

Figure 3.5 Within group analysis: sentence completion versus rest

Sentence completion versus rest analysis within groups, (a) controls (*n*=21), (b) high risk without symptoms (*n*=42), (c) high risk with symptoms (*n*=27). Activations displayed on 3D 'glass brain'; sagittal, axial, and coronal sections. Left side of coronal section represents left side of brain. Blue circle highlights increased activation in parietal lobe in high risk with symptoms. Maps thresholded at p<0.0001 uncorrected voxel level, extent threshold 50 voxels.

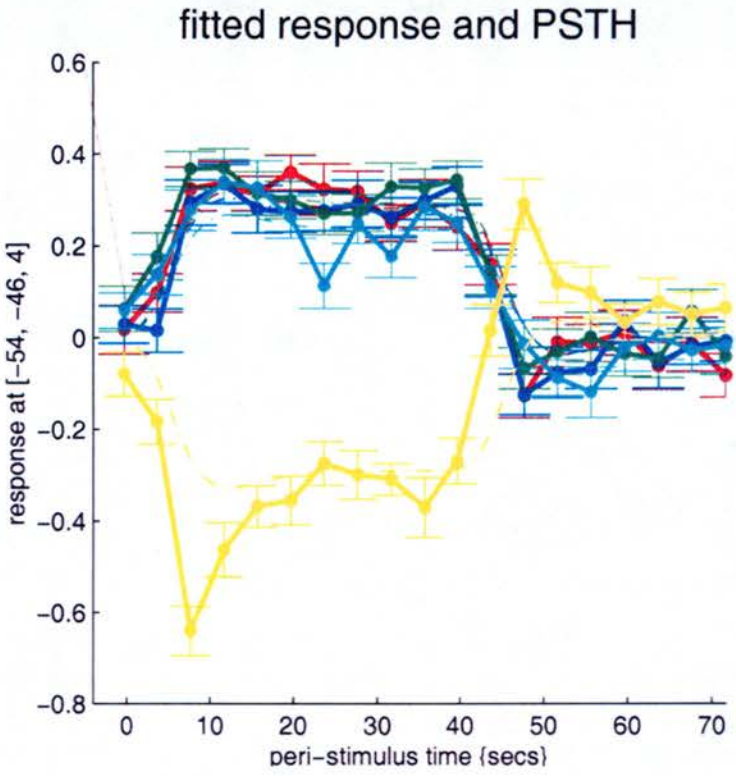

Figure 3.6 Example of BOLD response in left posterior middle temporal gyrus (BA 21), demonstrating sentence completion versus rest.

Fitted response and peri-stimulus time histogram: BOLD response averaged over 42 subjects (high risk without symptoms) for sentence completion (red = low constraint, blue = medium low constraint, green = medium high constraint, cyan = high constraint) and rest (yellow).

3.4.3.2 *Rest versus sentence completion*

Results for the rest versus sentence completion are shown in Tables 3.8-10. These indicated that in all three groups mainly posterior brain regions, extending from the posterior cingulate to parietal/occipital lobes were activated more in the visual rest condition than in the sentence completion condition, including the cuneus, precuneus, lingual gyrus and middle occipital gyrus, along with prefrontal area (BA 10) and the left superior temporal cortex.

Table 3.8 Rest versus sentence completion: controls (*n*=21)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	203	5.80	-24 -49 -6	L occipital/temporal: lingual g, BA 19
<0.001	8275	5.75	4 -63 26	L occipital: precuneus, BA 31
			47 -75 21	L occipital: middle occipital g, BA 19
			-2 -62 36	L occipital: precuneus, BA 7
<0.001	324	5.23	60 -28 32	R parietal: inferior parietal lobule, BA 40
			53 -28 19	R parietal: inferior parietal lobule, BA 40
			47 -19 25	R parietal: postcentral g, BA 2
<0.001	799	5.11	28 -53 -8	R occipital/temporal: fusiform g, BA 37
			9 -85 -4	R occipital: lingual g, BA 18
			22 -34 -7	R temporal: parahippocampal g, BA 35
0.001	95	5.08	-42 -76 28	L occipital: middle occipital g, BA 19
<0.001	165	4.80	2 40 16	R frontal: anterior cingulate g, BA 32
			2 55 5	R frontal: medial frontal g, BA 10
			4 41 16	R frontal: anterior cingulate g, BA 32
<0.001	250	4.69	30 28 51	R frontal: middle frontal g, BA 8/9
			28 31 45	R frontal: middle frontal g, BA 8
			21 6 51	R frontal: superior frontal g, BA 6
0.007	59	4.38	-8 -89 1	L occipital: cuneus/lingual g, 17
0.007	58	4.35	-38 -30 13	L temporal: insula
			-46 -23 5	L temporal: superior temporal g, BA 42

Table 3.9 Rest versus sentence completion: high risk without symptoms (*n*=42)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	18474	Inf	14 -85 38	R occipital: cuneus, BA 19
			-9 -89 -10	L occipital: lingual g, BA 17
			8 -38 -5	R occipital/temporal: lingual g, BA 30
<0.001	618	6.17	0 38 14	Midline: anterior cingulate g, BA 24
			0 47 2	Midline: medial frontal g, BA 10
			2 51 5	R frontal: medial frontal g, BA 10
<0.001	1808	6.01	59 -30 34	R parietal: inferior parietal lobule, BA 40
			38 -6 -7	R insula
			31 -10 15	R insula
<0.001	151	5.98	-42 -76 28	L occipital: middle occipital g, BA 19
0.001	195	5.82	26 51 7	R frontal: middle frontal g, BA 9/10
<0.001	809	5.82	24 10 55	R frontal: superior frontal g, BA 6
			24 21 50	R frontal: superior frontal g, BA 6/8
			23 28 38	R frontal: middle frontal g, BA 8
<0.001	540	5.38	-40 -16 2	L insula
			-48 -29 11	L temporal: superior temporal g, BA 42
			-33 -5 -7	L insula
<0.001	170	5.29	-26 33 39	L frontal: middle frontal g, BA 8
0.002	107	4.94	-12 -56 -39	L cerebellum
			-8 -49 -38	L cerebellum

Table 3.10 Rest versus sentence completion: high risk with symptoms (*n*=27)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	9963	6.94	4 -65 32 16 -102 16 8 -52 51	R parietal/occipital: precuneus, BA 31 R occipital: gyrus orbitales, BA 18 R parietal: precuneus, BA 7
<0.001	538	5.93	26 20 51 24 32 47 22 9 38	R frontal: middle frontal g, BA 8 R frontal: middle frontal g, BA 8 R frontal: middle frontal g, BA 6
<0.009	63	4.99	-28 -51 -3	L temporal: fusiform/parahippocampal g, BA 19
<0.001	163	4.77	-8 48 -2 9 47 2 -11 40 7	L frontal: medial frontal g, BA 10 L frontal: medial frontal g, BA 9/32 L frontal: anterior cingulate g, BA 32
<0.001	123	4.72	-40 -20 6 -48 -7 -7 -30 -23 11	L insula L temporal: superior temporal g, BA 42 L temporal: superior temporal g, BA 41
0.017	53	4.63	59 -26 27	R parietal: inferior parietal lobule, BA 40

3.4.3.3 *Parametric contrast*

For the parametric contrast, each group presented two main regions of increasing activation with increasing task difficulty: the left superior/medial frontal gyrus (BA 6) and the left inferior frontal gyrus (BA 44/47) see Figure 3.7 a-c (further details contained in Tables 3.11-13). An example of the BOLD response for one of the regions responding to increasing levels of difficulty (medial frontal gyrus, BA 6) is shown in Figure 3.8.

Table 3.11 Parametric contrast: controls (*n*=21)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	841	4.36	-4, 18, 45	L frontal: medial frontal gyrus, BA6
			2, 16, 40	R limbic: cingulate gyrus, BA 32
			-6, 10, 47	L frontal: medial frontal gyrus, BA 6/32
0.002	211	3.87	-46, 19, -6	L frontal: inferior frontal gyrus, BA 45/47
			-32, 19, -11	L frontal: inferior frontal gyrus, BA 47
			-53, 9, -9	L temporal: anterior superior temporal gyrus, BA 22
0.017	142	3.86	-20, 5, 13	L sub-lobar: lentiform nucleus
			-22, 10, 5	L sub-lobar: lentiform nucleus
			-16, -5, 8	L sub-lobar: lentiform nucleus
0.023	134	3.81	32, 18, 8	R cerebrum: insula
			50, 21, -11	R frontal: inferior frontal gyrus, BA 47
			46, 15, -2	R frontal: inferior frontal gyrus, BA 47

For Tables 3.11-16 analyses thresholded at 0.001 uncorrected cluster level, extent threshold=50. Co-ordinates represent the three maxima within one cluster in order to give an indication of the spatial extent of the cluster.

Table 3.12 Parametric contrast: high risk without symptoms (*n*=42)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	461	5.17	-50, 17, 25	L frontal: inferior frontal gyrus, BA 44
			-57, 18, 16	L frontal: inferior frontal gyrus, BA 44
			-53, 23, 3	L frontal: inferior frontal gyrus, BA 45
<0.001	396	5.11	-2, 14, 53	L frontal: medial/superior frontal gyrus, BA 6
0.004	204	4.23	-38, 23, -6	L frontal: inferior frontal gyrus, BA 47
			-50, 15, -2	L frontal: inferior frontal gyrus, BA 47
			-59, 19, -11	L temporal: superior temporal gyrus, BA 38

Table 3.13 Parametric contrast: high risk with symptoms (*n*=27)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	671	4.62	-38, 27, -1	L frontal: inferior frontal gyrus, BA 47
			-40, 23, -8	L frontal: inferior frontal gyrus, BA 47
			-52, 37, -5	L frontal: inferior frontal gyrus, BA 45/47
0.007	170	4.02	-51, 22, 10	L frontal: inferior frontal gyrus, BA 45
			-53, 27, 26	L frontal: middle frontal gyrus, BA 46
			-53, 30, 15	L frontal: inferior frontal gyrus, BA 46
0.008	167	4.00	-4, 9, 59	L frontal: superior frontal gyrus, BA 6
			-12, 11, 64	L frontal: superior frontal gyrus, BA 6

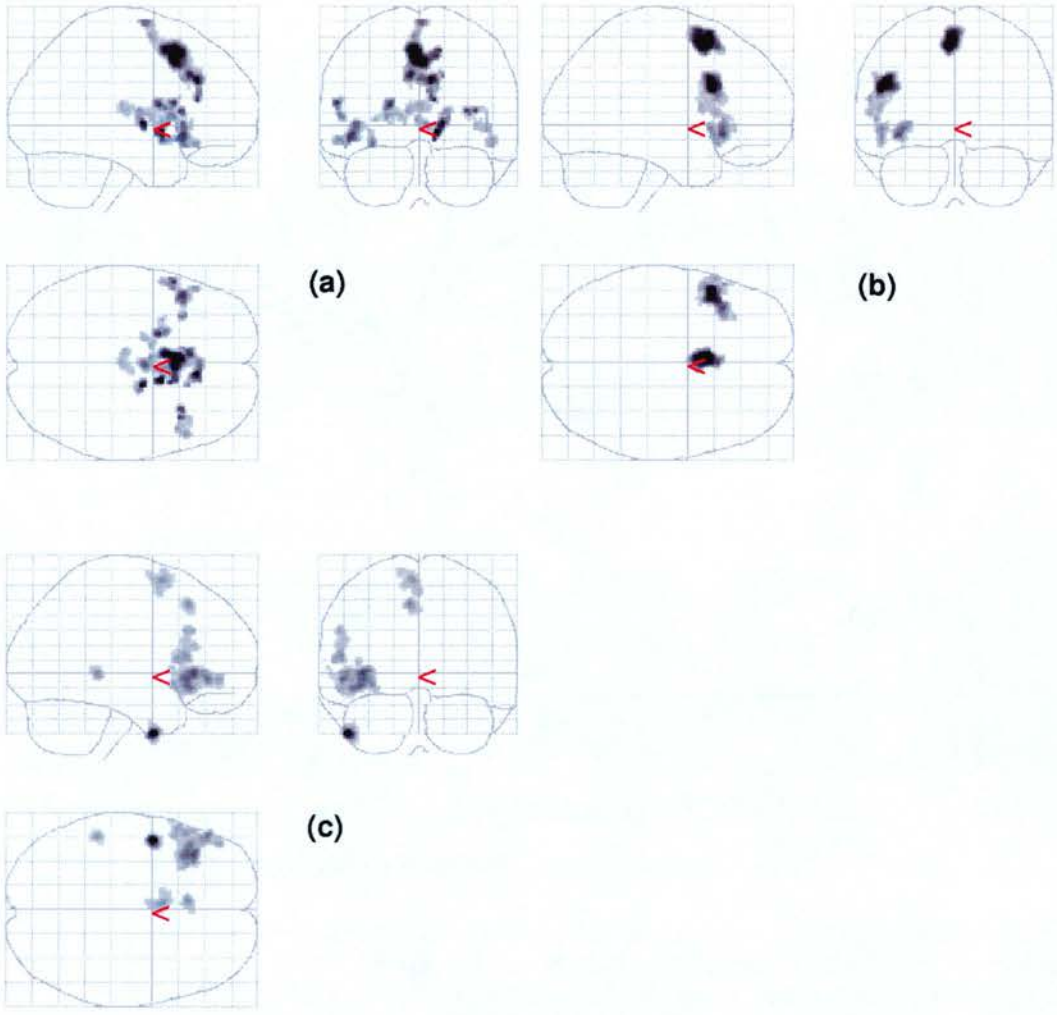

Figure 3.7 Within groups analysis: parametric contrast

Parametric within groups analysis, (a) controls (*n*=21), (b) high risk without symptoms (*n*=42), (c) high risk with symptoms (*n*=27). Activations displayed on 3D 'glass brain'; sagittal, axial, and coronal sections. Left side of coronal section represents left side of brain. Maps thresholded at p<0.001 uncorrected voxel level, extent threshold 50 voxels.

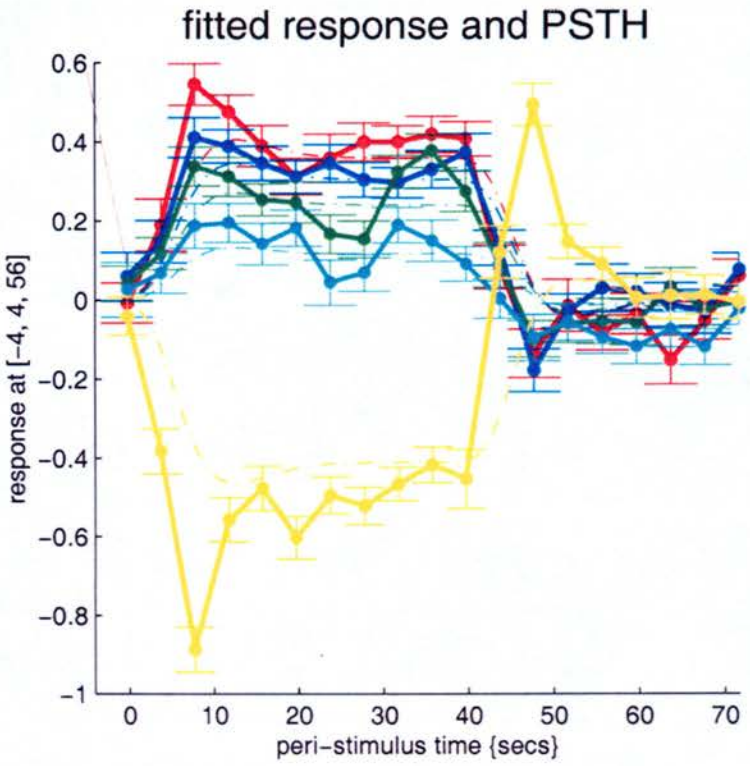

Figure 3.8 BOLD response in medial frontal gyrus, BA6, demonstrating different levels of difficulty and rest.

Fitted response and peri-stimulus time histogram: BOLD response averaged over 42 subjects (high risk without symptoms) for different conditions. Red = low constraint, blue = medium low constraint, green = medium high constraint, cyan = high constraint, yellow = rest. Low to high represents increasing task difficulty.

3.4.3.4 *Inverse parametric contrast*

Few regions were found to show increasing activation with decreasing task difficulty; the precuneus/posterior cingulate, supramarginal gyrus, occipitotemporal regions and anterior medial prefrontal regions. These were seen only in the high risk without symptoms group at the chosen statistical threshold (Table 3.14), potentially due to larger numbers in this group.

Table 3.14 Inverse parametric contrast: high risk without symptoms (*n*=42)

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	3505	5.32	-14 -69 32 4 -64 36 5 -44 28	L parietal: precuneus, BA 7 R parietal: precuneus, BA 7 R limbic: posterior cingulate g, BA 23
0.032	134	4.49	-44 -69 44 -53 -61 44 -41 -54 29	L parietal: superior parietal lobule, BA 7/19 L parietal: supramarginal g, BA 40 L parietal: supramarginal g, BA 40
<0.001	409	4.39	4 63 8 6 55 11 4 49 -4	R frontal: anterior medial frontal g, BA 10 R frontal: anterior medial frontal g, BA 10 R frontal: medial frontal g, BA 10
<0.001	352	4.29	63 -10 4 49 -7 17 55 -27 25	R temporal: superior temporal g, BA 22 R insula R parietal: inferior parietal lobule, BA 40
<0.001	602	4.12	55 -61 26 39 -76 20 48 -47 8	R parietal: supramarginal g, BA 39 R occipital: middle occipital g, BA 19 R temporal: middle temporal g, BA 21

Analyses thresholded at 0.001 uncorrected cluster level, extent threshold =50. Co-ordinates represent the three maxima within one cluster in order to give an indication of the spatial extent.

3.4.4 Between groups results

Between group results will only be presented for the contrast [sentence completion versus rest] and the parametric contrast [increasing activation with increasing difficulty]. Group analyses for the reverse contrasts, [rest > sentence completion], and the inverse parametric contrast [examining decreasing activation with increasing difficulty] are not reported since two tailed group analysis of the original contrasts were conducted. In essence these would be duplicating analyses already presented. For example, [sentence completion > rest] for the group comparison [controls > high risk] is identical to [rest > sentence completion] for [high risk > controls], and likewise for the parametric contrast [increasing activation with increasing task difficulty] for the group comparison [controls > high risk] is identical to the inverse parametric contrast for [high risk > controls].

3.4.4.1 *Main 'trait' effects*

Between group differences for potential trait effects (controls versus high risk) for both sentence completion versus rest, and the parametric contrast, are shown in Table 3.15.

3.4.4.1.1 Sentence completion versus rest

No significant between group differences regarding trait effects were found between the controls and all the high risk subjects for the sentence completion versus rest contrast.

3.4.4.1.2 Parametric contrast

Analysis of group differences for the parametric contrast revealed greater increases in activation with increasing task difficulty in the controls compared to the high risk group as a whole, in several regions: one involving the right medial frontal gyrus and to a lesser extent the anterior cingulate gyrus ($x = 14$, $y = 47$, $z = -1$; $p = 0.033$), another in the left posterior lobe of the cerebellum ($x = -2$, $y = -78$, $z = -11$; $p = 0.004$), and finally in the thalamic nuclei ($x = 8$, $y = -13$, $z = 6$; $p = 0.001$) see

Figure 3.9 a, b and Table 3.15. No other group differences were observed. Although these regions were not activated in the within group parametric contrast described in the tables above, activation in these regions became evident at a lower threshold of 0.005 p uncorrected in the control group.

Table 3.15 Between groups random effects analysis: main trait effects

P value	Extent	Z score	Peak height co-ordinates	Region
Sentence completion versus rest:				
Controls (n = 21) > all high risk (n = 69): n/s				
Controls (n = 21) < all high risk (n = 69): n/s				
Parametric:				
Controls (n = 21) > all high risk (n = 69)				
0.033	145	4.01	14 47 -1	R frontal: medial frontal gyrus, BA10
			2 53 5	R frontal: medial frontal gyrus, BA10
0.004	223	3.97	-2 -78 -11	L cerebellum
			-14 -78 -15	L cerebellum: posterior lobe, declive
			-22 -73 -17	L cerebellum: posterior lobe, declive
0.001	279	3.89	8 -13 6	R sub-lobar: thalamus
			-6 -15 12	L sub-lobar: thalamus
			18 -11 10	R sub-lobar: thalamus
Controls (n = 21) < all high risk (n = 69): n/s				

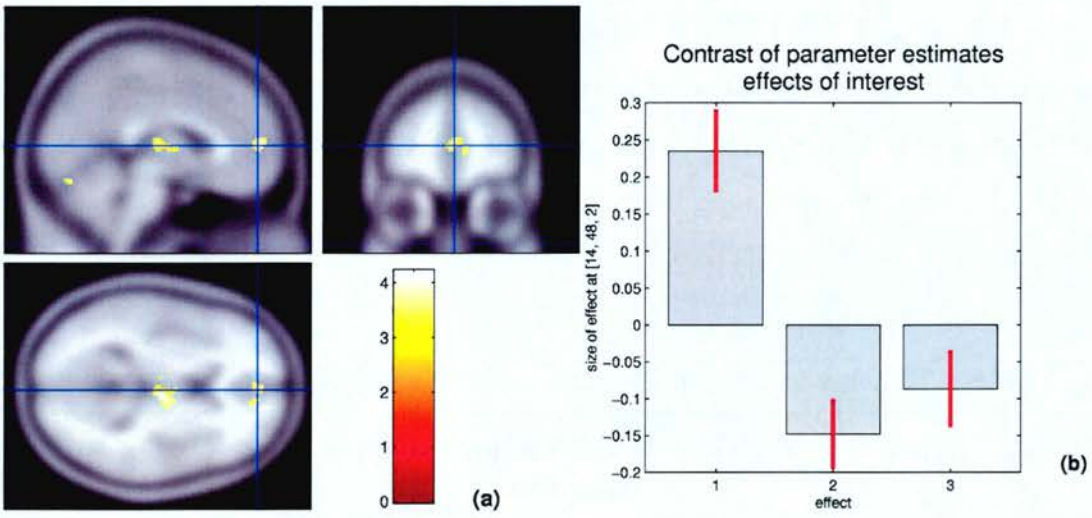

Figure 3.9 Between group differences, trait effects: parametric contrast

Parametric between group analysis, (a) group comparison showing greater increases in activation with task difficulty in controls versus all high risk subjects in medial frontal, thalamic and cerebellar regions. Maps thresholded at p<0.001 uncorrected voxel level, extent threshold 100 voxels. Colour bar represents Z score (b) effect size at peak co-ordinate for medial frontal region, (1=controls, 2=high risk without symptoms, 3=high risk with symptoms).

In order to determine whether these group differences met the more strict criteria for a 'trait' related effect as described in the methods section, further *post-hoc* examination of these activation differences were performed between the three groups, Table 3.16.

The largest number of differences were seen between the controls and high risk subjects without symptoms in several regions including: the right medial frontal gyrus (x = 6 y = 53, z = 12, p<0.001), and left posterior lobe of the cerebellum (x = -14, y = -79, z = -22, p=0.001). While differences were not found in the thalamus at the standard threshold of p=0.001 uncorrected, at a lower threshold of p=0.005 uncorrected differences also emerged between the controls and high risk subjects without symptoms in this region (x = 8, y = -13, z = 10, p<0.001). Also at this lower threshold, significant differences between controls and high risk with symptoms were seen in the thalamus (x = 6, y = -14, z = 4, p<0.001) and left posterior lobe of the

cerebellum (x = -4, y = -81, z = -14, p=0.033). No differences were seen between the two high risk groups at either statistical threshold.

The masking procedure produced consistent findings, whereby regions of overlap were seen in controls versus high risk with symptoms, and high risk without symptoms, but not between high risk with and without symptoms, in the region of the cerebellum (x = -4, y = -80, z = -8, p=0.049), and at a trend level, in the thalamus (x = 8, y = -14, z = 4, p=0.075).

Table 3.16 Random effects analysis: further examination of potential 'trait' related effects

P value	Extent	Z score	Peak height co-ordinates	Region
Parametric:				
Controls (n=21) > high risk without symptoms (n=42)				
<0.001	819	4.95	6 53 12	R frontal: medial frontal gyrus, BA 10
			12 49 2	R frontal: medial frontal gyrus, BA 10
			-2 42 9	L limbic: anterior cingulate gyrus, BA 32
<0.001	675	4.79	-2 -42 30	L limbic: cingulate gyrus, BA 31
			4 -53 33	R parietal: precuneus, BA 31
			5 -39 39	R limbic: cingulate gyrus, BA 31
0.001	308	4.08	-14 -79 -22	L cerebellum: posterior lobe, declive
			-2 -83 -20	L cerebellum
			-5 -63 -7	L cerebellum: anterior lobe
0.008	211	4.02	0 18 40	Midline: cingulate gyrus, BA 32
			0 -5 48	Midline: cingulate gyrus/ medial frontal gyrus
0.005	236	4.01	38 -69 20	R temporal: middle temporal gyrus, BA 39
			45 -71 35	R parietal: precuneus/angular g, BA 19/39
			37 -55 39	R parietal: inferior parietal lobule, BA 40
Controls (n=21) > high risk with symptoms (n=27):				
0.120	109	3.82	6 -14 4	R sub-lobar: thalamus
High risk without symptoms (n=42) > high risk with symptoms (n=27): n/s				

3.4.4.2 *'State' effects*

Between group differences for potential state effects for both sentence completion versus rest and the parametric contrast are shown in Table 3.17.

3.4.4.2.1 Sentence completion versus rest

As suggested by the within group maps for the sentence completion versus rest contrast described above (Figure 3.5), significantly greater activation was seen in the high risk subjects with psychotic symptoms versus the non-symptomatic groups in the left parietal lobe (x = -42, y = -48, z = 48, p = 0.001 see Figure 3.10 a, b and Table 3.17). This activation was located in the intraparietal sulcus, contained mainly within the inferior parietal lobule (BA 40) and to a lesser extent involved the superior parietal lobule (BA 7). No regions were shown to be relatively less active in the symptomatic group versus the non-symptomatic groups for this contrast.

Table 3.17 Between groups random effects analysis: state effects

P value	Extent	Z score	Peak height co-ordinates	Region
Sentence completion versus rest:				
Controls, high risk without (n=63) > high risk with symptoms (n = 27): n/s				
Controls, high risk without (n=63) < high risk with symptoms (n = 27)				
0.001	353	4.50	-42 -48 48	L parietal: inferior parietal lobule, BA 40
			-48 -49 54	L parietal: inferior parietal lobule, BA 40
			-30 -46 36	L parietal
Parametric:				
Controls, high risk without (n=63) > high risk with symptoms (n = 27): n/s				
Controls, high risk without (n=63) < high risk with symptoms (n = 27): n/s				

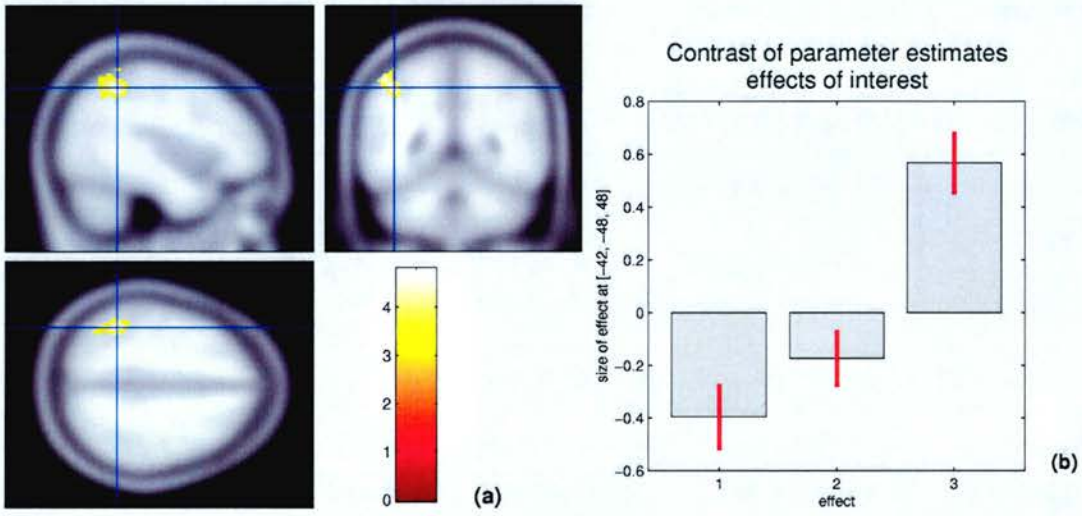

Figure 3.10 Sentence completion versus rest: between group differences

Sentence completion versus rest between groups analysis, (a) group comparison showing relatively greater activation in left inferior parietal lobule in high risk subjects with psychotic symptoms versus controls and high risk without symptoms. Maps thresholded at p<0.001 uncorrected voxel level, extent threshold 50 voxels. Colour bar represents Z score (b) effect size at peak co-ordinate, (1=controls, 2=high risk without symptoms, 3=high risk with symptoms).

In order to determine whether these group differences met the more strict criteria for 'state' related effects, a more detailed analysis between groups was performed, Table 3.18. These group comparisons indicated that there was relatively greater activation in the left inferior parietal lobule in the high risk with symptoms versus controls (x = -42, y = -50, z = 48, p=0.001), and versus the high risk without symptoms (x = -42, y = -48, z = 48, p=0.007), but not between the controls and high risk subjects without symptoms (nearest cluster at x=-34, y=-44, z=46; T score=1.74). The masking procedure confirmed these findings, where regions of overlap were seen in the left inferior parietal lobule in high risk with symptoms versus controls, and versus the high risk without symptoms, but not between controls and high risk without symptoms (x = -42, y = -50, z = 48, p<0.001).

Table 3.18 Random effects analysis: further examination of potential 'state' related effects

P value	Extent	Z score	Peak height co-ordinates	Region
Sentence completion versus rest				
Controls (n=21) < high risk with symptoms (n=27)				
0.001	334	4.17	-42 -50 48	L parietal: inferior parietal lobule, BA 40
			-48 -48 55	L parietal : inferior parietal lobule, BA 40
			-31 -36 56	L parietal: inferior parietal lobule/postcentral g
Controls (n=21) < high risk without symptoms (n=42): n/s				
High risk without symptoms (n=42) < high risk with symptoms (n=27)				
0.007	235	4.40	-42 -48 48	L parietal: inferior parietal lobule, BA 40
			-40 -62 54	L parietal: inferior parietal lobule, BA 40
			-32 –46 34	L parietal

3.4.4.2.2 Parametric contrast

There were no significant differences regarding state effects for the parametric contrast (Table 3.13).

3.4.5 Interactions between trait/state effects

3.4.5.1 *Sentence completion versus rest*

The analysis examining interactions between trait and state effects across the three groups for sentence completion versus rest suggested that the parietal lobe activity was lowest in the controls, followed by the high risk without symptoms, and greatest in high risk with symptoms (x = -42, y = -50, z = 48, p=0.019). There were no significant findings for the reverse contrast.

3.4.5.2 *Parametric contrast*

The analysis examining interactions between trait and state effects across the three groups for the parametric contrast suggested thalamic increases in activity were greatest in the controls, followed by the high risk without symptoms, and lowest in high risk with symptoms (x = 6, y = -18, z = 6, p=0.003). There were no significant findings for the reverse contrast.

3.4.6 Genetic liability: categorical

Results for the categorical measure of genetic liability are shown in Table 3.19 for both contrasts.

3.4.6.1 *Sentence completion versus rest*

The analysis with respect to degree of genetic risk (categorical) indicated only one region of difference between those with first and those with second degree relatives for the sentence completion versus rest analysis in the medial occipital lobe. There were no significant differences in the parietal lobe (see Table 3.19).

3.4.6.2 *Parametric contrast*

For the parametric contrast, results indicated greater increases in activation with increasing difficulty in those with first degree relatives versus those with second degree in the right medial frontal gyrus (x = 16, y = 32, z = 40, p=0.021), there was also a trend in the medial parietal lobe. No significant differences were observed for the reverse contrast.

Results using the restricted search volume did not confirm that the trait related findings above (medial prefrontal, thalamic, cerebellar) were associated with the categorical measure of the degree inherited of risk, i.e. there was no overlap between the trait related regions identified by the contrast [controls versus high risk] and the contrast examining associations with categorical genetic liability [first degree versus second degree relatives].

Table 3.19 Genetic liability: categorical

P value	Extent	Z score	Peak height co-ordinates	Region
Sentence completion versus rest:				
High risk with first degree relatives (n=48) > high risk with second degree relatives (n=21)				
0.003	274	4.23	-6 -99 4	L occipital: cuneus, BA 17/18
			10 -93 14	R occipital: cuneus, BA 18
			7 -72 2	R occipital: lingual g, BA18
High risk with second degree relatives (n=21) > high risk with first degree relatives (n=48): n/s				
Parametric:				
High risk with first degree relatives (n=48) > high risk with second degree relatives (n=21)				
0.021	173	4.47	16 32 40	R frontal: medial frontal g, BA8
			24 32 35	R frontal: middle frontal g, BA8/9
			21 19 30	R frontal
0.063	132	4.40	6 -42 52	R parietal: paracentral lobule, BA5/7
			5 -50 60	R parietal: precuneus, BA5
			16 -34 43	R parietal : paracentral lobule,
High risk with second degree relatives (n=21) > high risk with first degree relatives (n=48): n/s				

3.4.7 Genetic liability: continuous

Results for the continuous measure of genetic liability are shown in Table 3.20 for both contrasts. Activation in the medial occipital cortex was found to be positively associated with the continuous measure of genetic liability, although this was more superior to the region implicated by the categorical measure. The results using the restricted search volume to determine if the trait related findings above were associated with this continuous measure of degree of risk indicated no consistent regions.

Table 3.20 Genetic liability: continuous

P value	Extent	Z score	Peak height co-ordinates	Region
Sentence completion versus rest:				
Positive association (increasing activation with increasing risk)				
0.044	167	3.92	0 -82 41	Interhemispheric/occipital: precuneus
Negative association (decreasing activation with increasing risk): n/s				
Parametric:				
Positive association (increasing activation with increasing risk): n/s				
Negative association (decreasing activation with increasing risk): n/s				

3.5 DISCUSSION

This study used fMRI in combination with a sentence completion test to examine trait and state effects of brain activation in subjects at high risk of schizophrenia. Task-related activations were seen in areas consistently reported to be involved in self generated word production tasks i.e. lateral and medial prefrontal regions and superior/middle temporal gyrus (Frith *et al.*, 1995a; Nathaniel-James *et al.*, 1997; Lawrie *et al.*, 2002a; Indefrey and Levelt 2002). The effects of constraint were also consistent with similar studies reporting involvement of lateral and medial prefrontal regions with increasing task difficulty (Nathaniel-James and Frith 2002; Lawrie *et al.*, 2002a; Fu *et al.*, 2002; Barch *et al.*, 2000).

In terms of group differences, the controls showed significantly greater task related increases in activation in medial prefrontal, thalamic and cerebellar regions compared to the high risk group as a whole. The high risk subjects with isolated psychotic symptoms demonstrated significantly increased activation in the left inferior parietal lobule compared to controls and non-symptomatic high risk subjects. No group differences were reported in lateral prefrontal or lateral temporal regions. Importantly none of these subjects were considered ill at the time of testing; those referred to as high risk with psychotic symptoms had reported isolated or transient psychotic symptoms in the setting of unimpaired function. They did not meet diagnostic criteria for any psychotic disorder, and all subjects were anti-psychotic naïve at the time of investigation.

The differences in brain activation patterns occurred against a background of closely similar task performance. This has important implications in relation to interpretation of the data. Many functional imaging studies examining patient populations (often medicated) reveal activation differences alongside performance differences. Thus it is difficult to determine whether activation differences are due to a core neuronal abnormality, or whether they are a secondary effect of poor performance (and/or medication). Functional imaging studies have often reported hypofrontality (in dorsolateral prefrontal regions) in schizophrenic subjects,

particularly with executive tasks in which the patients perform worse than controls. Others have suggested a more complex relationship between performance and either decreased or even increased prefrontal activation, particularly with reference to increasing working memory load (see Manoach 2003). Here is it suggested that prefrontal activation can be described as an inverted 'U' (Figure 3.11). In this model prefrontal activation increases with increasing task demand until cognitive capacity is exceeded, further increases in load lead to decreased prefrontal activation. It is speculated that the patient group reaches their performance capacity before the control group. In this model the situation of hyperfrontality may occur when patients are at or near their maximum capacity (the peak of the inverted 'U'), while controls, who are further from their maximum, are still demonstrating increasing activation with increasing difficulty. Similarly, hypofrontality may occur when cognitive capacity in the patient group is exceeded and decreased activation takes precedence, whereas the controls are still displaying increasing or maximum activation.

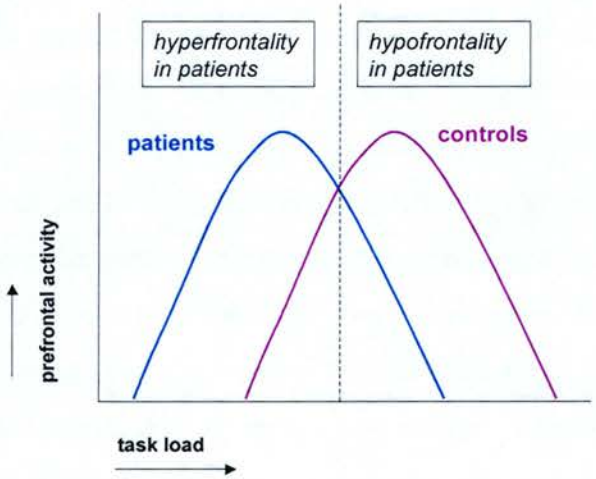

Figure 3.11 Schematic demonstrating model of hyper- and hypofrontality in patients in relation to task load.

Studies which have controlled for task performance suggest hypofrontality may only become evident when performance fails (Fletcher *et al.,* 1998; Frith *et al.,* 1995a), and this is in agreement with the current findings where there was no

evidence for decreased dorsolateral prefrontal activity in the presence of matched task performance.

An alternative explanation is that abnormal dorsolateral prefrontal activation may be specifically associated with deficits relating to the established illness. Results from other functional imaging studies of high risk subjects are at present inconsistent. One study of clinically unaffected relatives reported increased activation of the right dorsolateral prefrontal cortex in those at increased risk in the absence of performance deficits during a working memory paradigm (Callicott *et al.,* 2003). This was reported to involve Brodmann's area 9/10/46, so would appear to be more lateral than the prefrontal region reported to be positively associated with increased categorical familial risk in this analysis (high risk subjects with first degree relatives versus second degree relatives, Brodmann's area 8). In contrast, other studies of unaffected relatives report no differences in prefrontal cortex activation during the Wisconsin card sorting test (Berman *et al.,* 1992), increases, which did not survive after controlling for task performance on an auditory verbal working memory task (Thermenos *et al.*, 2004) (although greater anterior cingulate activity remained), reductions in anterior cingulate and left inferior prefrontal perfusion at rest (Blackwood *et al.,* 1999), or no differences in prefrontal activation during a verbal fluency test (Spence *et al.,* 2000). It is however important to consider that the subjects in these studies were generally out-with the period of maximum risk (mean ages 34, 32, 35, 39, 55 respectively, and included a number of individuals over 40 years), and therefore unlikely to develop the disorder. Our younger high risk subjects will therefore presumably be in different risk strata than these older relatives, which may account for differences between these studies and the current findings.

A matter that requires consideration is where the symptomatic high risk subjects lie in relation to well asymptomatic individuals and individuals with schizophrenia. These subjects did not have schizophrenia according to any diagnostic criteria. Their psychotic symptoms were fleeting and non-disabling and their functioning good, but they do of course have an enhanced liability to the disorder. It has recently been

reported that fleeting psychotic symptoms like these occur in around 10% of the normal population at some time (Verdoux and van Os 2002). In this high risk sample the occurrence lies nearer 40% (reported over the previous 18 months). Some of that 40% may still go on to develop schizophrenia as it has been reported that the cases who do have often, but not always, had transient or partial psychotic symptoms prior to becoming ill (14 high risk subjects out of the total 21 who became ill over the course of the entire study to date had psychotic symptoms prior to becoming ill, Johnstone *et al.,* 2000). In essence, in this sample the presence of these transient or partial psychotic symptoms is perhaps indicative of a state of genetically induced vulnerability to schizophrenia which, in some cases, will translate into psychosis.

Although *lateral* prefrontal differences were not found, medial prefrontal-thalamic-cerebellar regions of reduced activation were shown in the high risk group as a whole compared to control subjects. More detailed group comparisons also indicated differences between controls and both high risk groups (but not between the two high risk groups) in thalamic and cerebellar regions (although some only became evident at lower thresholds). Medial prefrontal differences were however only observed between controls and high risk subjects without symptoms. Similar results were also reflected in the masked analysis. It is interesting and unexpected that there were more differences observed between the high risk without symptoms and controls, than between the high risk with symptoms versus controls. This may be due to the larger number of subjects in this group giving greater statistical power, particularly in a contrast examining incremental differences associated with increasing task difficulty, rather than in the simpler contrast of sentence completion versus rest. Nevertheless, as a whole, these results are consistent with findings that this group of regions may be dysfunctional in schizophrenia (Andreasen *et al.,* 1996), and in part with another study of unaffected relatives (Callicott *et al.,* 2003) which also reported decreased activation in medial frontal, thalamus and cerebellar regions in unaffected relatives. One interpretation of these findings is that as task difficulty increases, healthy controls are able to increase activation in a network of areas involving medial prefrontal-thalamic and cerebellar regions, but those at genetically

enhanced risk of schizophrenia were less able to do so. These results suggest that the presence of the schizophrenia genotype (i.e. the high risk trait) is associated with a restricted ability to activate this network, but this may only have behavioural consequences on more demanding tasks where the deficit can not be compensated. Although increasing or decreasing degree of risk was not found to be associated with activity in these specific regions within the high risk group using the categorical or continuous measure of inheritance described, greater familial risk (categorical) was found to be associated with increased activity in a region with the maxima located in the right medial/superior prefrontal region (Brodmann's area 8). This region was slightly more superior and posterior to the medial prefrontal region referred to above (Brodmann's area 10/32). It should also be considered that, although the finding of increasing activation with increasing task difficulty in the thalamus met criteria for a genetically mediated effect, the analysis examining linear differences across the groups suggested that this increased activation was greatest in the controls, followed by the high risk without symptoms, followed by high risk with symptoms. This indicates this effect may not be purely trait-related, and that there may be some state modulation of trait effects with regards this finding.

Other studies of the Hayling sentence completion test in normal subjects have reported areas of activation similar to those reported here (Lawrie *et al.,* 2002a; Nathaniel-James *et al.,* 1997). The study by Lawrie *et al.* (2002a) compared schizophrenic patients and controls (n = 8, n = 10 respectively), but did not report any functional localization differences between groups. However, Lawrie *et al.* (2002a) examined medicated subjects with established schizophrenia who were not specifically selected to be at enhanced genetic risk of the disorder. Alternatively, the present much larger study was perhaps able to distinguish groups due to increased statistical power.

The verbal initiation section of the Hayling test was used rather than the verbal suppression condition because it had previously been shown to demonstrate differences between schizophrenic and control subjects (in terms of functional

connectivity: Lawrie *et al.* 2002a), and is considered to be a refinement of the verbal fluency test, which is commonly found to elicit functional imaging abnormalities in schizophrenia (Curtis *et al.,* 1998; Frith *et al.,* 1995a; Spence *et al.,* 2000; Yurgelun-Todd *et al.,* 1996). The verbal initiation section of the Hayling task also has advantages over the suppression condition which mean it is more easily adapted to functional imaging paradigms. It has reduced and less variable response latencies than the harder verbal supression section. In addition, good performance on the verbal inhibition section of the test requires implementation of strategies, which may not be uniform across the scanning session (for example a subject may take few trials to develop a strategy, or may switch strategies at any time) which poses problems for scanning paradigms where responses across trials/blocks are examined together.

As described in the previous chapter, PET studies of verbal fluency report a relative failure to deactivate the left superior temporal gyrus in schizophrenic subjects compared to controls (Frith *et al.,* 1995a), while fMRI studies report decreased left lateral prefrontal activation (Curtis *et al.,* 1998; Yurgelun-Todd *et al.,* 1996). Differences in scanning methodologies, particularly with respect to the requirement for covert responses in fMRI, have been suggested to account for inconsistencies in temporal lobe activations (Curtis *et al.,* 1998). In the current study no groups differences were found in either the left lateral prefrontal cortex or left superior temporal cortical region, consistent with another study of relatives of schizophrenic subjects (Spence *et al.,* 2000). Rather, parietal lobe over-activation was found in symptomatic high risk subjects. Further examination of this over-activation suggested that this was a 'state' related effect. Differences were found between controls and high risk subjects with psychotic symptoms, and between the two high risk groups, but *not* between controls and asymptomatic high risk subjects, even at more lenient thresholds. These results were also confirmed using the masked analysis. However, although the parietal lobe hyperactivity fulfilled criteria for state-specificity, the analysis regarding linear differences across the three groups suggested that the parietal activation was greatest in the high risk with symptoms, followed by

high risk without symptoms, followed by controls. Hence there may also be an interaction between genetically mediated and symptom related effects in this region.

The majority of imaging studies in schizophrenia have focused on prefrontal and temporal brain abnormalities, but there are a number that report abnormalities of parietal lobe regions. In an early study investigating the relationship between hippocampal pathology and prefrontal hypofunction in monozygotic twins discordant for schizophrenia, there was an inverse relationship between hippocampal volume and activation in the parietal cortex in the affected twin group (Weinberger *et al.,* 1992). Several other functional imaging studies have reported increased activation in parietal lobe regions in schizophrenic subjects compared to controls during a variety of paradigms, including memory tasks (Crespo-Facorro *et al.,* 1999), verbal fluency (Curtis *et al.,* 1998), decision making (Paulus *et al.,* 2002), and in a study of voluntary movements in patients with symptoms specifically attributed to self-monitoring failures (Spence *et al.,* 1997).

One interpretation of the relative over-activation of the parietal lobe in the symptomatic high risk subjects is that this region may be recruited to enable them to perform the task at a similar level to the other groups. Since activation in the left intraparietal area was not found to be linearly related to task difficulty, this suggests that the relative over-activation in this region represents a general dysfunction in those at high risk with psychotic symptoms. In other words, the presence of the early symptomatic state is associated with a compensatory over-activation of the parietal lobe at all levels of task engagement. The intraparietal area is considered to form part of the semantic/lexical language network (Mesulam 1990, and see Niznikiewicz *et al.*, 2000) and is considered to be involved in attentional maintenance (Corbetta *et al.,* 2000; Hopfinger *et al.,* 2000), sentence comprehension (Carpenter *et al.*, 1999), response preparation (Snyder *et al.,* 1997), and response monitoring (Garavan *et al.,* 1999; Menon *et al.,* 2001b); i.e. consistent with findings that some of these functions are abnormal in schizophrenia (Frith 1992; Schatz 1998). A compensatory additional activation in the parietal lobe in the high risk subjects with symptoms may therefore

be related to attentional aspects of the task, and the preparation and monitoring of suitable verbal responses. This interpretation is consistent with reports that subjects in the prodrome to schizophrenia commonly report difficulties focusing attention and a reduced sense of control of behaviour (Kosterkötter *et al.,* 1997; McGhie and Chapman 1961). Regions of the posterior parietal cortex have also been implicated in the role of distinguishing between self and others (Meltzoff and Decety 2003). Hyperactivity in this region in our symptomatic high risk subjects may therefore suggest disruptions of these neural systems. The misattribution of internally generated actions as being externally generated are considered to be a potential neurophysiological basis of positive symptomatology (Frith 1992). Indeed, findings of hyperactivity in parietal areas in subjects with the established illness experiencing passivity phenomena are consistent with this hypothesis (Spence *et al.*, 1997). Regardless of the origin of this abnormality (cause, consequence or compensation, Lewis 2000; Fletcher 2004), a relative over-activation of the parietal lobe could represent one of the earliest pathological changes by which the trait of high genetic risk of schizophrenia switches to the state of incipient psychosis.

It is conceivable that the differences in male:female ratios across the groups could be contributing to the reported findings, especially since there is evidence to suggest gender differences in brain activation during language based tasks (Shaywitz *et al.,* 1995). However, there were no statistically significant differences in gender between the groups, furthermore, there were no demonstrable differences in activation due to gender at the second level.

Overall, these results indicate that there are state and trait features of schizophrenia that can be demonstrated with functional imaging. No group differences were found in lateral prefrontal or temporal regions. However differences of apparent genetic cause were found in medial prefrontal-thalamic-cerebellar regions, and additional differences were found in subjects at high risk with isolated psychotic symptoms in the parietal lobe. These patterns of activation reveal information about the pathophysiology of the state of vulnerability to schizophrenia,

4.1 INTRODUCTION

From the previous chapter, baseline fMRI in the EHRS subjects has shown that those at high genetic risk of schizophrenia demonstrate deficits in prefrontal-thalamic-cerebellar activation, and in addition those at high genetic risk with isolated psychotic symptoms demonstrate altered inferior parietal cortex activity. However the modern view is that the symptoms of schizophrenia could arise from a disruption of integrated brain networks, consistent with models of connectivity in brain imaging.

As described in chapter two, studies of connectivity in subjects with established schizophrenia have reported altered connectivity with prefrontal regions: including interactions with temporal (Friston *et al.*, 1996; Lawrie *et al.*, 2002a), thalamic-cerebellar (Schlösser *et al.* 2003), and parietal regions (Kim *et al.* 2003), and between lateral and medial prefrontal regions (Spence *et al.,* 2000). In addition there is increasing evidence for disrupted white matter in the disorder (see Spalletta *et al.*, 2003; Burns *et al.*, 2003). However, it is unclear whether these deficits are related to inherited vulnerability to the disorder, or to the presence of symptoms, or medication effects. Furthermore, it is possible that patterns of connectivity may be dependant on the type of tasks used. This chapter will focus on functional connectivity in high risk subjects to address the first aim detailed in chapter 3, to examine state and trait effects in these individuals.

Three specific hypotheses regarding altered connectivity in the high risk subjects were tested; (i) state-related differences in dorsolateral prefrontal to superior/middle temporal gyrus connectivity in those at high risk with symptoms, as reported in a previous study in established schizophrenia (Lawrie *et al.*, 2002a). Fronto-temporal disconnectivity was hypothesised to be a state-related, rather than trait related effect, since it had previously been associated with symptoms (Lawrie *et al.*, 2002a), and had *not* been found in asymptomatic subjects at increased genetic risk (Spence *et al.*, 2000). (ii) genetically mediated reduced connectivity in a medial prefrontal-thalamic-

cerebellar network, and (iii) increased connectivity between dorsolateral prefrontal to inferior parietal lobule in high risk subjects, to a greater extent in those with symptoms. Hypotheses (ii) and (iii) were based on the previous localization study in high risk subjects (chapter three), and on previous findings in the literature (Schlösser *et al.*, 2003; Kim *et al.*, 2003).

Since the EHRS subjects represent such an unusual and valuable data set, the opportunity was also taken to perform exploratory analysis using a comprehensive range of seed regions. These seeds were defined according to three main criteria; language based seeds, schizophrenia regions of interest, and finally a set of empirically derived seeds. This study sought to test the results from the Hayling task, from the hypothesis driven and exploratory results, in two additional tasks performed by the subjects during the same scanning session (verbal encoding and retrieval task). This was primarily conducted to address multiple comparison issues. In order to permit this cross task comparison, and in view of the fact that inter-regional connectivity may be influenced by task effects (chapter 1), the current study will examine connectivity with the variance associated with task activations modelled and removed from the data. This comparison across tasks provided the opportunity to identify task invariant effects, rather than abnormalities that might just reflect differences on particular experimental paradigms.

4.2 METHODS

4.2.1 Study populations

The study populations examined in this chapter are identical to those described in the previous functional localisation chapter. Briefly, out of the first 100 subjects, 6 declined or were unable to participate in scanning. For the Hayling task four subjects were subsequently excluded: two due to minor vascular malformations and two due to excessive movement. A total of 90 subjects provided usable fMRI scans, comprising 21 normal controls and 69 high risk subjects. On detailed interview (the Present State Examination, Wing *et al.*, 1974) none of the EHRS subjects or controls

met criteria for any psychotic disorder. Twenty seven high risk subjects reported isolated psychotic symptoms; the remainder of the high risk group (n=42) and all of the controls had no psychotic symptoms. None of the subjects were on anti-psychotic medication, seeking treatment, or indeed saw themselves as unwell. Demographic details are presented in the previous chapter, Table 3.1.

For the encoding and retrieval tasks, in addition to the two subjects excluded due to vascular abnormalities, three subjects were excluded: two due to loss of behavioural data, and one due to excessive movement. It should be noted however that the two subjects excluded due to movement in the Hayling analysis are not the same as the subjects excluded due to movement and loss of behavioural data in the encoding and retrieval analysis. In the Hayling analysis the excluded subjects consisted of one male left hander and one female right hander. For the encoding and retrieval analysis excluded subjects consisted of one male right hander and two female right handers. All the excluded subjects belonged to the high risk without symptoms group. For the encoding and retrieval tasks therefore the control and high risk with symptom group were identical to those described for the Hayling task. For the high risk without symptoms demographic details for the encoding and retrieval tasks are as follows: n=41, age (26.6 years, sd 3.3); gender (male:female=17:24); handedness (right:left:mixed=37:3:1).

4.2.2 Scanning procedure and experiments

For the Hayling task, scanning and experimental details are presented in the previous chapter. Briefly, subjects were shown sentences with the last word missing and were asked to think of an appropriate word to complete the sentence (without speaking the word), and press a button when they had done so.

The encoding and retrieval paradigms were originally adapted for use in the Edinburgh High Risk Study by Dr S Lawrie. The paradigms were programmed in E-Prime (Psychology Software Tools (PST), Pittsburgh, PA) by Ms S Flett and Dr E Simonotto. For the encoding and retrieval experiments functional data were acquired

aligning the scanning planes to the AC-PC direction (near axial) using the following scanning parameters: TR/TE= 2000/40 ms; matrix 64x64; FOV 22x22 cm^2; 24 slices; 5 mm thickness; no gap. A total of 104 volumes were acquired for the encoding experiment, and 204 volumes were acquired for the retrieval task. The first four volumes of each acquisition were discarded. For the encoding a 'living versus non-living' classification task was used to elicit the semantic encoding of 36 words. For the retrieval task subsequent explicit memory for these words was tested by presentation of 36 studied ('old') words intermixed with 36 similar distractor ('new') words of similar complexity and use frequency, in an 'old versus new' word discrimination task. For the encoding task, single words were presented on the screen for 2 s followed by a fixation cross for a variable duration of 2-10 s. Subjects were requested to classify the item as either living or non-living by pressing the thumb or index finger button respectively to signify the response. Subjects could respond at any time during the presentation of the stimuli and subsequent fixation period. In total, 36 words were randomly presented, consisting of 18 words referring to living things, and 18 referring to non-living items. Similarly for the retrieval task, subjects were shown single words for 2 s followed by a fixation cross for a variable duration of 2-10 s. In total 72 words were randomly presented, consisting of the same 36 words presented during the encoding task intermixed with 36 matched similar new words. Subjects were requested to classify the item as a new or old word by pressing the thumb or index finger button respectively. Subjects could respond at any time during the presentation of the stimuli and subsequent fixation period. For both the encoding and retrieval tasks, subjects were given a practice session with feedback of their responses immediately before starting the actual task. All three tasks were collected in the same session and for all subjects the order for each session was identical; the Hayling task, followed by the encoding and the retrieval task.

Since there are common methodologies regarding scan processing and analysis for all three tasks they are described together below. However, it should be appreciated that the main experiment in the current study was the Hayling task; the additional encoding and retrieval tasks were only used to test significant results from

this task. Only those exploratory results that were replicated across tasks were considered significant.

4.2.3 Scan processing

Analysis was carried out using SPM99 (The Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, http://www.fil.ion.ucl.ac.uk/spm/). Pre-processing of the Hayling data was described in detail in the previous chapter. Briefly, data were first realigned to the first scan in the series to correct for head movement, normalised to the standard SPM EPI template, and smoothed with a 6x6x6 mm^3 FWHM Gaussian filter. For the encoding and retrieval tasks the data was first corrected for slice timing using Fast Fourier Transform interpolation. Data was then realigned to the first volume in the series, normalised to the SPM EPI template using linear and non-linear transformations, and smoothed with a 6x6x6 mm^3 FWHM Gaussian filter.

4.2.4 Functional connectivity

4.2.4.1 *Seed locations: Hayling task*

To address the three specific hypotheses, voxels in bilateral dorsolateral prefrontal cortex, superior/middle temporal gyrus, medial frontal gyrus, thalamus, and the inferior parietal lobule were identified based on previous findings (Lawrie *et al.*, 2002 and locations identified in the previous chapter).

For the exploratory analysis three main groups of seeds were selected. Present knowledge of the neural underpinnings of language comprehension and production were used as criteria to place a group of 'language' based seeds (n=18, comprising; inferior frontal gyrus (pars triangularis), rolandic operculum, frontal operculum, caudate, planum temporale, anterior and posterior superior temporal sulcus, inferior temporal gyrus, and fusiform gyrus, bilaterally). These were selected by an expert in the fields of linguistics and neuroimaging (Dr M Meyer), co-ordinates were

determined using a combination of the SPM structural template and the Talairach and Tournoux brain atlas. An additional 6 seeds were identified, which were not already included in the above list, on the basis of the schizophrenia literature as summarised in chapter two (anterior cingulate, anterior and posterior amygdala-hippocampal regions, bilaterally). As before co-ordinates were determined using a combination of the SPM structural template and the Talairach and Tournoux brain atlas. Finally a group of empirically derived seeds were selected based on regions activated in the Hayling task according to our previous localisation results, (n=12: caudal medial prefrontal gyrus, anterior and posterior middle temporal gyrus, precentral gyrus, inferior frontal gyrus, and lingual gyrus, bilaterally). Co-ordinates for all seed locations are detailed in the relevant tables below.

4.2.4.2 *First level analysis: Hayling, encoding and retrieval*

The methods for determining functional connectivity were devised by Dr E Simonotto, and have been published previously (Deary *et al.*, 2004). Maps of cross correlation coefficients were computed by measuring the correlation (in time) between each 'seed' brain region (i.e. a small sphere of 6 mm radius), and all the other brain voxels. Before computing the cross correlations, the voxel time courses were filtered in time (high pass filter: cut off period of 400 s, 150 s, 150 s for Hayling, encoding, retrieval respectively; low pass filter: convolution with a Gaussian with 4 s FWHM) and corrected for global signal fluctuations by global scaling of the images.

In order to reduce the amount of cross correlation purely induced by task related effects, and to permit testing the Hayling task in the additional paradigms, the task conditions were modelled with either standard block effects (Hayling) or event related responses (both encoding and retrieval), all convolved with canonical haemodynamical response functions. This model (which also included the six regressors for the estimated head movement) was fitted to the time filtered data and the residuals of this procedure were used to compute the cross correlations maps.

Since the distribution of cross correlation values are not normally distributed, the functional connectivity maps were transformed using the *r*-to-*z* Fisher transform to permit further statistical analysis:

$$z = [1/2]\log([(1+r)/(1-r)])$$

where r = Pearson's correlation

The mean and standard deviation of each corrected map were then estimated and each map was rescaled to zero mean and unity standard deviation (Lowe *et al.*, 1998; Hampson *et al.*, 2002). Corrected maps were finally smoothed with a Gaussian kernel of 6x6x6 mm^3. After the processing steps these values are now in arbitrary units; where positive values refer to positive correlations, zero refers to no observed correlation, and negative values refer to negative correlations. Batch scripts written by Dr E Simonotto were used to perform the first level statistical analysis.

4.2.4.3 *Second level analysis: Hayling task*

4.2.4.3.1 Main trait and state effects

Group analyses were performed for each one of the seeds in the study by entering the cross correlation maps (after the processing steps described above) into an ANOVA. As in the previous chapter, differences in activation due to 'trait' effects were initially examined by comparing controls versus all high risk subjects (and vice versa). Symptomatic 'state' effects were initially examined by comparing the non-symptomatic groups (controls plus high risk without symptoms) versus high risk subjects with symptoms (and vice versa). The contrast maps computed for the Hayling task were first thresholded at $p < 0.01$ (uncorrected for multiple comparison) and minimum cluster size k=50. Regions were considered significant at $p < 0.05$, cluster level, corrected for multiple comparisons. This analysis structure was chosen to simplify the reporting of results and to minimize the number of group comparisons.

4.2.4.3.2 Post-hoc trait and state effects

In order to further clarify any trait/state related findings, *post-hoc* group comparisons were conducted as in the previous chapter. The criteria followed for differences to be identified as potential trait-specific effects were that similar differences should be found between controls versus high risk with symptoms, and between controls versus high risk without symptoms, but *not* between high risk with, versus high risk without symptoms. Criteria for any potential state-specific effects were that similar differences should be found between high risk with symptoms versus controls, and between high risk with, versus high risk without symptoms, but *not* between high risk without symptoms versus controls.

4.2.4.3.3 Genetic liability

Although the trait contrast described above addressed issues relating to the presence/absence of genetic vulnerability, the study also sought to determine whether there were any associations with the degree of that risk within the high risk group. Categorical and continuous measures of risk were therefore used to examine associations between measures of functional connectivity and inherited vulnerability in the high risk group. These analyses were performed primarily to clarify whether trait effects found using the above contrasts were indeed influenced by the degree of inherited liability. For the categorical measure, high risk individuals were classified as having any first degree relatives with the disorder, or only second degree relatives, details in Table 3.1. Group comparisons were made by examining differences between those with any first versus those with only second degree relatives. The regression with the continuous measures of the degree of inherited risk was performed only on the high risk subjects using a single covariate of interest. The technique for generating this measure of genetic liability in high risk subjects was developed by Professor P Sham, and has been described previously (see Chapter 3, and Figure 3.1). Since the primary interest was to determine if any of the trait-related findings were associated with inherited vulnerability, the analyses for both categorical and continuous measures of genetic liability were performed using

inclusive masks determined by the significant clusters reported in the Hayling trait contrasts. Unlike the previous chapter, analysis regarding association with the degree of risk was not preformed at the whole brain level due the large number of comparisons this would involve.

4.2.4.3.4 Functional connectivity across tasks

Significant connectivity results from the Hayling paradigm were tested in the two additional paradigms, the encoding and retrieval tasks. This was to address issues relating to multiple comparisons due to the large number of seeds tested. To reduce the possibility of false positive findings only those exploratory results that were replicated across tasks were considered significant. To determine if there were consistent differences across tasks, a region based on the Hayling cluster(s) of interest was used as an inclusive mask to restrict the analysis in the additional tasks.

All p values quoted are at the corrected cluster level. Co-ordinates were converted from MNI (Montreal Neurological Institute) to Talairach co-ordinates using a non-linear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging).

4.3 RESULTS

4.3.1 Behaviour

There were no significant differences in mean age, gender, mean NART IQ, or handedness between the groups (Table 3.1). There were also no significant differences between the high risk groups (with and without psychotic symptoms) in terms of genetic liability. Performance on the Hayling task is detailed in the previous chapter (Table 3.3). Performance on the encoding and retrieval tasks is outlined below (Tables 4.1-2). There will be no further description of the behavioural measures or functional localisation results for the encoding and retrieval tasks since they are the subject of another PhD thesis within this department (Ms MC Whyte), however in general these measures indicated, as in the Hayling task, that subjects

were performing the tasks appropriately in the scanner, and there were no significant differences in performance between the groups.

Table 4.1 Behavioural results: encoding task

	Controls	High risk without symptoms	High risk with symptoms
Number of no responses	0.1 (0.6)	0.1 (0.8)	0.1 (0.3)
Number of incorrect responses	0.5 (1.2)	0.7 (1.1)	0.9 (1.4)
Number of correct responses	35.4 (1.2)	35.2 (1.1)	35.0 (1.4)
Reaction time in (ms)	928 (459)	1003 (353)	1006 (420)

Values represent mean number of responses (std dev)

Table 4.2 Behavioural results: retrieval task

	Controls	High risk without symptoms	High risk with symptoms
Number of no responses	6.2 (6.9)	5.1 (6.9)	5.1 (4.5)
Total number incorrect	14.5 (4.8)	13.5 (4.5)	15.2 (5.7)
Total number correct	51.3 (10.6)	53.3 (9.1)	51.6 (8.5)
Reaction time in (ms)	1322 (385)	1254 (339)	1313 (353)

Values represent mean number of responses (std dev)

4.3.2 Within group results (Hayling)

The main focus of this chapter concerns functional connectivity differences in the high risk subjects attributable to trait and state effects. However, to be confident of the validity of the between group differences it is also important to examine within group results. Due to the considerable amount of data, only the results for the hypothesised seeds are reported here. The remaining patterns of connectivity for the exploratory seeds are summarised below, full details are contained within the appendix.

4.3.2.1 *Hypothesis-driven seeds*

Results of the within group functional connectivity analyses for the Hayling task for the hypothesised seeds are presented in Tables 4.3-5. For clarity the clusters centred on the seed locations are not reported in the tables, and unlike other tables in this thesis, only the first maxima per cluster is recorded. Visual inspection of the within group maps indicated similar areas of connectivity across the groups, however the cluster sizes for the high risk without symptoms group were consistently larger than for the controls, and high risk with symptoms, potentially due to the larger number of subjects in this group (for example see Appendix Figure 2). Reporting only the first maxima per cluster in the high risk without symptoms group was therefore not considered to reflect the true extent of connectivity in this group. Since these within group results were primarily descriptive, it was decided to select a more stringent threshold for the high risk without symptoms group in order to divide the larger clusters into sub-regions. However, for the second level group comparisons all groups were treated identically, and it should be stressed that at the second level differences in group sizes are not considered to invalidate results in SPM[1],[2]

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[1] Dr Penny: http://www.jiscmail.ac.uk/cgi-bin/wa.exe?A2=ind0205&L=spm&P=R3408&D=0&I=-1
[2] Dr Nichols: http://www.jiscmail.ac.uk/cgi-bin/wa.exe?A2=ind0205&L=spm&P=R2693&D=0&I=-1

The data presented in the tables and text below indicate regions where there were positive correlations between the time courses in the seed region and the clusters reported. The seeds located in the dorsolateral prefrontal region (BA 9/46) demonstrated functional connectivity with the insula in the control group. In the two high risk groups connectivity was seen with contralateral prefrontal regions (BA 9/46), and with parietal regions (BA40). The seeds located in the middle temporal gyrus (BA 21) demonstrated connectivity with homologous contralateral regions across all three groups, with additional connectivity with prefrontal regions (BA 6 and 45) in the high risk groups. Seeds located in rostral medial prefrontal regions (BA 10/32) demonstrated connectivity with superior/medial prefrontal regions (BA 8/32) and superior temporal gyrus (BA 22) across all three groups. Seeds located in the thalamus demonstrated connectivity with the anterior cingulate across all three groups, with additional connectivity to inferior frontal gyrus (BA 45) in the controls. Finally seeds located in the parietal lobe (BA7/40) demonstrated connectivity with homologous contralateral regions, precentral gyrus (BA 6), and cingulate (BA 24/32) across all three groups, with additional connectivity with lateral prefrontal regions (BA 45, BA44/9) and lateral temporal regions (BA 21) in the two high risk groups.

Since previous studies have reported negative or inverse connectivity between dorsolateral prefrontal regions and lateral temporal cortex, for the seeds located in the bilateral dorsolateral prefrontal cortex negative associations were also examined. Across all three groups there was evidence of negative connectivity between left dorsolateral prefrontal cortex and right middle temporal gyrus (BA21), in the controls ($x = 58$, $y = -12$, $z = -15$, $Z = 6.60$), high risk without symptoms ($x = 50$, $y = -8$, $z = -13$, $Z = 5.90$), high risk with symptoms ($x = 53$, $y = -9$, $z = -18$, $Z = 4.23$). For the seed located in the right dorsolateral prefrontal cortex there was negative connectivity with bilateral middle temporal gyrus (BA21), in the controls ($x = -46$, $y = 3$, $z = -20$, $Z = 5.08$; $x = 59$, $y = -11$, $z = -16$, $Z = 4.62$), high risk without symptoms ($x = -57$, $y = -7$, $z = -22$, $Z = 6.91$; $x = 57$, $y = 1$, $z = -27$, $Z = 5.89$) and high risk with symptoms ($x = -42$, $y = -2$, $z = -22$, $Z = 4.93$; $x = 61$, $y = -9$, $z = -18$, $Z = 4.84$).

Table 4.3 Controls (*n*=21)

Seed location	P value	Z score	Peak height co-ordinates	Region
L dorso-lateral prefrontal, BA9/46 (-40 32 21)	<0.001	5.66	-34 16 8	L insula
R dorso-lateral prefrontal, BA9/46 (40 32 21)				*No clusters at 0.05 or 0.01 corrected threshold other than that centred on seed location.*
L posterior middle temporal g, BA21 (-53 -44 6)				*No clusters at 0.05 or 0.01 corrected threshold other than that centred on seed location*
R posterior middle temporal g, BA21 (53 -44 6)	<0.001	6.45	-50 -46 12	L temporal: middle temporal g, BA 21
L rostral medial frontal g, BA10/32 (-14 47 0)	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001	6.60 6.12 5.95 5.93 5.49 5.33	-20 18 41 20 22 50 -20 22 17 -36 -44 19 28 24 21 38 -7 24	L frontal: superior/medial frontal g, BA 8 R frontal: superior/medial frontal g, BA 8 L sub-lobar L temporal: superior temporal g, BA 22 R frontal: inferior/middle frontal g, BA 9 R frontal: precentral g, BA 4
R rostral medial frontal g, BA10/32 (14 47 0)	<0.001 <0.001	6.84 6.21	18 22 47 -14 47 0	R frontal: superior/middle frontal g, BA 8 L frontal: medial frontal/anterior cingulate g, BA 10/32
L thalamus (-8 -13 6)	<0.001 <0.001	6.10 5.53	-36 25 4 14 8 9	L frontal: inferior frontal g, BA 45 R sub-lobar: thalamus
R thalamus (8 -13 6)	<0.001	6.72	-2 14 38	L limbic: anterior cingulate g, BA 32
L inferior parietal lobule, BA40/7 (-42 -48 48)	<0.001 <0.001	6.65 5.44	55 -33 48 -14 2 40	R parietal: inferior parietal lobule, BA 40/7 R limbic: cingulate g, BA24/32
R inferior parietal lobule, BA40/7 (42 -48 48)	<0.001 <0.001 <0.001	6.79 6.15 5.62	-28 -58 50 10-10 35 16 5 57	L parietal: sup/inf parietal lobule, BA7/40 R limbic: cingulate g, BA 24 R frontal: superior frontal g, BA 6

Analyses thresholded at 0.05 corrected cluster level, extent threshold =100.

Table 4.4 High risk without symptoms (*n*=42)

Seed location	P value	Z score	Peak height co-ordinates	Region
L dorso-lateral prefrontal, BA9/46 (-40 32 21)	<0.001	Inf	28 32 22	R frontal: middle frontal g, BA 9/46
	<0.001	7.29	-36 -39 33	L temporal: fusiform g, BA 40
R dorso-lateral prefrontal, BA9/46 (40 32 21)	<0.001	7.58	-32 23 26	L frontal: inferior/middle frontal g, BA 9
	<0.001	7.12	51 -31 40	L parietal: inferior parietal lobule, BA 40
L posterior middle temporal gyrus, BA21 (-53 -44 6)	<0.001	7.18	55 -31 0	R temporal: middle temporal g, BA 21
	<0.001	6.60	-8 -1 61	L frontal: medial frontal g, BA 6
	<0.001	6.53	-38 17 -3	L frontal: inferior frontal gyrus/ frontal operculum, BA 45/47
R posterior middle temporal gyrus, BA21 (53 -44 6)	<0.001	6.69	-50 -46 10	L temporal: superior/middle temporal g, BA 22/21
L rostral medial frontal gyrus, BA10/32 (-14 47 0)	<0.001	610	-18 0 -2	L sub-lobar: lentiform nucleus
	<0.001	6.00	-27 30 -6	L frontal: orbito/inferior frontal g, BA47
	<0.001	5.99	11 10 -5	R sub-lobar: border caudate/lentiform
R rostral medial frontal gyrus, BA10/32 (14 47 0)	<0.001	Inf	-16 23 36	L frontal: superior/medial frontal g, BA32
	<0.001	Inf	20 25 39	R frontal: superior frontal g, BA 6/8
	<0.001	7.52	-38 -48 12	L temporal: superior temporal g, BA 22
	<0.001	6.86	32 -20 30	R parietal: postcentral g, BA 2
L thalamus (-8 -13 6)	<0.001	6.15	-2 3 53	L limbic: cingulate g, BA 32/6
R thalamus (8 -13 6)	<0.001	6.07	8 14 40	R limbic: cingulate g, BA 32
L inferior parietal lobule, BA40/7 (-42 -48 48)	<0.001	6.82	-32 18 3	L frontal operculum/insula, BA45
	<0.001	6.35	-53 -53 -2	L temporal: mid/inf temporal g, BA 21/37
	<0.001	5.79	55 -51 -6	R temporal: mid/inf temporal g, BA21/37
	<0.001	5.77	-20 6 7	L sub-lobar: lentiform nucleus
R inferior parietal lobule, BA40/7 (42 -48 48)	<0.001	Inf	-38 -46 47	L parietal: inf parietal lobule, BA40/7
	<0.001	Inf	46 4 37	R frontal: precentral g, BA 6
	<0.001	7.44	51 -51 -6	R temporal: inferior temporal g, BA 37
	<0.001	7.03	-8 -10 37	L limbic: cingulate g, BA 24
	<0.001	7.00	10 -31 40	R limbic: cingulate g, BA 31
	<0.001	7.00	-24 -3 54	L frontal: superior frontal g, BA 6
	<0.001	6.24	30 20 5	R frontal: frontal operculum, BA 45
	<0.001	6.08	8 22 45	R frontal: superior frontal g, BA 8/32

Analyses thresholded at 0.005 corrected cluster level, extent threshold =100.

Table 4.5 High risk with symptoms (*n*=27)

Seed location	P value	Z score	Peak height co-ordinates	Region
L dorso-lateral prefrontal, BA9/46 (-40 32 21)	<0.001	6.63	40 38 15	R frontal: inferior frontal g, BA 46
	<0.001	6.29	-24 -55 34	L parietal: superior parietal lobule, BA 7/40
R dorso-lateral prefrontal, BA9/46 (40 32 21)	<0.001	7.06	-36 32 17	L frontal: middle frontal g, BA 9/46
	<0.001	6.18	42 -45 35	R parietal: inferior parietal lobule, BA 40
L posterior middle temporal g, BA21 (-53 -44 6)	<0.001	6.50	-38 0 31	L frontal: precentral g, BA 6
	<0.001	6.37	48 -37 7	R temporal: superior temporal g, BA 22
	<0.001	5.61	-24 8 0	L sub-lobar: lentiform nucleus
	<0.001	5.30	-44 26 15	L frontal: inferior frontal g, BA 45
R posterior middle temporal g, BA21 (53 -44 6)	<0.001	6.47	-53 -50 4	L temporal: middle temporal g, BA 21
L rostral medial frontal g, BA10/32 (-14 47 0)	<0.001	7.12	-2 18 22	L limbic: cingulate g, BA 24
	<0.001	6.27	19 21 45	R frontal: superior/middle frontal g, BA 8/6
	<0.001	6.12	29 -14 12	R sub-lobar: lentiform nucleus
R rostral medial frontal g, BA10/32 (14 47 0)	<0.001	6.97	-20 -57 27	L parietal: precuneus, BA 7
	<0.001	6.19	-32 -41 2	L temporal: fusiform/parahippocampal g
	<0.001	5.70	38 -46 12	R temporal: superior temporal g, BA 22
L thalamus (-8 -13 6)	<0.001	7.27	18 13 3	R sub-lobar: lentiform nucleus (also overlaps thalamus)
R thalamus (8 -13 6)				*Cluster centred on seed location overlaps L thalamus*
	<0.001	5.78	6 12 45	R frontal: cingulate/medial frontal g, BA 32
L inferior parietal lobule, BA40/7 (-42 -48 48)	<0.001	Inf	44 -37 39	R parietal : inferior parietal lobule, BA 40
	<0.001	6.95	48 7 18	R frontal: inferior frontal g, BA 44
	<0.001	5.78	-20 4 11	L sub-lobar: lentiform nucleus
	<0.001	5.76	-55 -54 1	L temporal: middle temporal g, BA 21
R inferior parietal lobule, BA40/7 (-42 -48 48)	<0.001	Inf	-36 -45 35	L parietal: inferior parietal lobule, BA 40/7
	<0.001	7.44	55 11 18	R frontal: inferior frontal g, BA 9/44
	<0.001	6.90	-26 -9 48	L frontal: sup frontal/precentral g, BA 4/6
	<0.001	6.29	38 34 28	R frontal: middle frontal g, BA 9/46
	<0.001	6.20	26 -3 11	R sub-lobar: lentiform nucleus

Analyses thresholded at 0.05 corrected cluster level, extent threshold =100.

4.3.2.2 *Exploratory analysis*

Results for the exploratory seeds are detailed in the appendix. In the main, the results across all three groups indicated positive connectivity between the various seed locations and homologous contralateral regions. Additional results are summarised below, grouping seeds into the main cortical regions.

4.3.2.2.1 Lateral prefrontal cortex

For the seeds located in lateral prefrontal regions, connectivity across groups was seen between the pars triangularis, superior/middle frontal gyrus and superior/middle temporal gyrus. Seeds located in the frontal operculum and inferior frontal gyrus (BA44) showed a somewhat different pattern of connectivity across the groups, where the controls showed connectivity to cingulate regions (BA 32), whereas the high risk groups showed connectivity with parietal regions (BA 40).

4.3.2.2.2 Medial prefrontal cortex

Seeds located in the anterior cingulate demonstrated different patterns of connectivity across the groups. In the controls there was connectivity with superior/medial prefrontal regions (BA 8), in the high risk groups connectivity was seen with subcortical structures and inferior frontal regions (BA 47/45). A similar pattern was seen with seeds in caudal medial prefrontal regions, where connectivity in the high risk group also involved subcortical structures.

4.3.2.2.3 Lateral temporal neocortex

Seeds located in lateral temporal regions (superior temporal gyrus, inferior temporal gyrus and fusiform gyrus) in general demonstrated connectivity to homologous contralateral regions and superior and medial prefrontal regions (BA 8/6 and 32) across groups. In addition connectivity was observed between anterior superior temporal gyrus and parietal regions across the groups.

4.3.2.2.4 *Medial temporal regions*

At the thresholds chosen, there were no regions connected with seeds located in medial temporal regions other than homologous regions in the contralateral hemisphere.

4.3.3 Between groups (Hayling)

4.3.3.1 *Hypothesis-driven seeds*

For all analyses, only those results which fulfilled the more strict criteria for trait- or state-related effects are presented. Results for the hypothesis driven seeds are presented in Table 4.6. Regarding the first hypothesis of state-related differences in connectivity between dorsolateral prefrontal cortex and superior/middle temporal gyrus, there was no evidence for either increased or decreased connectivity between these regions in the high risk subjects with symptoms, consistent with the within group maps.

For the hypothesis of trait-related reductions in medial prefrontal-thalamic-cerebellar connectivity, decreases were seen in high risk subjects versus controls between right medial frontal gyrus and left cerebellum, Figure 4.1(a,c). The graphical display in Figure 4.1(c) indicates that both high risk groups manifested larger negative correlation values than the control group. Decreases in connectivity were also seen between the left thalamus and prefrontal regions, although these were lateral, rather than medial prefrontal.

Regarding the hypothesis of increased connectivity between prefrontal-parietal regions in high risk subjects, as reflected by the within group patterns of connectivity, increases were seen between left inferior parietal regions and left inferior frontal gyrus, Figure 4.1(b). This occurred in all high risk subjects, not specifically in those with symptoms. Closer inspection indicated significant increases across the three groups. Using a contrast weighting of [-3 1 2], representing increases across groups, this connectivity was found to be lowest in the controls, higher in

those at high risk without symptoms and greatest in those at high risk with symptoms ($p=0.021$, $x = -52$, $y = 30$ $z = 12$), suggesting the presence of additional state components associated with this finding. Figure 4.1(d) indicates that all three groups presented positive correlations between these regions, however the high positive correlations in the high risk with symptoms group may be influenced by one subject. As a point of note, this particular subject later made the transition to schizophrenia, which is the focus of following chapters.

Table 4.6 Hypothesis driven seeds

Seed location	P value (Z score)	Peak height co-ordinates	Region of connectivity difference	Post-hoc P values
Trait contrast: controls > high risk				***(c>hr_n); (c>hr_p); (hr_n>hr_p)***
L rostral medial frontal, BA10/32 (-14 47 0) (ii)	n/s	-	-	-
R rostral medial frontal , BA10/32 (14 47 12) (ii)	<0.001 (4.28)	-30 -51 -16 -16 -54 -16 -6 -66 -16	L cerebellum: anterior lobe L cerebellum: anterior lobe L cerebellum: posterior lobe	<0.001; <0.001; n/s
L thalamus (-8 -13 6) (ii)	0.056 (4.65)	34 26 -24 36 18 -16 24 10 -11	R frontal: inferior frontal g, BA11 R frontal: inferior frontal g, BA47 R frontal: inferior frontal g	0.003; 0.004; n/s
R thalamus (8 -13 6) (ii)	n/s	-	-	-
Trait contrast: controls < high risk				***(c<hr_n); (c<hr_p); (hr_n<hr_p)***
L inferior parietal lobule, BA40/7 (-42 -48 48) (iii)	0.008 (3.75)	-50 30 14 -45 27 17 -38 32 -3	L frontal: inferior frontal g, BA9/46 L frontal: inferior frontal g, BA45 L frontal: inferior frontal g	0.001; 0.007; n/s
R inferior parietal lobule, BA40/7 (42 -48 48) (iii)	-	-	-	-
State contrast: no symptoms > symptoms				***(c>hr_p); (hr_n>hr_p); (c>hr_n)***
L dorso-lateral prefront, BA9/46 (-40 32 21) (i)	n/s	-	-	-
R dorso-lateral prefront, BA9/46 (40 32 21) (i)	n/s	-	-	-
L posterior middle temporal g, BA21 (-53 -44 6) (i)	0.049 (4.58)	9 18 24 6 38 23 -5 20 25	R limbic: anterior cingulate, BA24 R limbic: anterior cingulate, BA32 L limbic: anterior cingulate, BA24	0.002; 0.035; n/s
R posterior middle temporal g, BA21 (53 -44 6) (i)	n/s	-	-	-
State contrast: no symptoms < symptoms				***(c>hr+); (hr->hr+); (c>hr-)***
L inferior parietal lobule, BA40/7 (-42 -48 48) (iii)	n/s	-	-	-
R inferior parietal lobule, BA40/7 (-42 -48 48) (iii)	n/s	-	-	-

hr_n=high risk without, hr_p=high risk with symptoms. (i)-(iii) indicates respective hypotheses. For hypothesis (i) there were no significant state-related connectivity increases.

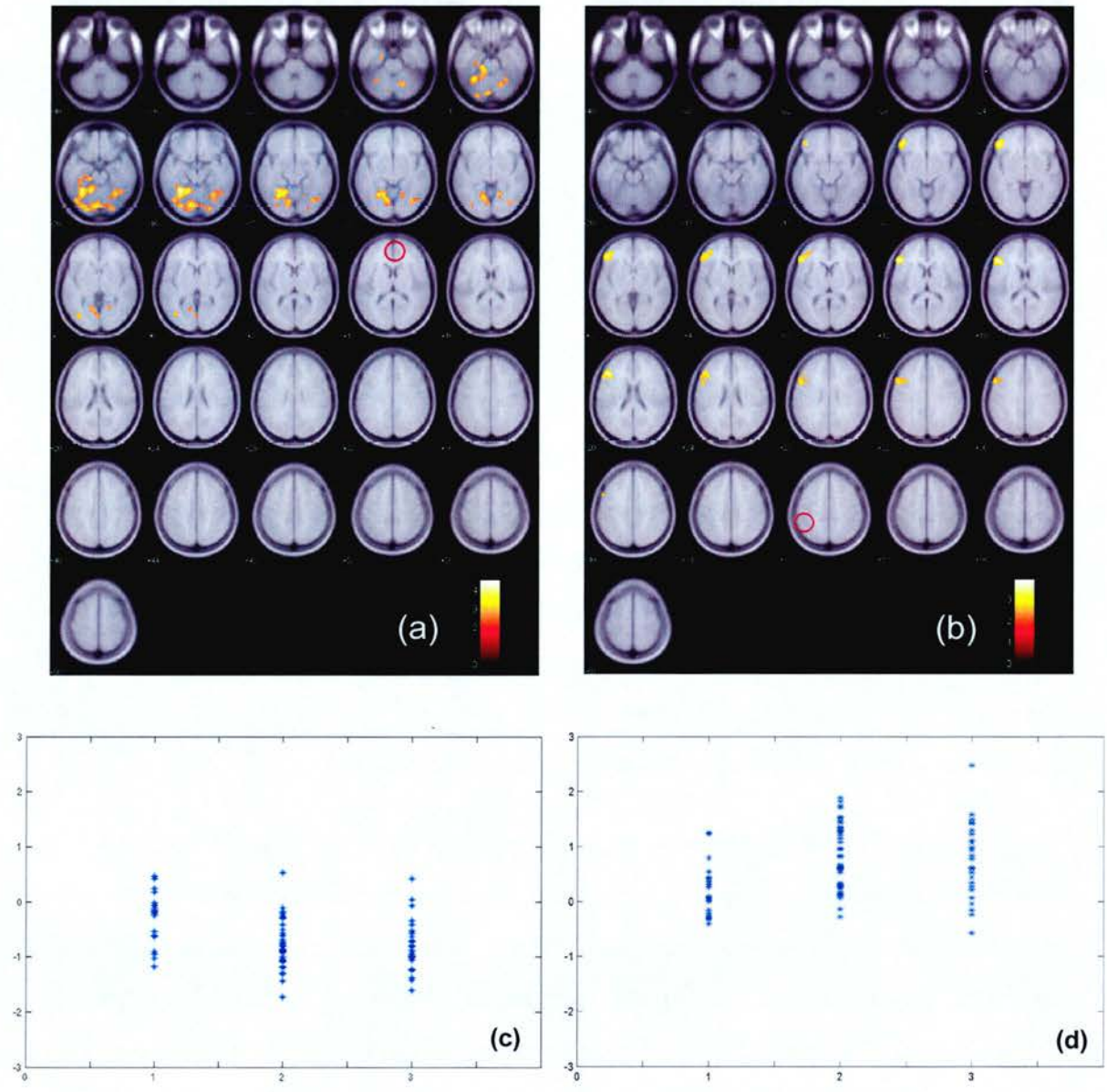

Figure 4.1 Hypothesis-driven seeds

Figure shows connectivity differences in high risk subjects for the hypothesized seeds. Red circle illustrates approximate seed position. The left side of image represents the left side of brain. Colour bar indicates Z score. Maps thresholded at $p<0.01$ uncorrected voxel level, $k=800$. (a) and (c) reduced connectivity in high risk subjects versus controls between right medial frontal gyrus (seed) and left cerebellum, consistent with hypothesis (ii); (b) and (d) increased connectivity in high risk subjects versus controls between left inferior parietal lobule (seed) and left inferior frontal gyrus, consistent with hypothesis (iii). Connectivity values on Y axis in graphs (c) and (d) in arbitrary units (see text), x axis represents 1 = controls; 2 = high risk without symptoms; 3 = high risk with symptoms.

4.3.3.2 *Exploratory analyses*

Trait-related findings from the exploratory analysis are summarised into categories according to findings reported in the literature i.e. lateral to medial prefrontal, prefrontal-temporal, prefrontal-thalamic-cerebellar, and prefrontal-parietal (Tables 4.7 and 4.8). These findings are in addition to the results from the hypothesised seeds described above.

Lateral to medial prefrontal connectivity deficits were seen in high risk subjects versus controls between right inferior frontal gyrus and bilateral superior/medial prefrontal region, between right rolandic operculum and bilateral anterior cingulate, and between left inferior frontal gyrus and left medial prefrontal regions. Prefrontal-temporal connectivity deficits were seen between right inferior frontal gyrus and left lateral temporal regions, and reciprocally between left medial prefrontal regions and left medial temporal regions. Prefrontal-thalamic-cerebellar deficits were seen between left inferior frontal gyrus and the contralateral cerebellum, and between left frontal operculum and right and left thalamus.

State-related findings from the exploratory analysis are also summarised into categories as above (Tables 4.9 and 4.10). Decreased connectivity in those with symptoms was seen between the left inferior frontal gyrus and bilateral medial temporal regions (uncus and parahippocampal gyrus). In terms of prefrontal-thalamic-cerebellar connectivity decreased connectivity in those with symptoms was seen between left inferior frontal gyrus and bilateral cerebellum. Increases in connectivity in those with symptoms was seen between left caudate, bilateral anterior cingulate and subcortical structures, and between right superior temporal gyrus and a regions located in the depths of the calcarine sulcus.

Only those findings that were replicated in the additional tasks were considered significant, these findings are discussed in a following section.

Table 4.7 Exploratory analysis, trait contrast: controls > high risk

Seed location	P value (Z score)	Peak height co-ordinates	Region of connectivity difference	Post-hoc P values (c>hr_n); (c>hr_p); (hr_n>hr_p)
Lateral-medial prefrontal				
R inferior frontal g, BA45 (55 21 1) [§]	0.015 (3.89)	-2 16 47 8 9 66 2 17 58	L frontal: medial frontal g, BA 8/6 R frontal: superior frontal g, BA 6 R frontal: superior frontal g, BA 6	<0.001; 0.128; n/s
R rolandic operculum, BA6 (59 6 5) [§]	0.017 (4.32)	14 34 -8 -18 31 -10 -6 39 -8	R limbic: anterior cingulate g, BA 32 L frontal: L limbic: anterior cingulate g, BA 32	0.002; 0.001; n/s
L caudal medial frontal g, BA6/32 (-4 6 51) [δ]	0.038 (4.14)	-55 30 -2 -43 50 -4 -42 25 -10	L frontal: inferior frontal g, BA 47 L frontal: middle frontal g, BA 10 L frontal; inferior frontal g, BA 47	0.02; 0.001; n/s
Prefrontal-temporal				
R inferior frontal g, BA45 (55 21 1) [§]	0.040 (4.24)	-53 -49 -11 -53 -40 -13 -38 -48 -18	L temporal: middle temporal g, BA 21 L temporal: middle temporal g, BA 21 L temporal: fusiform g, BA 37	0.002; 0.003; n/s
L anterior cingulate, BA 32 (-4 39 2) [$]	0.006 (3.69)	-20 -22 -18 -32 -39 -24 -14 4 -21	L limbic: parahippocampal gyrus L cerebellum: anterior lobe L limbic: uncus	0.005; 0.025; n/s
L amyg-hipp region (-18 -12 -16) [$]	*0.056* *(3.42)*	-22 48 22 -4 43 16 -5 34 40	L limbic: anterior sup frontal g, BA 9 L frontal: med frontal/anterior cingulate L frontal: med frontal/anterior cingulate	0.002; 0.026; n/s
Prefrontal-thalamic-cerebellar				
L inferior frontal g, BA45 (-55 23 3) [§]	0.001 (3.87)	45 -53 -40 28 -34 -37 23 -27 -12	R cerebellum: posterior lobe R cerebellum R limbic: parahippocampal g	0.047; <0.001; 0.083
L frontal operculum, BA45 (-38 23 3) [§]	0.003 (4.90)	4 -26 12 -14 -8 17 -3 4 -3	R sub-lobar: thalamus L sub-lobar: thalamus L frontal lobe: subcallosal g	0.002; <0.001; n/s
Other				
R caudate (10 14 3) [§]	0.009 (4.33)	-33 -85 -36 -36 -83 -22 -15 -70 -17	L cerebellum: posterior lobe L cerebellum: posterior lobe L cerebellum: posterior lobe	<0.001; 0.001; n/s
L anterior middle temporal g, BA21 (-51 -37 -3) [δ]	0.014 (4.57)	-40 18 -38 -31 4 -40 -44 2 -22	L temporal: temporal pole, BA38 L temporal: superior temporal g, BA 20 L temporal: sup/middle temporal g BA	0.001; 0.002; n/s
L posterior middle temporal g, BA21 (-53 -44 6) [δ]	0.047 (4.67)	-48 -19 40	L parietal: postcentral g	0.004; 0.001; n/s
R inferior parietal lobule, BA40/7 (-42 -48 48)	0.025 (3.92)	-32 -57 61 -16 -64 58 -18 -35 65	L parietal: inferior parietal lobule, BA 7 L parietal: sup parietal lobule, BA 7 L parietal: postcentral g, BA 4	0.001; 0.007; n/s

For all following tables [§] indicates language derived seeds, [$] indicates schizophrenia region of interest derived seeds, [δ] indicates data driven seeds. Co-ordinates indicate the three maxima within individual clusters.

Table 4.8 Exploratory analysis, trait contrast: controls < high risk

Seed location	P value (Z score)	Peak height co-ordinates	Region of connectivity difference	Post-hoc P values (c<hr_n); (c<hr_p); (hr_n<hr_p)
Other				
L frontal operculum, BA45 (-38 23 3) §	<0.001 (4.34)	40 -24 16 10 -63 26 11 -47 13	R insula R precuneus, BA 31 R limbic: posterior cingulate, BA 29	0.012; 0.001; n/s
R planum temporale, BA22 (65 -34 20) §	0.028 (4.00)	2 8 57 -2 0 67 0 31 22	R frontal: sup/medial frontal g,BA 6 L frontal: medial frontal g, BA 6 Interhemispheric	0.001; 0.014; n/s
L inferior temporal g, BA37 (-59 -51 -8) §	0.010 (3.29)	-10 -57 -16 15 -84 -5 -16 -46 -20	L cerebellum: anterior lobe, culmen R occipital: lingual g, BA 18 L cerebellum: anterior lobe, culmen	0.032; 0.004; n/s
L amyg-hipp region (-18 -12 -16) $	0.007 (3.78)	40 -79 -4 29 -90 -2 43 -58 2	R occipital: inf occipital g, BA 19 R occipital: inf occipital g, BA 18 R temporal: mid/inferior temporal g	0.003; 0.002; n/s

Table 4.9 Exploratory analysis, state contrast: no symptoms > symptoms.

Seed Location	P value (Z score)	Peak height co-ordinates	Region of connectivity difference	Post-hoc P values (c>hr_p); (hr_n>hr_p); (c>hr_n)
Prefrontal-temporal				
L inferior frontal g, BA 44 (-53 14 16) [δ]	0.002 (4.29)	38 16 -28 24 -13 -23 18 -7 -22	R temporal; anterior superior temp g R limbic: parahippocampal g, BA28/36 R limbic: uncus	<0.001; 0.007; n/s
	0.012 (3.95)	-20 -22 -42 -28 2 -38 -12 -8 -20	L brainstem L temporal: uncus L temporal: parahippocampal g, BA 28	<0.001; 0.065; n/s
Prefrontal-thalamic-cerebellar				
L inferior frontal g, BA 45 (-55 23 3) [§]	0.001 (4.15)	30 -53 -21 22 -63 -10 24 -34 -10	R cerebellum: anterior lobe, culmen R occipital: fusiform g, BA 19 R limbic: parahippocampal g, BA 36	<0.001; 0.026; n/s
	0.003 (3.80)	-40 -63 -24 -16 -68 -5 -34 -48 -18	L cerebellum: posterior lobe L occipital: lingual g, BA 18/19 L cerebellum	0.004; 0.031; n/s
Other				
R planum temporale, BA 22 (65 -34 20) [§]	0.013 (4.50)	48 6 -28 35 15 -38 31 -4 -25	R temporal: middle temporal g, BA 21 R temporal: superior temporal g, BA 38 R temporal: inferior temporal g, BA 20	0.001; 0.001; n/s
R thalamus (8 -13 6) [δ]	<0.001 (4.95)	2 -2 -14 -4 -24 -1 -8 -16 -10	R hypothalamic region L midbrain L midbrain	<0.001; 0.005; n/s
L pre-central g, BA 6 (-50 -2 44) [δ]	0.006 (4.13)	-32 28 -6 -51 32 -14 -45 24 -1	L frontal: inferior frontal g, BA47 L frontal: inferior frontal g, BA 47 L frontal: inferior frontal g, BA 45	<0.001; 0.004; n/s
R pre-central g, BA6 (50 -2 44) [δ]	0.039 (5.79)	-38 -70 27	L occipital: middle occipital g, BA 39	<0.001; 0.002; n/s

Table 4.10 Exploratory analysis, state contrast: no symptoms < symptoms.

Seed Location	P value (Z score)	Peak height co-ordinates	Region of connectivity difference	Post-hoc P values (c<hr_p); (hr_n<hr_p); (c<hr_n)
Other				
R inferior frontal g, BA45 (55 21 1) §	0.030 (3.81)	24 46 16 16 26 45 16 12 38	R frontal: superior frontal g, R frontal: superior frontal g, BA 8 R limbic: anterior cingulate, BA 32	0.017; 0.031; n/s
L caudate (-8 14 3) §	0.003 (4.78)	-8 35 6 10 37 7 6 26 15	L frontal: anterior cingulate, BA 24 R frontal: anterior cingulate, BA 24 R frontal: anterior cingulate, BA 24	0.002; <0.001; n/s
	0.002 (4.42)	-48 -7 7 -28 -4 4 -50 -22 18	L frontal: precentral g, L sub-lobar: lentiform nucleus L insula	<0.001; <0.001; n/s
	0.003 (3.95)	36 6 7 50 -6 6 24 -5 9	R sub-lobar: claustrum R temporal: superior temporal g/insula R sub-lobar: lentiform nucleus	0.005; 0.001; n/s
R posterior superior temporal s, BA22 (63 -46 21) §	0.016 (4.62)	18 -81 8 2 -72 4 -5 -70 11	R occipital: cuneus/ calcarine s, BA17 R occipital: lingual g/cuneus, BA 17 R occipital: cuneus/calcarine sulcus, BA 17	0.027; 0.002; n/s

4.3.3.3 *Association with genetic liability*

The trait related findings described in tables 4.7 and 4.8 (n=16) were investigated to determine if any of these findings were related to the degree of inherited risk within the high risk group. None of the trait-related findings distinguished between those with first degree relatives versus those with only second degree relatives. For the continuous measures of genetic liability only one trait-related finding showed any association with heritability to the disorder (increasing genetic liability associated with decreasing connectivity); this was at the trend level between the right inferior frontal gyrus and bilateral medial/superior frontal gyrus (p=0.069, x = 8, y = 23, z = 41).

4.3.3.4 *Functional disconnectivity across tasks*

All of the Hayling results presented in Tables 4.6-4.10 were tested in the encoding and retrieval tasks. Those which were replicated in one or more of these additional tasks are presented in Tables 4.11 and 4.12, corresponding to trait- and state-related effects respectively.

The only significant findings across all three tasks were trait-related reductions in positive correlation values between lateral-medial prefrontal regions in the high risk groups (right inferior frontal gyrus to bilateral medial frontal gyrus, Figure 4.2(a,c) and 4.3; and left medial frontal gyrus to left inferior frontal gyrus). Other findings were replicated in only one of the additional tasks. These included trait-related decreases in connectivity between prefrontal-cerebellar regions (left inferior frontal gyrus to right cerebellum, replicated in the retrieval task only) and state-related increases in connectivity between right posterior superior temporal sulcus and ipsilateral cuneus/calcarine sulcus (Figure 4.2b,d). Other findings were only replicated at the trend level. A number of these findings are however of interest: trait-related increased connectivity between left lateral parietal lobule to left inferior frontal gyrus was replicated in the encoding task (as hypothesised), and trait-related decreased connectivity was seen between bilateral parietal regions, replicated in the retrieval task.

Table 4.11 Analysis across tasks; trait contrast.

Summary of Hayling results tested in additional tasks				Encoding		Retrieval	
Seed location	**P value (Z score)**	**Co-ordinates**	**Region**	**P value (Z score)**	**Co-ordinates**	**P value (Z score)**	**Co-ordinates**
			controls > high risk				
Lateral-medial prefrontal:							
R inferior frontal g, BA45 (55, 21, 1) [§]	**0.015** (3.89)	-2 16 47 8 9 66 2 17 58	L medial frontal g, BA8/32 R superior frontal g, BA6 R superior frontal g, BA8/6	**0.008** (4.02)	6 24 48 4 28 42 3 21 55	**0.004** (4.43)	2 26 59 4 19 62 3 25 30
L medial frontal g, BA6/32 (-4, 6, 51) (ii)	**0.038** (4.14)	-55 30 -2 -43 50 -4 -42 25 -10	L inferior frontal g, BA45/47 L middle frontal g, BA10 L inferior frontal g, BA45/47	**0.048** (3.90)	-40 52 -8	*0.056* (3.84)	-57 28 6 -50 41 3
Prefrontal-temporal:							
L anterior amygdala/ hippocampus (-18, -12, -16) [$]	*0.056* (3.42)	-22 48 22 -4 43 16 -5 34 40	L superior frontal g, BA9 L med frontal/ant cing, BA9/32 L med frontal/ant cing, BA8/32	*0.067* (3.73)	-6 36 31 -4 39 42	-	-
Prefrontal-thalamic-cerebellar:							
L inferior frontal g, BA45 (-55, 23, 3) [§]	**0.001** (3.87)	45 -53 -40 28 -34 -37 23 -27 -12	R cerebellum; posterior lobe R cerebellum R limbic; parahippocampal g	-	-	**0.048** (3.80)	34 -57 -34 42 -61 35 29 -51 -44
Other:							
R inferior parietal lobule, BA40/7 (-42 -48 48) (iii)	**0.025** (3.92)	-32 -57 61 -16 -64 58 -18 -35 65	L inferior parietal lobule, BA40 L superior parietal lobule, BA7 L postcentral g, BA 1	-	-	0.066 (3.08)	-28 -48 54 -28 -42 54 -30 -40 57
			controls < high risk				
Prefrontal-parietal:							
L inferior parietal lobule, BA 40/7 (-42, -48, 48) (iii)	**0.008** (3.75)	-50 30 14 -45 27 17 -38 32 -3	L inferior frontal g, BA9/46 L inf/middle frontal g, BA45 L inferior frontal g, BA47	*0.066* (3.19)	-44 43 2	-	-
Other:							
R planum temporale, BA 22 (65 -34 20) [§]	0.028 (4.00)	2 8 57 -2 0 67 0 31 22	R sup/medial frontal g, BA6/32 L medial frontal g, BA6 Interhemispheric	*0.169 (2.82)*	2 11 57	*0.064* ***(3.74)***	10 7 62
L amyg-hipp region (-18 -12 -16) [$]	0.007 (3.78)	40 -79 -4 29 -90 -2 43 -58 2	R inferior occipital g, BA19 R inferior occipital g, BA18 R inferior temporal g, BA37	-	-	*0.138* ***(4.37)***	32 -78 -3

Table 4.12 Analysis across tasks; state contrast

	Summary of Hayling results			Encoding		Retrieval	
Seed location	**P value (Z score)**	**Co-ords**	**Region**	**P value (Z score)**	**Co-ords**	**P value (Z score)**	**Co-ords**
			No symptoms > symptoms				
Other:							
R planum temporale, BA22 (65 -34 20) §	0.013 (4.50)	48 6 -28 35 15 -38 31 -4 -25	R middle temporal g, BA21 R superior temporal g, BA38 R inferior temporal g, BA20	-	-	*0.070* (3.94)	54 6 -32
			No symptoms < symptoms				
Other:							
R posterior superior temporal s, BA22 (63 -46 21) §	0.016 (4.62)	18 -81 8 2 -72 4 -5 -70 11	R cuneus/ calcarine s, BA17 R lingual g/cuneus, BA17 R cuneus/calcarine s, BA17	-	-	**0.035** (4.72)	18 -81 6 16 -87 10

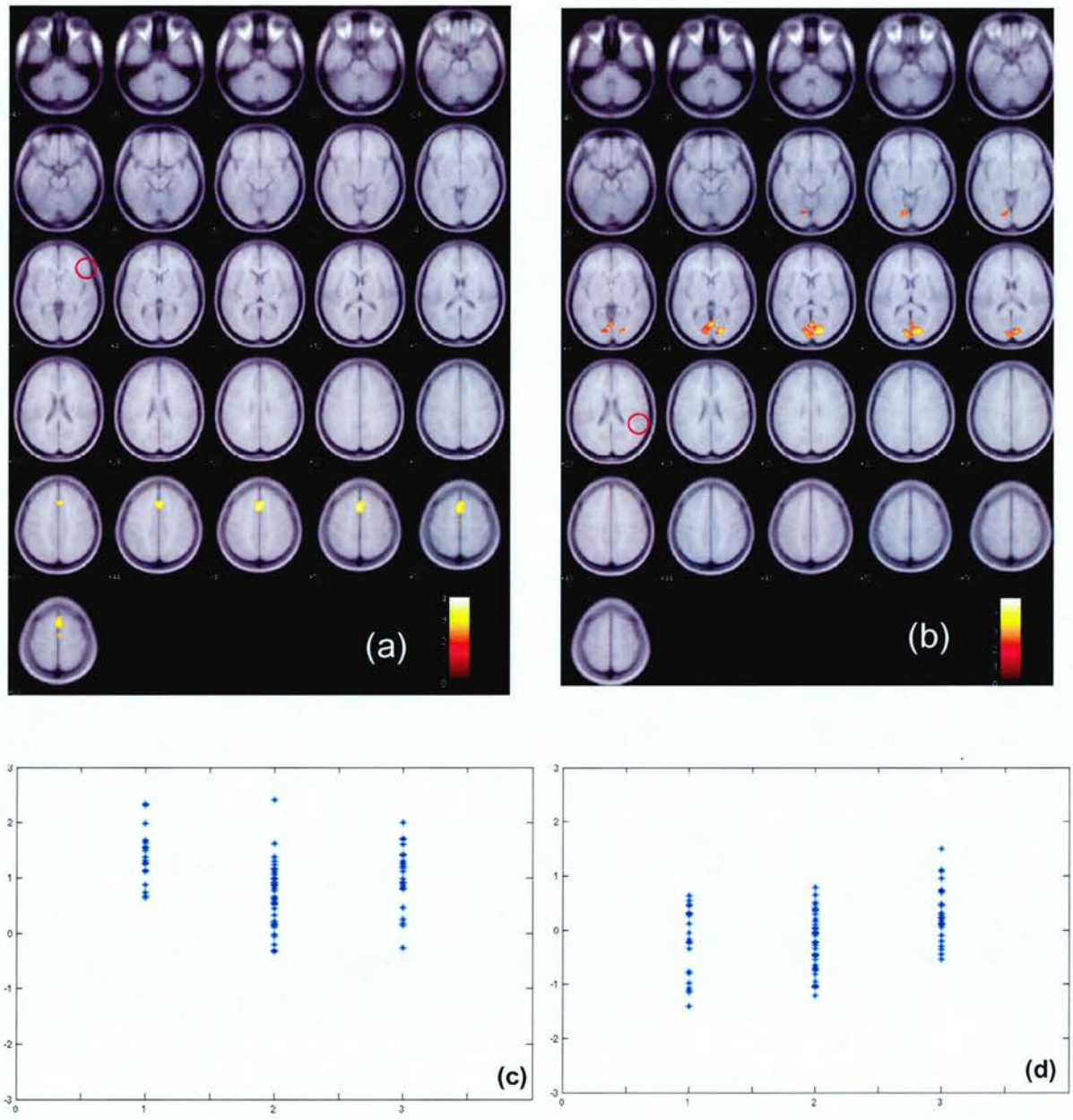

Figure 4.2 Exploratory seeds: results replicated across tasks.

Figure shows connectivity differences in high risk subjects for the exploratory seeds that were replicated across tasks. Red circle illustrates approximate seed position. The left side of image represents the left side of brain. Colour bar indicates Z score. Maps thresholded at $p<0.01$ uncorrected voxel level, $k=800$. (a) and (c) reduced connectivity in high risk subjects versus controls between right inferior frontal gyrus (seed) and left superior/medial frontal gyrus, replicated across all three tasks; (b) and (d) increased connectivity in those with symptoms between right superior temporal sulcus (seed) and right cuneus, replicated in retrieval only. Connectivity values on Y axis in graphs (c) and (d) in arbitrary units (see text), x axis represents 1 = controls; 2 = high risk without symptoms; 3 = high risk with symptoms.

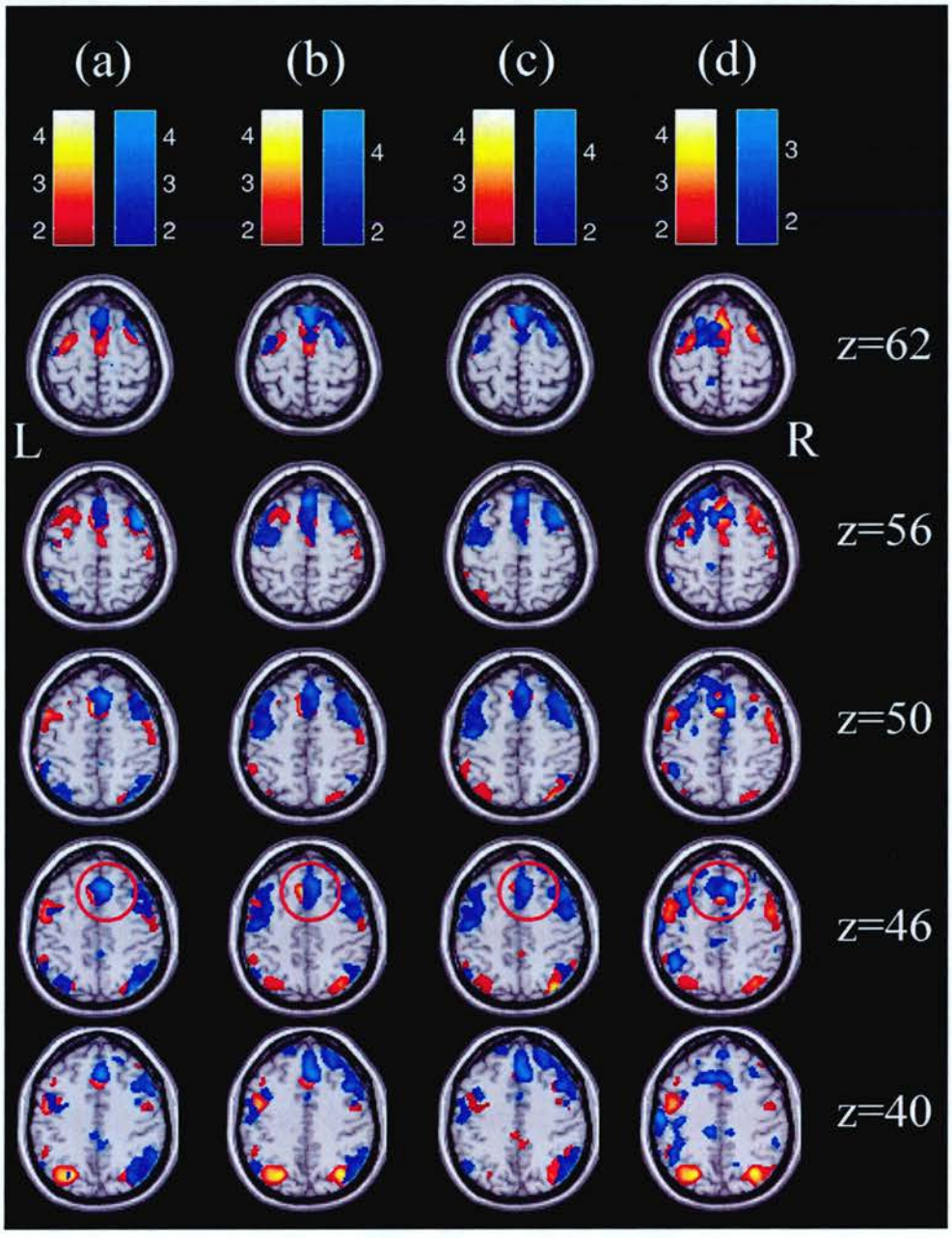

Figure 4.3 Lateral to medial prefrontal disconnectivity across tasks and associated with genetic liability.

Regions of decreased connectivity for the seed located in right inferior frontal gyrus.(a) red=Hayling task, blue=encoding task, (b) red=Hayling task, blue=retrieval task, (c) red=encoding task, blue=retrieval task, (d) red=Hayling task, blue=association with degree of inherited risk (continuous). Red circles highlight overlaps in medial/superior frontal gyrus at height of 46mm on the z-axis. Maps thresholded at $p<0.01$ uncorrected voxel level.

4.4 DISCUSSION

This chapter has examined functional connectivity across three different paradigms in a large group of anti-psychotic naïve individuals at high genetic risk of schizophrenia. Regarding the within group maps, in general connectivity was demonstrated between the seed region and homologous regions in the contralateral hemisphere. This is consistent with other studies of functional connectivity in normal subjects at rest (Koch *et al.*, 2002; Stein *et al.*, 2000; Biswal *et al.*, 1995). Regarding additional connectivity to other regions, it is difficult to draw firm conclusions in relation to anatomical studies in non-human primates due to inter-species differences, and since the results in the present study, by necessity, were summarised based on general rather than specific seed regions and across groups. However, the overall patterns of connectivity seen here between lateral prefrontal and parietal regions, between lateral prefrontal and lateral temporal regions, and between medial prefrontal regions and the thalamus are generally consistent with anatomical tracer studies in non-human primates (Pandya and Kuypers 1969; Petrides and Pandya 1984; Pandya and Yeterian 1990; Bachevalier *et al.*, 1997).

With regards the group difference findings, this discussion will focus on the analyses which were hypothesis driven and those that were replicated in one or more of the additional tasks. A figure summarising the main findings is presented below.

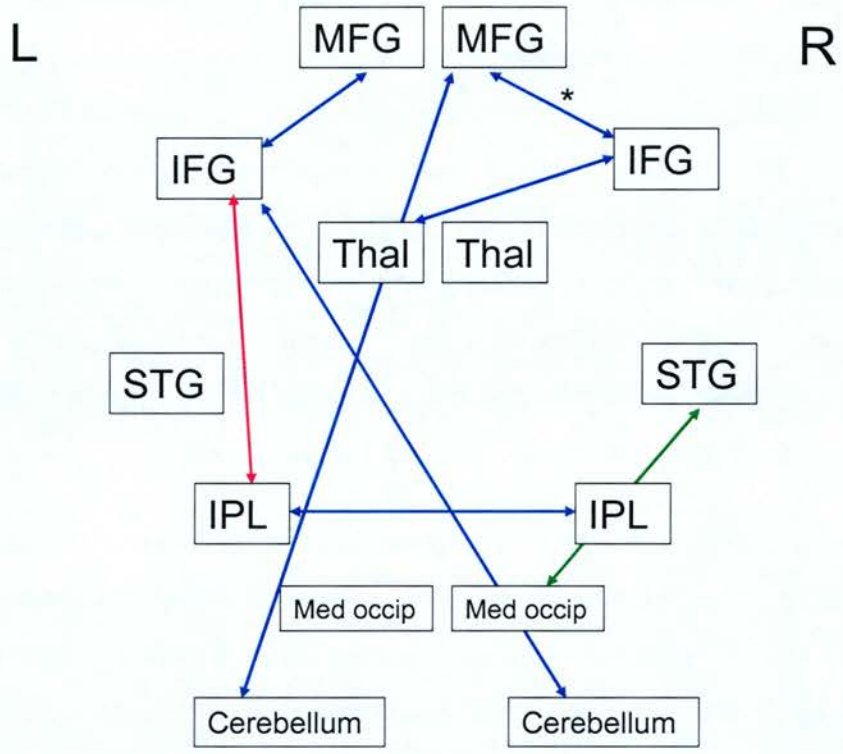

Figure 4.4. Schematic showing main functional connectivity results

Main hypothesised results and findings replicated across tasks. Blue lines indicate trait related decreased connectivity, red line indicates trait/state related increased connectivity, green line indicates state related increased connectivity. * indicates significant across all three tasks. MFG = medial frontal gyrus, IFG = inferior frontal gyrus, STG = superior temporal gyrus, Thal = thalamus, IPL = inferior parietal lobule, Med occip = medial occipital cortex. Right side of brain represented on right side of figure.

Unlike previous findings in the established state using a similar version of the verbal initiation task (Lawrie *et al.*, 2002a) disconnectivity between left sided lateral prefrontal to lateral temporal disconnectivity was not found in the present study. Since none of our high risk subjects met criteria for schizophrenia, this finding may indicate that this disconnectivity is only found in those with the established condition. Indeed there is evidence to suggest that lateral prefrontal to lateral temporal disconnectivity may be specifically associated with the presence of active symptomatology in the established state, and in particular with the manifestation of

auditory hallucinations, as described in chapter two (Lawrie *et al.*, 2002a; Shergill *et al.*, 2003; Ford *et al.*, 2002). Alternatively, prefrontal-temporal disconnectivity may not be evident since task effects have been removed from the data. As suggested in a previous chapter, the preponderance of studies reporting prefrontal-temporal disconnectivity described in the literature may be due to the majority of studies using language related paradigms, without removing task-associated activations. Analysis of the current data without task effects removed, or subdividing the high risk with symptoms group into those with delusions, hallucinations, or both, may be able to clarify this issue. Both of these analyses are planned for future work.

As hypothesised, trait-related decreased medial prefrontal-(thalamic)-cerebellar connectivity was reported in our high risk subjects (between right medial prefrontal regions and left cerebellum). This is consistent with previous reports of altered functioning in these regions in schizophrenia during a variety of tasks (Andreasen *et al.*, 1999), and with results from the previous localisation study of high risk subjects (chapter three). It is also in line with the phenomenon of crossed cerebellar diaschisis, where decreased cerebellar metabolism, brain perfusion, or neuronal activity is reported following damage to the contralateral neocortex. Fronto-cerebellar networks are increasingly implicated in a variety of cognitive tasks (Desmond and Fiez 1998), and dysfunction in this network may result in the abnormal synchrony or co-ordination of mental processing underlying several features of schizophrenia (Andreasen *et al.*, 1999). Differences in connectivity involving the thalamus implicated *lateral* rather than medial prefrontal regions (left thalamus – right inferior frontal gyrus). Also, only left *lateral* prefrontal-right cerebellar deficits were found to be replicated across tasks. These results therefore also implicate the involvement of lateral prefrontal regions in this network. This latter finding is compatible with another study which reported reduced connectivity between left lateral prefrontal and right cerebellar regions in medicated subjects in the established state (Schlösser *et al*, 2003). Since it is reported here in unmedicated high risk individuals indicates that this is not an effect attributable to antipsychotic medication. The study by Schlösser and colleagues in addition reported increased

connectivity from the thalamus to left lateral prefrontal regions, and this was considered to compensate for the decreased prefrontal-cerebellar connectivity. Such increases in prefrontal-thalamic connectivity were not seen here. Unlike the present study however, the subjects in the report by Schlösser and colleagues exhibited significant performance differences between groups, which may account for this discrepancy. Perhaps it is the case that a compensatory increase in thalamic input to prefrontal cortex only becomes apparent when significant performance deficits emerge.

As hypothesised, increased connectivity between left dorsolateral prefrontal and inferior parietal regions was also found in high risk subjects during the Hayling task. This finding was replicated at the trend level in the encoding task. The expected state-related increases in prefrontal-parietal connectivity specifically in those with symptoms were not found, but the increases across groups indicated an interaction between trait and state effects. Activity in these two regions has been reported to be inter-dependent, and they are strongly connected anatomically (Petrides and Pandya 1984). Significant volumetric correlations between these regions have also been reported previously in schizophrenic subjects but not in controls (Niznikiewicz *et al.*, 2000). This is of interest as it has been proposed that positive correlations between regional brain volumes further strengthen the notion of a functional relationship between them (see Bullmore *et al.,* 1998). Increased prefronto-parietal connectivity is consistent with the previous functional imaging finding in the high risk individuals where activation in the parietal cortex was elevated in high risk subjects (particularly, but not exclusively, in those with symptoms). Since there were no differences in performance between the groups, this was interpreted as compensatory, and the increased connectivity seen here may represent neural processes associated in this mechanism. This suggestion is consistent with another functional imaging study in normal controls reporting increased connectivity between inferior frontal and parietal regions in response to increasing working memory load and consequently increased demand on executive resources (Honey *et al.,* 2002a), and with another study suggesting that increases in parietal cortex activation in schizophrenic subjects acts

as compensatory mechanism for prefrontal cortex dysfunction in a working memory task (Quintana *et al.*, 2003). An earlier study also reported connectivity between these structures in normal controls during a semantic decision task, and suggested this fronto-parietal connection may be important in mediating subvocal articulatory rehearsal in working memory or self-monitoring tasks (Bullmore *et al.*, 2000 and see Becker *et al.*, 1999).

It is also of interest that, although only replicated at the trend level, decreased connectivity between right and left inferior parietal lobule was reported in the high risk subjects. The causal sequence of events within this bilateral parietal and left lateral prefrontal network in the high risk subjects is impossible to determine with functional connectivity approaches, but effective connectivity techniques may be able to provide more insight into this abnormal pattern of connectivity.

The most robust finding across the different tasks however was reduced connectivity between lateral-medial prefrontal regions in all those at high risk. This finding is similar to deficits previously reported in the established state (Spence *et al.*, 2000). That this deficit is present in those at enhanced risk, and therefore cannot be confounded by anti-psychotic medication or prolonged illness, indicates it may fundamentally be associated with inherited vulnerability to the disorder. Decreased connectivity between these regions was also the only finding which was associated (at the trend level) with increased genetic liability to the disorder. It should be mentioned however that the study by Spence and colleagues did not find lateral-medial prefrontal functional connectivity differences between their obligate carriers and normal controls (Spence *et al.,* 2000). Perhaps this study of three groups of 10 subjects (controls, obligate carriers and schizophrenic subjects), which reported lateral-medial connectivity differences between patients with schizophrenic versus normal controls, was unable to detect the more subtle lateral-medial connectivity difference in those at enhanced genetic risk due to smaller group numbers.

In the current study, the seed positioned in the right inferior frontal gyrus was located in the pars triangularis region, BA45, which has been consistently reported to

be activated as part of a larger distributed network involved in response inhibition (Menon *et al.*, 2001b; Aron *et al.*, 2004), and has known anatomical connections with medial prefrontal regions (Pandya and Yeterian 1996) which have also been associated with such functions (Smith and Jonides 1999). Such deficits are thought to play a critical role in self-monitoring processes, which have in turn been postulated as the primary psychological mechanism underlying schizophrenia (Frith 1992). The current findings in anti-psychotic naïve high risk individuals support this view.

There was only one significant state-related finding that was replicated in the additional tasks. This was increased connectivity in those with symptoms between right lateral temporal regions and right cuneus/calcarine sulcus. This may indicate abnormal interactions between auditory/language and visual areas in those with symptoms. This was an exploratory finding, and unlike the above, the author is not aware of any other connectivity studies that have reported this abnormality in those at high risk, or in the established condition. Abnormal activity of lateral temporal regions has however been previously linked with the presence of positive symptoms (Lawrie *et al.*, 2002a; McGuire *et al.*, 1995), and in two studies examining activation patterns in subjects who experienced auditory hallucinations, activation of this region was accompanied by increased activity in the cuneus/calcarine sulcus (McGuire *et al.*, 1995; Lennox *et al.*, 1999). Activity in the medial occipital cortex has also been found to be related to the severity of Schneiderian first rank symptoms, such as those seen in our symptomatic high risk subjects (Franck *et al.*, 2002). In the future it may be possible to determine if these state-related findings (lateral temporal-medial occipital and prefrontal-parietal) are associated with the severity, or type of symptoms, or in subjects who make the transition to schizophrenia, if they are predictive of those who go on to develop the disorder.

There are two main methodological issues to consider regarding the current study. Firstly, the relatively novel approach of removing variance associated with task effects was used in this analysis. This was to permit testing of the Hayling results in the additional paradigms, to identify differences that were not simply task

specific, and to identify differences that were over and above those attributable to the localisation data. It has also been suggested that examining connectivity that is not modulated by transient activity may be more related to underlying anatomical connectivity (Koch *et al.*, 2002). As discussed in Calhoun *et al.*, (2004) however, connectivity data with task effects removed could represent truly task-independent effects, or could reflect effects which are modulated to some degree by the task. Since in the current study the results were replicated across different paradigms, this would indicate the former situation to be more likely. However, it should be considered that there are some commonalities across the paradigms. For example, all three tasks involved verbal components, visual presentation of stimuli, and button pressing to signify responses. It is also not possible to exclude the situation that there may be task-by-connectivity interactions occurring in the data, along the lines of the PPI (psychophysiological interaction) approach. One way to address this issue, which may form part of future work, could be to examine task-unfiltered connectivity data partitioned into the different difficulty levels of the task rather than collapsing the time series data across all conditions.

Secondly, with regard to hypotheses two and three, it could be argued that the specific hypotheses tested were based on seeds derived from the localisation data obtained on the same subjects (chapter three). Hence, they might not be considered strictly 'a priori'. However, there existed previous evidence for abnormal connectivity between these regions in the literature as described in chapter one (prefrontal-thalamic-cerebellar: Andreasen *et al.*, 1998; Schlösser *et al.*, 2003; prefrontal-parietal: Kim *et al.*, 2003). Furthermore, since variance associated with task effects had been removed, the data which formed the basis of the localisation results was no longer present in the connectivity analysis.

Finally it is also worth mentioning that although the variance associated with task related activations have been removed, it is unlikely the current data was equivalent to examining subjects in the resting state, since at rest mental activity is completely unconstrained. In effect, this study has identified at least some functional

disconnectivities during 'constrained rest' in subjects at high risk of schizophrenia which are unlikely to be attributed to the potential confounders of task stimuli, performance, medication, or chronic disease effects.

In summary, no evidence was found for state-related fronto-temporal disconnectivity in high risk subjects. However these findings did support the hypothesis of genetically mediated reduced connectivity in medial prefrontal-(thalamic)-cerebellar circuits, and trait/state related increased connectivity between prefrontal-parietal regions. In addition these results also implicated genetically mediated lateral-medial prefrontal connectivity deficits and state-related increases in connectivity between lateral temporal and medial occipital regions. With regards to the trait related findings, since these are found in subjects at enhanced risk, are not confounded by anti-psychotic medication, by the effects of the illness, or by performance differences between groups, these connectivity abnormalities may represent the inherited neurophysiological basis of the diverse deficits seen in the established state and in individuals at high risk of schizophrenia (Byrne *et al.*, 2003). The additional state-related differences in connectivity may reflect the earliest changes associated with the development of psychotic symptoms.

5 BASELINE PREDICTORS: LOCALISATION

5.1 INTRODUCTION

As described in previous chapters, the established condition of schizophrenia has been associated with structural and functional brain abnormalities, most notably in the prefrontal and temporal lobes (Fletcher *et al.,* 1998; Frith *et al.,* 1995a; Lawrie and Abukmeil 1998; Shenton *et al.,* 2001), but the timing of these abnormalities in relation to development of the illness remains unclear. Prospective studies of young individuals at high genetic risk of the disorder allow the investigation of whether abnormalities predate the development of the illness, and if present, have the potential to identify those most likely to become ill.

The early phase of the disorder has been described as constituting three phases (McGlashan *et al.*, 2003); pre-morbid, prodromal, and first episode. The pre-morbid asymptomatic phase has been associated with subtle neurodevelopmental deficits which may reflect vulnerability to the disorder, as discussed in previous chapters, however as yet these have not provided any firm predictive value regarding subsequent development of the established condition. The prodromal phase begins when developmental changes become expressed pathologically. The mechanisms by which this phase translates into psychosis are however not known.

Methodologies employed in the past to examine the prodromal phase of the disorder have included retrospective examination of case notes from patients who subsequently became ill and interviews with patients in the early phase of the illness regarding recent changes (McGhie and Chapman 1961). Also, assessments performed on male adolescents undertaken to determine eligibility for military service have been examined in order to compare those who later develop schiziophrenia with those that do not (see Davidson *et al.*, 1999; David *et al.*, 1997). More recently prospective study of cohorts already presenting some of the classical features of the disorder have been conducted with the aim of creating assessment scales for defining prodromal diagnostic criteria. These include the Bonn Scale for the Assessment of Basic Symptoms (BSABS; Klosterkotter *et al.*, 1997, 2002), the Comprehensive Assessment of At-Risk Mental States (CAARMS; Yung *et al.,* 1998) and the

Structured Interview for Prodromal Signs and the Scale of Prodromal Symptoms (SIPS, SOPS, Miller et al., 1999).

These studies have indicated that there are potential predictive markers during the prodromal phase of the illness. Interviews with patients in early stages of the disorder indicated disturbances in the broad categories of attention, perception, cognition, motility and bodily movements, and affect (McGhie and Chapman 1961). Studies on male conscripts have indicated that predictive markers for subsequent hospitalisation were deficits in social functioning, organisational ability, and a decline in intellectual functioning (Davidson *et al.*, 1999; David *et al.*, 1997). From the Bonn scale the signs most predictive of subsequent development of schizophrenia have been reported to be thought interference, disturbance of receptive language, and visual distortions (Klosterkotter *et al.*, 2001). The neuropsychological abnormalities reported in prodromal patients from this research group were reported to be deficits in attention, verbal and visual memory, and particularly verbal fluency (Hambrecht *et al.*, 2002). The Comprehensive Assessment of At-Risk Mental States (CAARMS; Yung *et al.*, 1998) defines three prodromal subgroups; functional decline plus genetic risk, recent onset of subthreshold psychotic symptoms, and the onset of brief transient psychotic symptoms. Significant predictors of psychosis were found to be long duration of psychotic symptoms, poor functioning, low grade psychotic symptoms, depression and reduced attention (Yung *et al.*, 2003). A combination of the presence of a positive family history, a recent decrease in functioning, and a recent experience of psychotic symptoms was also predictive of subsequent psychosis (Yung *et al.*, 2003). In our own high risk study subsequent development of schizophrenia is often but not always been preceded by the presence of positive psychotic symptoms (Johnstone *et al.*, 2002a), and is commonly associated with increased levels of anxiety, depression and negative symptoms (Miller *et al.*, 2001). Hence, although evidence is emerging for possible predictive markers for the disorder, at present these are rather variable and non-specific. It is also uncertain whether imaging abnormalities reflect these functional deficits, and whether they can predict those likely to become ill. Regardless of whether such studies can reliably

identify vulnerability or predictive markers for subsequent development of the disease, at the very least they contribute valuable information regarding the early phase of the disorder about which little is known.

Functional magnetic resonance imaging (fMRI) in the EHRS subjects using a covert verbal initiation paradigm has revealed localised abnormalities in those at high genetic risk in medial prefrontal, thalamic, and cerebellar regions, with additional differences in those with isolated psychotic symptoms in the left inferior parietal cortex (chapter three). Similarly, state and trait correlates of functional connectivity disturbances have also been reported (chapter four). It should be stressed that although some of these subjects were found to have psychotic symptoms at the time of the detailed psychiatric assessment, none of the subjects were on anti-psychotic medication, nor did they fulfil diagnostic criteria for any psychiatric disorder at the time of the scan.

Subsequent to the baseline fMRI data collection, four subjects developed schizophrenia. Although this number is small it should be appreciated that having functional imaging data on such subjects before illness develops is extremely rare. This reflects both the relatively low incidence of the disease, and the difficulty in undertaking prospective studies in high risk populations, as discussed in chapter three.

This chapter concerns the baseline functional imaging findings in those who have subsequently developed schizophrenia compared to normal controls and those at high risk with, and without, psychotic symptoms who have not gone on to develop the disorder. This was specifically to address the second aim of the study, i.e. whether it is possible, using functional imaging techniques, to distinguish those who subsequently become ill versus those who remain well and normal controls. It was hypothesised, based on findings described in chapter three, that left inferior parietal lobe activation would be elevated in these subjects. The findings reported here are based on clinical data available at the end of April 2004.

5.2 METHODS

5.2.1 Subject details

This report presents the results from 21 normal controls and 69 high risk subjects, as presented previously. At the time of recruitment, all participants regarded themselves as being in good health and functioning well. Around the time of scanning all subjects underwent a structured psychiatric interview (the Present State Examination, (Wing *et al.,* 1974)). Twenty seven high risk subjects reported psychotic symptoms (usually isolated delusions or hallucinations); the remainder of the high risk group and all of the controls had no such symptoms.

Four of the high risk subjects were subsequently found to have developed schizophrenia after the baseline scan according to standard diagnostic criteria (referred to below as subjects 1-4). Diagnosis was made when psychotic symptoms were sufficiently severe or sustained to satisfy categorisation in terms of PSE/Catego (Wing *et al.*, 1974) and ICD-10 (World Health Organisation, 1993) criteria. Regarding these four cases, subject one, a young woman, had the fMRI scan 18 months before becoming ill. At interview (PSE) at the time of the first scan no symptomatology was reported and her mental state was normal. Subject two, a young man, was scanned 1 month before he was found to be ill. When interviewed at the time of the scan he was not forthcoming, but had a number of concerns about his body image about which he said he was not convinced, together with some referential ideas secondary to these, about which again he said he was not convinced. He was anxious and somewhat depressed and it was the impression of the interviewer that he was trying to make light of his symptoms but he persisted in the view that these were fleeting ideas and he knew that these were not really true. However, a month later the situation deteriorated, he lost insight, and required treatment. Two further subjects, both young males, were found to be ill at the time of their second fMRI scan, approximately one year after their first fMRI scan (15 and 12 months respectively). At the time of the first fMRI scan subject three, who led an alternative lifestyle, expressed overvalued ideas that the media sought to influence people to conform

with authorities, but these ideas were ill defined and thought to be partially sub-cultural. Finally, at the time of the first scan the fourth subject was well other than he was excessively concerned that the words said by a child with whom he had had contact might cause others to think he had been speaking inappropriately.

In early 2004, letters were sent to all of the subjects GPs in order to determine the symptomatic status of the remaining individuals in the study. The GPs were asked 'Has there been any contact with you in the past 18 months regarding mental health problems?' and 'To the best of your knowledge, does the above remain mentally well?'. Out of the remaining 86 subjects with a baseline fMRI scan whose GPs were contacted, 56 replied and reported no psychotic symptoms, 21 replied to say the subject was no longer registered with the practice, one subject gave no GP details, and only 8 had not replied by the end of April 2004. Of the 30 subjects who had moved GP, provided no details, or had not replied, 25 had last been seen in 2003/2002, and three had been last seen in 2001/2000, and we had no reason to believe that these subjects had become unwell. The status of two other subjects remained uncertain. One subject had had transient psychotic symptoms in the past, and her brother and sister contacted the department to say that they thought she was less well, although they could not be specific about the nature of her deterioration. Arrangements were made for her to be assessed, but before she could be seen she had gone abroad for an extended period. The other subject had withdrawn from the study having been known to have transient psychotic symptoms. She was known to remain in psychiatric care and had ongoing difficulties with some self harming behaviour. She had been treated with chlorpromazine (approximately 1 year after the scan), but was not regarded as having a psychotic illness. Her case note diagnosis was borderline personality disorder. Since it was not possible to definitely confirm the status of these cases, and since the study was specifically concerned with subjects who met ICD-10 criteria for schizophrenia, the approach was taken not to include these subjects in the 'subsequently ill' group (they remained in the 'high risk with psychotic symptoms' group). For completeness however, these subjects are identified individually in the group comparison figures below (referred to as subjects A and B).

It should be stressed that the subdivision of the groups was based on the clinical data available at the time of writing and that subsequent clinical investigations may alter the diagnosis of some of the participants. Basic demographic variables are presented in Table 5.1. The categorical measure of genetic liability refers to those subjects with any first degree relatives, or only second degree relatives. The continuous measure of genetic liability has been described elsewhere in this thesis and in Lawrie *et al.*, (2001).

5.2.2 Scanning procedure, experiment and image processing

These are identical to that described in chapter 3.

5.2.3 Statistical analysis

Statistical analysis was performed using the general linear model approach as implemented in SPM. At the individual subject level the data was modelled with 5 conditions (the four difficulty levels and the rest condition), each modelled by a boxcar convolved with a synthetic haemodynamic response function. The estimates of the subject's movement during the scan were also entered as 'covariates of no interest'. Before fitting the model, the subject's data was filtered in the time domain using both a low pass (Gaussian kernel, 4 s FWHM) and a high pass filter (400 s cutoff). Contrasts were constructed to examine all four sentence completion conditions versus rest, and areas of increasing activation with increasing task difficulty (the parametric contrast).

In order to examine differences between groups, contrast images for each subject (for both sentence completion versus rest and the parametric contrast) were entered into a second level random effects analysis (ANOVA). Analysis focused on group differences between the four subjects who subsequently became ill and the remaining three groups (controls, high risk without, and high risk with psychotic symptoms). Since the analysis involved small subject numbers in the group who subsequently became ill, a lower statistical threshold was chosen to that implemented in chapter

three (p=0.005 uncorrected). Regions were considered significant at p<0.05 cluster level, corrected for multiple comparisons.

All p values quoted in the text are at the corrected cluster level. Co-ordinates were converted from MNI (Montreal Neurological Institute) to Talairach co-ordinates using a non-linear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging).

5.3 RESULTS

5.3.1 Demographic details

Demographic details are presented in Table 5.1. There were no significant differences between the groups in terms of gender, mean NART IQ, handedness, or either measure of genetic liability (categorical or continuous). However, those who subsequently became ill were significantly younger than the other groups (p=0.019). Details of the movement parameters estimated from the realignment state of pre-processing are shown in Table 5.2. From these figures it is clear that those who subsequently became ill demonstrated more within scanner movement than the other groups. These differences were significant for the mean absolute maximum movement in the x direction (p=0.017). Both age and maximum movement in the x direction were therefore entered as nuisance covariates into the statistical model.

Table 5.1 Demographic details

	Controls (n = 21)	High risk without symptoms (n = 41)	High risk with symptoms (n = 24)	High risk, subsequently ill (n=4)
Mean age (std dev)	26.8 (2.7)	27.0 (3.2)	25.2 (3.0)	22.8 (4.5)
Gender (male:female)	13:8	17:24	10:14	3:1
Mean NART IQ (std dev)	97.95 (24.02)	99.78 (18.01)	97.58 (10.09)	97.25 (16.33)
Handedness (R:L:mixed)	19:2:0	38:2:1	18:4:2	4:0:0
Genetic liability ($1^{st°}$:$2^{nd°}$)*	N/A	31:10	15:9	2:2
Genetic liability (continuous)	N/A	0.30 (0.19)	0.24 (0.17)	0.25 (0.20)

*1^{st} or 2^{nd} degree relatives with schizophrenia

Table 5.2 Within scanner movement

	Controls (n = 21)	High risk without symptoms (n = 42)	High risk with symptoms (n = 27)	High risk, subsequently ill (n=4)
Max movement in x (mm), (std dev)	0.80 (0.51)	1.10 (0.84)	1.34 (0.81)	2.04 (1.37)
Max movement in y (mm), (std dev)	0.81 (0.47)	0.79 (0.30)	1.03 (0.46)	1.08 (0.90)
Max movement in z (mm), (std dev)	1.11 (0.74)	1.05 (0.66)	1.11 (0.55)	2.09 (1.62)

Estimate of movement parameters determined from realignment stage of pre-processing.

5.3.2 Behavioural measures

Behavioural measures are presented in Table 5.3. These indicated that the subjects were performing the task appropriately in the scanner. There were no significant differences between the groups in terms of word appropriateness scores or reaction time measures, however those who subsequently became ill showed consistently lower reaction times and produced marginally less appropriate words at debriefing than the other groups at the highest difficulty (lowest constraint) level.

Table 5.3 Behavioural measures

Group	**Mean word appropriateness (std dev)**				**Mean reaction time (ms) (std dev)**			
Constraint levels	Low	Med low	Med high	High	Low	Med low	Med high	High
Controls	6.40 (1.25)	3.25 (0.53)	1.93 (0.32)	1.10 (0.08)	2643 (528)	2543 (605)	2413 (615)	2375 (630)
High risk without symptoms	6.27 (0.99)	3.17 (0.60)	1.96 (0.35)	1.13 (0.09)	2510 (555)	2365 (600)	2237 (595)	2237 (628)
High risk with symptoms	6.38 (0.81)	3.35 (0.60)	2.00 (0.40)	1.11 (0.07)	2543 (723)	2459 (721)	2363 (754)	2356 (756)
High risk subsequently ill	6.76 (0.90)	3.37 (0.48)	2.09 (0.39)	1.13 (0.15)	2452 (82)	2343 (140)	2256 (102)	2221 (128)

Constraint levels high to low represent increasing difficulty

5.3.3 Within group results

Activation maps for sentence completion versus rest for each of the individuals who subsequently became unwell are presented in Figure 5.1. For comparison, the control group is also presented in the figure. It should be considered that activations seen in the controls initially appear to be more clearly defined than the individual subject maps since the analysis is performed at the group, rather than individual level. This figure suggests that the high risk individuals who later became ill, with the exception of subject 1, activated the left inferior frontal gyrus, left middle/superior temporal gyrus, bilateral precentral gyrus and medial/superior frontal gyrus, and cerebellum, i.e. similar to regions activated in this task in the control group. Subject 1 showed little inferior frontal or lateral temporal lobe activation at the threshold of p 0.0001 uncorrected, however these became evident at a lower statistical threshold (0.001 uncorrected p value; not shown).

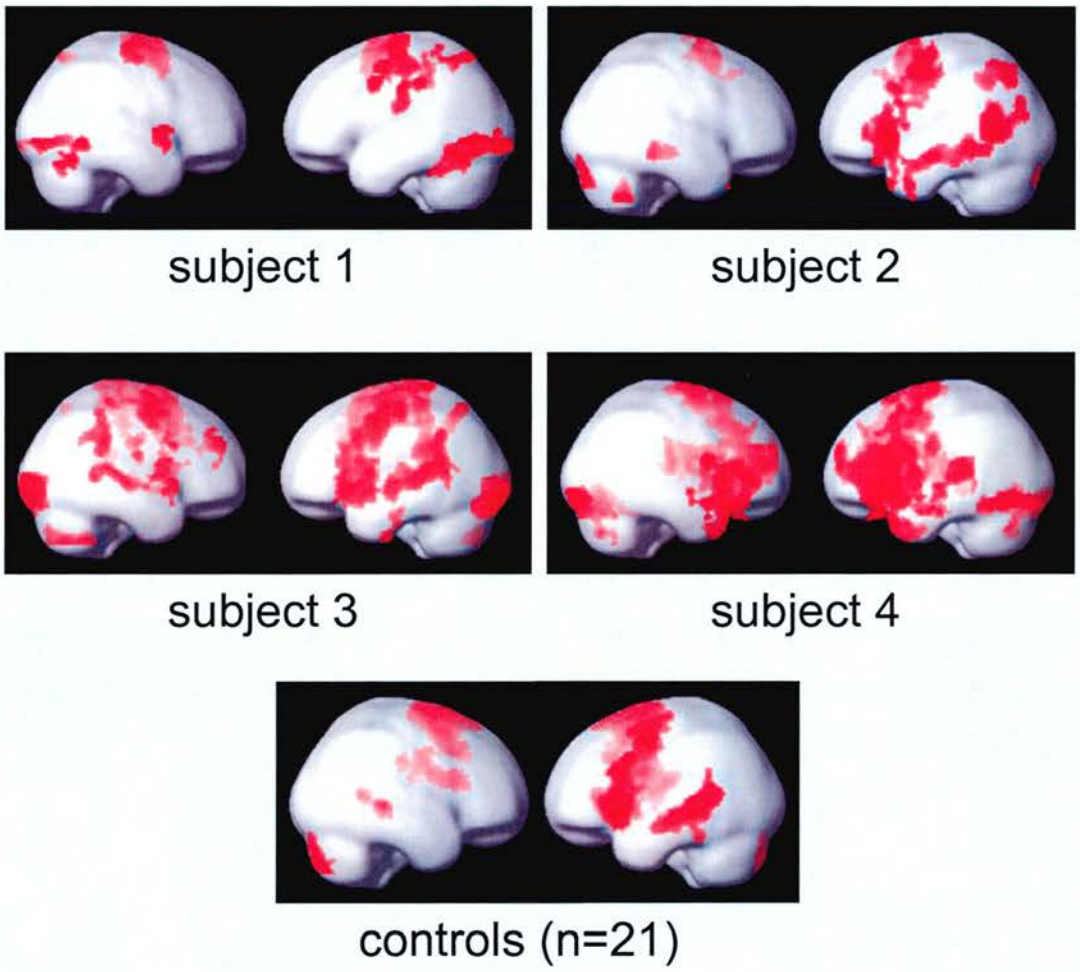

Figure 5.1 Sentence completion versus rest for subjects 1-4 and controls

Activation maps for 'sentence completion (all levels) versus rest' displayed on rendered image. Figures represent activation in each of the four subjects who subsequently became ill listed in the order they are described in the main text, maps thresholded at $p<0.0001$ uncorrected voxel level, extent = 200 voxels. For comparison activation in the control group presented below, map thresholded at $p<0.01$ uncorrected voxel level, extent = 200 voxels

5.3.4 Between group results

5.3.4.1 *Sentence completion versus rest*

For the sentence completion versus rest contrast, those who subsequently became ill demonstrated reduced activation of the anterior cingulate gyrus compared to the other groups (Table 5.4, Figure 5.2). However the figure indicated that this finding was strongly influenced by reduced activation in subject 4. When the analysis was performed excluding subject 4, a peak of reduced activation in comparison with the other groups remained at this location ($x = 2$, $y = 28$, $z = -4$) with relatively high Z scores, (against controls, high risk with, and without symptoms, $Z > 4$), but this was only statistically significant when the ill group was compared against the control group.

For the reverse contrast there was a suggestion of increased activity of the left postcentral gyrus and left inferior/superior parietal lobule in those who subsequently became ill versus the controls, however this was not significant at the chosen level of statistical significance (Table 5.4, Figure 5.3).

Table 5.4 Sentence completion versus rest

Controls > ill			**High risk without symptoms > ill**		**High risk with symptoms > ill**	
P value (Z score)	Co-ordinates	Region	P value (Z score)	Co-ordinates	P value (Z score)	Co-ordinates
<0.001	2 28 -4	R limbic: anterior cingulate g, BA24	<0.001	4 28 -4	<0.001	4 28 -4
(4.73)	-18 31 -14	L frontal: inf frontal/ant cing, BA11/32	(5.22)	-15 31 -16	(5.25)	13 52 -16
	-16 28 -3	L frontal		14 46 -13		-14 27 -13
Controls < ill			**High risk without symptoms < ill**		**High risk with symptoms < ill**	
n/s	-52 -13 21	L parietal: postcentral g, BA 43	n/s	-50 -14 22	-	-
(5.31)	-48 -5 26	L pre/postcentral g, BA4/6	(5.23)	-47 -5 29		
				26 -13 6		
n/s	-38 -50 46	L inf/sup parietal lobule, BA40/7	-	-	-	-
(3.42)	-29 -41 46	L inf/sup parietal lobule, BA7				
	-29 -40 59	L superior parietal lobule, BA7				

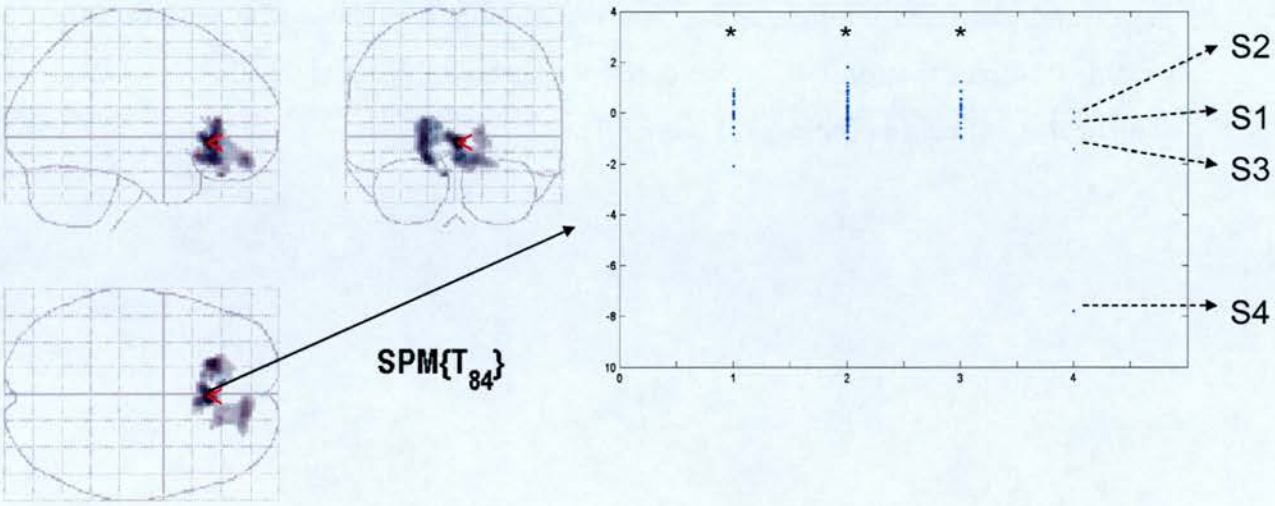

Figure 5.2 Sentence completion versus rest: group comparison, controls>ill

Activation differences for [controls > ill] displayed on 3D 'glass brain', maps thresholded at p<0.005 uncorrected voxel level, extent = 200 voxels. Graph represents size of response (rescaled data from contrast images for each subject entered into the second level analysis in arbitrary units) for the cluster in the right anterior cingulate gyrus, (on x axis 1=controls, 2=high risk without symptoms, 3=high risk with symptoms, 4=subsequently ill). Numbers on right correspond to subjects 1-4 who later became ill. * indicates significantly different versus the ill group (Table 5.4). (Values for subject A=-0.16, subject B=0.37)

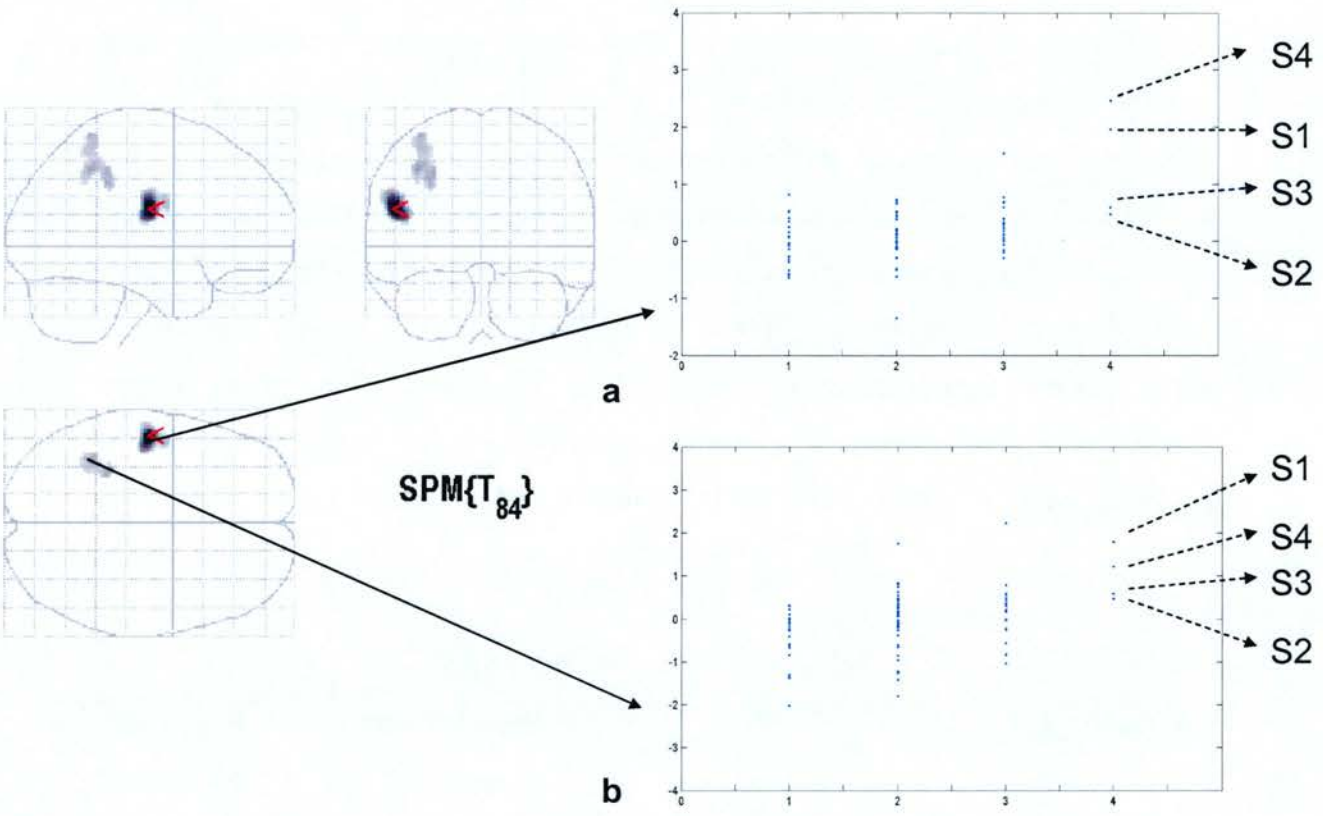

Figure 5.3 Sentence completion versus rest: group comparison, controls<ill

Activation differences for [controls < ill] displayed on 3D 'glass brain', maps thresholded at p<0.005 uncorrected voxel level, extent = 200 voxels. Graphs represent size of response (rescaled data from contrast images for each subject entered into the second level analysis in arbitrary units) for clusters in (a) left postcentral gyrus, and (b) left inferior/superior parietal lobule, (on x axis 1=controls, 2=high risk without symptoms, 3=high risk with symptoms, 4=subsequently ill) (Table 5.4). Numbers on right correspond to subjects 1-4 who later became ill. (Values for subject A= -0.06,-0.43, subject B=0.31, 0.19 for regions a,b respectively)

5.3.4.2 *Parametric contrast*

For the parametric contrast those who subsequently became ill demonstrated smaller increases in activation with increasing task difficulty compared to the controls in the regions of the bilateral medial temporal lobe (encompassing the medial aspect of the anterior superior temporal gyrus, uncus, amygdala and anterior hippocampus), the right cerebellum, and the right lingual gyrus (Table 5.5, Figure 5.4). The activation differences in the right medial temporal region and right lingual gyrus were also significant against the other high risk groups. There were no regions where those who subsequently became ill demonstrated greater increases in activation with increasing task difficulty compared to the other groups.

Table 5.5 Parametric contrast

Controls > ill			**High risk without symptoms > ill**		**High risk with symptoms > ill**	
P value (Z score)	Co-ordinates	Region	P value (Z score)	Co-ordinates	P value (Z score)	Co-ordinates
<0.001	30 -6 -18	R limbic: amygdala	0.045	30 20 -32	0.015	30 20 -32
(4.78)	32 22 -32	R temporal: superior temporal g, BA38	(4.30)	50 12 -42	(4.87)	48 12 -40
	22 6 -22	R limbic: uncus		21 7 -21		25 9 -20
0.029	4 -83 -26	R cerebellum: posterior lobe	-	-	-	-
(4.18)	-7 -81 -42	L cerebellum				
	10 -58 -21	R cerebellum: anterior lobe				
<0.001	6 -58 -2	R cerebellum/lingual g, BA18	0.014	8 -58 -2	0.006	8 -55 0
(4.01)	16 -59 9	R lingual gyrus/cuneus, BA BA18	(4.10)	18 -59 6	(3.91)	9 -64 10
	18 -51 18	R depth of parieto-occipital sulcus		6 -51 9		16 -46 5
0.019	-28 14 -26	L temporal: superior temporal g, BA38	-	-	0.053	-44 22 -30
(3.86)	-44 21 -30	L temporal: superior temporal g, BA38			(4.02)	-40 14 -32
	-32 -6 -17	L limbic: amygdala				-17 9 -20

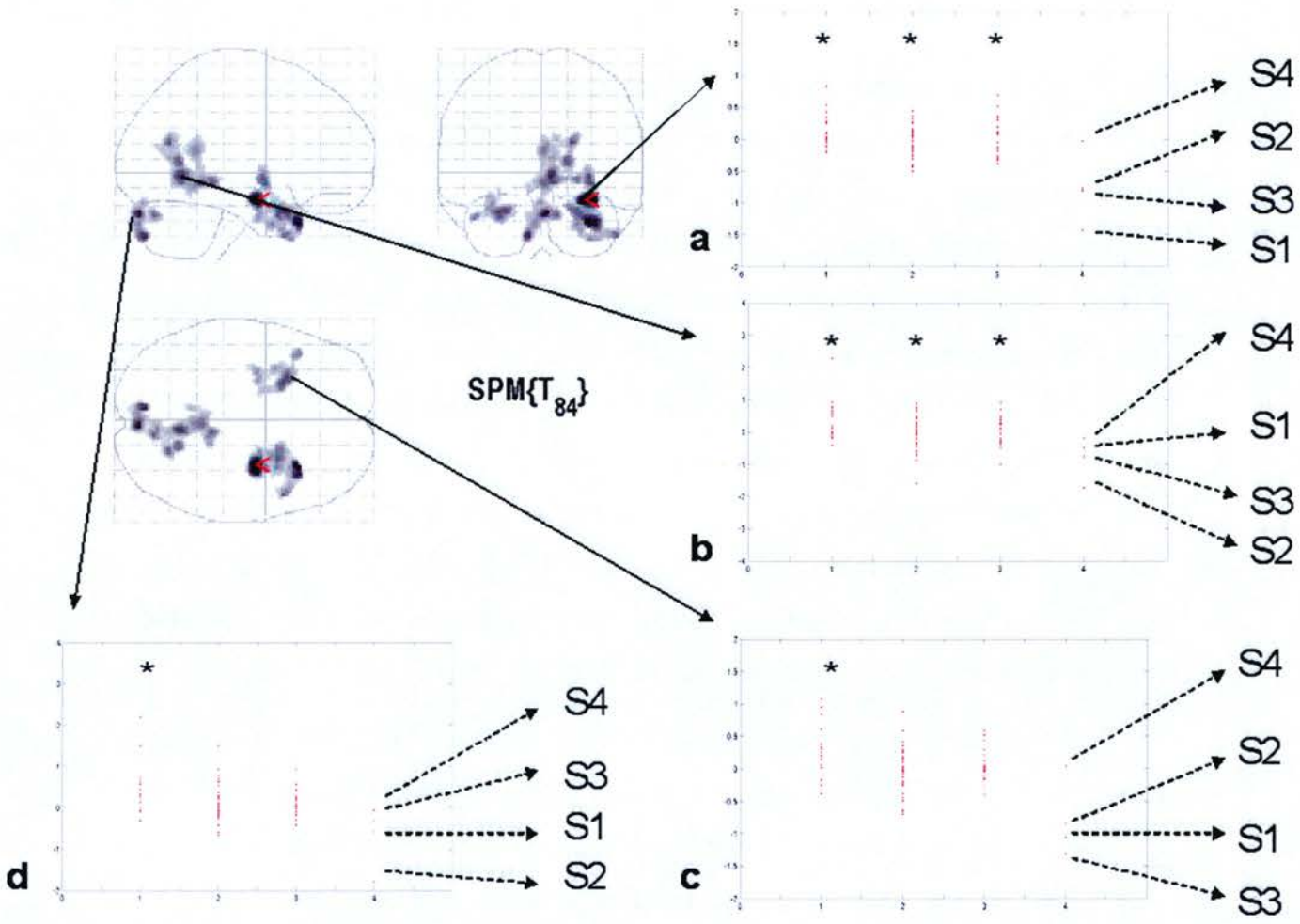

Figure 5.4 Parametric contrast, group comparison

Activation differences for [controls > ill] displayed on 3D 'glass brain', maps thresholded at p<0.005 uncorrected voxel level, extent = 200 voxels. Graphs represent size of response (rescaled data from contrast images for each subject entered into the second level analysis in arbitrary units) for clusters in (a) right medial temporal lobe, (b) right lingual gyrus, (c) left medial temporal lobe, (d) left cerebellum, (on x axis 1=controls, 2=high risk without symptoms, 3=high risk with symptoms, 4=subsequently ill). Numbers on right correspond to subjects 1-4 who later became ill. * indicates significantly different versus the ill group (Table 5.5). (Values for subject A=, -0.31, 0.38, 0.13, 0.45 subject B=0.16, -0.20, -0.27, -0.08 for regions a-d respectively).

5.4 DISCUSSION

These results indicate that subjects at high genetic risk who later develop schizophrenia demonstrate reduced activation of the subgenual portion of the anterior cingulate in comparison to the other groups. In addition, these subjects did not show the normal pattern of increasing activation with increasing task difficulty in the bilateral medial temporal lobe, medial posterior cerebellum, and right lingual gyrus. There was also a suggestion of the expected increased activation of left parietal regions in these subjects, although this did not meet the pre-specified levels of statistical significance. The subject who went on to develop schizophrenia sooner than the other subjects (subject 2; 1 month), did not appear to display greater activation differences than the other subjects who became ill later (after 12-18 months). It is important to note that these results are not confounded by antipsychotic medication as none of the subjects were treated at the time of the scan.

Even taking into consideration that subject 4 may be strongly influencing the results seen in the anterior cingulate, the other three subjects also showed lower levels of activation in this region compared to the other groups. It is also *not* the case that subject 4 was an outlier in all other regions described, and therefore it was considered appropriate to leave this subject in the analysis, having no solid grounds for exclusion. Activation of the anterior cingulate has been reported by others to be involved in verbal initiation in normal subjects (Nathaniel-James *et al.*, 1997), consistent with the current (chapter three), and our previous study (Lawrie *et al.*, 2002a). This region is also considered to be involved in aspects of performance monitoring (Paus 2001; Bush *et al.*, 2000). If this finding of decreased anterior cingulate activity is genuine, dysfunction of this region may therefore fit well with the patterns of task performance seen here. Although those subjects who later became ill did not demonstrate statistically significant differences in performance, they did present a somewhat different pattern of behavioural measures to the other groups. Pre-morbid participants tended to respond quicker, and at debriefing produced less appropriate words at the most difficult level of the task. These measures suggest the beginnings of a deficit in inhibitory control and self-monitoring, consistent with

previous findings that those with the established illness perform less well on tasks requiring such functions (Frith 1992). However it should be considered that in the current study the functional activation deficits in the anterior cingulate occurred at all levels of task engagement, whereas the behavioural differences occurred only at the highest difficulty level. Perhaps it is the case that this functional deficit manifests as a behavioural deficit only on the more demanding levels of the tasks.

Differential activations between the groups on increasing task difficulty were seen in temporolimbic regions. The verbal initiation section of the Hayling test was used in this project because is considered to be a refinement of the verbal fluency test which is commonly found to elicit functional imaging abnormalities in schizophrenia (Frith *et al.*, 1995; Yurgelun-Todd *et al.*, 1996; Curtis *et al.*, 1998; Spence *et al.*, 2000). Activation of medial temporal lobes structures are not commonly reported in such studies, but have however been implicated in a number of verbal fluency studies specifically examining semantic verbal fluency tasks (Pihlajamäki *et al.*, 2000; Gleissner and Elger 2001). Also subjects with focal temporal lobe damage have been reported to demonstrate larger deficits on semantic verbal fluency tests than with letter fluency (Henry and Crawford 2004). Activation in these regions were also found in our previous study of subjects with schizophrenia versus controls using the sentence completion paradigm (Lawrie *et al.*, 2002a), and there was an indication of right medial temporal lobe involvement in the parametric contrast in the control subjects in the current study (although this only became evident at lower thresholds, x = 36, y = 9, z = -22; Z score = 2.72). The differences reported here may therefore represent deficits in functions associated with the retrieval of suitably semantically related words, which is consistent with reports that disruption in the organisation of semantic memory may underlie deficits seen in verbal fluency tasks in schizophrenia (Bokat and Goldberg 2003).

Structural and functional deficits in the frontal and temporal lobes, including the anterior cingulate, and medial temporal lobe structures, have been repeatedly implicated in the established illness (Shenton *et al.*, 2001; Fletcher *et al.*, 1999), and in those in their first episode of the disorder (Job *et al.*, 2002; Kubicki *et al.*, 2002). Similarly located but less marked abnormalities are also evident in those at high

genetic risk of schizophrenia (Lawrie *et al.*, 1999; Keshavan *et al.*, 2002; Job *et al.*, 2003). In our own high risk study, structural abnormalities in these regions have been observed in many more subjects than are likely to develop the disorder, and are considered to reflect a state of inherited vulnerability which may or may not translate into schizophrenia (Lawrie *et al.*, 1999; Job *et al.*, 2003). It is also interesting to note that the region of difference located in the cerebellum was similar to the region identified as a trait related abnormality described in chapter three. Taken together therefore these findings may suggest that more extreme values of what may be continuously distributed measures could differentiate those who later become ill versus those who remain well, but it is equally possible that some additional change is required.

Although there are a number of reports examining potential predictive markers with regards clinical features of the illness (Klosterkötter *et al.*, 1997; McGlashan *et al.*, 2003; Yung *et al.*, 2003), there are only a very small number of studies that have investigated whether brain abnormalities prior to illness can distinguish those who later become unwell. A recent structural imaging study by Pantelis and colleagues (2003) reported grey matter reductions in the right medial temporal and cingulate cortex in those who later developed psychosis versus those who did not, which is highly consistent with the current functional imaging findings. To the best of the author's knowledge there are no other studies that have examined whether functional imaging abnormalities can distinguish individuals who later become ill versus those who remain well. These novel findings therefore require replication. Furthermore, whether the functional or structural abnormalities are primary or develop simultaneously requires further investigation.

Activation differences in response to increasing task difficulty in those who became ill versus the other groups were also seen in the medial occipital cortex (lingual gyrus, BA18). Activation in this secondary visual area has been seen in our own and other word generation studies (Lawrie *et al.*, 2002a; Nathaniel-James *et al.*, 1997), in response to reading versus rest (Collette *et al.*, 2001), and has been associated with the processing of word form (Petersen *et al.*, 1999). A similar area was also implicated in the state-related connectivity analysis in the previous chapter

(increased connectivity between right superior temporal sulcus and medial occipital cortex, BA18). As mentioned previously, activation in this region has been reported to be negatively correlated with severity of Schneiderian first-rank symptoms (such as the positive psychotic symptoms observed in our high risk subjects) (Franck *et al.*, 2002). It is unclear at present how dysfunction in this region may be associated with the subsequent development of the disorder, but is consistent with the finding that visual perceptual disturbances are commonly reported in prodromal subjects who later make the transition to schizophrenia (Klosterkotter *et al.*, 1997).

Increased activation in the parietal lobe did not meet the chosen level of statistical significance. For the region of difference located in the left inferior parietal lobule this did however appear to be highest in those who became ill, followed by high risk subjects with and without symptoms, followed by the normal controls. As the finding of increased activation in high risk subjects with symptoms led to the hypothesis that this may be increased to a greater extent in those who subsequently became ill, it could be argued that a multiple comparison correction based on this smaller region of interest would be appropriate. However, since these results are based on the same pre-processed baseline scans as the previous findings, the conservative 'whole brain' approach was considered to be more suitable.

The main limitation of the current study is that there are only as yet four subjects who have met criteria for schizophrenia after having a baseline fMRI scan. It is interesting to note however that each subject was an outlier on at least one of the regions described. This low number of subjects reflects the difficulty in obtaining prospective data on such individuals. Due to the experimental design it is not the case that subjects can simply be recruited to increase this group number, only time will tell if more subjects will make the transition to illness. The decision was made therefore to examine the data as it stood at the end of April 2004 when the clinical status of the majority of subjects had been determined by contacting the subject's GPs. The status of two subjects remained questionable, however, and it was decided not to include these subjects in the subsequently ill group since it was not possible to confirm whether they met criteria for the disorder. Values for these subjects are detailed in the figures. It should be noted however that in the main these values lay

within the overlap between the high risk groups and those that subsequently became ill. It is difficult to draw firm conclusions regarding these subjects since clinical status could not be confirmed.

With such a small sample size these preliminary findings should be considered cautiously. It should also be appreciated that these findings are based on clinical data available at the time of writing which may change in the future. It is also the case that there were significant differences in movement between the groups. Although this was corrected for at the first and second level of the analysis, the possibility that there is some residual effect, perhaps interacting with susceptibility artefacts (seen particularly in orbitofrontal regions) cannot be excluded. However, these subjects did not meet our criteria for exclusion regarding movement, and close examination of the data indicated that the image quality in these four subjects was equivalent to the other groups, revealing the typical language related patterns of activity associated with this task (Figure 5.1). Furthermore, our results are consistent with abnormalities in these regions reported in the schizophrenia literature.

The current findings indicate that functional localisation abnormalities are present before the development of the disorder, which are able to distinguish these subjects from normal controls and those at high risk who have not developed the illness. The following chapter will address whether these subjects also have abnormal functional connectivity.

6 BASELINE PREDICTORS: INTEGRATION

6.1 INTRODUCTION

The aim of the work described in this chapter is to determine if any of the subjects who became ill over the course of the study demonstrated differences in functional connectivity at baseline in addition to the functional localisation abnormalities described in the preceding chapter. It was generally hypothesised that a greater extent of the connectivity abnormalities seen in chapter four would be present in those who subsequently become ill. To address this aim the hypothesis-driven seeds from chapter four were selected, along with seeds based on the state-related findings as described in chapter four, and finally seeds located in accordance with the findings described in chapter five were selected. The latter group of seeds constitute analysis pertaining specifically to the localisation predictor regions identified in the previous chapter.

It was therefore specifically hypothesised that those who became ill would demonstrate decreased connectivity between prefrontal-thalamic-cerebellar regions, increased connectivity between prefrontal-parietal regions, and increased lateral temporal-medial occipital connectivity. If, as previously suggested, prefrontal-temporal connectivity is only disrupted in the established condition in those with active symptomatology, this would not be expected to be present in subjects who become ill months to years after the scan.

6.2 METHODS

The basic methods have been covered in preceding chapters. Subject details are presented in chapter five. As previously, the values for the two questionable subjects (subjects A and B) are detailed in the figures. The scanning procedure, experiment, and image processing have been described in detail in chapter three, and functional connectivity analysis methods have been presented in chapter four.

6.2.1 Functional connectivity

6.2.1.1 *Seed locations*

The seeds tested in this analysis were the hypothesised seeds as described in chapter four (bilateral dorsolateral prefrontal cortex, superior/middle temporal gyrus, medial frontal gyrus, thalamus, and inferior parietal lobule). Also from chapter four, seeds based on additional state related findings were selected (bilateral superior temporal sulcus), and finally bilateral seeds located in the anterior cingulate, amygdalo-hippocampal complex and lingual gyrus were selected in line with results described in chapter five. No new seeds were generated; only those previously pre-processed for the analysis described in chapter four were selected.

6.2.1.2 *Functional connectivity analysis*

The first level analysis has been described in chapter four. For all seeds the second level analysis focused on group differences (increases and decreases) between those subjects who subsequently became ill and the remaining three groups (controls, high risk without symptoms, and high risk with symptoms). The analysis was thresholded at $p=0.01$ uncorrected, and regions were considered significant at $p<0.05$ cluster level, corrected for multiple comparisons. As in the previous chapter, since there were significant differences between the groups in terms of age and maximum movement in the x direction, these were entered as nuisance covariates in the statistical model.

All p values quoted in the text are at the corrected cluster level. Co-ordinates were converted from MNI (Montreal Neurological Institute) to Talairach co-ordinates using a non-linear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging).

6.3 RESULTS

6.3.1 Demographic details and behavioural measures

These are identical to those previously presented in chapter five.

6.3.2 Within group results

From the behavioural and activation data reported in the previous chapter it was demonstrated that the four subjects who became ill were performing the task in the scanner, and that were activating the general pattern of regions associated with this task (Figure 5.1).

In terms of the within group patterns of connectivity for those subjects that subsequently became ill, as before, most seeds demonstrated positive connectivity to contralateral homologous regions. For seeds located in the bilateral dorsolateral prefrontal cortex, left middle temporal gyrus, left medial frontal gyrus and right lingual gyrus, these were the only significant clusters. The seed located in the right middle temporal gyrus showed additional connectivity to the left postcentral gyrus ($p<0.001$, $Z=4.06$). The seed located in the left medial frontal gyrus showed additional connectivity to the contralateral posterior cingulate ($p<0.001$, $Z=4.33$). The seed located in the left thalamus demonstrated additional connectivity to left precentral gyrus ($p<0.001$, $Z=4.19$) and right paracentral lobule ($p<0.001$, $Z=4.79$), and the right thalamus with the left medial frontal gyrus ($p<0.001$, $Z=4.19$). Seeds located in the bilateral inferior parietal lobule showed additional connectivity with ipsilateral middle frontal gyri ($p<0.001$, $Z=4.51$; $p<0.001$ $Z=4.40$ for left and right respectively). Bilateral seeds located in the anterior cingulate regions demonstrated additional connectivity to left superior temporal gyrus ($p<0.001$, $Z=4.34$; $p<0.001$, $Z=4.41$, left and right respectively), to right insula/frontal operculum ($p<0.001$, $Z=4.22$; $p<0.001$, $Z=4.35$, left and right respectively), to contralateral pre/postcentral gyri ($p<0.001$, $Z=4.31$; $p<0.001$, $Z=3.69$, left and right respectively), and the seed in the right anterior cingulate demonstrated connectivity to the left posterior cingulate

($p<0.001$, $Z=3.61$). Seeds located in the bilateral amydalo-hippocampal regions demonstrated connectivity to the left thalamus ($p<0.001$, $Z=4.81$; $p<0.001$, $Z=4.55$, left and right respectively). The seed located in the left lingual gyrus showed connectivity to the left anterior cingulate ($p<0.001$, $Z=5.08$), the right superior temporal gyrus ($p<0.001$, $Z=4.59$) and bilateral parahippocampal gyri ($p<0.001$; 4.58, $p<0.001$, $Z=4.32$, left and right respectively). Finally, neither of the seeds located in the superior temporal region showed connectivity to contralateral regions at this threshold, they both however demonstrated connectivity with ipsilateral inferior frontal gyri ($p<0.001$, $Z=4.03$; $p<0.001$, $Z=4.11$ left and right respectively).

6.3.3 Between group results

Functional connectivity differences between the groups are presented in Table 6.1, and Figures 6.1 and 6.2. For frontal-thalamic-cerebellar differences (Figure 6.1), the only significant finding between those who subsequently became ill and the other groups was reduced connectivity between left dorsolateral prefrontal cortex and a cluster covering sub-cortical structures (lentiform/thalamus) and extending to the insula. From the graph depicted in Figure 6.1(c) this indicated the controls and high risk who did not develop schizophrenia presented positive correlations between these regions, whereas those who became ill demonstrated negative values. Decreased connectivity between right medial frontal gyrus and bilateral thalamic nuclei was also found, but this did not reach the chosen levels of significance. Those who became ill presented marginally more negative connectivity values between these regions.

Increased prefrontal-parietal connectivity was also seen in those who subsequently became ill between bilateral inferior parietal lobules and the left inferior frontal gyrus, BA9/44 (Figure 6.2), but these connectivity differences did not reach statistical significance. All groups appeared to show positive correlation values between these regions, but in the case of the left inferior parietal lobule the values for those who subsequently become ill were strongly influenced by the high value demonstrated by subject 4 (as seen in chapter four). For the right inferior parietal

lobule those who became ill presented marginally higher positive correlation values between prefrontal and parietal regions.

No significant differences in connectivity were found between dorsolateral prefrontal regions and the lateral temporal cortex, however increased connectivity was found in those who became ill between the left posterior middle temporal gyrus and a cluster located in the contralateral parietal/middle temporal gyrus, and between the left medial temporal region and the right middle frontal gyrus, however these were only significant against the high risk without symptoms group. There was no evidence for disconnectivity between lateral temporal and medial occipital regions, or between lateral prefrontal and lateral temporal regions.

Table 6.1 Functional connectivity differences between groups

Controls > ill			**High risk without symptoms > ill**		**High risk with symptoms > ill**	
P value (Z score)	Co-ordinates	Region	P value (Z score)	Co-ordinates	P value (Z score)	Co-ordinates
Prefrontal-thalamic-cerebellar:						
Seed location: R dorsolateral prefrontal cortex BA9/46 (40 32 21)						
0.022	28 -16 8	R border lentiform/thalamus	**0.015**	28 -14 8	**<0.001**	28 -16 8
(4.66)	31 12 5	R sub-lobar: border	(4.45)	25 13 8	(4.43)	29 -42 22
		lentiform/insula		28 -2 5		26 15 4
Seed location: R rostral medial frontal gyrus (14 47 12)						
n/s	-6 -24 2	L sub-lobar: thalamus	n/s	-4 -24 2	n/s	12 -23 9
(3.29)	6 -20 14	R sub-lobar: thalamus	(2.93)	10 -24 6	(2.89)	
	-5 -19 12	L sub-lobar: thalamus		7 -17 12		
Controls < ill			**High risk without symptoms < ill**		**High risk with symptoms < ill**	
Prefrontal-parietal:						
Seed location: L inferior parietal lobule (-42 -48 48)						
n/s	-34 10 28	L inferior frontal g, BA9/44	n/s	-33 32 -17	n/s	-34 11 25
3.48	-51 12 17	L inferior frontal g, BA44	(2.84)	-38 26 -20	(3.23)	-46 10 12
	-41 10 23	L inferior frontal g, BA9/44				
Seed location: R inferior parietal lobule (42 -48 48)						
n/s	-34 11 23	L inferior frontal g, BA44	n/s	-34 9 25	n/s	-34 11 23
(3.15)	-34 13 36	L inferior frontal g, BA9	(3.25)		(2.60)	-26 13 36
Other:						
Seed location: L posterior middle temporal g, BA21 (-53 -44 6)						
n/s	55 -24 27	R inferior parietal lobule, BA40	**0.022**	57 -26 30	n/s	63 -27 3
(3.59)	63 -22 29	R postcentral g, BA1/2	(3.83)	64 -38 6	(3.68)	55 -24 25
		R temporal: mid temporal g, BA21		45 -57 2		
Seed location: L anterior amyg-hipp region (-24 -1 -18)						
n/s	28 26 27	R frontal: middle frontal g, BA9/46	**0.048**	28 24 38	n/s	28 25 30
(3.37)	17 18 38	R frontal: middle frontal g, BA 8	(3.51)	17 22 36	(2.94)	16 21 32
	27 14 24	R frontal: middle frontal g, BA 8		27 15 24		

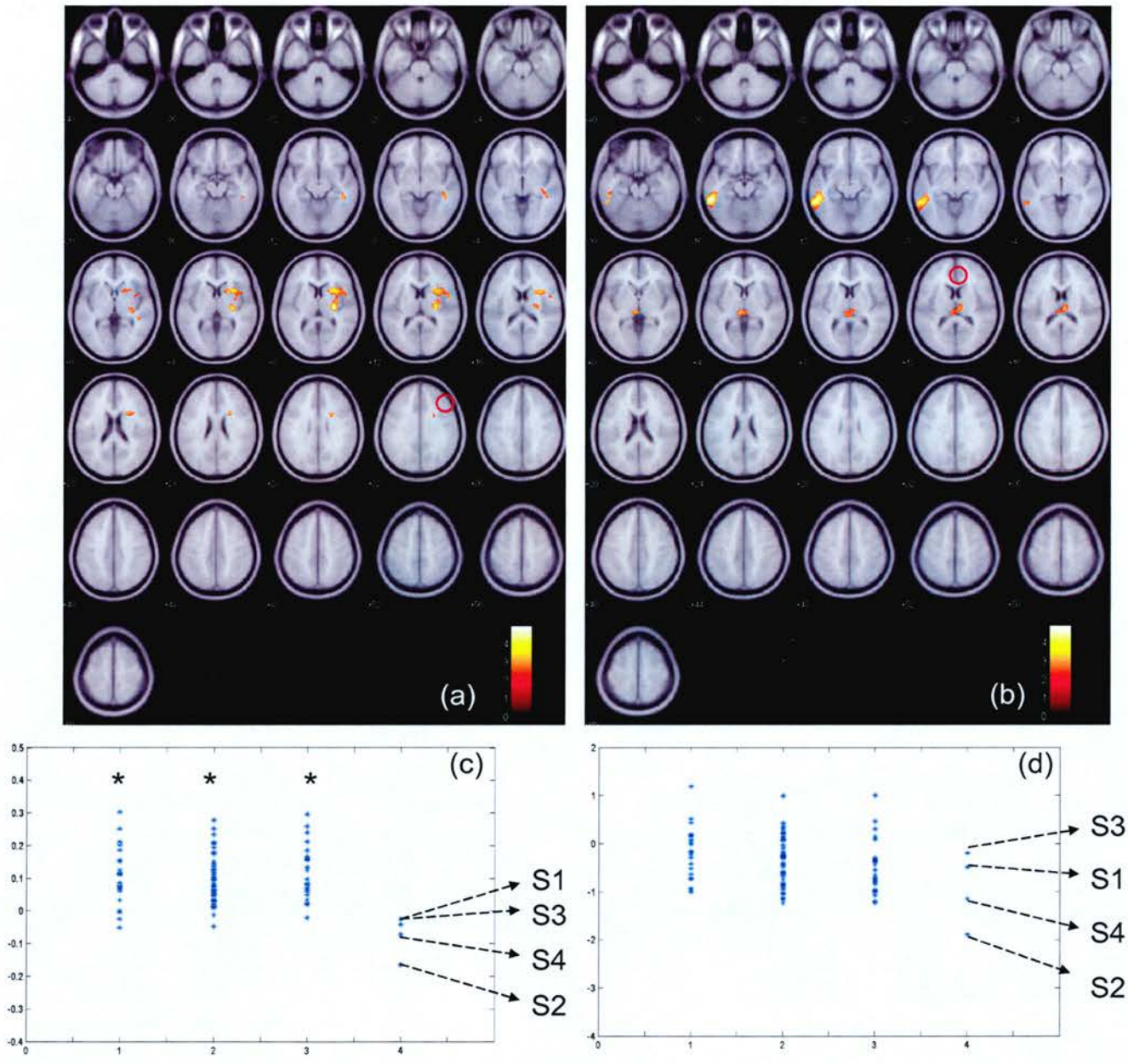

Figure 6.1 Decreased prefrontal-thalamic-cerebellar connectivity

Connectivity differences for [controls > ill]. (a) and (c) seed located in right dorsolateral prefrontal cortex, (b) and (d) seed located in right medial frontal gyrus. Maps thresholded at p<0.01 uncorrected voxel level, extent = 300 voxels. Red circle indicates approximate position of seed location. Graphs represent functional connectivity in arbitrary units (cross correlation values after processing, transformed using Fisher's r-z, and rescaled; zero represents no observed correlation, positive values represent positive correlations, and negative values represent negative correlation). On x axis 1=controls, 2=high risk without symptoms, 3=high risk with symptoms, 4=subsequently ill). Numbers on right correspond to subjects 1-4 who later became ill. (Values for subject A=0.05, -0.72 subject B=0.17, 0.31 for regions a, and b, respectively).

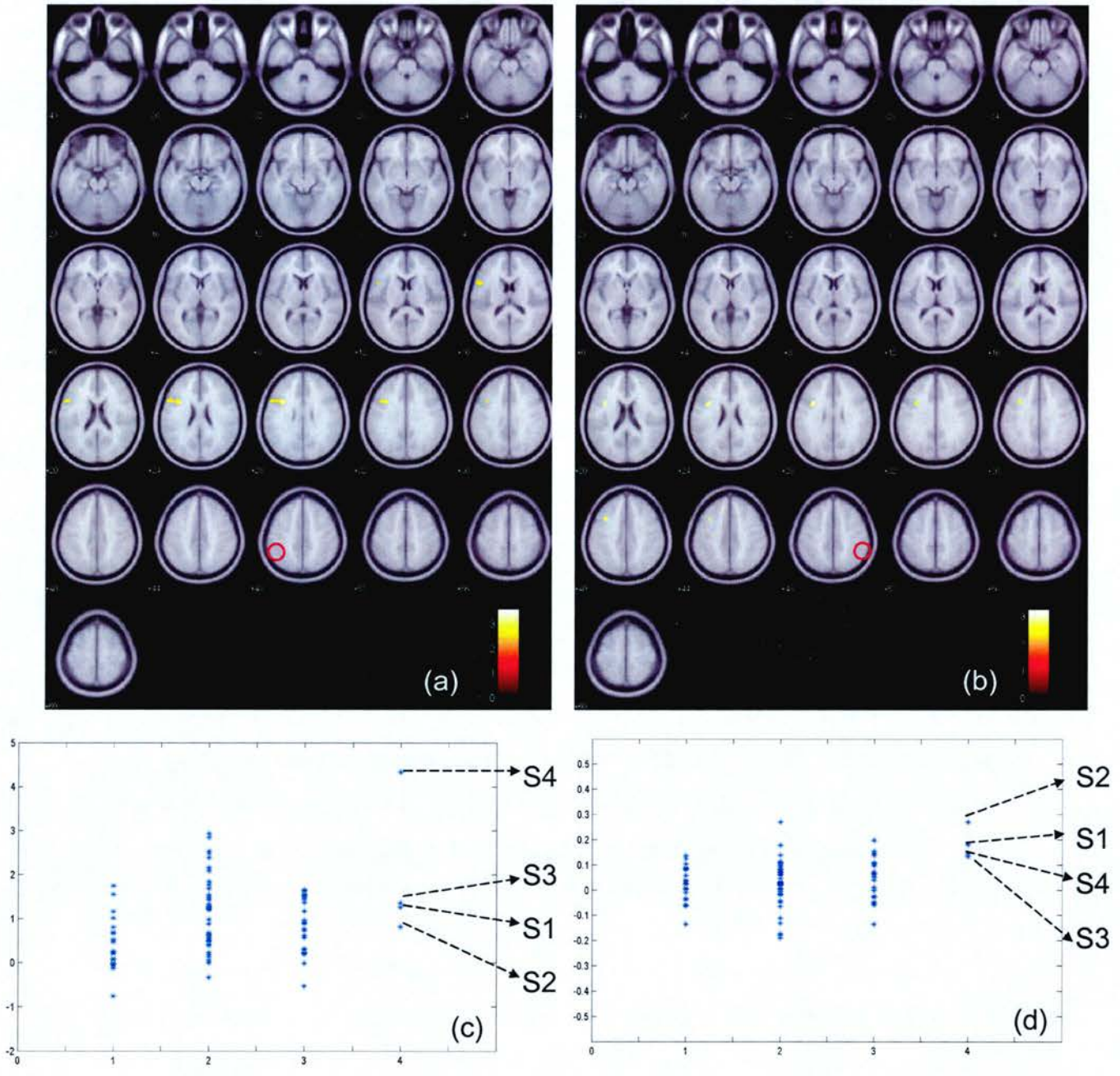

Figure 6.2 Increased prefrontal-parietal connectivity

Connectivity differences for [controls < ill]. (a) and (c) seed located in left inferior parietal lobule, (b) and (d) seed located in right inferior parietal lobule. Maps thresholded at p<0.01 uncorrected voxel level, extent = 300 voxels. Red circle indicates approximate position of seed location. Graphs represent functional connectivity in arbitrary units (cross correlation values after processing, transformed using Fisher's r-z, and rescaled; zero represents no observed correlation, positive values represent positive correlations, and negative values represent negative correlation). On x axis 1=controls, 2=high risk without symptoms, 3=high risk with symptoms, 4=subsequently ill). Numbers on right correspond to subjects 1-4 who later became ill. (Values for subject A=1.55, 0.01 subject B=0.31, 0.04 for regions a, and b, respectively).

6.4 DISCUSSION

This study has examined functional connectivity abnormalities in subjects who subsequently developed schizophrenia with the aim of determining if it is possible to distinguish these subjects from normal controls and high risk subjects who remain well using functional integration approaches.

Overall the patterns of within group maps for these subjects are similar to those reported previously in chapter four, in that the main regions of connectivity for the majority of seeds were with contralateral homologues. Other patterns of connectivity in these four subjects appeared to be less systematic than for the other groups, and this discrepancy is perhaps due to the small number of subjects in this group. That said however, there was evidence for within group connectivity between lateral prefrontal and parietal regions, seen previously in both the high risk groups and in keeping with anatomical tracer studies in non-human primates (Petrides and Pandya 1984), and between the thalamus and medial prefrontal cortex, seen previously across all three groups and in keeping with known anatomical connections (Bachevalier *et al.*, 1997). The lack of connectivity between seeds located in superior temporal sulcus and contralateral homologous regions had not previously been seen in any of the previous groups, but could be due to the difference in thresholding of this analysis.

Regarding the between groups results, a figure summarising the main findings are presented in Figure 6.3. No evidence was found for altered dorsolateral prefrontal to lateral temporal connectivity in those who became ill, consistent with suggestions that this may be associated with the established state, (or task-activation connectivity deficits). Reduced connectivity between right dorsolateral prefrontal to right sub-cortical structures was however clearly seen against all of the other study groups, which is in line with the connectivity findings described in chapter four, indicating the importance of the lateral prefrontal cortex in this prefrontal-thalamic-cerebellar network. There was also a subtle indication of reduced medial prefrontal-thalamic connectivity in those who became ill, consistent with the hypothesis described in the

introduction. Subject two, who made the transition to illness much sooner than the other subjects presented the largest deficit in both lateral and medial prefrontal-thalamic connectivity. It should be appreciated however that unlike the findings described in chapter four, no differences involving the cerebellum were found in those who made the transition to schizophrenia. Overall, these findings suggest that perhaps extremes of this disconnectivity between nodes in the prefrontal-thalamic-cerebellar network may indicate those most vulnerable to developing the disorder. This is in keeping with the notion that the variety of symptoms seen in schizophrenia may originate from a disruption in this circuit (Andreasen *et al.,* 1996, 1998), and further suggest that these abnormalities are present prior to disease onset, and are greater in those who subsequently become ill.

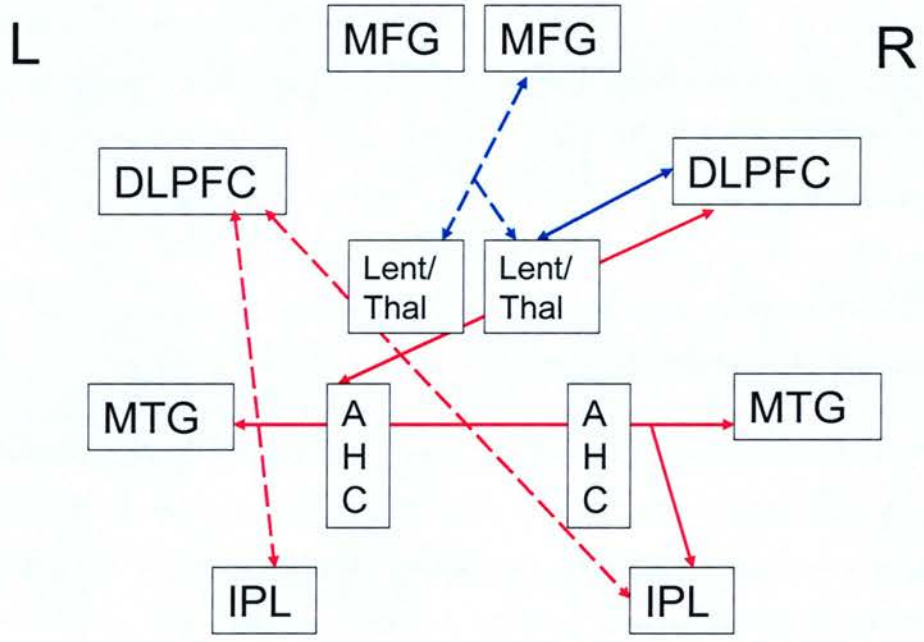

Figure 6.3 Schematic showing main functional connectivity results

Solid blue line indicates significantly decreased connectivity in those who subsequently became ill versus one or more of the other three groups. Solid red line indicates significantly increased connectivity in those who subsequently became ill versus one of more of the other three groups. Dashed lines represent hypothesised connectivity differences that did not meet significance. MFG = medial frontal gyrus, DLPFC=dorsolateral prefrontal cortex, MTG = middle temporal gyrus, Lent/Thal = lentiform/thalamus, AHC=amygdalo-hippocampal complex, IPL = inferior parietal lobule. Right side of brain represented on right side of figure.

There was also an indication of increased prefrontal-parietal connectivity in those who became ill, again suggesting that perhaps extremes of the previous connectivity abnormalities may be manifest in those who go on to develop the disorder. However, the prefrontal-parietal findings did not meet the prespecified criteria for statistical significance. As in the above chapter it was not considered appropriate to use small volume corrections to focus the analysis on the hypothesised regions as they were essentially based on the same preprocessed baseline data (chapter four) which partly formed the basis for the current hypotheses. For this reason the more conservative 'whole brain' approach was considered to be appropriate. In the case of the left inferior parietal lobule to left lateral prefrontal cortex, the results appeared to be strongly influenced by the increased connectivity seen in subject 4. For the seed located in the right inferior parietal lobule values for those who subsequently became ill showed less variation. It is interesting to note that both the left and right inferior parietal lobules displayed disrupted connectivity to a similar region in the left dorsolateral prefrontal cortex. This is similar to the network of regions implicated in the analysis described in chapter four. However as previous, the direction of influence cannot be determined with functional connectivity approaches. Effective connectivity techniques may allow more inference regarding the causal sequence of events in this bilateral parietal-inferior frontal network.

In terms of the analysis of the seeds derived from the localisation predictor results, it may have been expected that there would be evidence of abnormal connectivity between limbic structures i.e. between medial temporal and medial prefrontal regions, however the only significant finding was increased connectivity between the left medial temporal region and right dorsolateral prefrontal region (middle frontal gyrus, BA9/46). This abnormality was only significant against the high risk without symptoms group. Plots of the correlation values indicated similar values for the controls, and high risk with symptoms group, suggesting that perhaps this result only reached statistical significant against the high risk subjects without symptoms because this was the largest study group (not shown). Abnormal lateral prefrontal to medial temporal connectivity is consistent with previous reports of

disturbances in connectivity between these regions in the established illness (Meyer-Lindenberg *et al.*, 2001 and see Weinberger *et al.*, 1992), and perhaps indicates that abnormal activation of the medial temporal region reported in the previous chapter is the result of aberrant connectivity with lateral prefrontal regions. Indeed disruption in the prefrontal-medial temporal network has been proposed to underlie deficits in verbal memory in schizophrenia and in particular is considered to be involved in making semantic associations between items (Weiss *et al.*, 2003). One could perhaps speculate therefore that the disconnectivity between these regions may underlie the subtle performance differences seen in these subjects in terms of retrieving suitably semantically related words (also see chapter five).

The only other significant finding was increased connectivity between the left and right posterior middle temporal gyrus (extending also to parietal regions). Again this was only significant against the high risk subjects without symptoms, potentially due to the increased statistical power offered by this larger group. Another study has reported a similar finding of disrupted bilateral lateral temporal lobe connectivity in task-uncorrelated data (Calhoun *et al.*, 2004). However this was reported as disrupted connectivity between superior rather than middle temporal gyrus connectivity, where patients showed greater synchrony in ventral and medial regions, and controls had greater synchrony between dorsal and lateral regions. The regions reported to be presenting increased synchrony here did however encompass regions of the superior temporal gyrus and insula reported as increased in patients with schizophrenia by Calhoun and colleagues (2004). This indicates that task-uncorrelated auditory cortex disconnectivity is present in those with the disorder (Calhoun *et al.*, 2004), and seen here, in those who later go on to develop schizophrenia.

On the whole the results described here are consistent with previous findings in that they suggest extremes of the abnormalities reported in previous chapters are subtly evident in those that later go on to develop the disorder (i.e. decreased prefrontal-thalamic-cerebellar and increased prefrontal-parietal connectivity). Further, these results suggest there are additional differences between regions

commonly reported to be abnormal in the established illness, namely between dorsolateral prefrontal-medial temporal regions and between contralateral lateral temporal lobes. However, these additional findings were only significant against the largest study group. Small group numbers may be restricting these results, particularly in detecting subtle differences in connectivity over and above task-related effects.

7 HIGH RISK CHANGE OVER TIME: LOCALISATION

7.1 INTRODUCTION

The previous chapters have indicated that functional abnormalities are present in those at high genetic risk of schizophrenia, and that those with symptoms display additional differences. There are also cautious indications that those who later go on to develop the disorder are distinguishable from the other groups at baseline. It is uncertain however whether such abnormalities are fixed, or whether there are further changes as individuals at high risk of schizophrenia develop the disorder.

7.2 BACKGROUND

As previously described, the neurodevelopmental model suggests that a pre- or peri-natal insult is present from early in life which later becomes manifest by subsequent developmental processes (Marenco and Weinberger 2000; Murray and Lewis 1987; Weinberger 1986). However, there is increasing evidence, although still controversial, that there may be progressive elements in schizophrenia; a view consistent with Kraepelin's notion of a premature progressive clinical deterioration in the disorder. These longitudinal studies are discussed below, for a detailed account see Okubo *et al* (2001).

Regarding studies that have examined progressive changes, early longitudinal computerised tomography (CT) reports in general failed to show ventricular enlargement over time in schizophrenia (Illowsky *et al.*, 1988; Nasrallah *et al.*, 1986; Sponheim *et al.*, 1991; Vita *et al.*, 1988). Subsequent MRI studies have examined ventricular volumes, total brain volume, or specific regional changes. Regarding ventricular enlargement, findings with MRI have been less consistent than with CT; some report no ventricular enlargement (Degreef *et al.*, 1991; Jaskiw *et al.*, 1994; Lieberman *et al.*, 2001; Puri *et al.*, 2001), others find progression in adults (Nair et al., 1997; DeLisi *et al.*, 1995, 1997; Saijo *et al.*, 2001) and in childhood-onset schizophrenia (Rapoport *et al.*, 1997, 1999; Giedd *et al.*, 1999). Progressive loss in whole brain volume has been reported in patients in their first episode of schizophrenia (DeLisi *et al*, 1995, 1997; Cahn *et al.*, 2002) and in chronically ill

patients (Woods *et al.*, 2001). In terms of regional changes, reductions have been reported in frontal regions in adults (Gur *et al.*, 1998; Mathalon *et al.*, 2001), and in childhood-onset schizophrenia (Rapoport *et al.*, 1999). In the temporal lobe and hippocampus, some studies fail to find progressive loss (DeLisi *et al.*, 1997; Wood *et al.*, 2001; Lieberman *et al.*, 2001), while other report reductions over time in patients in their first episode and in those previously treated (Gur *et al.*, 1998; Mathalon *et al.*, 2001; Kasai *et al.*, 2003), and reductions in childhood-onset cases (Rapoport *et al.*, 1997; Jacobsen *et al.,* 1998; Giedd *et al.*, 1999).

In general therefore, although progressive structural changes have been reported in particular regions by more than one study, few studies have reported similar patterns of changes across different regions (see Weinberger and McClure 2002). It should be appreciated however, that methodological issues may contribute to the inconsistencies seen in the above results; in particular, differences in inter-scan intervals (time periods may be too short to detect subtle changes), confounds relating to anti-psychotic medication, head re-positioning (and associated problems with serial registration of images), and the reliability of semi-automated measurement methods. The use of fully automated analysis methods, for example voxel based morphometry (VBM), may in the future provide more consistent and regionally specific findings. One automated study by Thompson and colleagues constructed three dimensional spatiotemporal maps of the patterns of brain loss in childhood-onset schizophrenic patients (Thompson *et al.*, 2001). These subjects were scanned every 2 years at three time points. The resulting patterns of grey matter loss indicated that the earliest changes occurred in parietal regions and over the following five years deficits were found to move anteriorly, encompassing temporal lobe and dorsolateral prefrontal areas. In another voxel based analysis reduced volumes of the right medial temporal lobe, medial cerebellum, bilateral anterior cingulate and right posterior segment of the internal capsule were all found to be negatively correlated with illness duration, suggesting progressive loss in these regions following onset of the disorder (Velakoulis *et al.*, 2002).

The lack of unequivocal findings, particularly in first episode subjects (aside from the methodological issues described above) could also perhaps indicate that the period leading up to the onset of the illness may be the point of greatest change in schizophrenia. In other words, high risk subjects in the prodromal phase of the disorder may show the largest morphological and functional changes.

In our own structural imaging study of high risk subjects, relatively greater reductions in temporal lobe volume were reported in those that developed symptoms versus those that did not (Lawrie et al., 2002b). In another structural imaging study Pantelis and colleagues examined a group of ultra high risk subjects (n=21), ten of whom developed a psychotic disorder over a period of around 12 months, the remainder did not. This study reported localised structural grey matter reductions in the left parahippocampal, fusiform, orbitofrontal, cingulate gyrus and cerebellum in those who became ill. In those that did not make the transition structural changes were restricted to the cerebellum. Therefore there appear to be morphological changes in high risk subjects who develop symptoms and in ultra-high risk subjects who develop a psychotic disorder. It is unclear however whether these structural changes are accompanied with functional imaging abnormalities. Perhaps functional imaging changes may be considered to be more closely coupled to the changes in neurophysiology associated with developing and fluctuating symptoms than structural imaging.

The overall purpose of this chapter is therefore to address the third aim of this thesis; to determine whether progressive functional changes were present in high risk subjects using fMRI. This was broken down into two further goals. Firstly to determine if there were changes over time seen in those subjects who later made the transition to schizophrenia relative to subjects who remained well. It was hypothesised that if differences in lateral prefrontal and lateral temporal lobe functioning become apparent at or around the time of onset of the disorder, then there may be changes in these regions over time in those that become ill. Secondly, since there were a number of subjects who developed psychotic symptoms who were

previously asymptomatic, and conversely a number of subjects whose symptoms remitted, it was possible to investigate whether patterns of brain activity varied with the development or remission of psychotic symptoms. It was predicted, based on the previous functional imaging results, that parietal lobe activation levels may change with the development or remission of psychotic symptoms in those at high risk of the disorder.

7.3 METHODS

7.3.1 Study populations

This chapter presents the results from those of the first 100 baseline analysis subjects who returned for a second scan before the end of 2003. As described in the summary at the beginning of the thesis, there was a delay in the completion of collection of the second visit scans due to technical problems with the IFIS equipment. Hence not all subjects who formed part of the first 100 baseline analysis completed their second assessment before the end of this data collection period. These results should therefore be considered preliminary.

Basic demographic variables are presented in Table 7.1. Individuals were classed as with or without psychotic symptoms at the time of each scan based on the PSE assessments, described in chapter 3. Groups were then based on the symptomatic status of the individuals at both time points.

A total of 62 subjects returned for a second scan by the end of 2003. This included 16 control subjects, three of whom were found to have psychotic symptoms at the time of the second assessment. Out of the 46 high risk subjects to return for a second visit, 23 were found to be without symptoms at both assessments, 6 were found to have developed symptoms who were previously asymptomatic, 11 subjects moved from the symptomatic group to the asymptomatic group, 4 were found to be symptomatic at both time points, and 2 subjects were classed as schizophrenic at the time of their second scan (who had been in the group with psychotic symptoms at the

time of their first assessment). Although these two individuals met criteria for schizophrenia at the time of their second scan they were antipsychotic naïve on both occasions. The latter two subjects are also represented in previous baseline predictors chapters. In terms of the symptomatology of these particular subjects, subject 1 was reported at the time of the first scan to have partially held delusions, but these were ill defined and thought to be partially sub-cultural. At the time of the second scan the symptom profile indicated more firmly held delusions. At the time of the first scan, subject 2 was reported to have vague referential ideas. At the time of the second scan this subject presented firmly held delusions. Overall, subject 1 gave some cause for concern at the time of the first assessment, at the second assessment the morbid features he presented were an intensification and deterioration of those present at the first assessment. In subject 2 the features at assessment one were very minor and in ordinary clinical circumstances would probably have been disregarded, but at the second assessment he was clearly unwell with features which were definitely abnormal and which would have been indicative of schizophrenia in any circumstances. These were quite different from the features he presented at the first assessment.

In all, these subjects comprised seven groups. In the tables and text below the nomenclature of 'n' will be used to refer to those *negative* for psychotic symptoms, and 'p' will be used to refer to those *positive* for psychotic symptoms at each time point. For example controls(n_p) refer to those controls without psychotic symptoms at time one, but who did have psychotic symptoms at time two. These should not be confused with actual positive or negative psychotic symptoms.

The mean time in days between the time of the second PSE and the second scan was 6 (sd 13.5), 10 (sd 11.7), 7 (sd 14.8), 3 (sd 6.0), 6 (sd 10.5), 0.5 (sd 0.7) for the controls(n_n), controls(n_p), high risk(n_n), high risk(n_p), high risk(p_n), high risk(p_p), high risk(p_ill), respectively. The majority of subjects had the PSE assessment within 3 days of the scan (n=46; 74%). The mean time intervals in years between the first and second assessments were 1.4 (sd 0.3), 1.4 (sd 0.6), 1.4 (sd 0.4),

1.7 (sd 0.1), 1.4 (sd 0.3), 1.2 (sd 0.3), 1.1 (sd 0.2) for the controls(n_n), controls(n_p), high risk(n_n), high risk(n_p), high risk(p_n), high risk(p_p), high risk(p_ill), respectively. There were no significant differences between the groups for either of these variables.

Table 7.1 Demographic details.

	Controls (n_n) (*n* =13)	**Controls (n_p)** (*n*=3)	**High risk (n_n)** (*n*=23)	**High risk (n_p)** (*n*=6)	**High risk (p_n)** (*n*=11)	**High risk (p_p)** (*n*=4)	**High risk (p_ill)** (*n*=2)
Mean age (std dev)	27.8 (1.82)	29.2 (2.08)	29.1 (2.94)	26.8 (3.00)	27.3 (3.16)	27.4 (1.06)	23.7 (5.42)
Gender (male:female)	9:4	1:2	11:12	2:4	5:6	1:3	2:0
Mean NART IQ (std dev)	96.92 (29.95)	98.67 (9.01)	97.52 (22.76)	106.17 (5.60)	95.64 (10.43)	100.00 (11.58)	90.50 (6.36)
Handedness (R:L:A)	11:1:1	3:0:0	21:1:1	6:0:0	9:0:2	2:2:0	2:0:0
Categorical genetic liability ($1^{st°}$:$2^{nd°}$)*	n/a	n/a	18:5	5:1	7:4	3:1	0:2
Continuous genetic liability	n/a	n/a	0.3278 (0.20)	0.3272 (0.16)	0.2548 (0.19)	0.2231 (0.16)	0.0477 (0.02)

*any 1^{st}, or only 2^{nd} degree relatives with schizophrenia; n=negative for psychotic symptoms, p=positive for psychotic symptoms, referring to first and second assessment

7.3.2 Scanning procedure and experiment

The scanning procedure, experiment, collection and analysis of the behavioural data are identical to that previously described in detail in chapter 3. As before, a number of subjects recorded no reaction time measures in the scanner (n=3, n=4, n=1, n=1; for controls(n_n), high risk(n_n), high risk(n_p) and high risk(p_n) respectively). Since these subjects presented activation patterns consistent with performance of the task, and since they indicated at debriefing that they had indeed performed the task in the scanner, they were included in the analysis.

7.3.3 Scan processing

Since the analysis of the baseline scans there had been considerable refinement to the SPM software. This is inevitable in such a rapidly developing field of research. Hence it was proposed to analyse the change over time data using a newer version of the statistical parametric mapping software, SPM2. However difficulties arose with the quality of normalisation seen with the newer version of the software, and for this reason pre-processing was performed in SPM99 and statistical analysis in SPM2. The opportunity was also taken at this time for other methodological refinements which were not possible at the beginning of the study. For readability these methodological issues are only summarised in the text below, full details are contained in the appendix (and are referenced in the following text where appropriate).

7.3.3.1 *Summary of preprocessing steps*

For each subject, EPI volumes were realigned to the mean volume in the series using rigid body transformations (see appendix). Details of within scanner movement are presented in Table 7.2. Movement parameters were checked using the graphical output in SPM, and by using scripts, as previously described. None of the subjects presented significant motion artefact and therefore all were included in the analysis. There were no significant differences in movement between the groups.

As before, the mean image created during the realignment step was used in the normalisation stage of pre-processing. The mean image was selected as the 'image to determine parameters', and then all images in the run were selected as the 'images to write normalised'. The images were normalized to a new study specific EPI template (see appendix) using a linear affine transformation followed by non-linear deformations, and resampled using sinc interpolation to cubic voxels of size 8 mm^3 (2x2x2mm). Normalized images were spatially smoothed with an 8x8x8 mm^3 FWHM Gaussian filter to minimize residual inter-subject differences, and in order to meet assumptions for statistical analysis regarding the distribution of residuals.

Table 7.2 Movement parameters

	Controls (n_n) (n=13)	Controls (n_p) (n=3)	High risk (n_n) (n=23)	High risk (n_p) (n=6)	High risk (p_n) (n=11)	High risk (p_p) (n=4)	High risk (p_ill) (n=2)
Max movement in x (mm), (std dev)	0.46 (0.37)	0.29 (0.12)	0.73 (0.67)	0.57 (0.28)	0.63 (0.40)	0.48 (0.34)	0.43 (0.12)
Max movement in y (mm), (std dev)	0.86 (0.70)	1.05 (1.30)	0.88 (0.72)	0.84 (0.45)	0.79 (0.46)	0.82 (0.42)	0.65 (0.32)
Max movement in z (mm), (std dev)	0.75 (0.61)	0.90 (0.77)	1.14 (0.91)	1.25 (0.63)	0.85 (0.76)	0.84 (0.21)	0.57 (0.02)

The estimate of movement parameters determined from realignment stage of pre-processing

7.3.4 Statistical analysis

7.3.4.1 *First level analysis*

Statistical analysis was performed in SPM2 using the general linear model approach as implemented in SPM. At the individual subject level the data was modelled with 7 conditions (the four difficulty levels, the rest condition, plus two additional task switching conditions, see appendix), each modelled by a boxcar function convolved with a synthetic haemodynamic response function. The estimates of the subject's movement during the scan were also entered as 'covariate of no interest'. Before fitting the model the AR-1 technique (autoregressive model) was used to address the issue of temporal autocorrelations in the data, and the data was was filtered in the time domain using a high pass filter, 200s cutoff. Contrasts were constructed to examine all four sentence completion conditions versus rest, and areas of increasing activation with increasing task difficulty (the parametric contrast).

7.3.4.2 *Change over time*

To determine changes over time, the following calculation was performed for each subject for the sentence completion versus rest and the parametric contrasts:

([contrast:session 2]- [contrast:session 1])/time interval between sessions in days

The resulting 'difference images' were then entered into the second level analysis to examine differences between the groups.

7.3.4.3 *Batch scripting*

As previous, first level statistical analysis was performed using batch scripts devised and tested by Dr Enrico Simonotto and the author, based on examples provided with SPM. These scripts are detailed in the appendix.

7.3.4.4 *Second level analysis*

To address the first aim in the chapter, it was originally proposed to perform group comparisons to examine changes over time in those subjects who became ill versus the groups who remained asymptomatic at both time points [high risk(n_n) + controls(n_n)]. However, since there were only two subjects who developed schizophrenia with two scans, and since these two subjects presented different magnitudes of symptomatic change between assessments, the decision was made to examine these two cases individually in comparison to the other groups. This type of 'single subject versus group' approach has been used previously with both fMRI (Maguire *et al.*, 2001), and PET (Kertzman *et al.*, 1997), but it should be appreciated that, since the common variance estimate is computed by pooling data from all groups, 'single subject versus group' analysis are conducted based on the assumption that the variance is the same across groups.

To address the second aim, to examine changes in activation with the development of psychotic symptoms, the high risk (n_p) group were compared with the high risk and controls who remained asymptomatic at both time points [high

risk(n_n) + controls(n_n)]. To examine changes in activation with the remission of psychotic symptoms the high risk(p_n) group were compared with the high risk who remained asymptomatic at both occasions [high risk(n_n) + controls(n_n)].

These second level analyses were performed by entering the difference images into the ANOVA model. Statistical maps were thresholded at a level of $p=0.001$ uncorrected. Regions were considered significant at the $p<0.05$ corrected cluster level. All p values quoted in the text are at the corrected cluster level, and co-ordinates were converted from MNI (Montreal Neurological Institute) to Talairach co-ordinates using a non-linear transformation, as described in (http://www.mrc-cbu.cam.ac.uk/Imaging).

7.4 RESULTS

7.4.1 Demographics

There were no statistically significant differences in mean age, gender, handedness or mean NART IQ between the subject groups. It is interesting to note however that the hr(p_ill) group were non-significantly younger, both male, and had the lowest IQ scores, consistent with the general findings in schizophrenia that males develop the illness before females, and that lower IQ scores are a risk factor for schizophrenia (David *et al.*, 1997). There were no significant differences between the high risk groups on either measure of genetic liability (Table 7.1).

7.4.2 Behaviour

All groups showed the expected pattern of quicker reaction time and higher word appropriateness scores with greater contextual constraint at the time of the second assessment (Table 7.3). This pattern of reaction time confirms that the subjects were performing the task appropriately during the scanning session. There were no significant differences between the groups in terms of their performances on word appropriateness scores, the numbers of words, or reaction time measures recorded, or the number of asterisks reported (Table 7.3). There was however a significant difference between reaction time measures at the hardest constraint level (low, $p = 0.032$), which was significantly lower in the high risk asymptomatic to symptomatic group (high risk(n_p)). The high risk(p_ill) group also presented the highest word appropriateness scores (produced less appropriate words) at the most difficult constraint level, however this was not statistically significant

Table 7.3 Behavioural measures at second visit

Group	Mean word appropriateness (std dev)				Mean reaction time (ms) (std dev)			
Constraint	Low	Med Low	Med High	High	Low*	Med Low	Med High	High
Controls (n_n)	6.02 (1.08)	3.08 (0.42)	2.00 (0.33)	1.12 (0.12)	2650 (645)	2553 (705)	2485 (749)	2400 (734)
Controls (n_p)	7.14 (2.08)	3.22 (0.85)	1.76 (0.39)	1.15 (0.10)	2276 (612)	2357 (888)	2220 (770)	2286 (615)
High risk (n_n)	5.84 (0.95)	3.04 (0.67)	1.80 (0.29)	1.12 (0.06)	2398 (533)	2342 (513)	2183 (506)	2213 (483)
High risk (n_p)	6.27 (0.79)	2.94 (0.52)	1.70 (0.24)	1.10 (0.05)	1977 (566)	1979 (495)	1912 (538)	1912 (492)
High risk (p_n)	6.99 (3.08)	3.10 (0.57)	1.89 (0.31)	1.11 (0.06)	3032 (707)	2883 (710)	2795 (749)	2772 (736)
High risk (p_p)	6.83 (0.49)	3.08 (0.51)	1.97 (0.24)	1.09 (0.07)	2192 (247)	2095 (205)	1963 (362)	1962 (323)
High risk (p_ill)	7.33 (0.99)	3.88 (0.88)	2.13 (0.13)	1.16 (0.13)	2347 (25)	2234 (18)	2119 (43)	2109 (138)
	Mean number of words recorded (std dev) ***(Mean number of asterisk reported)***				**Mean number of reaction time measures recorded (std dev)**			
Controls (n_n)	7.84 (0.42) *(1.15)*	7.81 (0.42) *(0.44)*	7.99 (0.07) *(0.23)*	7.96 (0.09) *(0.15)*	7.58 (0.46)	7.90 (0.17)	7.85 (0.13)	7.78 (0.28)
Controls (n_p)	8.00 (0.00) *(1.15)*	8.00 (0.00) *(0.58)*	8.00 (0.00) *(0.50)*	8.00 (0.00) *(0.33)*	7.25 (0.66)	7.67 (0.38)	7.41 (0.52)	7.58 (0.72)
High risk (n_n)	7.58 (1.33) *(1.48)*	7.80 (0.57) *(0.79)*	7.91 (0.28) *(0.60)*	7.98 (0.07) *(0.45)*	7.42 (0.67)	7.74 (0.40)	7.74 (0.39)	7.81 (0.29)
High risk (n_p)	7.92 (0.13) *(0.96)*	8.00 (0.00) *(0.63)*	7.84 (0.30) *(0.58)*	7.92 (0.13) *(0.25)*	7.95 (0.11)	7.95 (0.11)	7.80 (0.33)	7.65 (0.42)
High risk (p_n)	7.54 (1.00) *(0.42)*	7.80 (0.42) *(0.18)*	7.68 (0.75) *(0.14)*	7.77 (0.68) *(0.05)*	7.60 (0.36)	7.73 (0.42)	7.58 (0.47)	7.53 (0.46)
High risk (p_p)	7.94 (0.13) *(1.19)*	8.00 (0.00) *(0.38)*	7.94 (0.13) *(0.00)*	8.00 (0.00) *(0.06)*	7.38 (0.48)	7.89 (0.25)	8.00 (0.00)	7.88 (0.25)
High risk (p_ill)	8.00 (0.00) *(1.38)*	8.00 (0.00) *(1.38)*	8.00 (0.00) *(0.75)*	8.00 (0.00) *(0.50)*	7.75 (0.35)	7.63 (0.18)	7.88 (0.18)	8.00 (0.00)

* $p<0.05$

Differences between the first and second assessments for the behavioural measures are reported in Table 7.4. Most groups presented minimal differences between assessments. The largest difference however was seen for the controls(n_p) group (n=3) where reaction times were reduced by > 1000ms between assessments. This originated from two subjects within this group who demonstrated high reaction times (of the order of >3000ms) at the time of the first assessment. The largest difference in terms of word appropriateness scores was seen in the high risk(p_ill) group at hardest difficulty level (produced less appropriate words at the time of the second assessment). After excluding the control(n_p) group (this group was not included in any further analyses), there were no significant differences between the groups in terms of the change in behavioural measures between assessments.

Table 7.4 Difference in behavioural measures between visits

Group	**Difference in mean word appropriateness over time* (std dev)**				**Difference in mean reaction time over time* (ms) (std dev)**			
Constraint	Low	Med Low	Med High	High	Low	Med Low	Med High	High
Controls (n_n)	-0.33 (1.04)	-0.06 (0.36)	0.23 (0.28)	0.03 (0.09)	-70 (396)	-44 (310)	3 (308)	13 (285)
Controls (n_p)	0.51 (20.3)	-0.60 (0.58)	-0.53 (0.73)	-0.05 (0.10)	-1459 (21)	-1559 (114)	-1357 (448)	-1305 (276)
High risk (n_n)	-0.45 (1.01)	-0.20 (0.70)	-0.18 (0.35)	-0.03 (0.06)	-186 (390)	-130 (358)	-157 (374)	-170 (400)
High risk (n_p)	0.80 (1.25)	-0.10 (0.46)	-0.18 (0.48)	0.01 (0.04)	-257 (91)	-37 (419)	-134 (217)	8 (110)
High risk (p_n)	0.67 (3.12)	-0.36 (0.88)	-0.04 (0.40)	-0.01 (0.05)	65 (500)	-65 (572)	-29 (648)	-83 (524)
High risk (p_p)	0.22 (0.46)	-0.31 (0.20)	-0.08 (0.49)	0.01 (0.08)	-183 (113)	-139 (116)	-191 (126)	148 (56)
High risk (p_ill)	1.23 (0.27)	0.18 (0.55)	0.15 (0.48)	-0.09 (0.04)	-53 (106)	-105 (218)	-89 (42)	-191 (39)

*(visit 2 – visit1); negative values represent decreases, positive values represent increases.

Closer inspection of the two subjects who became ill at the time of the second scan is presented in Table 7.5. Both subjects produced less appropriate word scores at the most difficult levels of the task at the time when they were classed as ill. Subject 2 however showed the largest difference between assessments particularly at the two hardest constraint levels.

Table 7.5 Behavioural measures for subjects 1 and 2: hr(p_ill)

Group	**Mean word appropriateness (std dev)**				**Mean reaction time (ms) (std dev)**			
Constraint	Low	Med Low	Med High	High	Low	Med Low	Med High	High
Subject 1								
Visit 1	5.59	3.46	1.54	1.19	2343	2506	2209	2231
Visit 2	6.63	3.25	2.03	1.06	2364	2247	2150	2012
Difference (visit2-visit1)	1.04	-0.21	0.49	-0.13	21	-259	-59	-219
Subject 2								
Visit 1	6.61	3.94	2.40	1.31	2457	2172	2208	2370
Visit 2	8.03	4.50	2.22	1.25	2329	2220	2089	2207
Difference (visit2-visit1)	1.42	0.56	-0.18	-0.06	-128	48	-119	-163

7.4.3 Within group results

7.4.3.1 *Activations at time one and time two*

Figures 7.1 and 7.2 show the within group activation maps for the sentence completion versus rest and the parametric contrasts. These are presented separately for each assessment. Since the control(n_p) only contained 3 subjects, and are not

included in any group comparisons, they are not detailed in the figures. The left hand column represents the groups with no change in symptomatic status between visits, and the right hand column represents groups that did present changes in symptom status between visits. The subjects who became ill are presented individually.

For sentence completion versus rest (Figure 7.1) qualitative inspection of these maps indicated consistent areas of activation at both time points, particularly in the larger and more stable groups: controls(n_n) and high risk(n_n). Regions activated at both assessment included left lateral prefrontal, lateral temporal, superior/medial frontal, and cerebellar regions, consistent with areas reported to be involved in this task in previous chapters. Groups with smaller numbers ($n<12$) presented less widespread activations, likely due to smaller subject numbers in these groups, but visual inspection suggests that these were generally consistent at both occasions. Regarding the two subjects who were ill at the time of the second scan, qualitative inspection indicated that subject 1 presented slightly more activation at time two than at time one, but at both occasions the regions activated were consistent with the task activated areas described above. Conversely, subject 2 presented stronger activations at time one than at time two with much more anterior prefrontal involvement than that seen in the other groups at the first occasion.

For the parametric contrast (Figure 7.2) the patterns of activation over time also appear to be generally consistent. On the whole, all groups presented predominantly left lateral prefrontal activation (mostly inferior) along with superior/medial prefrontal activations. The exception to this is subject two who at the threshold $p<0.01$ did not present this pattern of response, however at a lower threshold of $p<0.05$, a similar pattern became apparent at both time points.

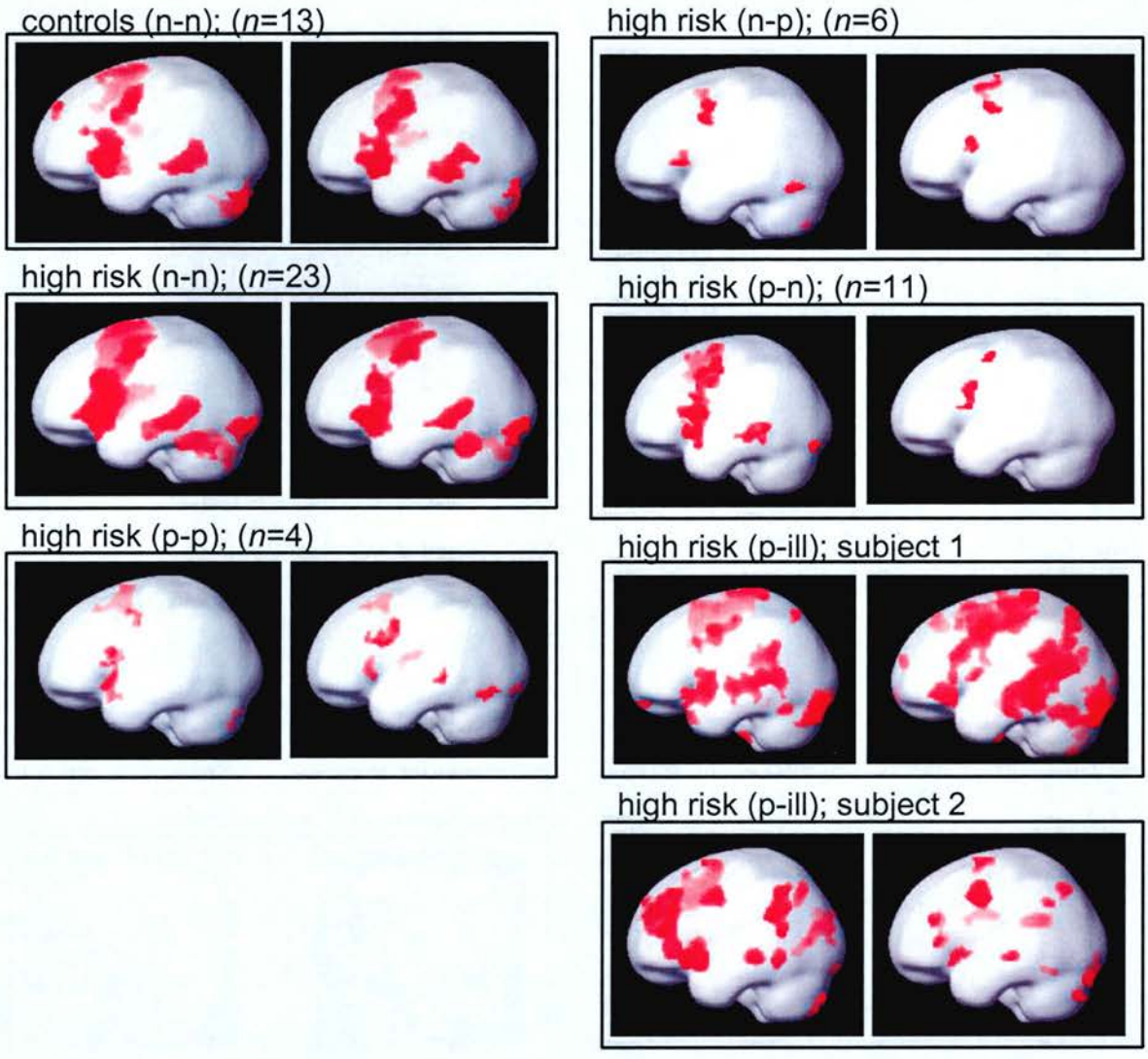

Figure 7.1 Activation patterns at time 1 and time 2 for sentence completion versus rest

Sentence completion versus rest for each of the groups; the two high risk subjects who were ill at the time of the second scan are shown individually. Control(n_p) group not shown. For each group the left image represents the activation pattern at time 1 and the right at time 2. The left column represents subjects that did not change symptomatic status between scans, those on the right underwent worsening or improvement in symptomatic status. Maps thresholded at p=0.001 uncorrected, extent threshold=50 voxels.

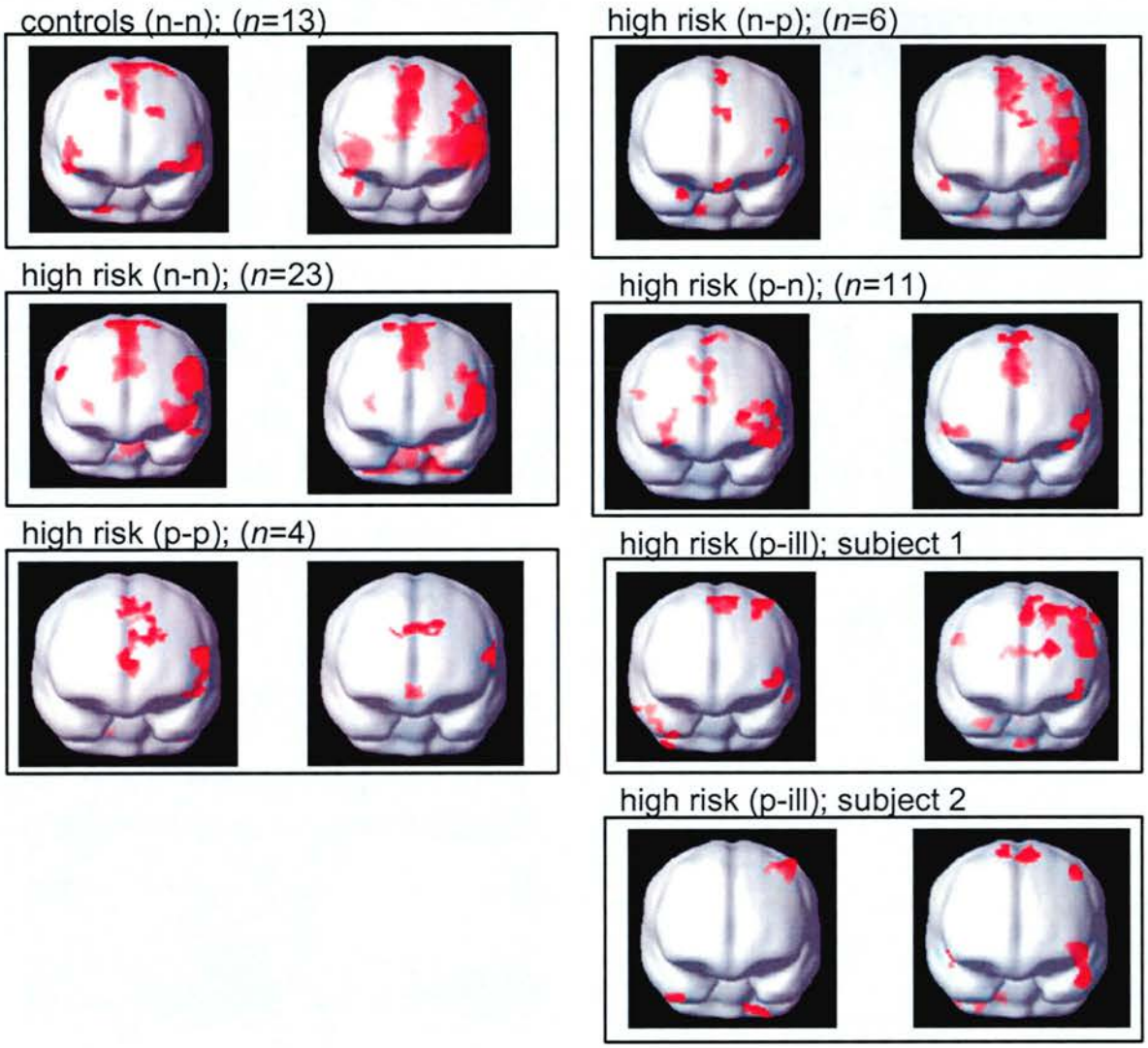

Figure 7.2 Activation patterns at time 1 and time 2 for the parametric contrast

Increasing activations with increasing tasks difficulty for each of the groups; the two high risk subjects who were ill at the time of the second scan are shown individually. Control(n_p) group not shown. For each group the left image represents the activation pattern at time 1 and the right at time 2. The left column represents subjects that did not change symptomatic status between scans, those on the right underwent worsening or improvement in symptomatic status. Maps thresholded at p=0.01 uncorrected, extent threshold=50 voxels.

7.4.4 Between group results

7.4.4.1 *Changes in activation with development of schizophrenia*

The changes over time in the two subjects who developed schizophrenia with two scans versus the subjects who were asymptomatic at both time points are described below for both the sentence completion versus rest and the parametric contrasts (Tables 7.6 and 7.7).

7.4.4.1.1 Sentence completion versus rest

For sentence completion versus rest no significant differences in the change of activation over time, increases or decreases, were found for high risk(p_ill) subject 1, consistent with the within group maps described above. High risk(p_ill) subject 2 however demonstrated relative decreases in activation over time in comparison to the stable asymptomatic groups in regions including the bilateral anterior superior temporal gyrus, inferior frontal gyrus, and posterior cingulate/precuneus. Subject 2 also demonstrated larger increases in activation over time compared to the asymptomatic groups in the right middle frontal gyrus, posterior middle temporal/lingual gyrus and caudate, Figure 7.3.

Table 7.6 Sentence completion versus rest: change over time, hr(p-ill)

P value	Z score	Peak height co-ordinates	Region
[controls(n_n)+high risk(n_n)] > hr(p_ill) subject 1:n/s			
[controls(n_n)+high risk(n_n)] < hr(p_ill) subject 1:n/s			
[controls(n_n)+high risk(n_n)] > hr(p_ill) subject 2:			
<0.001	inf	40 24 -16 45 23 -30 52 3 -14	R inferior frontal g, BA47/11 R inferior frontal g, R anterior superior temporal g
<0.001	6.86	-48 14 -36 -42 22 -36 -21 6 -23	L anterior superior temporal g, L inferior frontal, BA47 L uncus
<0.001	6.25	-6 -46 12 2 -55 39 -3 -51 37	L posterior cingulate R posterior cingulate/precuneus L precuneus
0.005	6.12	-14 -7 17 -14 3 14	L sub-lobar: border mid-caudate/thalamus L sub-lobar: border mid-caudate/thalamus
<0.001	6.03	-63 0 8 -63 -12 26 -49 2 19	L superior temporal g, BA22 L postcentral g. L precentral g,
0.014	5.75	12 -6 18 12 4 14 7 11 -1	R sub-lobar: border mid-caudate/thalamus R sub-lobar: border mid- caudate R sub-lobar: border caudate
<0.001	5.66	-54 20 -10 -56 13 -16 -33 25 -9	L inferior frontal g, BA45/47 L anterior superior temporal g L inferior frontal g, BA11
0.009	5.37	-4 -29 46 -4 -30 31	L posterior cingulate L posterior cingulate
[controls(n_n)+high risk(n_n)] < hr(p_ill) subject 2			
<0.001	7.80	22 53 -14 48 47 17 34 51 -1	R middle frontal g, BA10 R middle frontal g, BA10 R middle frontal g, BA10
<0.001	6.60	34 -63 14 18 -79 4	R posterior middle temporal g, BA39 R lingual g/depths calcarine s, BA17
0.005	6.16	-12 20 10 -3 5 12 9 20 10	L sub-lobar: anterior caudate L sub-lobar: caudate R sub-lobar: anterior caudate

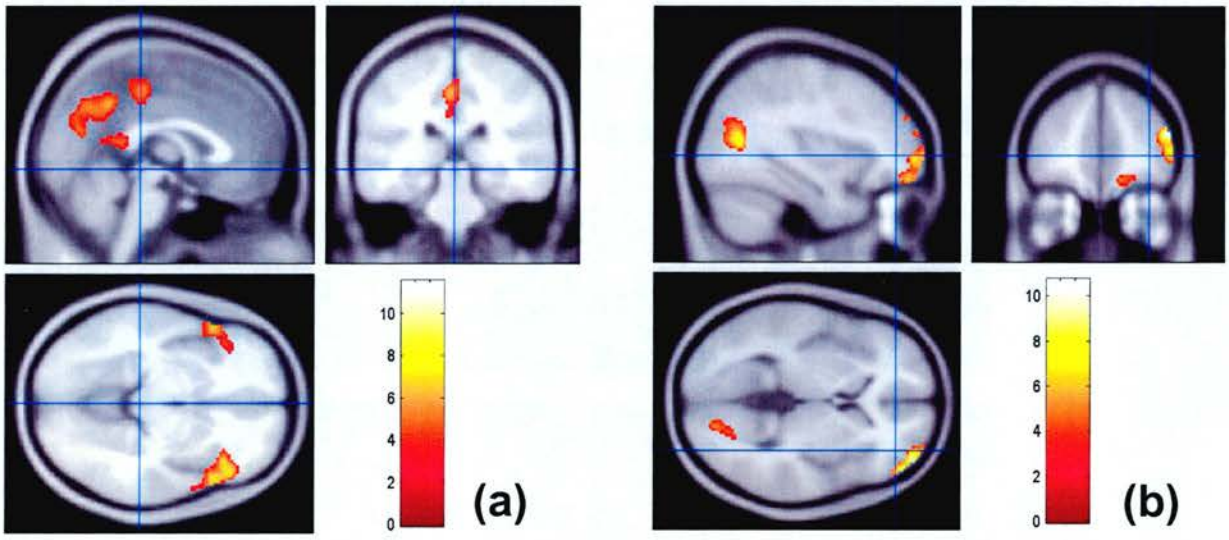

Figure 7.3 Changes over time, asymptomatic groups versus subject 2: sentence completion versus rest

(a) decreases in activation over time (b) increases in activation over time in subject 2 relative to controls and high risk who were asymptomatic at both occasions. Maps thresholded at 0.001 p uncorrected, extent 200 voxels. Colour bar represents Z score.

In order to better understand the origins of these group differences, activation maps at both time points were visually assessed. Figure 7.4 below presents the visit one and visit two activation maps for the group high risk(n_n) and high risk(p_ill) subject 2 focussing on these regions. As can be seen from this figure, in the case of the inferior frontal, thalamic and lateral temporal lobe differences, these appear to originate from reduced activation at time two for the subject who was ill at the time of the second scan. In terms of the increases, additional activation in subject two in the lingual gyrus at time two is also evident in these figures. Subject 1 is not shown in these figures as the previous analysis indicated there were no significant differences between this subject and the asymptomatic groups.

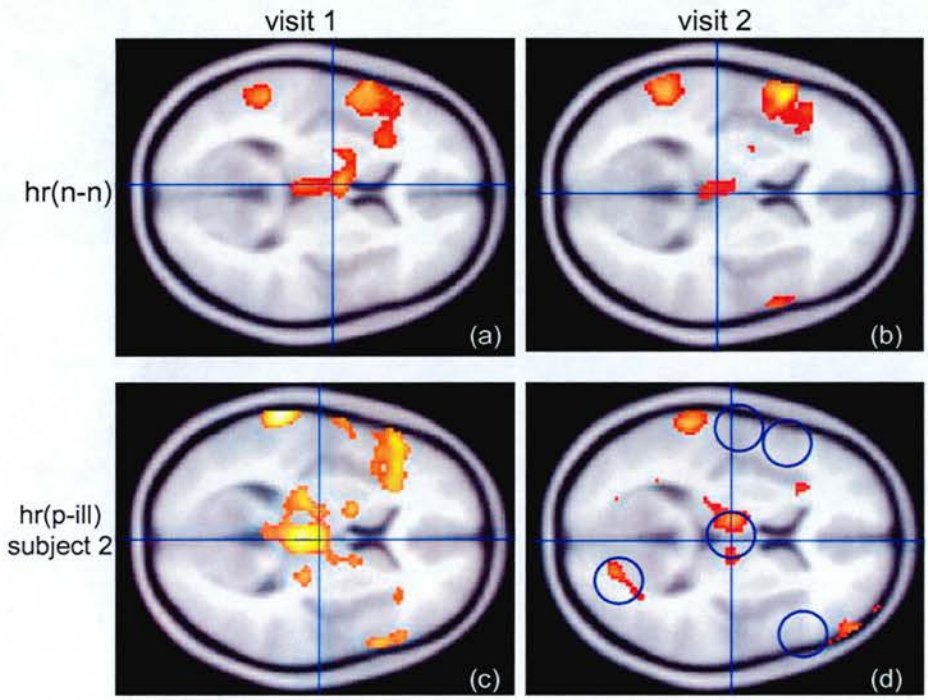

Figure 7.4 Examining origins of activation differences: sentence completion versus rest

(a) and (b) high risk(n_n) at time one and two respectively; (c) and (d) subject two at time one and two respectively. Circles indicate possible origins of the groups differences Maps thresholded at 0.001 uncorrected, extent 50 voxels.

One of the main omissions from these activation maps was the difference located in the posterior cingulate. Since this region was found to be activated in the rest versus sentence completion contrast (see chapter three), the possibility that this difference could originate from the reverse contrast was investigated, see Figure 7.5. It appears from this figure that the origin of the posterior cingulate/precuneus difference was reduced activation at rest (relative to task activation) at the time of the first assessment for high risk(p_ill) subject two.

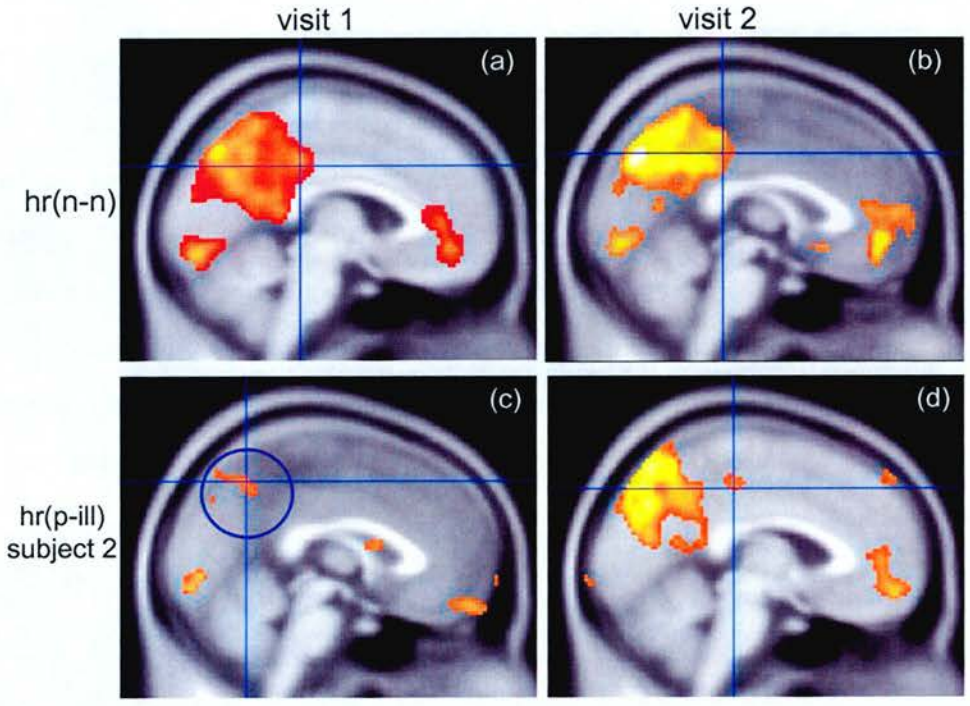

Figure 7.5 Examining origins of group differences: rest versus sentence completion

(a) and (b) high risk(n_n) at time one and two respectively; (c) and (d) subject two at time one and two respectively. Circle indicates possible origins of the groups differences Maps thresholded at 0.001 uncorrected, extent 50 voxels.

7.4.4.1.2 Parametric contrast

Results for the parametric contrast are shown in Table 7.7. As in the previous contrast, there were no significant differences in activation over time for high risk(p_ill) subject 1 versus the stable asymptomatic groups. High risk(p_ill) subject 2 however demonstrated relatively larger decreases in activation over time for the parametric contrast in right middle and inferior frontal gyrus, bilateral superior parietal lobule, posterior cingulate and bilateral hippocampus (Figure 7.6). Subject 2 did not demonstrate significant increases in activation versus the asymptomatic groups for this contrast.

Table 7.7 Parametric contrast: change over time, hr(p-ill)

P value	Z score	Peak height co-ordinates	Region
[controls(n_n)+high risk(n_n)] > subject 1:n/s			
[controls(n_n)+high risk(n_n)] < subject 1:n/s			
[controls(n_n)+high risk(n_n)] > subject 2:			
0.038	6.06	12 60 -3 18 61 10	R anterior medial frontal g, BA10 R middle frontal g, BA10
<0.001	5.71	32 18 57 20 26 52 19 24 38	R middle frontal g, BA8 R superior/middle frontal g, BA8 R superior/middle frontal g, BA8
0.017	5.46	-28 -21 -19 -42 -24 -21	L hippocampus L parahippocampal/fusiform g
<0.001	5.35	34 18 28 41 34 23 37 22 34	R inferior frontal g, BA9/44 R inf/middle frontal g, BA9/46 R middle frontal g, BA9
0.042	5.10	42 -70 39 42 -74 30	R superior parietal lobule BA7 R middle occipital g, BA19
0.015	5.00	6 20 14 -8 20 10	R cingulate g, L cingulate g
0.010	4.95	26 -16 -14 16 -7 -18	R hippocampus R parahippocampal g
0.002	4.81	-28 -50 63 -18 -48 74 -13 -26 46	L superior parietal lobule, BA7 L superior parietal lobule, BA7 L paracentral lobule, BA5
0.008	4.69	4 -45 35 -6 -35 35	R posterior cingulate L posterior cingulate
[controls(n_n)+high risk(n_n)] < subject 2 :n/s			

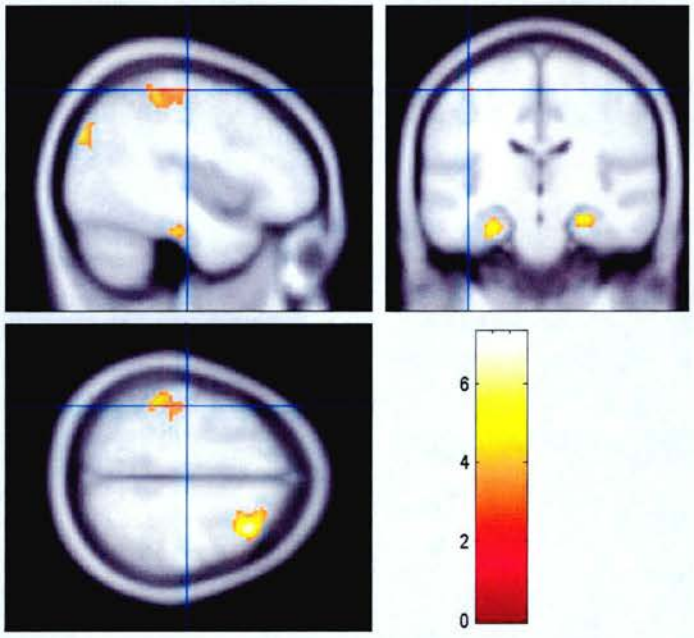

Figure 7.6 Changes over time, asymptomatic groups versus subject 2: parametric contrast

Decreases in activation over time in subject 2 relative to controls and high risk who were asymptomatic at both occasions. Maps thresholded at 0.001 p uncorrected, extent 200 voxels. Colour bar represents Z score.

As before, in order to examine the origins of these parametric group differences, the group maps at both time points were visually assessed. A figure highlighting the differences in the medial temporal and superior parietal regions is presented below (Figure 7.7). This figure indicates that high risk(p_ill) subject two presented less bilateral medial temporal lobe activity at the time of the second assessment when this subject was classified as ill. At time one when this subject was classed as 'high risk with psychotic symptoms', this subject presented greater activation in bilateral superior parietal regions, and in right prefrontal regions (not shown). Again, differences in the posterior cingulate were not seen in this parametric contrast, however examining the inverse parametric contrast (decreasing activation with increasing difficulty) revealed that this was reduced at the time of the first assessment (not shown).

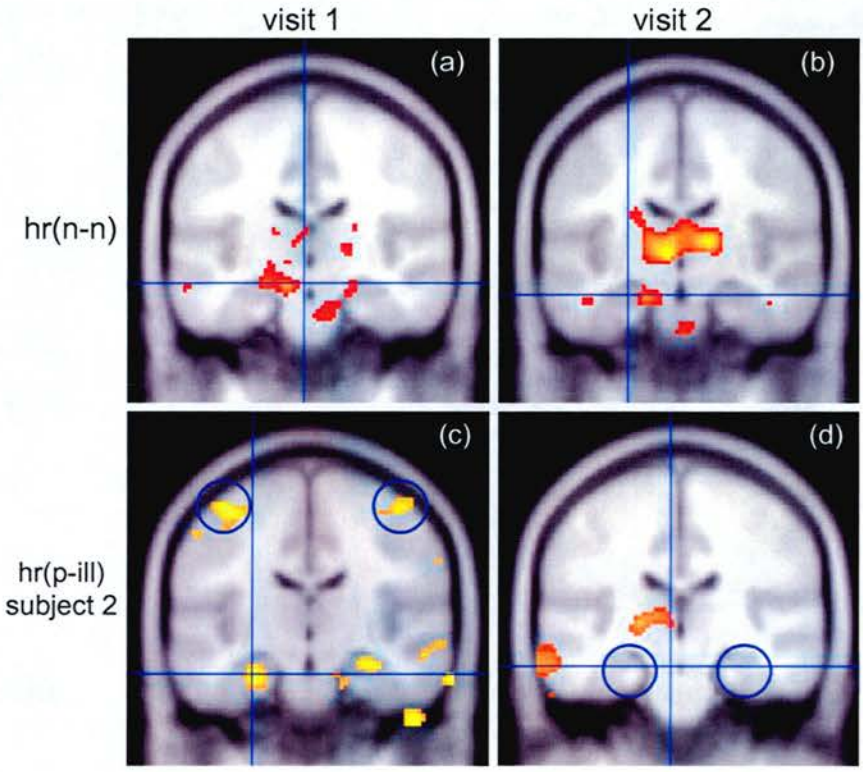

Figure 7.7 Examining origins of group differences: parametric contrast

(a) and (b) high risk(n_n) at time one and two respectively; (c) and (d) subject two at time one and two respectively. Circle indicates possible origins of the groups differences Maps thresholded at 0.05 uncorrected, extent 50 voxels.

7.4.4.2 *Changes in activation with development/remission of symptoms*

7.4.4.2.1 Sentence completion versus rest

For the sentence completion versus rest contrast there were no significant differences between those at high risk who developed psychotic symptoms between time 1 and time 2 relative to those that remained asymptomatic at both occasions. Similarly, there were no significant differences between the high risk with remitting symptoms relative to those that remained asymptomatic at both occasions. There was however an indication in the high risk(p_n) group of decreased activation in the parietal lobe over time compared to those who remained asymptomatic at both occasions (x = -36, y = -71, z = 49, superior/inferior parietal lobule, BA7/40; Z score=3.79), although this was not significant.

7.4.4.2.2 Parametric contrast

For the parametric contrast no significant differences were seen between the stable asymptomatic groups and the high risk(n_p) group, however differences were seen against the high risk(p_n) group in the depths of the right sylvian fissure/superior temporal gyrus and the posterior cingulate, Table 7.8 and Figure 7.8.

Table 7.8 Parametric contrast: change over time, hr(n-p) and hr(p-n)

P value	Z score	Peak height co-ordinates	Region
[controls(n_n)+high risk(n_n)] > high risk(n_p):n/s			
[controls(n_n)+high risk(n_n)] < high risk(n_p):n/s			
[controls(n_n)+high risk(n_n)] > high risk(p_n):			
0.003	4.24	16 -40 32 0 -23 24 6 -29 19	R posterior cingulate, BA23 R posterior cingulate, BA23 R posterior cingulate, BA23
<0.001	3.86	42 -36 22 56 -40 12 41 -18 16	R depths of sylvian fissure R superior temporal g, BA 22 R insula
[controls(n_n)+high risk(n_n)] < high risk(p_n):n/s			

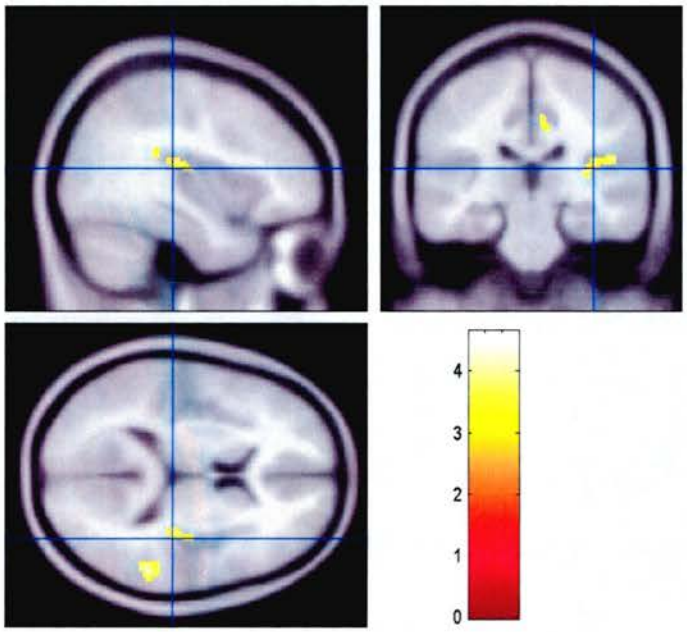

Figure 7.8 Changes over time, asymptomatic groups versus high risk(p_n):parametric contrast

Decreases in activation over time in posterior cingulate and superior temporal gyrus in high risk with remitting symptoms versus high risk and controls who were asymptomatic at both occasions. Maps thresholded at 0.001 p uncorrected, extent 300 voxels. Colour bar represents Z score.

As above, visual assessment of the activation maps at each time point were conducted to determine the origins of these group differences. For the reverse parametric contrast there appeared to be reduced activation of these regions at the time of the first assessment when the high risk(p_n) group were classed as 'with psychotic symptoms'(Figure 7.9).

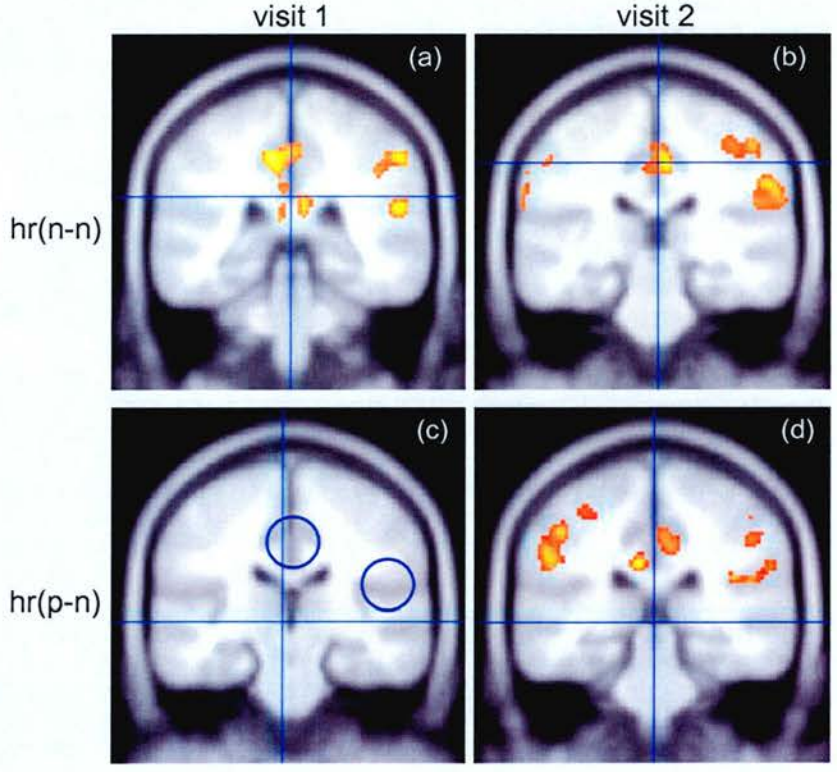

Figure 7.9 Examining origins of group differences: inverse parametric contrast

(a) and (b) high risk(n_n) at time one and two respectively; (c) and (d) high risk(p_n) at time one and two respectively. Circle indicates possible origins of the groups differences Maps thresholded at 0.01 uncorrected, extent 50 voxels.

7.5 DISCUSSION

On the whole these preliminary longitudinal results are encouraging. Although no direct quantitative examination of repeatability was performed on this data visual inspection of within group maps, particularly for the larger groups, suggested highly consistent patterns of activation over the two time points. This has obvious benefits for interpreting the current findings and is encourgaging for future longitudinal fMRI studies. It is also in line with other repeatability studies, where it has been reported that while longitudinal fMRI studies on individual subjects may remain questionable, longitudinal studies on groups of subjects seem to be less vulnerable to inter-session differences (McGonigle *et al.*, 2000)

The main findings from this study indicated that changes in activation over time were found in one out of the two high risk subjects who developed schizophrenia in frontal and temporal regions, and the posterior cingulate. Secondly, differences in activation over time were also found with the change in symptomatic status of the high risk subjects in the sylvian fissure/insula and posterior cingulate.

Due to the differing symptom course in the two subjects who developed schizophrenia they were considered separately. Subject 1 underwent a much less dramatic change in the severity of symptoms between scan one and scan two compared to the second subject. Subject 2 also appeared to undergo larger decrements in performance between the two assessments, particularly at harder levels of the task. These differences may indicate that they were at different stages in the transition to illness, or may reflect heterogeneity of the illness course. From these clinical and behavioural measures it may have been expected that the second subject would show the largest change in patterns of activation between the two scans. The current findings would however have been much strengthened if similar differences were observed for both subjects who became ill at the time of the second scan. Furthermore, differences at the level of single subjects should obviously be taken with caution. They are based on the assumption of equal variance across groups, and

issues of repeatability at an individual subject level should also be considered (see above and chapter one). Therefore the results detailed here are considered provisional and essentially descriptive.

What seems clear however is that the differences seen in inferior frontal, lateral and medial temporal regions in subject 2 are principally due to reductions in activation at the time of the second assessment when this subject was classified as ill. Differences in these regions fit well with structural and functional deficits consistently reported in patients with schizophrenia (Shenton *et al.*, 2001; Niznickiewicz *et al.*, 2003), and add support to the notion that functional deficits in lateral prefrontal and lateral temporal regions may only become apparent around the time of onset of the disorder, or when performance begins to fail. Focussing on activation of the frontal lobes in this subject, at time 1 there appears to be 'hyperfrontality' (Figure 7.1), whereas at time two (when performance is impaired) this subject demonstrates 'hypofrontality' (in bilateral inferior frontal gyrus BA45/47). This pattern is consistent with the hypothesised relationship between prefrontal functioning and behavioural performance discussed in chapter three (described as the inverted 'U', Manoach *et al.*, 2003). One could speculate that, as symptoms worsen and performance on the task begins to fail, patterns of prefrontal activation change from being hyper- to hypoactive. It is impossible to say however, whether the behavioural and clinical changes reflect the cause or consequence of the activation differences.

Other interesting findings in this particular subject are the reductions over time in limbic regions (the bilateral medial temporal lobes and posterior cingulate). From chapter five it was seen at baseline that those subjects who subsequently became ill presented smaller increases in activation than the other groups in bilateral medial temporal lobes in response to increasing task difficulty. The results presented here suggest that in one of these subjects, there were further reductions over time in this region for the parametric contrast compared to the stable asymptomatic groups, and these were associated with further decrements in task performance. As described

in chapter five, deficits in medial temporal lobe regions, particularly at the structural level, have been consistently reported in those with the disorder (Shenton *et al.*, 2001), in those in their first episode of the illness (Job *et al.*, 2002), in those at high genetic risk (Lawrie *et al.*, 1999, 2001; Job *et al.*, 2003), and in those at risk who later become ill (Pantelis *et al.*, 2003). These findings perhaps suggest further impairment in this region, which may be associated with subsequent development of the illness or with further performance decrements.

Deficits in the posterior cingulate are less often reported than the anterior portion of this region, however there are indications of structural abnormalities in early onset schizophrenia (Sowell *et al.*, 2000), in ultra-high risk subjects who later developed psychosis (Pantelis *et al.*, 2003), and they have been associated with poor outcome in the established illness (Mitelman *et al.*, 2004). In terms of functional imaging, the severity of first rank symptoms has also been reported to be negatively associated with regional cerebral blood flow to this region (Franck *et al.*, 2002). Functionally, this region has been implicated in the "default mode" hypothesis (Raichle *et al.*, 2001b) or the 'network of the resting brain'. It has been considered to form part of the activation pattern observed during unstructured periods of thought or reasoning. Indeed this type of response can be seen in the current findings in chapter three, where activation of posterior brain regions, including the posterior cingulate/precuneus, were seen in the rest versus sentence completion, and in the inverse parametric contrast. In other words this region is more active during the baseline condition, and at the easier less cognitively demanding levels of the task. The origin of this change over time difference in subjects 2 appeared to stem from a relative reduction of activation in this region at rest (and a relative reduction in activation with decreasing difficulty), at the time of the first assessment when this subject was classed as 'high risk with symptoms'. It is at present unclear how to interpret these results. One could speculate that in terms of the parametric finding, a relative reduction in the activation of the posterior cingulate with decreasing difficulty indicates this subject is not presenting the normal default mode pattern of response (increasing activation with decreasing difficulty) in reaction to a task which

is perhaps experienced as being more cognitively challenging than it is for the asymptomatic subjects. Following on from this, perhaps as capacity is exceeded and task performance begins to fail (at the second assessment), the default mode pattern of response re-emerges. This finding could however equally be interpreted as a failure to decrease activation in this region during the task, or with increasing difficulty, at the time of the first assessment.

With regards the second aim of this chapter, this study reports changes in the patterns of activation with changes in the symptomatic status of the high risk subjects. Specifically, reductions in right sylvian fissure/superior temporal gyrus and posterior cingulate in high risk subjects who were symptomatic at time one but asymptomatic at time two for the parametric contrast. The origins of these results were similar to the posterior cingulate finding above. In other words, these regions were found to be activated in the reverse parametric contrast (more active at easier levels of the task) for the asymptomatic groups at both occasions, and for the high risk(p_n) at the time of the second assessment when they reported no psychotic symptoms. However there was a relative reduction of activation in these regions for the inverse parametric contrast at the time of the first assessment when the high risk(p_n) reported symptoms. As above, perhaps when the high risk subjects present psychotic symptoms and find the task more challenging, they 'switch off' the default-mode type response at easier levels of the task, and when symptoms remit, the normal pattern of response is restored. Equally, as above, when the subjects are symptomatic (at time one) this pattern of response could indicate that they fail to undergo deactivation of these regions during the task.

It may have been expected from the previous results, that parietal activation levels would fluctuate with the presence of symptoms. Although not significant, there was an indication in the high risk(p_n) group of a decrease in activation in this region over time compared to those who remained asymptomatic at both occasions. As in the previous chapter this analysis was performed at the 'whole brain' level for reasons outlined previously. More weight could have been placed on this finding if

the high risk(n_p) group also showed increasing activation in this parietal region with the worsening of psychotic symptoms, but this was not found. In fact no differences were found in high risk subjects who were asymptomatic at time one and symptomatic at time two compared to the stable asymptomatic groups. However, the high risk(n_p) group was considerably smaller (n=6) than the group that made the reverse change (high risk(p_n), n=11).

At present time the author is not aware of any other fMRI studies that have examined changes over time with the development of schizophrenia, or with the worsening or remission of psychotic symptoms, in un-medicated subjects. Indeed from the point of view of the current study it was a matter of good fortune that the opportunity existed to conduct a second scan in two people at the time when it was first evident that they clearly did have schizophrenia and when they were un-medicated. There are however a number of structural imaging studies that have examined the relationship between progressive structural changes and the clinical course in subjects in early stages of the illness, for example poorer outcome has been associated with greater ventricular enlargement (see Lieberman *et al.*, 2001). In general however, these studies have yielded inconsistent findings, some counter-intuitively reporting greater clinical improvement with increased loss of brain parenchyma (see Weinberger and McClure 2002). Furthermore, such studies can not distinguish the effects of the disease itself and medication effects.

There are reports from our own and other high risk studies reporting structural changes in relation to symptom change in un-medicated individuals. In our high risk cohort structural decreases were seen over time in the high risk subjects who developed psychotic symptoms in the temporal lobe (Lawrie *et al.*, 2002b). The study by Pantelis and colleagues also reported structural grey matter reductions in temporal regions, with additional reductions in orbitofrontal regions and posterior cingulate in those who developed psychosis versus those that did not (Pantelis *et al.*, 2003). It is unclear at this stage however how these functional abnormalities fit with the structural deficits reported in high risk individuals. Further longitudinal studies in

prodromal patients at frequent time intervals may be the only way to establish whether the structural or functional changes are primary.

In terms of methodological considerations, it should be appreciated that technical difficulties with the IFIS software delayed follow-up scanning of the study participants. This chapter has therefore outlined preliminary findings only. For simplicity the analysis focussed on two specific aims: changes in those who become ill, and changes in those with developing or remitting psychotic symptoms. It has therefore focussed on two specific sets of contrasts, each in comparison to the stable asymptomatic subject groups. This was performed by collapsing the asymptomatic controls and asymptomatic high risk subjects into one group, this approach was used to increase group numbers and reduce the number of group comparisons. Ideally these should be treated separately however, and once the collection of second round scans is fully complete, a re-analysis of the data with the groups separated will be conducted which may be able to clarify and extend the current findings. With greater group numbers it may also be possible to perform other group comparisons, for example involving subjects who were symptomatic at both time points but have not made the transition to schizophrenia. If it is possible to distinguish those who have consistent underlying psychotic symptoms but who do not develop the disorder versus those that do, this may have potential clinical relevance.

It is also interesting to note that out of the 17 subjects with two scans who were classed as 'with psychotic symptoms' at the time of the first scan, the majority (n=11) did not report symptoms at the time of the second assessment. Only four remained symptomatic at the second assessment, and only two made the transition to illness. This indicates that it is not the case that the presence of psychotic symptoms at a single time point is deterministic for subsequent conversion to illness. However, in line with previous findings (Johnstone *et al.*, 2002a), those that do make the transition usually report psychotic symptoms prior to onset of the illness.

On the whole these findings indicate that patterns of brain activation fluctuate with the changes in symptomatic status of individuals, and that changes may be greater in those showing the greatest symptomatic change during early stages in the development of the disorder. Such findings may have important implications for the understanding of the pathogenesis of the disorder, and could provide possible targets for early intervention.

8 GENERAL DISCUSSION

8.1 INTRODUCTION

The work described in this thesis has used fMRI to examine patterns of functional localisation and functional integration in subjects at high genetic risk of schizophrenia. As described in the introduction, fMRI is increasingly becoming the tool of choice for the study of human brain function. It has a favourable balance between spatial and temporal resolution, and it is particularly suited to this series of experiments since it allows repeated scanning of study participants.

Neuroimaging studies of the established condition have indicated subtle structural and functional abnormalities in a variety of brain regions, particularly but not exclusively in frontal and temporal brain regions. Disrupted interactions between these brain regions are also thought to be important in the pathophysiology of the disorder. However, the extent to which these abnormalities were related to inherited vulnerability (and whether they are present before disease onset), medication, or disease effects was not clear. Nor was it clear whether imaging abnormalities could distinguish individuals who later make the transition to schizophrenia, or whether patterns of activation would change with symptomatic status. Hence, the aims of this thesis were to (i) identify the neural correlates of trait and state effects in young subjects at high genetic risk of schizophrenia, (ii) to determine if it is possible to distinguish those who become ill versus those that remain well, and (iii) to determine if changes in the patterns of brain activity occur with the transition to illness, or with the development or improvement of psychotic symptoms.

Since each of the experimental chapters in this thesis contains a separate discussion section, the aim of this chapter is to offer a more general discussion, integrating the main results from the previous chapters, discussing limitations of the current findings, and suggesting possible avenues for future work. It should be remembered that these results were based on clinical data at the time of the scan, or clinical data available at the time of writing, and due to the nature of this longitudinal

study, changes in the status of these individuals may still occur, which could alter some of these findings.

8.2 SUMMARY OF MAIN RESULTS

To aid this discussion a figure summarising the main findings is presented below (Figure 8.1). The main results from this thesis were that fMRI can detect trait and state effects in subjects at high genetic risk for the disorder using functional localisation and functional integration analysis techniques. These trait and state effects were considered to reflect inherited abnormalities and symptom-related abnormalities respectively. These results occurred in a situation unconfounded by anti-psychotic medication, prolonged illness, or performance differences between groups and are novel findings. Abnormal prefrontal-thalamic-cerebellar activations were found in all those at high risk of the disorder (chapter three), and were presumed therefore to be a genetically mediated deficit. Abnormal patterns of connectivity between these regions, over and above those due to localisation differences, were also found in high risk subjects (chapter four), and in addition there may be some subtle indications that these deficits are larger in those who subsequently become ill (chapter six).

Hyperactivity of the left inferior parietal lobe was found in high risk subjects, more so in those with symptoms (chapter three), and was therefore considered, at least partially, to be a symptom-related effect. Abnormal patterns of connectivity were also seen in high risk subjects between this region and the dorsolateral prefrontal cortex (chapter four), and again there were cautious indications that these effects were stronger in those with symptoms, and in those who go on to develop the disorder (chapters five and six).

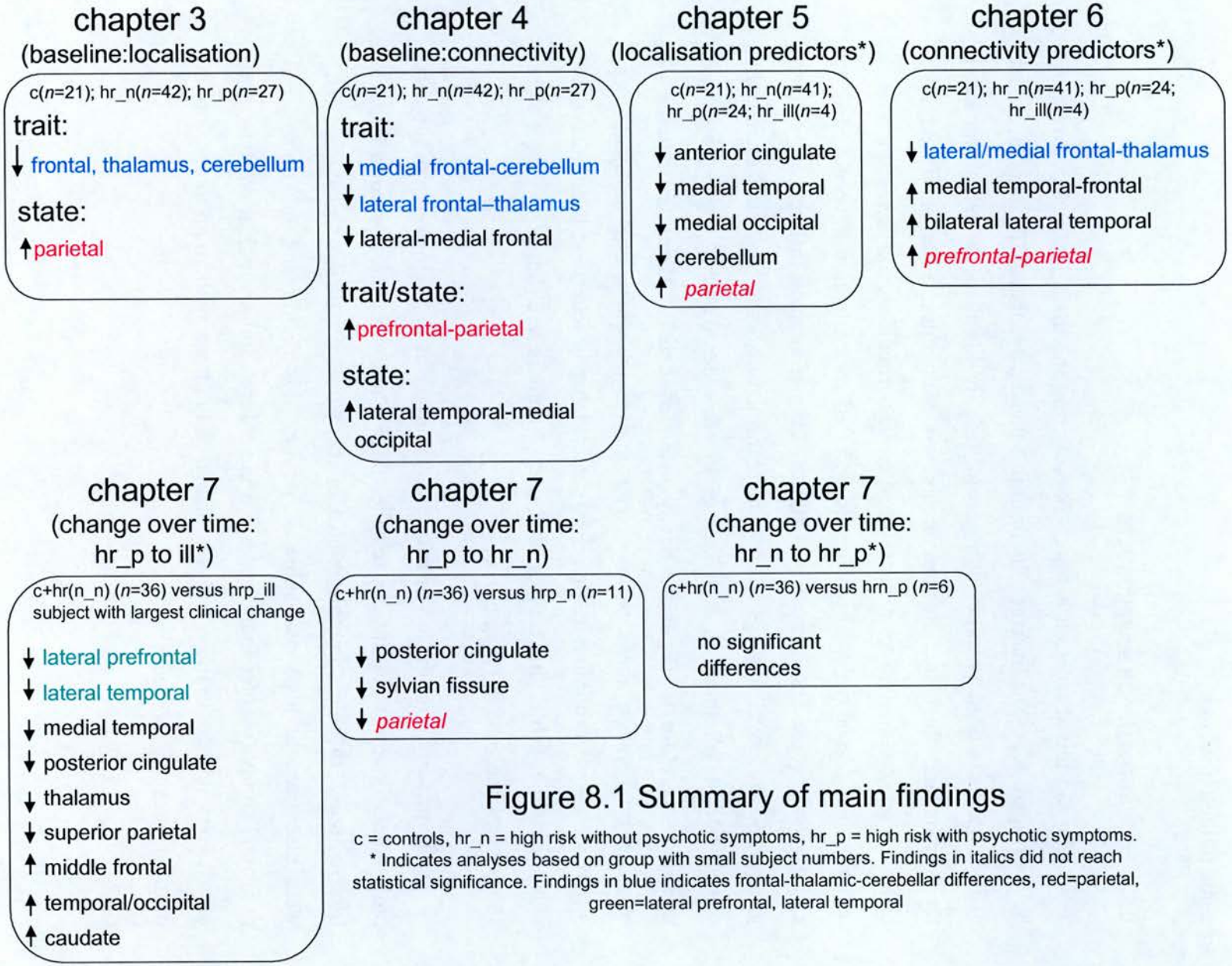

Figure 8.1 Summary of main findings

c = controls, hr_n = high risk without psychotic symptoms, hr_p = high risk with psychotic symptoms. * Indicates analyses based on group with small subject numbers. Findings in italics did not reach statistical significance. Findings in blue indicates frontal-thalamic-cerebellar differences, red=parietal, green=lateral prefrontal, lateral temporal

Other results of note were decreased lateral to medial prefrontal connectivity in those at high risk, a finding which was consistent across all three of the tasks tested, and the only trait related finding to be associated with the degree of risk within the high risk cohort (although at a marginal level), and increased lateral temporal to medial occipital connectivity which was predominantly seen in those with symptoms.

Taken together therefore these findings suggest that prefrontal-thalamic-cerebellar, and medial-lateral prefrontal deficits may represent the inherited neurophysiological basis of the disorder, and indeed evidence exists in the schizophrenia literature regarding deficits in these regions (described in chapters three and four): prefrontal-thalamic-cerebellar deficits have been hypothesised as underlying abnormalities in the co-ordination of mental processing ('cognitive dysmetria': Andreasen *et al.*, 1996, 1998, 1999), and medial-lateral prefrontal deficits, also reported by Spence *et al.*, (2000), have been associated with functions such as response inhibition (Menon *et al.*, 2001b; Aron *et al.*, 2004). These findings are also consistent with studies reporting abnormalities in the white matter tracts connecting these regions (Sigmundsson *et al.,* 2001; Zhou *et al.*, 2003; Wang *et al.*, 2004), and fit with the diverse deficits seen in the disorder (Andreasen *et al.,* 1996, 1999; Frith 1992).

Expanding on the model of cognitive dysmetria proposed by Andreasen and colleagues (1996, 1999), this is based on considering cognitive functions as an extension of motor processes (a concept also described by Feinberg and Guazzelli 1999, discussed below). This is the origin of the term 'dysmetria' - from 'motor dysmetria' describing a disruption in the co-ordination of motor activity. Cognitive dysmetria is therefore used to describe a disruption in the fluid co-ordination of mental activity. In the paper by Feinberg and Guazzelli (1999) the authors put forward the model that the abnormalities seen in schizophrenia could result from deficits in corollary discharge and feedforward systems (also extensively written about by Frith 1992). They suggest that 'thinking' or cognitive processes can be

thought of as a complex motor act, and therefore subject to the same corollary discharge systems. With regards to the neural circuitry involved, they speculate that since nature is in general opposed to duplication, the same circuits involved in sensori-motor integration would also be involved in integrating motor mechanisms of cognition. In particular they implicate the thalamus and cerebellum (along with Andreasen *et al.*, 1998, 1999). In other words, in the same way that these structures regulate the speed, timing and accuracy of movement they could also regulate the speed, timing and accuracy of cognitive processes. It is proposed that abnormalities in the circuit linking these regions to those involved in higher-order functioning, such as prefrontal regions, may lead to the diverse cognitive impairments seen in the disorder.

Turning to the parietal lobe findings, these may reflect early pathological changes associated with the presence of psychotic symptoms. It is not possible to say whether these represent primary brain disturbances (cause), secondary events (consequence), or a response designed to restore normal functioning (compensatory) (Lewis 2000; Fletcher 2004). However, one possible explanation is that since these subjects were not presenting performance deficits, the parietal regions and associated networks may be recruited to allow subjects to perform the task at similar levels to the other groups, as discussed in chapters three and four. It has been suggested that the parietal cortex in humans may be organised into functional zones similar to those seen in non-human primates, however at present detailed functional mapping of this higher order association cortex in humans is still far from clear (see Culham and Kanwisher 2001). Indeed, activation in this multimodal region is seen in a wide variety of paradigms, for example tasks involving spatial representations and co-ordination of motor responses, response preparation, response monitoring, response inhibition, sustained attention, and subvocal rehearsal, amongst others. It is therefore difficult to unequivocally conclude the exact nature of this increased activity and connectivity observed in these high risk subjects. In the context of the current task it would seem likely however that this region is involved in maintaining attention and the preparation and monitoring of suitable covert verbal responses (as discussed in

chapters three and four). This is in line with reports that prodromal subjects report difficulties focussing attention and a reduced sense of control of behaviour (Klosterkötter *et al.*, 1997). This may therefore suggest deficits in self-monitoring, and indeed parietal regions have been implicated in functions such as distinguishing between the self and others (Meltzoff and Decety 2003). This is discussed in more detail below regarding the context of delusional phenomena. As an extension to the cognitive dysmetria concept outlined above, one could speculate that other regions where sensori-motor functions are integrated could equally contribute to the variety of symptoms seen in schizophrenia, for example in association areas such the parietal cortex.

The other main state-related finding was increased connectivity between lateral temporal regions and the medial occipital cortex. Interestingly, abnormalities in the medial occipital cortex, particularly in the region of the calcarine sulcus/lingual gyrus, are evident several times in this thesis. In addition to the increased connectivity with lateral temporal cortex, this region appears to be more active during rest conditions than during the task (chapter three), and shows a positive relationship with genetic liability (greater activation in those with first degree relatives versus those with second degree, also in chapter three), suggesting it is not purely a state-related finding. In chapter five, reduced activity in this region in response to increasing task difficulty was seen at baseline in those who later become ill. Finally, the results presented in chapter seven indicate increased activation this region at the time of the second scan when one of the two subjects who made the transition to schizophrenia was classified as ill. The interpretation of this state/trait related abnormality is at present unclear, but dysfunction of this region in those with symptoms and in those who later become ill is perhaps linked with reports that visual perceptual disturbances are often described in the prodromal or early phases of the illness (Klosterkötter *et al.*, 1997; Johnstone *et al.*, 1988; Chapman 1966).

It was originally hypothesised that symptom related abnormalities would be primarily found in lateral prefrontal and temporal regions, or in the connectivity

between these regions (dorsolateral prefrontal and lateral temporal cortex), based on literature of the established state. No evidence for such deficits were found in terms of the localisation or functional connectivity data in the high risk subjects. Findings from other high risk studies regarding abnormalities of activation in these regions are at present inconclusive, as discussed in chapter three, and the only other connectivity study in high risk relatives (to the authors knowledge) also failed to find dorsolateral prefrontal-lateral temporal disconnectivity (Spence *et al.*, 2000). There were however indications of activation deficits in these regions in one out of the two subjects with follow-up scans who made the transition to schizophrenia. These findings may therefore suggest that dorsolateral prefrontal and lateral temporal abnormalities may occur later in the disease progression and may be principally associated with the established state, equally they may only become manifest when performance deficits become apparent. On the whole these results are highly consistent with an automated structural longitudinal study of childhood onset schizophrenia, where early deficits were first reported in the parietal association cortex, whereas deficits in dorsolateral prefrontal and superior temporal regions only became apparent much later in the disease (Thompson *et al.*, 2001).

It was unexpected that only one of the trait-related findings described in chapters three and four were found to be related to the degree of risk within the high risk group. In general the results indicated that differences were attributable to the presence or absence of inherited risk, rather than the perhaps more subtle 'dose-response' relationship between the severity of abnormality and the degree of risk. It is difficult to account for this finding since little is known about the mode of genetic transmission of the disorder. What is clear however is that the nature of transmission is likely to be complex, perhaps involving multiple susceptibility genes, with varying degrees of expression, and maybe even involving different genes in different individuals. An exciting new approach to this problem however is beginning to be reported in the literature, this involves combining molecular genetic techniques and functional imaging methods to examine the association between specific genes and specific functional deficits (see Egan *et al.*, 2001). The collection of blood samples

from the Edinburgh High Risk Study participants and their relatives began recently, and similar analysis approaches are planned for the future.

The predictor findings as mentioned above hint that perhaps extremes of the prefrontal-thalamic-cerebellar and prefrontal-parietal abnormalities may distinguish those who later develop the disorder. However, it is likely that additional differences are also involved, since the predictor findings also included abnormal activation of the anterior cingulate, bilateral medial temporal lobe, medial posterior cerebellum, and medial occipital cortex, and abnormal connectivity between medial temporal lobe and lateral prefrontal regions, and between bilateral middle temporal gyrus. The first two regions in particular have been repeatedly implicated in the disorder - from histopathological studies through to structural and functional imaging. They have also been implicated in structural imaging studies in high risk individuals, and in those who later develop psychotic disorders (as discussed in chapter five). Hence these results are encouraging, but due to small group numbers should be considered with caution.

Although the change over time analysis should also be considered preliminary, the initial results are encouraging. Firstly, they indicated consistent patterns of activation over time in the larger groups, which is particularly important regarding the validity of longitudinal fMRI studies. Secondly, alterations in the patterns of activity were seen in the subject with the largest clinical and behavioural changes between assessments, and in high risk subjects with changes in symptomatic status (discussed in chapter seven).

Finally, it is worth highlighting the interesting pattern of behavioural responses across the different studies. At baseline (chapters three and four), when subjects were grouped into the broad categories of controls, and high risk with, and without symptoms, there were no performance differences between the groups. However, when the groups were subdivided into those subjects who later became ill (chapters five and six), and those who develop symptoms at the second scan (chapter seven), behavioural deficits, although not significant, began to emerge. These were

particularly evident at the harder levels of the task (low constraint) where the subjects produced less appropriate words, and/or decreased reaction times. At the low constraint level there is a larger choice of potential responses, i.e. the response is less specified by the sentence context than at easier levels. This is consistent with reports that subjects with schizophrenia show deficits in tasks where the required response is ambiguous or only partly specified (Frith 1995b), and the fact that the subjects are responding quicker, could be attributable to difficulties in inhibiting these inappropriate responses.

8.3 STRENGTHS AND LIMITATIONS OF THE CURRENT STUDY

One of the main strengths of the current study, particularly in relation to other functional imaging studies, was that it involved a relatively large group of well characterised individuals. In addition, these subjects were antipsychotic medication naïve at all times of assessment, and were on the whole performing the tasks at equivalent levels to the other groups, avoiding these commonly associated confounders.

Over the course of the study, a small number of subjects made the transition to schizophrenia (two of whom had two functional scans), which provided the unusual and valuable opportunity to examine changes in brain activation during the course of this transition. Although these findings are based on small numbers of subjects, close examination of this data was considered justified given the rarity of, and difficulty in, obtaining such data. Further replication however would naturally be desirable, particularly given the variability in single subject fMR images (see chapter one). One prospect for subsequent work could be to design criteria based on the current imaging data to determine if it is possible predict those most likely to become unwell in the future. From the main figures presented in this thesis however, it would appear that a range of abnormalities would need to be used, since there appeared to be incomplete group separation in any individual region. Difficulties associated with taking statistical group differences to form the basis for clinical inferences regarding individuals should also be appreciated. It should also be considered that fMRI is still

a relatively new technique, and particularly in patient populations, reproducibility has yet to be fully determined. Another issue pertinent to the current study is that the exact nature of neurovascular coupling and how this may be altered in disease states has yet to be established. Although these findings are in general encouraging in that they suggest there are measurable differences in subjects who subsequently become ill, at present the diagnostic accuracy of such a test remains uncertain.

In terms of the limitations of the current study which have not previously been described in preceding chapters, it should be remembered that the high risk cohort is a highly specialised group of individuals, recruited on the basis of the presence of a strong family history of the disorder, and of course on their willingness to participate in the study. It is also *not* the case that all schizophrenic subjects have a close relative with the disorder. It has been reported that approximately 60% of patients report neither having a first nor second degree relative with the disease (Gottesman and Erlenmeyer-Kimling 2001). Both of these issues restrict generalisability of these findings. Nevertheless, this study allows insight into a phase of the disorder rarely studied.

In terms of the actual task, one limitation of the current study is that, although within-scanner measures of performance by way of reaction time were recorded, on-line performance in terms of word appropriateness involved *covert* rather than *overt* word generation due to the artefacts induced by vocalisation in fMRI paradigms (see chapter one). At the time the study began novel paradigms which allow overt verbal responses cued in the gap between image acquisitions (see Henson *et al,*. 2002), were not readily available, or indeed widely used. In addition, word appropriateness scores in the current study were determined from Bloom and Fischlers set of sentence completion norms (1980), based on the responses of 100 undergraduate American college students. However, a group of UK based researchers examined responses in UK citizens and found that sentence completion responses may be subject to regional variation (Arcuri *et al.*, 2001). This paper was not available at the beginning of the study, and since not all of the sentences selected for use in the current study were

included in the article, the frequencies described in the UK based population could not be used to determine word appropriateness scores. However, since all the high risk subjects and controls are from similar demographic populations, it is unlikely that using frequencies based on the Bloom and Fischler paper will introduce any major bias in the behavioural results. Nonetheless, for future studies it may be advantageous to use a more up to date, UK derived frequency list for the sentence completion norms.

Also, it should also be considered that for the baseline analysis, in order to demonstrate presumed genetic influences or effects related to phenotypic expression it was necessary to show consistent differences between two combinations of groups but not in the third. For example, in terms of genetic influences it was necessary to demonstrate differences between high risk with and without symptoms versus controls, but not between the two high risk groups. However, the latter comparison brings about the problem of attempting to prove a negative hypothesis, which in statistical terms is impossible. This may be seen as an inherent limitation to this study.

8.4 SUGGESTIONS FOR FUTURE RESEARCH

Suggestions for future research include a number of studies which may help to clarify the current results. As described in previous chapters, since schizophrenia can have a variety of different symptom profiles, where two subjects diagnosed with schizophrenia may have no symptoms in common (see Honey *et al.*, 2002b), it may be a useful approach in this data set to characterise activation and connectivity differences based on symptom profile of the high risk subjects. Conversely it may also be useful to have a better understanding of the activation and connectivity patterns in those subjects at high genetic risk who have never had any psychotic symptoms in the past (see Johnstone et al., 2002b). It may be hypothesised, based on previous studies in the established state and cognitive models of specific symptoms, that those with auditory hallucinations may show abnormalities in superior/middle

temporal gyrus activation (McGuire et al., 1996; Woodruff et al., 1997b; Dierks et al., 1999) and/or abnormal connectivity with the prefrontal cortex (Lawrie *et al.*, 2002a; Shergill *et al.*, 2003; Ford *et al.*, 2002). It may also be hypothesised that those with delusions may show relatively greater abnormalities in parietal functioning and/or connectivity. Evidence for the association between parietal lobe dysfunction and delusions in schizophrenia is not as substantial as the association between lateral temporal lobe dysfunction and auditory hallucinations. However, there are reports of such relationships in schizophrenic subjects with delusions of alien control (Spence *et al.*, 1997), where psychological states or bodily movements are thought to be controlled by others. The origins of such delusions were hypothesised to result from deficits in feedforward models of intended actions, in line with the theory of deficits in self-monitoring in schizophrenia (Frith 1992, and see above), and may therefore reflect dysfunctional of fronto-parietal networks (Frith *et al.*, 2000). It may be hypothesised therefore that high risk subjects with delusional symptoms may present more severe abnormalities in parietal activation or fronto-parietal connectivity.

It is also interesting to note that associations between parietal dysfunction and delusions or delusional-related phenomena have been reported outwith the field of schizophrenia research. Patients with Alzheimer's disease manifesting delusions have been reported to present abnormal parietal cortex activation (Fukuhara *et al.*, 2001; Mentis *et al.*, 1995), and in a recent study electrical stimulation of the posterior parietal cortex in a preoperative epilepsy patient resulted in out-of-body experiences, which may be likened to delusions of control or passivity phenomena (see Tong 2003, and Danckert *et al.*, 2004). Also, patients with lesions to the parietal cortex have been shown to confuse the ownership of hand movements when shown someone else's hand performing similar movements to their own (see Blakemore 2003). Finally, functional imaging studies examining the neural correlates of imagined actions in normal subjects have indicated that when subjects are asked to imagine an action being preformed by themselves rather than by another individual, there is increased activity of the left inferior parietal lobule, suggesting this region has a role in distinguishing between the self and others (Melzoff and Decety 2003).

Hence it may be interesting to examine activation and connectivity patterns in the high risk subjects during tasks specifically designed to tap into such functional deficits, for example paradigms involving motor control or motor imagery (tasks that require the use of internal models of intended actions), see Danckert *et al.*, (2004).

Focussing on the connectivity findings, the relatively novel approach of examining activation data once task effects have been removed was used in the current study for reasons outlined previously. It may be beneficial however to examine the connectivity data presented here without the task effects removed. This may help elucidate whether there are additional deficits which are related to the type of task. Dividing the time series data into the different difficulty levels may also be a useful approach using the task-unfiltered data, in order to determine whether connectivity is modulated by the different levels of difficulty, and whether this is different between the subject groups. One might predict larger differences in connectivity at the harder constraint levels in those subjects beginning to present behavioural deficits.

Examining the changes in functional connectivity over time was considered beyond the scope of this thesis, but would be important to address in relation to the changes in functional localisation presented here. This is also planned for future work. Another major piece of work arising from this thesis is the examination of effective connectivity in the high risk cohort. This is currently underway in collaboration with Dr A Storkey at the School of Informatics at the University of Edinburgh. Finally, since these results implicate disconnectivity in the disorder, it may also be interesting to examine whether there are underlying abnormalities of white matter in the tracts connecting these regions in the high risk group with such techniques as diffusion tensor imaging (DTI) or voxel based morphometry of the white matter segments (VBM).

8.5 CONCLUSIONS

The results from this series of studies have indicated abnormalities in functional localisation and functional integration in subjects at high genetic risk of schizophrenia, strengthening theories that the basis of the illness may be due to disconnectivity between distributed brain regions. Deficits in connectivity were not however seen in dorsolateral prefrontal and lateral temporal regions, which may indicate these deficits are associated with the established illness, or when performance decrements become manifest. Abnormalities associated with inherited vulnerability were however seen in prefrontal-thalamic-cerebellar regions and lateral-medial prefrontal regions. That these deficits are seen in subjects at high genetic risk of the disorder implies that they are associated with inherited risk rather than medication or disease effects. These abnormalities were interpreted as underlying deficits in the co-ordination of mental processing and response inhibition. Abnormalities associated with symptoms were seen as increased activation of the parietal lobe, and increased connectivity between this region and lateral prefrontal regions (although this was not exclusively seen in those with symptoms), this was interpreted as compensatory in the light of the fact that there were no differences in performance between the groups.

On the whole the results reported here are consistent with previous findings from the Edinburgh High Risk Study – what is inherited by the high risk individuals is a state of heightened vulnerability, manifesting in the case of functional imaging, as abnormalities in activation and/or connectivity in prefrontal-thalamic-cerebellar and prefrontal-parietal regions. These findings also suggest that there are additional differences seen in those with psychotic symptoms, and to some extent in those who subsequently go on to develop the disorder. These may reflect the earliest changes associated with the psychotic state, and may offer the possibility of identifying what may be the earliest possible signs of the disorder. Together with evidence that suggests early intervention in the course of the illness may produce long term benefits in some patients (Wyatt and Henter 2001), this may have potential benefits regarding the ultimate outcome of the disorder.

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APPENDICIES

APPENDIX I: SUMMARY TABLES OF LITERATURE

Appendix table 1. Literature summary: Functional disconnectivity in schizophrenia

Author	Study population	Connectivity methods	Task	Main findings.
prefrontal-temporal:				
Friston *et al.*, 1996	3 groups of 6 patients with schizophrenia (categorised according to task performance), 6 healthy controls	(PET) Multidimensional scaling. Activation data used to identify regions.	Verbal fluency task	Normal subjects demonstrated negative fronto-temporal connectivity, this configuration differed in all schizophrenic groups (positive to independent correlations between dorsolateral prefrontal-lateral temporal regions) indicating abnormal fronto-temporal connectivity in schizophrenia.
Lawrie *et al.*, 2002	8 patients with schizophrenia, 10 healthy controls	(fMRI) Region of interest correlation analysis. Activation data on all subjects used to identify regions.	Hayling sentence completion test. Significant performance difference between groups	Reduced connectivity between dorsolateral prefrontal cortex and middle/superior temporal gyrus, correlations lowest in patients with auditory hallucinations
Shergill *et al.*, 2003	8 patients with schizophrenia (with history of auditory hallucinations), 8 healthy controls	(fMRI) Time series of voxel in left inferior frontal gyrus used as covariate of interest	Fast v slow covert articulation. Button pressing to signify response, no significant performance differences.	Patients demonstrated reduced correlations between inferior frontal gyrus and right middle/superior temporal gyri, right insula, right parahippocampus, inferior temporal and fusiform gyri, precentral gyrus, and medial parietal lobule. No regions of increased connectivity reported in patient group.
Calhoun *et al.*, 2004	17 patients with schizophrenia, 17 healthy controls	(fMRI) Analysis of synchronous haemodynamic independent maps (SHIMs). ICA used to identify task-uncorrelated coherent activity in auditory cortex	Auditory oddball task. No significant differences in performance although controls responded quicker than patients	Patients demonstrated greater synchrony in dorsolateral auditory cortex than the healthy controls. Healthy controls demonstrated greater synchrony in ventromedial auditory cortex than patients. Did not examine connectivity with prefrontal regions.
Meyer-Lindenberg *et al.*, 2001	13 patients with schizophrenia, 13 healthy controls	(PET) Canonical variates analysis	n-back working memory task. Patient performance was significantly worse than controls	Pattern in the patient group was characterised by loadings in infero-temporal, parahippocampus and cerebellum, in controls the pattern was characterised by loadings in dorsolateral prefrontal cortex and anterior cingulate, indicating disturbed fronto-(medial)temporal interactions in schizophrenia.
prefrontal-subcortical-cerebellar:				
Katz *et al.*, 1996	18 patients with schizophrenia, 22 healthy controls	(PET) Regional correlation patterns between cortical and subcortical structures comprising two circuits.	Continuous performance test. No performance measures presented	Patients demonstrated different patterns of correlation in both circuits, the largest difference was between thalamus and frontal cortex. Correlations between frontal lobe and other regions also more positive in control subjects
Stephan *et al.*, 2001	6 patients with schizophrenia (scanned off/on medication), 6 healthy controls	(fMRI) Seed voxel approach. Algorithm used to identify seed regions in anterior cerebellum across subjects	Finger tapping task. No significant performance differences between groups.	Olanzapine caused widespread changes in functional connectivity of the cerebellum with prefrontal cortex and thalamus. In the right cerebellum the effects were to normalise cerebellar connectivity, not seen to same extent in left cerebellum
Schlösser *et al.*, 2003	12 patients with schizophrenia (on typical and atypical medication), 6 healthy controls	(fMRI) Effective connectivity using SEM. Literature used to define nodes, individual activation maxima used to define co-ordinates	2-back working memory task. Significant difference in task performance between patients and controls	Both patient groups demonstrated reduced connectivity between prefrontal-cerebellum, and cerebellum-thalamus, and enhanced thalamo-cortical connectivity.
Jacobsen *et*	13 patients with	(fMRI) Seed voxel	n-back working	Examined effects of nicotine (patch) on performance +

Author	Study population	Connectivity methods	Task	Main findings.
al., 2004	schizophrenia (smokers), 13 healthy controls (smokers)	approach. Seed placed in right thalamus (seen to have significant diagnosis by nicotine interaction)	memory task. Patients performed more slowly and less accurately than controls, except in 2-back condition where nicotine improved patient performance but worsened control performance	functional connectivity. Positive connectivity between thalamus and inferior frontal gyrus, right precentral gyrus, bilateral middle temporal gyrus, bilateral putamen seen in patients during 2 back condition during the active patch condition, negative relationships between these regions seen in the placebo condition. In controls there was only a weak relationship between activity of these regions and the seed region, which was not modulated by the patch condition.
Menon *et al.*, 2001a	8 patients with schizophrenia, 12 healthy controls	(fMRI) Analysis of covariance. Regions of interest placed in caudate, lentiform, and thalamus.	Motor sequencing task. No movement differences between groups. No performance measures reported	In patients the deficits in thalamic activation were related to deficits in putamen and globus pallidus activation
prefrontal-parietal:				
Kim *et al.*, 2003	12 patients with schizophrenia, 12 healthy controls	(PET) Interregional correlations. Seed region in lateral prefrontal cortex.	n-back working memory task. Marginal differences in performance were reported between groups (p=0.07).	Activation in frontal pole and in bilateral inferior parietal cortex was significantly correlated with activity in right lateral prefrontal cortex in control subjects. No significant correlations were seen in the patient group.
lateral-medial prefrontal:				
Spence *et al.*, 2000	10 obligate carriers (OC) 10 stable patients with schizophrenia (Sc) 10 healthy controls (HC)	(PET) Covariates of interest analysis. Maximally activated voxel in left dorsolateral prefrontal cortex chosen across groups	Verbal fluency test. All subjects performed task appropriately (rate of stimulus presentation allowed satisfactory performance by patient groups)	HC v OC: OC demonstrated reduced connectivity between left dorsolateral prefrontal cortex and precuneus HC v Sc: patients demonstrated reduced connectivity between left dorsolateral prefrontal cortex and anterior cingulate cortex OC v Sc: patients demonstrated reduced connectivity between left dorsolateral prefrontal cortex and anterior cingulate cortex No evidence of abnormal dorsolateral prefrontal-superior temporal gyrus connectivity differences in patients or obligate carriers.
Fletcher *et al.*, 1999	12 patients with schizophrenia, 7 healthy controls	(PET) PPI (regression) approach. Examined anterior cingulate modulation of fronto-temporal interaction.	Learning and recall of word lists. Both groups showed perfect recall with lists up to 4 words, beyond this, patient performance declined to a greater extent than controls	In the control group the product of prefrontal and anterior cingulate activity significantly predicted the decreased activation of the superior temporal gyrus. This relationship was not seen in the patient group.
widespread network deficits:				
Mallet *et al.*, 1998	12 patients with schizophrenia, 10 patients with depression, 15 patients with obsessive-compulsive disorder, 18 healthy controls	(PET) Interregional correlation analysis. 11 regions of interest defined, covering main cortical, subcortical regions and cerebellum	Rest	Focussing on schizophrenic group compared to the control group, there was no interhemispheric correlation between the frontal lobes, correlation between the right frontal and right posterior associative region changed sign from negative in the control group to positive in the patient group, similarly for correlations between the right frontal lobe and medial occipital cortex, and between the left posterior association areas and the right thalamus. Correlation between the right temporal lobe and medial occipital cortex changed from positive in the control group to negative in the patient group.
Jennings *et al.*, 1998	8 patients with schizophrenia, 8 healthy controls	(PET) Effective connectivity using SEM. Regions of interest selected: left inferior frontal gyrus, left anterior cingulate, bilateral middle frontal, left	Semantic processing task	The patient group showed more positive correlations between: the left inferior frontal gyrus and left middle frontal gyrus, between right middle frontal gyrus and left anterior cingulate, between left anterior cingulate and left middle frontal gyrus, and between left superior temporal gyrus and left anterior cingulate. In addition the patient group showed more negative correlations between: left inferior frontal gyrus and right middle

Author	Study population	Connectivity methods	Task	Main findings.
		superior temporal gyrus		frontal gyrus, between left anterior cingulate and right middle frontal gyrus, between left anterior cingulate gyrus and left inferior frontal gyrus, between left inferior frontal gyrus and left superior temporal gyrus, and finally between left inferior frontal gyrus and left anterior cingulate.
Josin and Liddle 2001	16 patients with schizophrenia, 6 healthy controls	(PET) Neural network model of functional connectivity. Seed region (62 voxels) located in left lateral frontal cortex selected based on activation data from both subject groups.	Verbal fluency test, (as described in Frith *et al.*, 1995a)	Neural network trained on data from 7 patients and 2 controls, correctly classified the remaining subjects. The patients demonstrated less positive correlations between left lateral prefrontal cortex and thalamus, greater positive correlations between left lateral prefrontal cortex and anterior cingulate, more negative correlations between left lateral prefrontal cortex and left lingual gyrus and finally there were negative correlations in controls and positive correlations in patients between the left lateral prefrontal cortex and left lateral temporal cortex and left inferior parietal lobe.
Welchew *et al.*, 2002	19 patients with schizophrenia, 20 healthy controls	(fMRI) Multidimensional scaling.	Semantic categorisation with sub-vocal articulation of response. No performance measures reported	No significant global or local differences in the interregional configurations were found between groups. Greater variability in patient group noted.

Appendix table 2. Literature summary: High risk functional imaging studies

Author	Study population	Mean age (years)	Task	Performance	Main findings, notes and conclusions
PET/SPECT studies:					
Berman *et al.*, 1992	21 pairs of monozygotic twins: 10 discordant for schizophrenia (DSc) 8 concordant for schizophrenia (CSc) 3 concordant healthy controls(HC)	DSc: 32 (24-44) CSc: 32 (24-41) HC: 28 (26-32)	Wisconsin Card Sorting Test	For discordant pairs Mean no of categories: 7.2:3.1 % perseverative errors: 15.3:17.4 %conceptual level response:68.3:50.3 for unaffected and affected respectively	All twins with schizophrenia demonstrated hypofrontality compared with unaffected co-twin (10 out of the 10 pairs). No differences observed comparing unaffected twins with healthy controls (although only a qualitative assessment was performed in the latter comparison due to small numbers of normal control twins. Conclusion: Hypofrontality related to non-genetic factors since not seen in unaffected twins.
Blackwood *et al.*, 1999	36 unaffected siblings 19 patients with schizophrenia 34 healthy controls	siblings: 39.1 (23-60) patients: 32.3 (22-50) HC: 39.5 (23-64)	Rest	n/a	Decreased perfusion of inferior frontal cortex and anterior cingulate region in both the patient group and unaffected siblings. Conclusion: Suggests a possible genetic origin of prefrontal deficits
Spence *et al.*, 2000	10 obligate carriers (OC) 10 stable patients with schizophrenia (Sc) 10 healthy controls (HC)	OC: 55.4 (sd 6.5) Sc: 51.5 (sd 11.7) HC: 41.7 (sd 8.8)	Verbal fluency test	All subjects performed task appropriately (rate of stimulus presentation allowed satisfactory performance by patient groups)	Activation differences: HC v OC: no significant difference Sc v HC and OC: patients demonstrated overactivation of the precuneus versus both other groups, and overactivation of the occipital cortex BA18 versus healthy controls only. Functional connectivity differences: See previous table Conclusion: No evidence of abnormal dorsolateral prefrontal-superior temporal gyrus connectivity differences in patients or obligate carriers.
fMRI studies:					
Keshavan *et al.*, 2002b	4 unaffected relatives healthy controls	siblings: 13.3 (±2.2) HC: 12.5 (±3.5)	Memory guided saccade task (spatial working memory)	Out-with scanner no significant differences in performance between groups	Unaffected siblings demonstrated task-related reductions of activity in dorsolateral prefrontal cortex (BA8 + 9/46) and inferior parietal lobule (BA40). Notes: High field 3T study. No within-scanner measures of performance. Small pilot study.
Callicott *et al.*, 2003	2 independent groups 1st study group: 23 unaffected relatives 18 healthy controls 2nd study group: 25 unaffected relatives 15 healthy controls	1st study group: siblings: 34.4 (sd 9) HC: 29.6 (sd 7) 2nd study group: siblings: 36.6 (sd 9) HC: 27.9 (sd 8)	n-back working memory task 1st study: 0, 1, 2 back 2nd study: 0, 2 back.	In first and second study no significant group differences in performance in terms of accuracy. No significant differences in reaction time between groups in second study, no reaction time measures for first study.	Across both first and second studies: Siblings demonstrated increased response in 2 back condition in right dorsolateral prefrontal cortex (BA9/10/46), bilateral inferior frontal gyrus (BA44/45), bilateral anterior cingulate cortex (BA34/32), bilateral inferior parietal lobule (BA40), precuneus (BA7), right middle temporal gyrus (BA21/39), and siblings demonstrated reduced response in left medial frontal gyrus (BA10), posterior cingulate, bilateral thalamus, right hippocampus and bilateral cerebellum. Notes: first study thresholded at $p< 0.01$ uncorrected, second study thresholded at $p<0.05$ uncorrected. Conclusion: unaffected siblings demonstrated inefficient response of dorsolateral prefrontal cortex in absence of performance differences.

Author	Study population	Mean age (years)	Task	Performance	Main findings, notes and conclusions
Thermenos *et al.*, 2004	12 unaffected relatives 12 healthy controls	siblings: 35.5 (sd 6.0) HC: 32.3 (sd 7.7)	Auditory verbal working memory task	Relatives performed significantly worse and had a trend towards higher reaction time measures. Controls also had significantly higher educational levels and higher vocabulary scores.	Relatives demonstrated increased task-related response in left dorsolateral prefrontal cortex, anterior cingulate and thalamus. When task performance was controlled for, relatives showed increase response in anterior cingulate only. Notes: Thresholded at p=0.005 uncorrected. Small volume corrections used on locations of predicted peaks in prefrontal cortex, anterior cingulate, thalamus and hippocampus. Conclusion: Relatives demonstrate different patterns of functioning in prefrontal-limbic networks.
Fahim *et al.*, 2004	Case study: 1 pair of monozygotic twins discordant for schizophrenia	24	Encoding and retrieval of aversive and neutral pictures	Significant difference between affected and unaffected subjects for aversive stimuli (control subject responded slower to negative stimuli)	Brain activity associated with aversive stimuli versus neutral, For encoding: Sc>HC: right fusiform gyrus Sc<HC: orbitofrontal cortex For retrieval: Sc>HC: right anterior cingulate cortex and dorsolateral prefrontal cortex Sc<HC: cerebellum Notes: Case report, difficult to generalise findings.
fMRI and susceptibility genes:					
Egan *et al.*, 2001	Sub-sample selected for fmri study: 16 unaffected relatives (5 Met/Met; 6 Val/Met: 5 Val/Val) 11 patients with schizophrenia second relative group n=11, scanned using different sequence (3 Met/Met; 5 Met/Val; 3 Val/Val)	Figures only given for full sample: Siblings (n=219): 35.6 (sd 8.8) HC (n=55): 33.9 (sd 9.2)	n back working memory task (0 and 2 back)	No significant difference in terms of performance accuracy between groups	Investigated the effects of COMT polymorphism ($Val^{108/158}$Met) on frontal lobe functioning. In unaffected siblings fMRI activation in the dorsolateral prefrontal cortex (BA46) and anterior cingulate cortex (BA32) was greatest in Val/Val>Val/Met>Met/Met at 2 back condition Conclusion: Interpreted findings in that Met allele load produced a more efficient physiological response in frontal cortex

APPENDIX II: SCRIPTS USED IN IMAGE ANALYSIS

Script to examine cross correlation between movement and task parameters

```
function cc(cn_number,sess,exam)
global x y z

path_to_exam = ['S',sess,'_',exam];
path_to_data =
['/home/raidB/heatherw/new_Hayling
_analysis/',cn_number,'/'];
path_to_spm =
[path_to_data,'Analysis_Hayling.',pat
h_to_exam];
realign_file =
[path_to_data,path_to_exam,'/realig
nment_params_',path_to_exam,'.00
1.txt'];

mydir = pwd;

cd(path_to_spm);
load SPMcfg
cd(mydir);

x = xX.X(:,1);
y = x;
z = x;
```

```
pitch = x;
yaw = y;
roll = x;

fid = fopen(realign_file,'r');
for i = 1:length(x)
x(i) = fscanf(fid,'%f',1);
y(i) = fscanf(fid,'%f',1);
z(i) = fscanf(fid,'%f',1);
pitch(i) = fscanf(fid,'%f',1);
yaw(i) = fscanf(fid,'%f',1);
roll(i) = fscanf(fid,'%f',1);
end
fclose(fid);

a = size(xX.X);
b = a(2);

x = x - mean(x);
y = y - mean(y);
z = z - mean(z);
pitch = pitch - mean(pitch);
yaw = yaw - mean(yaw);
roll = roll - mean(roll);
```

```
for i = 1:b-1
aa = xX.X(:,i);
aa = aa - mean(aa);
x_cc(i) = mycc(x,aa);
y_cc(i) = mycc(y,aa);
z_cc(i) = mycc(z,aa);
pitch_cc(i) = mycc(pitch,aa);
yaw_cc(i) = mycc(yaw,aa);
roll_cc(i) = mycc(roll,aa);
end

for i = 1:b-1
myout = sprintf('%f %f %f %f %f
%f\n',x_cc(i),y_cc(i),z_cc(i),pitch_cc(i
),yaw_cc(i),roll_cc(i));
disp(myout);
end

function [aaa] = mycc(xxx,yyy)
aaa
```

Pre-processing and analysis scripts: baseline analysis SPM99

Preprocessing script:

```
global path_to_images
root_image_names my_working_dir
hayling

start_time = clock;
mydata(1) = struct(...
'CN_number',{'CN320065'},...
'series',{845},...
'exam',{3},...
'hayling',{1});

analysis_dir =
'/home/raidB/heatherw/new_Hayling
_analysis/';

mydata(1).CN_number = 'CN.....';
mydata(1).series = ....;
mydata(1).exam = ...;
mydata(1).hayling=...;

size_mydata = size(mydata);
numseries = size_mydata(2);

for i=1:numseries

create_working_dir = 1;
mypath =
[analysis_dir,mydata(i).CN_number,'
/'];
names=dir(mypath);
size_names = size(names);
```

```
root_image_names =
sprintf('S%d_%d',mydata(i).series,m
ydata(i).exam);
new_working_dir =
['Analysis_Hayling.mask.RP.','.',root
_image_names];
for iname=1:size_names(1)

if(isequal(names(iname).name,new_
working_dir))
create_working_dir = 0;
end
end
path_to_images =
[mypath,root_image_names];
my_working_dir =
[mypath,'Analysis_Hayling.mask.RP.
','.',root_image_names,'/'];
hayling = mydata(i).hayling;

if(isequal(create_working_dir,1))
cur_dir = pwd;
cd(mypath)
mkdir(new_working_dir);
cd(cur_dir);
end

spm_bch('process_hayling_mask_re
al_bch');
mypwd = pwd;
cd(my_working_dir);
load SPMcfg
```

```
xM.TH = ones(size(xM.TH))*(-Inf);

vol =
spm_vol('/home/raidB/heatherw/new
_Hayling_analysis/new_masks/mask
_150.img');

xM.VM=vol;
save SPMcfg xM -append;
load SPMcfg
spm_spm(VY,xX,xM,F_iX0,Sess,xs
Des)

spm_bch('contrasts_hayling_mask_r
eal_bch');
cd(mypwd)

end
end_time = etime(clock,start_time);

disp(' ');
disp('**********************************
**************************');
disp('');
message = sprintf('Processed %d
subjects',numseries);
disp(message);
message = sprintf('in %f
seconds',end_time);
disp(message);
disp('');
disp('**********************************
**************************');
```

preprocessing + analysis script, SPM99

```
global path_to_images
root_image_names hayling
p = path_to_images;
myroot_image_names =
[root_image_names,'*.img'];
registered_image_names =
['r',myroot_image_names];
normalized_image_names =
['n',myroot_image_names];
smoothed_images =
['sn',root_image_names,'*.img'];
F1 =
spm_get('files',p,myroot_image_nam
es);
F2 = spm_get('files',p,'mean*.img');
F3 =
spm_get('files',p,registered_image_n
ames);
F4 = spm_get('files',p,'*_sn3d.mat');
F5 =
spm_get('files',p,normalized_image_
names);
F6 =
spm_get('files',p,smoothed_images);
l = 1;
ml = 2;
mh = 3;
h = 4;
r = 5;
order_epochs = [l ml mh r h ml r l
mh h mh l r ml h r mh ml l h];
switch (hayling)
  case 1
    l = 1;
    ml = 2;
    mh = 3;
    h = 4;
    r = 5;
  case 2
    l = 4;
    ml = 1;
    mh = 2;
    h = 3;
    r = 5;
  case 3
    l = 3;
    ml = 4;
    mh = 1;
    h = 2;
  case 4
    l = 2;
    ml = 3;
    mh = 4;
    h = 1;
end
o_low   = find(order_epochs == l);
o_medlow  = find(order_epochs ==
ml);
o_medhigh = find(order_epochs ==
mh);
o_high    = find(order_epochs == h);
o_rest    = find(order_epochs == r);
o_low     = 10 * (o_low - 1);
o_medlow  = 10 * (o_medlow - 1);
o_medhigh = 10 * (o_medhigh -1 );
o_high    = 10 * (o_high - 1);
o_rest    = 10 * (o_rest -1);
cond_names =
{'low','medlow','medhigh','high','rest'};
z6 = [0 0 0 0 0 0];
c_active = [ 1  1  1  1 -4 z6 0];
c_rest   = [-1 -1 -1 -1  4 z6 0];
c_low    = [ 1  1 -1 -1  0 z6 0];
c_high   = [-1 -1  1  1  0 z6 0];
c_incr   = [-2 -1  1  2  0 z6 0];
c_decr   = [ 2  1 -1 -2  0 z6 0];
```

```
real_params =
[path_to_images,'/realignment_para
ms_',...

root_image_names,'.001.txt'];
realign_params =
spm_load(real_params);
for kk=1:6
 tmp1 = realign_params(:,kk);
 tmp1 = tmp1 - mean(tmp1);
 tmp1 = tmp1 / sqrt(tmp1'*tmp1);
 realign_params(:,kk) =  tmp1;
end
model(1) = struct( ...
 'types',         4, ...
 'global_effects', 'None', ...
 'burst_mode',    0, ...
 'HF_fil',        'specify',  ...
 'HF_cut',        [400], ...
 'LF_fil',        'Gaussian', ...
 'LF_cut',        4, ...
 'int_corr',      'none', ...
 'now_later',     0, ...
 'stop_writing',  0, ...
 'trial_fcon',    0, ...
 'RT',            4, ...
 'replicated',    0, ...
 'nsess',         1, ...
 'nscans',        [200], ...
 'files',         {{F6}}, ...
 'conditions_nb',  [5], ...
 'conditions',    [1], ...
 'regressors_nb',  [6], ...
 'regressors',    [1], ...
 'parametrics_type', {{'none'}}, ...
 'parametrics',   [], ...
 'stochastics_flag', [0], ...
 'stochastics',   [] ...
);

regressor_names =
{'xt','yt','zt','xr','yr','zr'};
regressors(1) = struct( ...
'names',  {regressor_names}, ...
'values', {realign_params});
conditions(1) = struct( ...
'names',   {cond_names}, ...
 'onsets',
{{o_low,o_medlow,o_medhigh,o_hig
h,o_rest}}, ...
 'types',
{{'epochs','epochs','epochs','epochs',
'epochs'}}, ...
 'bf_ep',   [1 1 1 1 1], ...
 'volterra',  0, ...
 'variable_dur', 0 ...
);

% bf_ev(1) = struct( ...
%  'ev_type', 2, ...
%  'win_len', [] ...
% );
% bf_ev(2) = bf_ev(1) ;
% bf_ev(2).ev_type = 1;

bf_ep(1) = struct( ...
  'ep_type', 4, ...
  'length',10, ...
  'conv',  1, ...
  'deriv', 0 ...
);
contrasts(1) = struct( ...
   'names',  {{'active vs rest','rest vs
active','low vs high',...
```

```
'high vs low','increase','decrease'}},
...
   'types',   {{'T','T','T','T','T','T'}}, ...
   'values',
{{c_active,c_rest,c_low,c_high,c_inc
r,c_decr}} ...
);

realign = struct(...
'subject_nb', 1, ...
'num_sessions', [1], ...
'sessions', [1], ...
'option', 3, ...
'modality', 1, ...
'reslice_method', 1, ...
'create', 4, ...
'mask', 0, ...
'adjust_sampling_errors', 0,...
'reg_quality',0.75);

sessions(1) = struct(...
'images', {{F1}});

normalize = struct(...
'option', 3, ...
'nbsubjects', 1, ...
'object_masking', 0, ...
 'template',
'/home/raid/enrico/spm99/templates/
EPI.img', ...
'type', 1, ...
'nonlin_func_nb', 8, ...
'func_nb', 0, ...
'nonlin_ite_nb', 12, ...
'nonlin_regular', 0.01, ...
'mask_brain', 0, ...
'interp', 1, ...
'bounding_box', 1, ...
'direction1', [-10;10], ...
'direction2', [-10;10], ...
'direction3', [-10;10], ...
'voxel_sizes', 4, ...
'voxel_sizes_custom', [2 2 2],...
'image', {{F2}}, ...
'objmask', {{''}}, ...
'images', {{F1}});

smooth = struct(...
'FWHMmm', [6 6 6], ...
'files', F5 ...
);
% defaults
defaults_edit = struct( ...
   'type_area', 5, ...
   'index',     1 ...
);

type_area =
{'Misc','Printing','Hdr','Statistics', ...

'Normalisation','RealignCoreg','Rese
t'};

Normalisation = struct( ...
   'defaults',  1, ...
   'estimates',  1, ...
   'custom_norm',  1, ...
   'nonlin_func_nb', 7, ...
   'func_nb',  0, ...
   'nonlin_ite_nb',  12, ...
   'nonlin_regular',  0.01, ...
   'mask_brain',  0, ...
   'mask_object_brain',  0, ...
   'bounding_box',  1, ...
   'voxel_sizes',  -1 ...)
```

Pre-processing and analysis scripts: change over time analysis SPM99/SPM2

Modified preprocessing script for SPM99:

```
spm_defaults
fprintf('Working on %s series %d
exam %d\n',CN,exam,series);
image_dir       =
sprintf('%s/%s/S%d_%d',experiment
_dir,CN,exam,series);
image_names     =
sprintf('S%d_%d.*.img',exam,series)
;
image_file_names =
spm_get('files',image_dir,image_na
mes);
mypwd = pwd;

cd(image_dir);

delete_these_files('*mean*',image_d
ir);
delete_these_files('n*',image_dir);
delete_these_files('sn*',image_dir);
delete_these_files('*txt',image_dir);
delete_these_files('pp*',image_dir);
delete_these_files('S*.mat',image_di
r);

% Realignment
fprintf('%s
Realignment\n',datestr(now));
FlagsC.quality = 0.95;
FlagsC.fwhm    = 8;
FlagsC.sep     = 4;
FlagsC.rtm     = 1;
FlagsC.hold    = -8;
spm_realign(image_file_names,Flag
sC);
FlagsR.mask  = 0;
FlagsR.mean  = 1;
FlagsR.which = 0;
```

```
FlagsR.hold  = -9;
spm_reslice(image_file_names,Flag
sR);

% Normalisation
fprintf('%s
Normalisation\n',datestr(now));
F_mean =
spm_get('files',image_dir,'mean*.img
');
global sptl_Ornt
sptl_Ornt = [0 0 0  0 0 0  1 1 1 0 0 0];
% Neurological Convention (R is R)
epi_template =
'Z:\enrico\create_templates\EPI_edi\
EPI_edi.img';
bb     = reshape([-80 80 -118 90 -72
100],2,3);
Vox    = [2 2 2];
params = [ 10 12 10 12 8 0.02];
Hold   = -9;
[pa,fi,ext] =
fileparts(deblank(F_mean));
matname =
sprintf('%s/%s_sn3d.mat',pa,fi);
spm_sn3d(F_mean,matname,bb,Vo
x,params,epi_template,".");
spm_write_sn(F_mean,matname,bb,
Vox,Hold);

spm_write_sn(image_file_names,m
atname,bb,Vox,Hold);
% Smoothing
fprintf('%s
Normalisation\n',datestr(now));
F_s =
spm_get('files',image_dir,'nS*.img');
S = [8 8 8];
```

```
for ii=1:size(F_s,1)
  fprintf('Smoothing %d\r',ii);
  [pa,fi,ext] =
fileparts(deblank(F_s(ii,:)));
  Q = sprintf('%s/s%s%s',pa,fi,ext);
  spm_smooth(F_s(ii,:),Q,S);
end

fprintf('
\r');

fprintf('%s Cleaning
up\r',datestr(now));
delete_these_files('nS*',image_dir);
fprintf('%s Done
\n',datestr(now));
cd(mypwd);

function
delete_these_files(file_mask,image_
dir)
tmp_files =
spm_get('files',image_dir,file_mask);
if (~isempty(tmp_files))
   for ii=1:size(tmp_files,1)
     delete(deblank(tmp_files(ii,:)));
     tmp_ii = ii - 4 * fix(ii/4);
     switch(tmp_ii)
       case 0
             fprintf('|\r');
       case 1
             fprintf('/\r');
       case 2
             fprintf('-\r');
       case 3
             fprintf('\\\r');
     end
   end
end
```

Analysis script, SPM2:

```
spm_defaults;
global defaults
defaults.stats.maxmem = 2^28;
subject_dir =
fullfile(experiment_dir,CN);
tmp_str =
sprintf('S%d_%d',exam,series);
data_dir =
fullfile(subject_dir,tmp_str);
tmp_str =
sprintf('Hayling.1st_level.%s',tmp_str
);
status =
mkdir(subject_dir,tmp_str);
analysis_dir =
fullfile(subject_dir,tmp_str);

mypwd = pwd;
cd(analysis_dir);

this_F =
spm_get('files',analysis_dir,'*');

for ii=1:size(this_F,1)
   delete(deblank(this_F(ii,:)));
end

l = 1;
ml = 2;
mh = 3;
h = 4;
r = 5;
order_epochs = [l ml mh r h ml r l
mh h mh l r ml h r mh ml l h];

switch (hayling)
  case 1
    l = 1;
    ml = 2;
    mh = 3;
    h = 4;
    r = 5;
  case 2
    l = 4;
    ml = 1;
    mh = 2;
    h = 3;
    r = 5;
   case 3
    l = 3;
    ml = 4;
    mh = 1;
    h = 2;
   case 4
    l = 2;
    ml = 3;
    mh = 4;
    h = 1;
end

o_low    = find(order_epochs == l);
o_medlow  = find(order_epochs ==
ml);
o_medhigh = find(order_epochs ==
mh);
o_high   = find(order_epochs == h);
o_rest   = find(order_epochs == r);

o_low    = 10 * (o_low - 1);
o_medlow  = 10 * (o_medlow - 1);
o_medhigh = 10 * (o_medhigh -1 );
o_high   = 10 * (o_high - 1);
o_rest   = 10 * (o_rest -1);

% This is based on an example by
% K. Friston


% Specify design

% number of scans and session,
%-----------------------------------------
SPM.nscan        = 200;
% basis functions and timing
parameters
%-----------------------------------------
% OPTIONS:'hrf'
%       'hrf (with time derivative)'
%       'hrf (with time and dispersion
derivatives)'
%       'Fourier set'
%       'Fourier set (Hanning)'
%       'Gamma functions'
%       'Finite Impulse Response'
%-----------------------------------------
SPM.xBF.name      = 'hrf';
SPM.xBF.length          =  32.2;
% length in seconds
SPM.xBF.order     = 2;          %
order of basis set
SPM.xBF.T               =  16;
% number of time bins per scan
SPM.xBF.T0        = 1;          %
first time bin (see slice timing)
SPM.xBF.UNITS           =  'scans';
% OPTIONS: 'scans'|'secs' for
onsets
SPM.xBF.Volterra  = 1;          %
OPTIONS: 1|2 = order of
convolution

% Trial specification: Onsets,
duration (UNITS) and parameters for
modulation
%-----------------------------------------
SPM.Sess(1).U(1).name           =
{'Low'};
SPM.Sess(1).U(1).ons      = o_low';
SPM.Sess(1).U(1).dur      = 10;
SPM.Sess(1).U(1).P(1).name      =
'none';

SPM.Sess(1).U(2).name           =
{'MedLow'};
SPM.Sess(1).U(2).ons            =
o_medlow';
SPM.Sess(1).U(2).dur      = 10;
SPM.Sess(1).U(2).P(1).name      =
'none';

SPM.Sess(1).U(3).name           =
{'MedHigh'};
SPM.Sess(1).U(3).ons            =
o_medhigh';
SPM.Sess(1).U(3).dur      = 10;
SPM.Sess(1).U(3).P(1).name      =
'none';

SPM.Sess(1).U(4).name           =
{'High'};
SPM.Sess(1).U(4).ons      = o_high';
SPM.Sess(1).U(4).dur      = 10;
SPM.Sess(1).U(4).P(1).name      =
'none';

SPM.Sess(1).U(5).name           =
{'Rest'};
SPM.Sess(1).U(5).ons      = o_rest';
SPM.Sess(1).U(5).dur      = 10;
SPM.Sess(1).U(5).P(1).name      =
'none';

SPM.Sess(1).U(6).name     = {'Task
On'};

SPM.Sess(1).U(6).ons      =

(10+o_rest)';
SPM.Sess(1).U(6).dur      = 1;
SPM.Sess(1).U(6).P(1).name      =
'none';

SPM.Sess(1).U(7).name     = {'Task
Off'};
SPM.Sess(1).U(7).ons      = o_rest';
SPM.Sess(1).U(7).dur      = 1;
SPM.Sess(1).U(7).P(1).name      =
'none';

% design (user specified covariates)
move_regre_file                 =
spm_get('files',data_dir,'r*.txt');
realign_params                  =
spm_load(move_regre_file);

for ii=1:size(realign_params,2)
  tmp = realign_params(:,ii);
  tmp = tmp - mean(tmp);
  tmp = tmp / norm(tmp);
  realing_params(:,ii) = tmp;
end

SPM.Sess(1).C.C                 =
[realign_params];          % [n x c
double] covariates
SPM.Sess(1).C.name  =  {'x','y','z','r
x','r y','r z'};  % [1 x c cell]  names

%       global      normalization:
OPTINS:'Scaling'|'None'
%-----------------------------------------
SPM.xGX.iGXcalc   = 'Scaling';

% low frequency confound: high-
pass cutoff (secs) [Inf = no filtering]
%-----------------------------------------
SPM.xX.K.HParam   = 200;

%       intrinsic      autocorrelations:
OPTIONS: 'none'|'AR(1) + w'
%-----------------------------------------
SPM.xVi.form      = 'AR(1) + w';

% specify data: matrix of filenames
and TR
SPM.xY.P                        =
spm_get('files',data_dir,'snS*.img');
SPM.xY.RT                       =  4;
% seconds

% Configure design matrix
SPM = spm_fmri_spm_ui(SPM);
% Apply apriori mask
for ii=1:length(SPM.xM.TH)
   SPM.xM.TH(ii) = -Inf;
end

%           path_to_spm           =
fileparts(which('spm'));
%           mask_file             =
fullfile(path_to_spm,'apriori','brainma
sk.mnc');
mask_file                       =
'C:\Heather\masks\spm_mask_thres
h_0.30.img';
Vmask    = spm_vol(mask_file);
SPM.xM.VM = spm_vol(Vmask);
```

```
% Estimate parameters
SPM = spm_spm(SPM);

% Add extra contrasts
% F-contrasts
%-----------------------------------------------
iX0             = [8:14];
cname           = 'Hayling';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'F','iX0',iX0,
SPM.xX.xKXs);

% T-contrasts
%-----------------------------------------------
c               = [1 1 1 1 -4 0 0 0 0 0 0
0 0 0 ];
cname           = 'Active vs Rest';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [-1 -1 -1 -1 4 0 0 0 0 0
0 0 0 0];
cname           = 'Rest vs Active';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [-2 -1 1 2 0 0 0 0 0 0
0 0 0 0];
```

```
cname           = 'Increase';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [2 1 -1 -2 0 0 0 0 0 0
0 0 0 0];
cname           = 'Decrease';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [0 0 0 0 0 1 0 0 0 0 0
0 0 0];
cname           = 'Task On (+)';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [0 0 0 0 0 -1 0 0 0 0 0
0 0 0];
cname           = 'Task On (-)';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [0 0 0 0 0 0 1 0 0 0 0
0 0 0];
cname           = 'Task Off (+)';
```

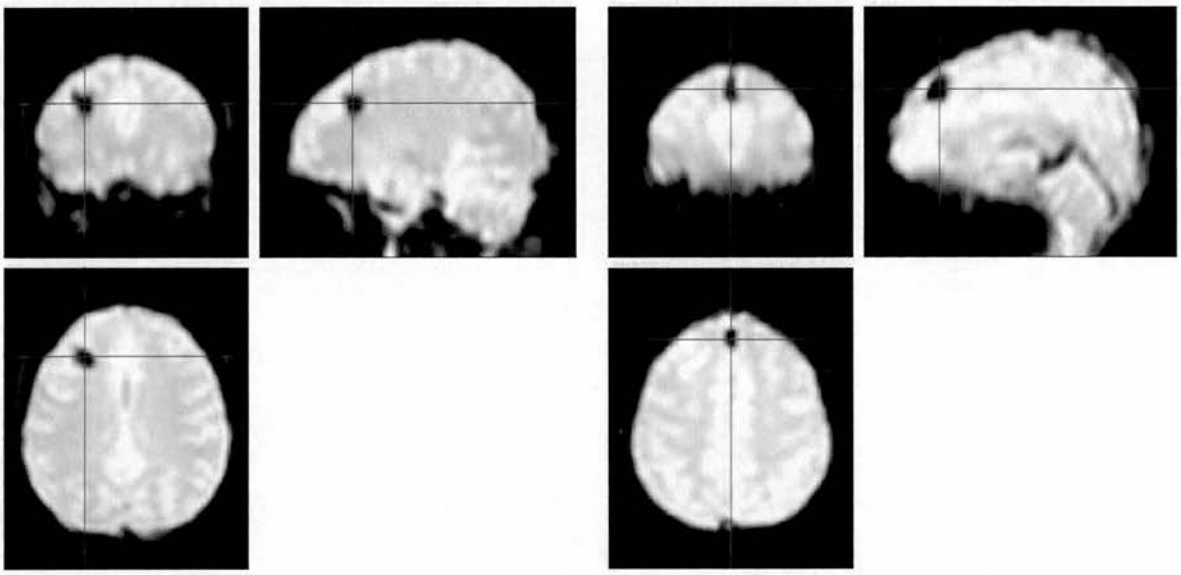
```
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [0 0 0 0 0 0 -1 0 0 0 0
0 0 0];
cname           = 'Task Off (-)';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [0 0 0 0 0 1 -1 0 0 0 0
0 0 0];
cname           = 'Task On vs Off';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

c               = [0 0 0 0 0 -1 1 0 0 0 0
0 0 0];
cname           = 'Task Off vs On';
SPM.xCon(end    +   1)          =
spm_FcUtil('Set',cname,'T','c',c(:),S
PM.xX.xKXs);

% and evaluate
spm_contrasts(SPM);
cd(mypwd);
```

changes over time script:

```
global defaults
spm_defaults;

contrast_name    = 'parametric';
contrast_number  = 14;
CN_masterfile;

base_analysis_dir                =
'C:\Heather\Changes_Over_Time\';

experiment_dir{1}                =
'C:\Heather\Hayling_data\';
experiment_dir{2}                =
'D:\Heather\Hayling_data\preproces
sing\';

status                           =
mkdir(base_analysis_dir,contrast_na
me);
analysis_dir                     =
fullfile(base_analysis_dir,contrast_n
ame);

target_dir{1}{1} = 'con_n__con_n';
target_dir{1}{2} = 'con_n__con_p';
target_dir{2}{1} = 'con_p__con_n';
target_dir{2}{2} = 'con_p__con_p';
target_dir{3}{3} = 'hr_n__hr_n';
target_dir{3}{4} = 'hr_n__hr_p';
target_dir{3}{5} = 'hr_n__ill';
target_dir{4}{3} = 'hr_p__hr_n';
target_dir{4}{4} = 'hr_p__hr_p';
target_dir{4}{5} = 'hr_p__ill';
target_dir{5}{5} = 'ill__ill';

contrast_image_name              =
sprintf('con_%04d.img',contrast_nu
mber);

for ii=1:length(CN)
   if (length(CN(ii).visit) > 1)
      if ((CN(ii).visit(1).group >= 0) &
...
         (CN(ii).visit(1).group <= 4) &
...
         (CN(ii).visit(2).group >= 0) &
...
         (CN(ii).visit(2).group <= 4))

         this_CN_1               =
CN(ii).visit(1).CN;
         this_exam_1             =
CN(ii).visit(1).exam;
         this_series_1           =
CN(ii).visit(1).series;
         this_group_1            =
CN(ii).visit(1).group+1;

         this_CN_2               =
CN(ii).visit(2).CN;
         this_exam_2             =
CN(ii).visit(2).exam;
         this_series_2           =
CN(ii).visit(2).series;
         this_group_2            =
CN(ii).visit(2).group+1;

         contrast_source_file_1  =
fullfile(experiment_dir{1},this_CN_1,.
..

sprintf('Hayling.1st_level.S%d_%d',t
his_exam_1,this_series_1),contrast_
image_name);

         if
(~exist(contrast_source_file_1,'file'))
            contrast_source_file_1  =
fullfile(experiment_dir{2},this_CN_1,.
..

sprintf('Hayling.1st_level.S%d_%d',t
his_exam_1,this_series_1),contrast_
image_name);
         end

         contrast_source_file_2  =
fullfile(experiment_dir{1},this_CN_2,.
..

sprintf('Hayling.1st_level.S%d_%d',t
his_exam_2,this_series_2),contrast_
image_name);

         if
(~exist(contrast_source_file_2,'file'))
            contrast_source_file_2  =
fullfile(experiment_dir{2},this_CN_2,.
..

sprintf('Hayling.1st_level.S%d_%d',t
his_exam_2,this_series_2),contrast_
image_name);
         end

         V_1                     =
spm_vol(contrast_source_file_1);
         V_2                     =
spm_vol(contrast_source_file_2);


fprintf('%s\n%s\n',V_1.fname,V_2.fn
ame);

         con_1                   =
spm_read_vols(V_1);
         Q = find (~isfinite(con_1));
         con_1(Q) = 0;

         con_2                   =
spm_read_vols(V_2);
         Q = find (~isfinite(con_2));
         con_2(Q) = 0;

         time_diff               =
(datenum(CN(ii).visit(2).scan_date) -
datenum(CN(ii).visit(1).scan_date))/
100;
         change_over_time = (con_2
- con_1) / time_diff;

         this_group_dir          =
target_dir{this_group_1}{this_group_
2};
         save_dir                =
fullfile(analysis_dir,this_group_dir);
         if (~exist(save_dir,'dir'))
            status               =
mkdir(analysis_dir,this_group_dir);
         end

         this_filename           =
sprintf('%s_%d_%d.img',this_CN_1,t
his_exam_1,this_series_1);
         X = V_1;
         X.fname                 =
fullfile(save_dir,this_filename);

spm_write_vol(X,change_over_time)
;
      end
   end
end
```

APPENDIX III. APPENDIX TO CHAPTER THREE

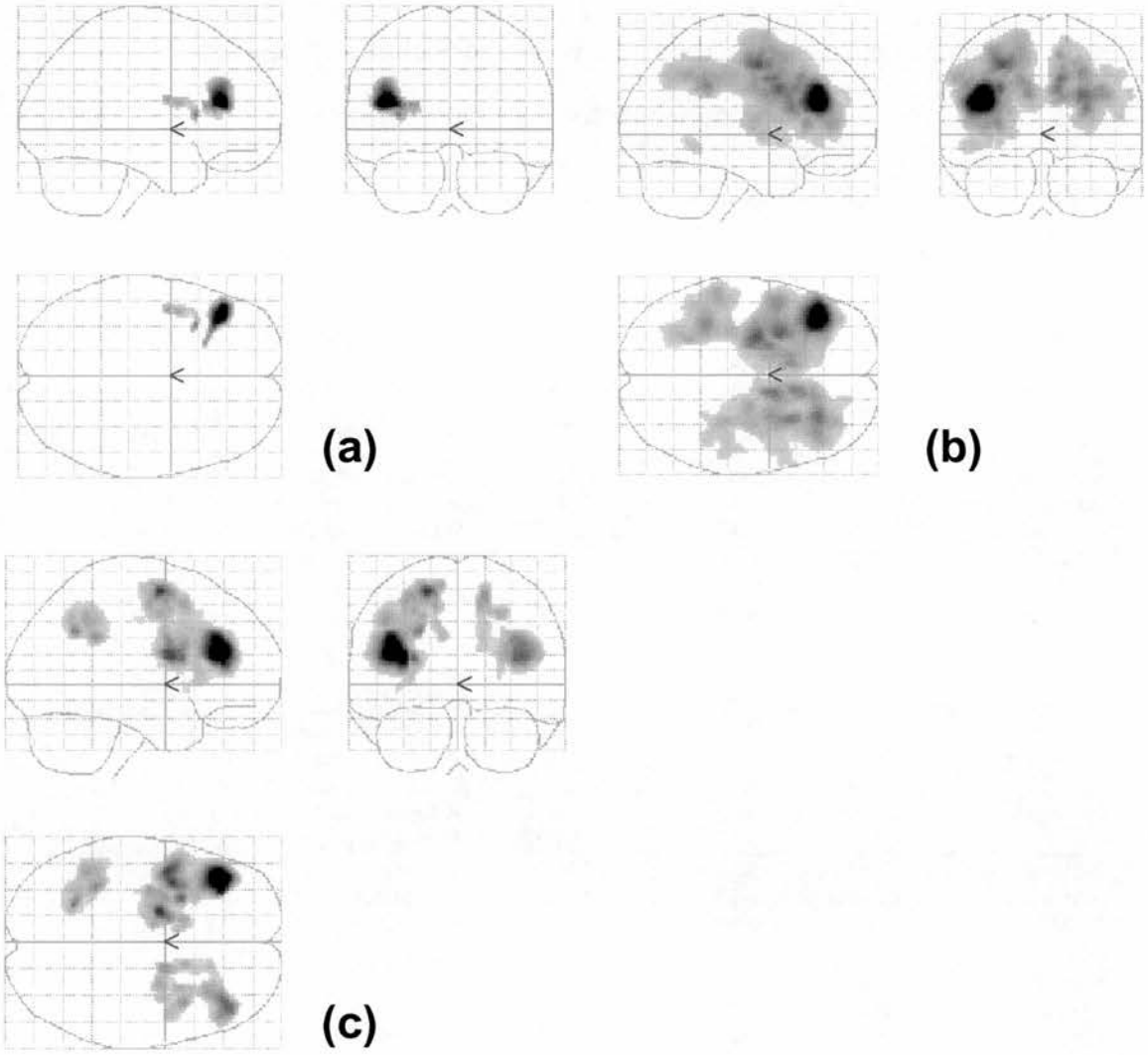
Excluded subjects



Appendix figure 1. Subjects excluded due to minor vascular malformations

APPENDIX IV. APPENDIX TO CHAPTER FOUR.

Additional within group functional connectivity results.



Appendix figure 2. Within group maps for seed in left dorsolateral prefrontal cortex.

To illustrate the reason behind selecting a higher statistical threshold for reporting the within group functional connectivity results. All three maps thresholded at 0.05 corrected cluster level, extent threshold 100 voxels, (a) controls, (b) high risk without symptoms, (c) high risk with symptoms. The connectivity displayed in the high risk without symptoms group consists of one large cluster. Reporting only the maximum therefore does not reflect the true extent of this region. For this reason a higher threshold was selected to divide this large cluster into smaller regions.

Additional within group functional connectivity results tables.

Detailed in the tables below are the within group functional connectivity results for the exploratory seeds. The cluster centred on seed location is not reported, and for brevity only the co-ordinate of the first maxima within each cluster is reported.

Appendix table 3. Controls (*n*=21)

Seed location	P value	Z score	Peak height co-ordinates	Region
Lateral prefrontal:				
L inferior	<0.001	6.20	-12 18 54	L frontal: superior frontal g, BA 8/6
frontal g; pars	<0.001	5.45	44 29 -5	R frontal: inferior frontal g, BA 47
triangularis,	<0.001	5.41	-57 -43 -5	L temporal: middle/inferior temporal g, BA 21
BA45	<0.001	5.26	-12 14 5	L sub-lobar: caudate nucleus
(-55 23 3) §				
R inferior	<0.001	7.57	-44 15 -9	L frontal: inferior frontal g, BA 47
frontal g; pars	<0.001	7.23	6 9 66	R frontal: medial frontal g, BA 6
triangularis,	<0.001	5.89	63 -28 -17	R temporal: middle temporal g, BA 21
BA45	<0.001	5.65	-53 -53 -16	L temporal: inferior temporal g, BA 37
(55 21 1) §	<0.001	5.48	0 61 28	Midline: superior frontal g, BA 9
L rolandic operculum, BA4/7 (-60 0 7) §	<0.001	5.39	50 8 0	R temporal: superior temporal g, BA 22
R rolandic	<0.001	7.36	-55 4 0	L temporal: superior temporal g, BA 22
operculum,	<0.001	6.33	48 -5 50	R frontal: precentral g, BA 4
BA4/7	<0.001	5.94	-57 -30 18	L temporal: superior temporal g, BA 22
(59 6 5) §	<0.001	5.92	6 -8 63	R frontal: medial frontal g, BA 6
	<0.001	5.47	-51 -19 51	L parietal: postcentral g, BA 1
L frontal	<0.001	6.36	24 16 3	R insula
operculum,	<0.001	6.25	-8 8 44	L frontal: medial frontal g, BA 32
BA45	<0.001	6.06	12 14 40	R frontal: medial frontal g, BA 32
(-38 23 3) §	<0.001	5.58	-16 -15 6	L sub-lobar: thalamic nucleus
R frontal	<0.001	7.17	38 14 10	R insula
operculum,	<0.001	6.51	-28 14 9	L insula
BA45	<0.001	5.83	36 -4 43	R frontal: middle frontal g, BA 6
(40 20 6) §				
L inferior frontal g, BA44 (-53 14 16) ô	<0.001	6.34	-10 10 46	L frontal: cingulate g, BA 32
R inferior frontal g, BA44 (53 14 16) ô				*No clusters at 0.05 or 0.01 corrected threshold other than that centred on seed location*
Medial prefrontal:				
L anterior	<0.001	5.84	12 17 -1	R sub-lobar : caudate nucleus
cingulate,	<0.001	5.81	18 35 44	R frontal: superior/medial frontal g, BA 8
BA32 (-4 39 2) $				
R anterior cingulate, BA32	<0.001	6.13	18 35 44	R frontal: superior/medial frontal g, BA 8

Seed location	P value	Z score	Peak height co-ordinates	Region
(4 39 2) [$]				
L caudal medial frontal g, BA6/32 (-4 6 51) [δ]	<0.001 <0.001 <0.001	6.46 6.39 5.58	32 19 1 -20 12 5 46 -5 52	R insula/frontal operculum, BA 45 L sub-lobar: lentiform nucleus R frontal: precentral g, BA 4
R caudal medial frontal gyrus, BA6/32 (4 6 51) [δ]	<0.001 <0.001	6.64 5.88	36 23 3 48 -3 50	R frontal: inferior frontal g, BA 45 R frontal: precentral g, BA 4
Lateral temporal:				
L planum temporale, BA22 (-61 -34 16) [§]	<0.001	5.75	59 -5 15	R temporal: superior temporal g, BA 22
R planum temporale, BA22 (65 -34 20) [§]	<0.001 <0.001 <0.001	6.79 6.28 5.39	-63 -37 30 -48 -7 8 36 -3 13	L parietal: inferior parietal lobule, BA 40 L temporal: superior temporal g, BA 22 R insula
L anterior superior temporal g, BA22 (-48 -2 -10) [§]	<0.001	6.27	50 11 -12	R temporal: superior temporal g, BA 22/38
R anterior superior temporal g, BA22 (60 13 -11) [§]	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001	6.64 6.39 6.26 6.09 5.82 5.57	-58 13 -7 0 12 44 4 -53 63 -63 -26 16 2 -68 -7 4 -3 65	L temporal: superior temporal g, BA 22/38 Mid anterior cingulate/medial frontal g BA32 R parietal: precuneus, BA 7 R temporal: post superior temporal g, BA 22 R cerebellum R frontal: medial frontal g, BA 6
L posterior superior temporal s, BA22 (-55 -50 19) [§]	<0.001	6.60	53 -46 13	R temporal: posterior superior/middle temporal g, BA, 22/21
R posterior superior temporal s, BA22 (63 -46 21) [§]	<0.001	5.36	-57 -39 26	*No clusters at 0.05 corrected threshold other than that centred on seed location. At 0.01 corrected:* L temporal: superior temporal g, BA 22
L inferior temporal g, BA37 (-59 -51 -8) [§]	<0.001	5.49	59 -49 -11	*No clusters at 0.05 corrected threshold other than that centred on seed location. At 0.01 corrected:* R temporal: inferior temporal g, BA 37
R inferior temporal g, BA37 (59 -51 -4) [§]	<0.001	6.81	-53 -55 -7	L temporal: inferior temporal g, BA 37
L fusiform g, BA37 (-40 -49 -13) [§]	<0.001	5.28	42 -40 -15	*No clusters at 0.05 corrected threshold other than that centred on seed location. At 0.01 corrected:* R temporal: fusiform g, BA37
R fusiform g, BA37 (40 -49 -13) [§]	<0.001	5.59	-36 -70 -3	L temporal: fusiform g, BA 19
L anterior	<0.001	5.90	-40 -22 -12	L med temporal

Seed location	P value	Z score	Peak height co-ordinates	Region
middle temporal g, BA21 (-51 -37 -3) [δ]	<0.001	5.75	-10 43 37	L frontal: superior/medial frontal g, BA 8
R anterior middle temporal g, BA21 (51 -37 -3) [δ]				*No clusters at 0.05 or 0.01 corrected threshold other than that centred on seed location*
Medial temporal:				
L anterior amyg-hipp region (-24 -1 -18) [$]	<0.001	6.60	28 3 -14	R med temporal: anterior amyg-hipp region
R anterior amyg-hipp region (24 -1 -18) [$]	<0.001 <0.001	6.56 5.85	-48 11 -19 -22 -3 -22	L temporal: superior temporal g, BA 38 L med temporal: anterior amyg-hipp region
L posterior amyg-hipp region (-18 -12 -16) [$]	<0.001	5.54	24 -12 -15	R med temporal: hippocampus
R posterior amyg-hipp region (18 -12 -16) [$]	<0.001	5.46	-16 -15 -18	L med temporal: hippocampus
Subcortical:				
L caudate (-8 14 3) [§]				*Large cluster centred on seed co-ordinates, overlapping right caudate*
R caudate (10 14 3) [§]				*Large cluster centred on seed co-ordinates, overlapping left caudate*
Other:				
L precentral g, BA 4 (-50 -2 44) [δ]	<0.001 <0.001 <0.001	6.28 5.61 5.28	-6 5 53 48 -4 41 -32 21 1	L frontal: cingulate/medial frontal g, BA 6/32 R frontal: precentral g, BA 4 L insula
R precentral g, BA 4 (50 -2 44) [δ]	<0.001	6.56	-44 -6 44	L frontal: precentral g, BA 4
L lingual g, BA18/17 (-28 -97 -3) [δ]	<0.001 <0.001 <0.001 <0.001 <0.001	7.03 5.90 5.84 5.76 5.58	28 -100 -2 6 58 34 32 62 -5 -37 59 8 26 -87 -21	R occipital: lingual g, BA 18 R frontal: superior frontal g, BA 9 R frontal: middle frontal g, BA 10 L frontal: middle frontal g, BA 10 R occipital; fusiform g, BA 18
R lingual g, BA 18/17 (28 -97 -3) [δ]	<0.001 <0.001 <0.001 <0.001	6.07 6.01 5.71 5.70	33 62 -1 -33 62 -3 0 62 28 -55 -11 -16	R frontal: middle frontal g, BA 10 L frontal: middle frontal g, BA 10 Midline: superior frontal g, BA9 L temporal: middle temporal g, BA 21

[§] indicates language derived seeds, [$] indicates schizophrenia region of interest derived seeds, [δ] indicates data driven seeds. Analyses thresholded at 0.05 corrected cluster level, extent threshold =100.

Appendix table 4. High risk without symptoms (*n*=42)

Seed location	P value	Z score	Peak height co-ordinates	Region
Lateral prefrontal:				
L inferior	<0.001	Inf	-4 43 46	L frontal: medial frontal g, BA 9
frontal g,	<0.001	7.55	55 21 1	R frontal: inferior frontal g, BA 45
BA45; pars	<0.001	6.87	12 6 11	R sub-lobar: caudate nucleus
triangularis	<0.001	6.83	-59 -41 2	L temporal: middle/sup temporal g, BA22/21
(-55 23 3) §	<0.001	6.68	14 8 11	R sub-lobar: caudate nucleus
	<0.001	6.22	26 -95 -5	R occipital: calcarine s, BA 17
	<0.001	6.07	42 -75 -16	R cerebellum
R inferior	<0.001	7.79	-59 16 1	L frontal: inferior frontal g, BA 45
frontal g,	<0.001	6.89	64 -50 14	R temporal: superior temporal g, BA 22
BA45; pars	<0.001	6.45	-28 58 -1	L frontal: middle frontal g, BA 10
triangularis	<0.001	6.44	2 13 58	R frontal: medial frontal g, BA 6
(55 21 1) §	<0.001	6.38	4 -97 10	R occipital: cuneus, BA 18
	<0.001	6.04	-55 -66 0	L temporal: inferior temporal g, BA 37
	<0.001	6.03	-14 -89 -26	L cerebellum
L rolandic	<0.001	Inf	42 -26 18	R temporal: superior temporal g, BA 22
operculum,	<0.001	7.03	4 -11 52	R frontal: medial frontal g, BA 6
BA4/7	<0.001	6.62	20 -24 66	R frontal: precentral g, BA 4
(-60 0 7) §				
R rolandic	<0.001	Inf	-46 -15 8	L temporal: superior temporal g, BA 41
operculum,	<0.001	7.70	-8 6 40	R frontal: cingulate/medial frontal g, BA 32
BA4/7	<0.001	6.58	50 -60 5	R temporal: middle temporal g, BA 21/37
(59 6 5) §	<0.001	5.82	26 -36 63	R parietal: postcentral g, BA 7
L frontal	<0.001	Inf	36 22 6	R insula/frontal operculum, BA 45
operculum,	<0.001	7.32	-50 -44 17	L temporal: superior temporal g, BA 22
BA45	<0.001	7.22	-8 14 45	L frontal: cingulate/medial frontal g, BA 32
(-38 23 3) §	<0.001	6.73	14 -6 4	R thalamus/lentiform nucleus
R frontal	<0.001	Inf	-36 18 3	L insula/frontal operculum, BA 45
operculum,	<0.001	6.18	57 -37 28	R parietal: inferior parietal lobule, BA 40
BA45	<0.001			
(40 20 6) §				
L inferior	<0.001	6.98	-59 -39 2	L temporal: superior temporal g, BA 22
frontal gyrus,	<0.001	6.75	57 17 23	R frontal: inferior frontal g, B 44
BA44	<0.001	5.26	24 -77 -31	R cerebellum
(-53 14 16) δ				
R inferior	<0.001	7.15	59 -35 39	R parietal: inferior parietal lobule, BA 40
frontal gyrus,	<0.001	6.83	-59 -28 29	L parietal: inferior parietal lobule, BA 40
BA44	<0.001	6.43	-50 5 15	L frontal: precentral g, BA 6
(53 14 16) δ	<0.001	5.86	-40 38 13	L frontal: inferior frontal g, BA 46
	<0.001	5.73	-28 -7 54	L parietal: postcentral g, BA 4
Medial prefrontal:				
L anterior	<0.001	7.40	36 8 2	R insula
cingulate, BA	<0.001	7.15	-40 -2 4	L insula
32	<0.001	6.88	-20 19 -6	L sub-lobar: border of caudate nucleus
(-4 39 2) $	<0.001	6.16	16 21 -6	R sub-lobar: border of caudate nucleus
R anterior	<0.001	7.38	-42 -2 4	L limbic: insula
cingulate,	<0.001	7.26	46 -6 4	R limbic: insula
BA32	<0.001	6.27	-18 21 -4	L frontal: inferior frontal g, BA 47
(4 39 2) $	<0.001	6.01	14 21 -4	R frontal: inferior frontal g, BA 47
L caudal medial	<0.001	Inf	20 14 3	R sub-lobar: lentiform nucleus
frontal gyrus,	<0.001	6.43	32 36 28	R frontal: middle frontal g, BA 9

Seed location	P value	Z score	Peak height co-ordinates	Region
BA6/32 (-4 6 51) [δ]	<0.001	5.89	-53 -40 22	L inferior parietal/superior temporal g, BA 22/40
R caudal medial frontal gyrus, BA6/32 (4 6 51) [δ]	<0.001 <0.00 <0.001	Inf 6.81 6.18	20 13 3 -10 -15 4 32 36 28	R sub-lobar: lentiform L sub-lobar: thalamus R frontal: middle frontal g, BA 9
Lateral temporal:				
L planum temporale, BA22 (-61 -34 16) [§]	<0.001 <0.001	Inf 6.27	58 -32 18 -46 -69 13	R temporal: superior temporal g, BA 22 L temporal: middle temporal g, BA 21/39
R planum temporale, BA22 (65 -34 20) [§]	<0.001 <0.001 <0.001 <0.001	Inf 6.52 6.33 6.22	-53 -32 20 -12 -35 48 16 -5 61 44 -60 10	L temporal: superior temporal g, BA22 L parietal: paracentral lobule, BA 4 R frontal: superior frontal g, BA 6 R temporal: middle temporal g, BA 21/37
L anterior superior temporal gyrus, BA22 (-48 -2 -10) [§]	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001	Inf 7.06 6.92 6.86 6.47 6.16	44 -4 -5 30 -33 0 36 -26 25 30 30 -10 -36 -14 25 -4 31 -3	R temporal: superior temporal g, BA 22 R med temporal: hippocampus R parietal: inferior parietal lobule, BA 40 R frontal: orbital gyrus, BA 11 L parietal: inferior parietal lobule, BA 40 L limbic: cingulate gyrus, BA 24/32
R anterior superior temporal gyrus, BA22 (60 13 -11) [§]	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001	7.77 7.18 7.11 7.01 6.69 6.38 6.28 6.27 5.47	-55 9 -6 -61 -24 16 12 -62 7 0 -12 67 53 -66 -5 6 -55 64 0 10 44 -53 -66 7 4 -80 26	L temporal: ant superior temporal g, BA 22 L temporal: superior temporal g, BA 22 R occipital: lingual g, BA 18 Midline medial frontal g, BA 6 R temporal: inferior temporal g, BA 37 R parietal: precuneus, BA 7 Midline: cingulate/medial frontal, BA 32 L temporal: middle temporal g, BA 39 R occipital: cuneus, BA 18
L posterior superior temporal s, BA22 (-55 -50 19) [§]	<0.001 <0.001	6.92 5.92	48 -44 21 -32 19 -4	R temporal: superior temporal g, BA 22 L frontal operculum/insula
R posterior superior temporal s, BA22 (63 -46 21) [§]	<0.001 <0.001	6.50 6.38	-57 -52 15 44 18 3	L temporal: superior temporal g, BA22 R frontal operculum/insula
L inferior temporal g, BA37 (-59 -51 -8) [§]	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001	Inf Inf 7.17 6.87 6.64 6.45	60 -41 -5 -32 -68 40 -26 7 62 40 -60 47 34 -72 -38 -46 32 15	R temporal: middle temporal g, BA 21 L parietal: superior parietal lobule, BA 7 L frontal: superior frontal g, BA 6 R parietal: superior parietal lobule, BA 7 R cerebellum L middle frontal g, BA 46
R inferior temporal g, BA37 (59 -51 -4) [§]	<0.001 <0.001 <0.001 <0.001	6.79 6.73 6.13 6.06	-50 -58 -4 38 -47 39 -38 -50 45 51 13 23	L temporal: inferior temporal g, BA 37 R parietal: superior parietal lobule, BA 7 L parietal: superior parietal lobule, BA 7 R frontal: inferior frontal g, BA 9/44
L fusiform g BA37 (-40 -49 -13) [§]	<0.001 <0.001	6.26 5.87	-57 -40 8 30 -76 33	L temporal: superior temporal g, BA 22 R parietal: superior parietal lobule, BA 7
R fusiform g, BA37 (40 -49 -13) [§]	<0.001	7.61	-38 -59 -7	L temporal: fusiform g, BA 37

Seed location	P value	Z score	Peak height co-ordinates	Region
L anterior middle temporal g, BA21 (-51 -37 -3) [δ]	<0.001	7.10	-30 19 -6	L frontal operculum/insula
	<0.001	7.02	48 -35 -5	R temporal: middle temporal g, BA 21
	<0.001	6.66	-34 13 21	L temporal: insula
	<0.001	6.01	14 13 -6	L sub-lobar: caudate/lentiform
	<0.001	5.91	16 17 38	R limbic: cingulate g, BA32
	<0.001	5.81	34 1 28	R frontal: precentral g, BA 6
	<0.001	5.57	26 -23 44	R parietal: post/precentral g, BA4
	<0.001	5.45	24 -69 -27	R cerebellum
R anterior middle temporal g, BA21 (51 -37 -3) [δ]	<0.001	6.81	-52 -37 -5	L temporal: middle/inf temporal g, BA 21/20
	<0.001	6.22	-18 12 40	L limbic: cingulate g, BA32
	<0.001	5.77	28 -31 37	R frontal/parietal: central s, BA 2/40
	<0.001	5.68	40 0 39	R frontal: precentral g, BA 6
	<0.001	5.68	30 -14 23	R frontal/temporal: lateral s/insula,
	<0.001	5.61	12 24 47	R frontal: superior frontal g, BA 6/8
		5.61	-22 -23 44	L frontal: precentral g, BA 4
Medial temporal:				
L anterior amyg-hipp region (-24 -1 -18) [$]	<0.001	Inf	26 1 -17	R med temporal: amyg-hippocampus
R anterior amyg-hipp region (24 -1 -18) [$]	<0.001	Inf	-25 -13 -18	L med temporal: amyg-hippocampus
L posterior amyg-hipp region (-18 -12 -16) [$]	<0.001	Inf	18 -7 -18	R med temporal: amyg-hippocampus
R posterior amyg-hipp region (18 -12 -16) [$]	<0.001	Inf	-16 -18 -16	R med temporal: hippocampus
Subcortical:				
L caudate (-8 14 3) [§]	<0.001	7.20	-8 -27 7	L sub-lobar: thalamus
	<0.001	6.67	14 37 4	R frontal: cingulate/medial frontal g, BA 32
	<0.001	6.63	-14 39 4	L frontal: cingulate/medial frontal g, BA 32
	<0.001	6.43	-10 14 49	L frontal: medial frontal g, BA 6/32
R caudate (10 14 3) [§]	<0.001	Inf	-10 37 9	L limbic: cingulate g, BA 32
	<0.001	7.46	10 39 4	R limbic: cingulate g, BA 32
	<0.001	6.32	28 36 29	R frontal: middle frontal g, BA 9
Other:				
L pre-central g, BA4 (-50 -2 44) [δ]	<0.001	7.41	50 2 33	R frontal: precentral g, BA 6
	<0.001	6.02	16 16 3	R sub-lobar: caudate/lentiform
	<0.001	5.62	-48 -57 -4	L temporal: inferior temporal g, BA 37
R pre-central g, BA4 (50 -2 44) [δ]	<0.001	5.99	-24 4 5	L sub-lobar: lentiform
	<0.001	5.87	32 -50 47	R parietal: superior parietal lobule, BA 7
L lingual g, BA18/17 (-28 -97 -3) [δ]	<0.001	7.28	-46 50 -9	L frontal: middle frontal g, BA 10/11
	<0.001	6.98	40 22 -31	R temporal: ant superior temporal g, BA 38
	<0.001	6.19	-2 5 57	L frontal: medial frontal g, BA 6
	<0.001	5.75	-63 -52 8	L temporal: middle temporal g, BA 21
R lingual g, BA 18/17	<0.001	Inf	-54 21 -9	L frontal: inferior frontal g, BA 45/47
	<0.001	7.56	40 22 -31	R temporal: ant superior temporal g, BA 38

Seed location	P value	Z score	Peak height co-ordinates	Region
(28 -97 -3)[δ]	<0.001	7.00	-2 2 66	L frontal: medial frontal g, BA 6
	<0.001	5.78	-2 -17 -19	L brainstem
	<0.001	5.55	-67 -39 0	L temporal: middle temporal g, BA 21

[§] indicates language derived seeds, [$] indicates schizophrenia region of interest derived seeds, [δ] indicates data driven seeds. Analyses thresholded at 0.005 corrected cluster level, extent threshold =100. A more stringent threshold was used for this larger group in order to keep the number of significant clusters to a practicable number.

Appendix table 5. High risk with symptoms (n=27)

Seed location	P value	Z score	Peak height co-ordinates	Region
Lateral prefrontal				
L inferior frontal g, BA45; pars triangularis (-55 23 3) [§]	<0.001	7.62	42 19 -1	R frontal: inferior frontal g, BA 45
	<0.001	5.81	-58 -24 12	L temporal: superior temporal g, BA 22
	<0.001	5.73	-4 27 43	L frontal: superior frontal g, BA 8
R inferior frontal g, BA45; pars triangularis (55 21 1) [§]	<0.001	6.85	0 25 37	Interhemispheric: cingulate g, BA 32
	<0.001	6.83	-60 20 6	L frontal: inferior frontal g, BA 45
	<0.001	5.95	-18 -87 -31	L cerebellum
	<0.001	5.49	-38 36 29	L frontal: middle frontal g, BA 9
	<0.001	5.42	44 -58 51	R parietal: inferior parietal lobule, BA 40
	<0.001			
L rolandic operculum, BA4/7 (-60 0 7) [§]	<0.001	7.25	55 -5 15	R frontal: precentral g, BA 4
	<0.001	6.07	4 -18 60	R frontal: medial frontal g, BA 6
	<0.001	5.48	44 -18 38	R parietal: postcentral g, BA 1
	<0.001	5.41	24 -32 59	R parietal: postcentral g, BA 1
R rolandic operculum, BA4/7 (59 6 5) [§]	<0.001	7.40	-57 -2 6	L frontal: precentral g, BA 6
	<0.001	5.95	4 -6 44	R limbic: cingulate g, BA 24
L frontal operculum, BA45 (-38 23 3) [§]	<0.001	7.77	40 14 7	R insula/frontal operculum, BA 45
	<0.001	6.29	14 10 40	R frontal: middle frontal g, BA 6
	<0.001	6.01	-10 -1 52	L frontal: medial frontal g, BA 6/32
	<0.001	5.59	-40 0 37	L frontal: precentral g, BA 6
R frontal operculum, BA45 (40 20 6) [§]	<0.001	7.13	-40 10 9	L frontal operculum/insula
	<0.001	6.95	-16 4 44	L frontal: cingulate/medial frontal g, BA 6/32
	<0.001	6.18	50 -41 35	R parietal: inferior parietal lobule, BA 40
	<0.001	6.13	20 48 18	R frontal: middle frontal g, BA 46
L inferior frontal g, BA44 (-53 14 16) [δ]	<0.001	6.22	-10 44 33	L frontal: medial frontal g, BA 9
	<0.001	6.21	42 12 16	R insula
	<0.001	5.61	-54 -49 -1	L temporal: middle temporal g, BA 21
	<0.001	5.43	-45 -45 37	L parietal: inferior parietal lobule, BA 40
R inferior frontal g, BA44 (53 14 16) [δ]	<0.001	7.27	51 -35 39	R parietal: inferior parietal lobule, BA 40
	<0.001	6.61	-36 -41 35	L parietal: inferior parietal lobule, BA 40
	<0.001	6.32	-57 10 12	L frontal: inferior frontal g, BA 44
	<0.001	6.30	12 12 51	R frontal: superior/medial frontal g, BA 6
	<0.001	5.96	22 6 7	R sub-lobar: lentiform nucleus
Medial prefrontal:				
L anterior cingulate, BA32 (-4 39 2) [$]	<0.001	6.31	18 18 5	R sub-lobar: caudate/lentiform nucleus
	<0.001	6.08	-12 16 1	L sub-lobar: caudate nucleus
	<0.001	6.03	-48 -10 4	L temporal: superior temporal g, BA 22
R anterior cingulate, BA32 (4 39 2) [$]	<0.001	6.66	-48 -8 2	L temporal: superior temporal g, BA 22
	<0.001	6.62	16 17 4	R sub-lobar: border caudate nucleus
	<0.001	6.41	42 -4 0	R frontal: frontal operculum, BA 45
L caudal medial frontal g, BA 6/32 (-4 6 51) [δ]	<0.001	6.33	-12 -13 6	L sub-lobar: thalamus
	<0.001	5.98	20 8 5	R sub-lobar: thalamus
	<0.001	5.87	60 -23 45	R parietal: postcentral g, BA 1
	<0.001	5.62	-32 35 31	L frontal: middle frontal g, BA 9
R caudal medial frontal	<0.001	6.97	59 -28 46	R parietal: inferior parietal lobule, BA 40
	<0.001	6.29	-45 -35 47	L parietal: inferior parietal lobule, BA 40/7

Seed location	P value	Z score	Peak height co-ordinates	Region
g, BA6/32 (4 6 51) [δ]	<0.001	6.13	-16 7 3	L sub-lobar: lentiform nucleus
Lateral temporal:				
L planum temporale, BA22 (-61 -34 16) [§]	<0.001	6.65	53 -24 21	R temporal: superior temporal g, BA 22
R planum temporale, BA22 (65 -34 20) [§]	<0.001 <0.001	7.16 6.07	-53 -38 18 38 4 3	L temporal: superior temporal g, BA 22 R insula
L anterior superior temporal g, BA22 (-48 -2 -10) [§]	<0.001 <0.001	7.35 6.36	48 -6 -8 -28 -33 5	R temporal: superior temporal g, BA 22 L temporal: border hippocampus
R anterior superior temporal g, BA22 (60 13 -11) [§]	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001	7.63 7.46 6.76 6.69 6.59 6.21 5.94 5.90 5.82 5.34	-53 15 -11 2 8 47 -10 -74 -10 -65 -28 27 6 -84 32 -2 27 -13 -48 -38 57 -55 -61 -10 -20 -43 -40 2 -19 14	L temporal: superior temporal g, BA 38 R frontal: medial frontal g, BA 6/32 L occipital: lingual gyrus/cerebellum L parietal: inferior parietal lobule, BA 40 R occipital: cuneus, BA 19 L frontal: rectal gyrus, BA 11 L parietal: inferior parietal lobule, BA 40/7 L temporal: inferior temporal g, BA 37 L cerebellum R thalamus
L posterior superior temporal s, B22 (-55 -50 19) [§]	<0.001 <0.001 <0.001	6.33 5.35 5.34	48 -50 17 46 -31 2 -44 22 8	R temporal: superior temporal g, BA 22 R temporal: superior temporal g, BA 22 L frontal: inferior frontal g, BA 45
R posterior superior temporal s, BA22 (63 -46 21) [§]	<0.001	6.39	-57 -53 30	L temporal: superior temporal g, BA 22
L inferior temporal g, BA37 (-59 -51 -8) [§]	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001	6.61 6.53 6.16 5.93 5.90 5.43 5.25	57 -53 -7 -46 13 27 -28 -72 42 18 -75 -23 -51 25 -11 32 -66 47 -42 -50 49	R temporal: inferior temporal g, BA 37 L frontal: inferior frontal g, BA 8/9 L parietal: superior parietal lobule, BA 7 R cerebellum L frontal: inferior frontal g, BA 45/47 R parietal: superior parietal lobule, BA 7 L parietal: superior parietal lobule, BA 7/40
R inferior temporal g, BA37 (59 -51 -4) [§]	<0.001 <0.001 <0.001	6.17 5.65 5.24	-53 -56 -1 28 -69 51 48 11 23	L temporal: inferior temporal g, BA 37 R parietal: superior parietal lobule, BA 7 R frontal: inferior frontal g, BA 9/44
L fusiform g, BA37 (-40 -49 -13) [§]	<0.001	6.16	38 -74 -6	R occipital: fusiform g, BA 18
R fusiform g, BA37 (40 -49 -13) [§]	<0.001 <0.001	6.29 5.64	-30 -65 -14 34 -73 20	L occipital: fusiform gyrus/cerebellum R occipital: middle occipital g, BA 19
L anterior middle temporal g, BA21	<0.001 <0.001 <0.001	6.47 6.39 5.54	40 -33 0 -22 1 15 -26 -29 38	R temporal: lateral border hippocampus L sub-lobar: lentiform nucleus L parietal: postcentral g, BA 1

Seed location	P value	Z score	Peak height co-ordinates	Region
(-51 -37 -3) [δ]				
R anterior middle temporal g, BA21 (51 -37 -3) [δ]	<0.001	6.45	-48 -33 -7	L temporal: middle/inferior temporal g, BA21/20
Medial temporal:				
L anterior amyg-hipp region (-24 -1 -18) [$]	<0.001	6.84	18 -1 -18	R med temporal: amyg-hippocampus
R anterior amyg-hipp region (24 -1 -18) [$]	<0.001	6.27	-20 -15 4	*Cluster centred on seed co-ordinates overlaps L amyg-hippocampus* L sub-lobar: lentiform/thalamus
L posterior amyg-hipp region (-18 -12 -16) [$]	<0.001	6.92	18 -11 -18	R med temporal: amyg-hippocampus
R posterior amyg-hipp region (18 -12 -16) [$]	<0.001	6.62	-20 -11 -20	L med temporal: amyg-hippocampus
Subcortical:				
L caudate (-8 14 3) [§]				*Large cluster centred on seed co-ordinates, overlapping right caudate*
R caudate (10 14 3) [§]				*Large cluster centred on seed co-ordinates, overlapping right caudate*
Other:				
L pre-central g, BA4 (-50 -2 44) [δ]	<0.001	6.60	-20 10 0	L sub-lobar: lentiform nucleus
	<0.001	6.51	-10 -1 57	L frontal: medial frontal g, BA 6
	<0.001	6.31	53 4 31	R frontal: precentral g, BA 6
R pre-central g, BA4 (50 -2 44) [δ]	<0.001	6.49	-14 -5 59	L frontal: superior/medial frontal g, BA 6
	<0.001	6.36	-40 -11 47	L frontal: precentral g, BA 4
L lingual g, BA18/17 (-28 -97 -3) [δ]	<0.001	5.89	33 62 -1	R frontal: ant middle frontal g, BA 10
	<0.001	5.80	51 14 -26	R temporal: ant superior temporal g, BA 38
	<0.001	5.66	-58 26 8	L frontal: inferior frontal g, BA 45
	<0.001	5.52	-50 23 -13	L frontal: inferior frontal g, BA 47
	<0.001	5.46	-59 -13 -26	L temporal: inferior temporal g, BA 20
	<0.001	5.44	-30 64 -1	L frontal: ant inferior frontal g, BA 10
R lingual g, BA18/17 (28 -97 -3) [δ]	<0.001	6.93	-36 -91 -2	L occipital: fusiform g, BA 18
	<0.001	6.41	-57 19 29	L frontal: middle frontal g, BA 9/45
	<0.001	6.22	-59 -10 -13	L temporal: middle temporal g, BA 21
	<0.001	5.85	52 4 -36	R temporal: middle temporal g, BA 21
	<0.001	5.68	-30 64 0	L frontal: ant middle frontal g, BA 10
	<0.001	5.43	4 55 5	R frontal: ant medial frontal g, BA 10
	<0.001	5.22	-48 8 -32	L temporal: middle temporal g, BA 21
	<0.001	5.19	-4 63 24	L frontal: ant medial frontal g, BA 10

[§] indicates language derived seeds, [$] indicates schizophrenia region of interest derived seeds, [δ] indicates data driven seeds. Analyses thresholded at 0.05 corrected cluster level, extent threshold =100.

APPENDIX V: APPENDIX TO CHAPTER SEVEN.

Change over time methodological issues

Methodological issues relating to the change over time analysis involved the transition to the newer version of the software, SPM2. Since this would also necessitate a re-analysis of the baseline images, the opportunity was also taken at this time for other methodological refinements to the analysis. These are outlined below. These included options which were not possible at the beginning of the study, for example normalisation to a study specific template.

The initial testing of the normalisation procedures seen with our data using SPM2 and matlab (version 6.5) however were considered to be inferior to those seen with the previous version, SPM99. The main issues were that the frontal lobes extended beyond the bounding box of the images, there were shape differences in posterior brain regions, and some internal structures appeared to be shifted. A message detailing these issues and the raising the possibility of using SPM99 to pre-process of the data, and then SPM2 for the statistical analysis was therefore sent to the email discussion list for SPM (http://www.jiscmail.ac.uk/lists/spm.html; 06/08/03; heading: 'EPI normalisation'). The reply from the SPM methods group indicated that this was an acceptable option. The conclusion was therefore to use SPM99 to the pre-process the data, and then use SPM2 for statistical analysis. There have since been reports from other groups regarding problems with normalisation in SPM2 listed on the mailbase. Later entries to the discussion list have suggested that normalisation differences could be due to conflicts with particular versions of Matlab's reshape function (versions 6 and above) and SPM2 (SPM mailbase; 27/01/04; heading 'normalisation and Matlab 6.5').

Methodological refinements:

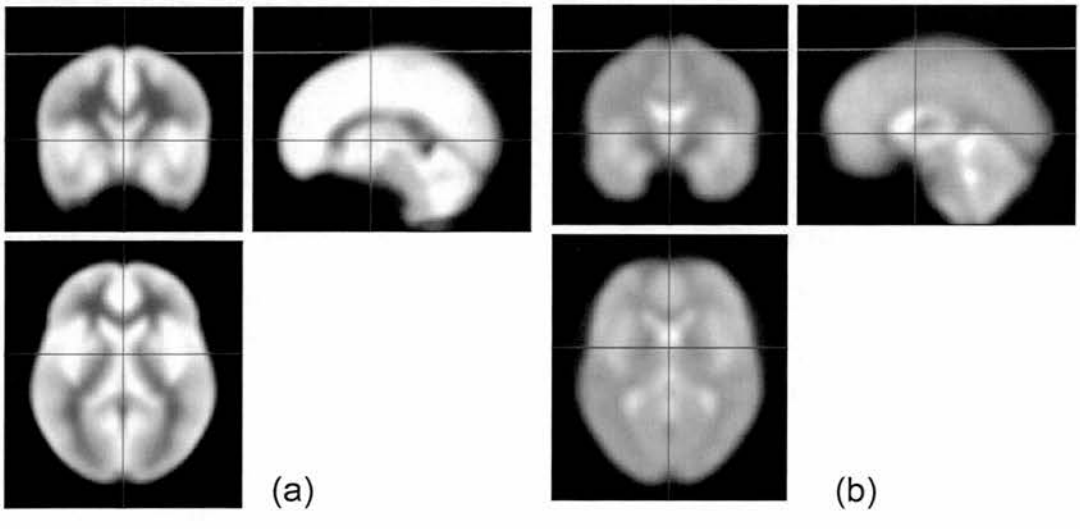
Realignment

Realigning images to the first volume in a series has in the past been the standard way to correct for movement over the course of a scanning session. It has since been

proposed by the SPM methods group that a better reference image would be the mean of all of the images in the series. Hence, for this analysis the EPI volumes for each subject were realigned to the mean of the series.

Normalisation

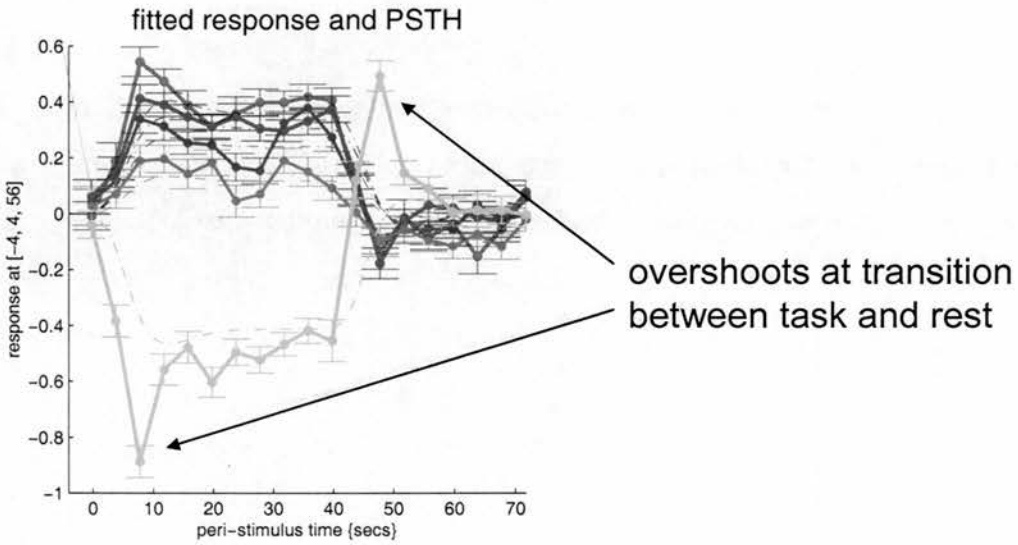
In the change over time analysis the images were normalized to a new study specific template, rather than the SPM EPI template. The use of a study specific template offers potential advantages to using the generic templates supplied with SPM. Firstly, scans are normalised to a template generated from the same scanner with the same imaging parameters, such as intensity profiles, and non-uniformities. Since varying degrees of susceptibility artefact can occur between scanners, it was considered that there may be considerable improvements offered by the use of a study specific EPI template. Secondly the template is generated from the same demographic population to that used in the study; this has indeed proved advantageous in our structural imaging studies (Job *et al.*, 2002). The study specific template was generated by Dr E Simonotto using scripts based on those supplied with SPM (Dr J Ashburner) from 121 Edinburgh High Risk subjects performing the Hayling task (appendix figure 3). This option was obviously not possible at the beginning of the study.



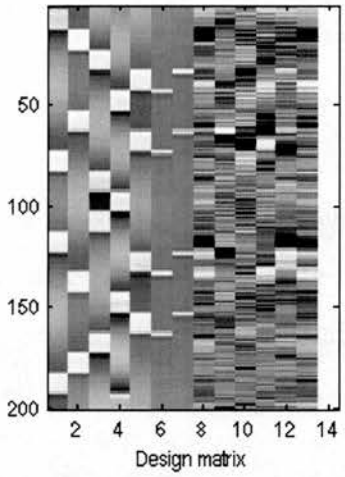
Appendix figure 3. New study specific EPI template (a), generic SPM EPI template (b).

First level statistical analysis

It had been noted from the data described in chapter three (also represented below in appendix figure 4), that there seemed to be a sizeable over-shoot in the measured BOLD response at the transition between blocks of task (sentence completion) and blocks of rest. Again this observation was only possible once data had been collected on a number of subjects. To refine the model therefore, additional 'task switching' components were entered into the design matrix. The data were now modelled with 7 conditions; the four sentence completion difficulty levels, the rest condition, and two additional regressors representing the transitions between 'task on' and 'task off'. The new design matrix is illustrated in appendix figure 5. Activation data associated with the new conditions are detailed below. In order to be consistent with the other analyses reported in this thesis however the analysis described in the main text refers only to the sentence completion versus rest and parametric contrasts.



Appendix Figure 4. Response at transition between task and rest



Appendix figure 5. New design matrix

Figure to illustrate new design matrix. Columns 1-4 represent the four task conditions, column 5 represents the rest condition, columns 6,7 represent the new regressors, i.e. the transition between sentence completion and rest, 'task on' and 'task off' respectively, and columns 8-13 represent the movement parameters.

New contrasts

Patterns of activation for the new contrasts 'task on' and 'task off' are shown below (appendix tables 4, 5 and appendix figure 6). These were determined based on the largest group with two scans (high risk without symptoms, n=23).

Task on

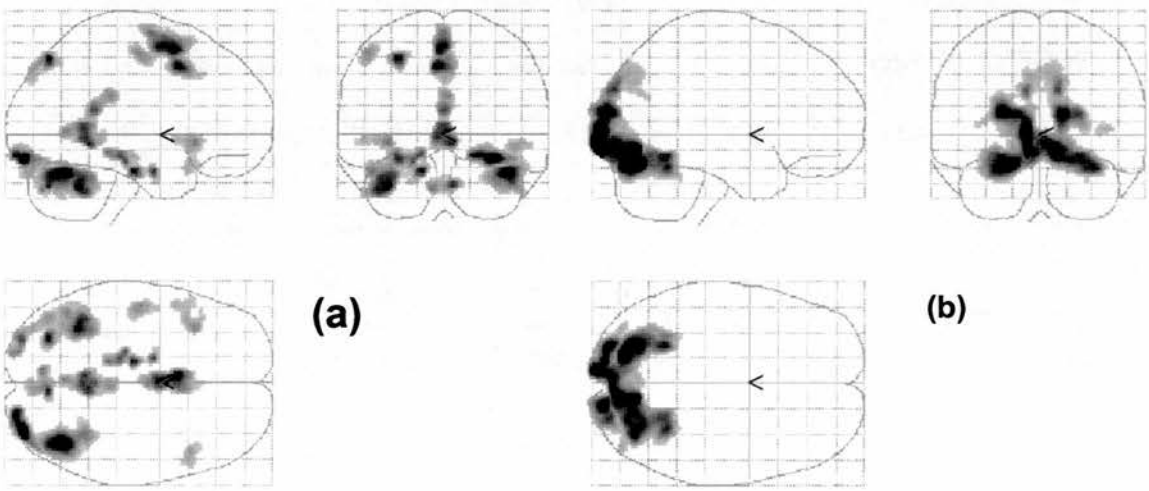
Appendix table 6. 'Task on' effects, high risk without symptoms

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	1426	5.86	32 -87 -16	R occipital: fusiform g, BA18
			40 -66 -26	R cerebellum
			18 -74 11	R occipital: calcarine s, BA17
<0.001	824	4.99	-4 10 46	L frontal: medial frontal g, BA6
			-2 8 57	L frontal: medial frontal g, BA6
			0 0 51	Midline: medial frontal /anterior cingulate, BA32
<0.001	1120	4.90	-36 -51 -34	L cerebellum
			-28 -91 -16	L cerebellum
			-15 -73 -13	L occipital: lingual g, BA 18
0.034	133	4.81	-30 -67 49	L parietal: superior parietal lobule, BA 7
0.001	255	4.72	-14 -18 -24	L temporal: parahippocampal g, BA28/36
			-16 -5 -24	L temporal: parahippocampal g, BA 28
			-16 -18 -14	L temporal: parahippocampal g, BA34/28
<0.001	716	4.69	6 -46 -6	R cerebellum
			-4 -48 -1	R cerebellum
			0 -35 -15	Brainstem
0.029	138	4.66	6 -71 -25	R cerebellum
			-8 -75 -27	R cerebellum
0.032	135	4.18	2 -69 50	R parietal: precuneus, BA7
			6 -75 46	R parietal: superior parietal lobule, BA 7
0.014	163	4.11	-36 22 -6	L frontal: frontal operculum, BA45
			-43 16 -4	L frontal operculum/insula
			-40 8 -3	L limbic: insula
0.038	130	3.80	46 20 -8	R frontal operculum, BA45
			52 18 -22	R temporal: anterior superior temporal g, BA38
			40 19 -14	R inferior frontal gyrus, BA47

Task off

Appendix table 7. 'Task off' effects, high risk without symtpoms

P value	Extent	Z score	Peak height co-ordinates	Region
<0.001	6307	6.11	-8 -93 4	L occipital: cuneus, BA18
			-9 -96 -12	L occipital: lingual g, BA17/18
			-15 -61 -16	L occipital: fusiform g, BA19/ cerebellum



Appendix figure 6. Task on (a), task off (b) for high risk without symptoms, (*n*=23).

APPENDIX VI: ADDITIONAL PUBLICATIONS

Conference abstracts:

Whalley HC, Flett S, Simonotto E, Goddard HN, Marshall I, Owens DGC, Ebmeier KP, Johnstone EC, Lawrie SM. 2001. Functional MRI of the Hayling sentence completion test in subjects at high genetic risk of schizophrenia: preliminary results. *Neuroimage, 6:S1117.* Presented at the Human Brain Mapping conference in 2001.

Whalley HC, Flett S, Simonotto E, Goddard HN, Marshall I, Owens DGC, Ebmeier KP, Johnstone EC, Lawrie SM. 2001. Functional MRI of the Hayling test in high-risk subjects: preliminary findings. *Schizophrenia Research, 53:s120.* Presented at the Biennial Winter Workshop on Schizophrenia Research in 2002.

Whalley HC, Simonotto E, Goddard HN, Marshall I, Flett S, Lakany H, Owens DGC, Ebmeier KP, Johnstone EC, Lawrie SM. Functional imaging in subjects at high risk of schizophrenia. *Schizophrenia Research, 60:s237.* Presented at the International Congress on Schizophrenia Research in 2003.

Whalley HC, Simonotto E, Meyer M, Whyte MC, Marshall I, Ebmeier KP, Owens DGC, Goddard HN, Johnstone EC, Lawrie SM. Functional disconnectivity in subjects at genetically enhanced risk of schizophrenia. *Schizophrenia Research 67:s93.* Presented at the Biennial Winter Workshop on Schizophrenia Research in 2004.

Whalley HC, Simonotto E, Marshall I, Ebmeier KP, Owens DGC, Goddard HN, Johnstone EC, Lawrie SM. Functional imaging predictors of schizophrenia. Presented at the Human Brain Mapping conference in 2004.

Other publications:

Moorhead TWJ, Job DE , Whalley HC, Sanderson TL, Johnstone EC, Lawrie SM. 2004. Voxel-based morphometry of comorbid schizophrenia and learning disability: analyses in normalized and native spaces using parametric and nonparametric statistical methods, *NeuroImage, In Press*

Job DE, Whalley HC, McConnell S, Glabus M, Johnstone EC, Lawrie SM. 2003 Voxel-based morphometry of grey matter densities in subjects at high risk of schizophrenia, *Schizophrenia Research, Volume 64: 1-13*

Burns J, Job D, Bastin ME, Whalley H, Macgillivray T, Johnstone EC, Lawrie SM. 2003. Structural disconnectivity in schizophrenia: a diffusion tensor magnetic resonance imaging study. *British Journal of Psychiatry. 182:439-43.*

Lawrie SM, Whalley HC, Job DE, Johnstone EC. 2003. Structural and functional abnormalities of the amygdala in schizophrenia. *Annals of the New York Academy of Sciences. 985:445-60.*

Byrne M, Clafferty BA, Cosway R, Grant E, Hodges A, Whalley HC, Lawrie SM, Owens DG, Johnstone EC. 2003. Neuropsychology, genetic liability, and psychotic symptoms in those at high risk of schizophrenia. *Journal of Abnormal Psychology, 112(1):38-48.*

Job DE, Whalley HC, McConnell S, Glabus M, Johnstone EC, Lawrie SM. 2002. Structural gray matter differences between first-episode schizophrenics and normal controls using voxel-based morphometry. *Neuroimage. 17(2):880-9.*

Lawrie SM, Whalley HC, Abukmeil SS, Kestelman JN, Miller P, Best JJ, Owens DG, Johnstone EC. 2002. Temporal lobe volume changes in people at high risk of schizophrenia with psychotic symptoms. *British Journal of Psychiatry. 181:138-43.*

Lawrie SM, Buechel C, Whalley HC, Frith CD, Friston KJ, Johnstone EC. 2002. Reduced frontotemporal functional connectivity in schizophrenia associated with auditory hallucinations. *Biological Psychiatry. 51(12):1008-11.*

Steel RM, Whalley HC, Miller P, Best JJ, Johnstone EC, Lawrie SM. 2002. Structural MRI of the brain in presumed carriers of genes for schizophrenia, their affected and unaffected siblings. *Journal of Neurology, Neurosurgery & Psychiatry, 72(4):455-8.*

Boyes J, Whalley HC, Lawrie SM, Johnstone EC. Best JJ. 2001. A MRI study of ocular hypertelorism in individuals at high risk of developing schizophrenia. *Schizophrenia Research. 50(1-2):1-2.*

Lawrie SM, Whalley HC, Abukmeil SS, Kestelman JN, Donnelly L, Miller P, Best JJ, Owens DG, Johnstone EC. 2001. Brain structure, genetic liability, and psychotic symptoms in subjects at high risk of developing schizophrenia. *Biological Psychiatry. 49(10):811-23.*

Whalley HC, Wardlaw JM. Accuracy and reproducibility of simple cross-sectional linear and area measurements of brain structures and their comparison with volume measurements. *Neuroradiology. 43(4):263-71.*

Whalley HC, Kestelman JN, Rimmington JE, Kelso A, Abukmeil SS, Best JJ, Johnstone EC, Lawrie SM. 1999. Methodological issues in volumetric magnetic resonance imaging of the brain in the Edinburgh High Risk Project. *Psychiatry Research. 91(1):31-44.*

Lawrie SM, Whalley H, Kestelman JN, Abukmeil SS, Byrne M, Hodges A, Rimmington JE, Best JJ, Owens DG, Johnstone EC. 1999. Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. *Lancet. 353(9146):30-3.*

APPENDIX VII: PUBLISHED PAPER.

Article published in 'Brain' in March 2004. Permission to include article has been sought and granted by the publisher Oxford University Press, and by the co-authors.

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fMRI correlates of state and trait effects in subjects at genetically enhanced risk of schizophrenia

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Summary

Schizophrenia is a highly heritable disorder that typically develops in early adult life. Structural imaging studies have indicated that patients with the illness, and to some extent their unaffected relatives, have subtle deficits in several brain regions, including prefrontal and temporal lobes. It is, however, not known how this inherited vulnerability leads to psychosis. This study used a covert verbal initiation fMRI task previously shown to elicit frontal and temporal activity (the Hayling sentence completion task) to examine this issue. A large (n = 69) number of young participants at high risk of developing schizophrenia for genetic reasons took part, together with a matched group of healthy controls (n = 21). At the time of investigation, none had any psychotic disorder, but on detailed interview some of the high-risk participants (n = 27) reported isolated psychotic symptoms. The study aimed to determine: (i) whether there were activation differences that occurred in all subjects with a genetic risk of schizophrenia (i.e. 'trait' effects); and (ii) whether there were activation differences that only occurred in those at high risk who had isolated psychotic symptoms ('state' effects). No activation differences were found in regions commonly reported to be abnormal in the established illness, namely the dorsolateral prefrontal cortex or in the temporal lobes, but group differences of apparent genetic cause were evident in medial prefrontal, thalamic and cerebellar regions. In addition, differences in activation in those with symptoms were found in the intraparietal sulcus. No significant differences in performance were found between the groups, and all subjects were antipsychotic naïve. These findings therefore suggest that vulnerability to schizophrenia may be inherited as a disruption in a fronto-thalamic-cerebellar network, and the earliest changes specific to the psychotic state may be related to hyperactivation in the parietal lobe.

Keywords: fMRI; high risk; schizophrenia; sentence completion

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Introduction

Schizophrenia is a highly heritable disorder that generally becomes manifest in early adult life. Many studies report that structural and functional abnormalities, principally in the prefrontal and temporal lobes, are associated with the established illness (Frith *et al.*, 1995; Fletcher *et al.*, 1998; Lawrie and Abukmeil, 1998; Shenton *et al.*, 2001). One of the most prominent findings reported in the functional imaging literature is of abnormal prefrontal activation, particularly in response to executive tasks in which patients perform worse than control subjects, such as working memory (see Manoach, 2003), and verbal initiation tasks (Curtis *et al.*, 1998). However, it has been suggested that findings of hypofrontality may be confounded by performance differences between groups (Ebmeier *et al.*, 1995; Weinberger and Berman, 1996; Ramsey *et al.*, 2002). In studies that have controlled for task performance, findings of hypofrontality are less consistently reported (Frith *et al.*, 1995; Fletcher *et al.*, 1998; Ramsey *et al.*, 2002).

Studies on the established illness, however, are not able to clarify the extent to which the abnormal findings relate to the presence of symptoms and/or medication effects, or to the presence of a schizophrenic predisposition or genotype. This has led to the prospective study of relatives of affected individuals, which allows the investigation of whether abnormalities predate development of the illness and reflect genetic vulnerability, or whether

there are changes specifically associated with the manifestation of symptoms. Previous structural and functional imaging studies have indeed suggested that some of the abnormalities associated with the established state are present to some extent in unaffected relatives (Lawrie *et al.*, 1999, 2001; Seidman *et al.*, 1999; Keshavan *et al.*, 2002a, b; Callicott *et al.*, 2003).

The Edinburgh High Risk Study is designed to address such issues and serially examines, in comparison with matched healthy controls, young people (aged 16–25 years at ascertainment) who are at enhanced risk of schizophrenia for genetic reasons (Johnstone *et al.*, 2000). Ten to fifteen percent were predicted to develop schizophrenia by the age of 30 years on the basis of the known frequency of schizophrenia in individuals with this degree of heredity, and the actual occurrence of schizophrenia by the age of 30 years (Johnstone *et al.*, 2002). The first phase of the study (conducted in 1994–1999) employed repeated clinical assessments that have shown increasing psychopathology. Isolated psychotic symptoms are occurring in two to three times as many subjects as are expected to develop schizophrenia, but in those who have done so (as predicted, ~10% at the end of 2002) the florid condition has almost always been preceded by such isolated psychotic symptoms (Johnstone *et al.*, 2002). In the current phase of the study (1999–2004), a covert verbal initiation fMRI activation paradigm previously shown to elicit fronto-temporal activity, the Hayling sentence completion test (Burgess and Shallice, 1997; Lawrie *et al.*, 2002), has been added to the tests used in the first phase. A number of high-risk participants in the current study reported, on direct questioning at standardized interview, isolated and/or transient psychotic symptoms in the presence of unimpaired functioning. Although it is likely that a few of the high-risk participants may still go on to develop schizophrenia or a related disorder, none of the participants in this current study met diagnostic criteria for any psychiatric disorder at the time of investigation. This study therefore allows consideration not only of the way in which regional brain function differs between normal controls and those at enhanced risk (i.e. 'trait' effects), but also how that function is altered in those with isolated psychotic symptoms (i.e. early 'state' effects), all in a situation uncontaminated by the effects of prolonged illness and/or antipsychotic medication.

Subjects and methods

Subject details

Participants were selected on the basis of being aged between 16 and 25 years when first recruited (1994–1999), and having two or more first or second degree relatives with schizophrenia. The control group had no family history of the illness. At the time of recruitment, all participants regarded themselves as being in good health and functioning well. As part of an ongoing study, this report presents the results from the first 100 subjects reviewed in the second phase of the study, between 1999 to the present. Basic demographic variables are presented in Table 1. Out of the first 100 subjects, six did not participate in scanning. A further four scans were subsequently excluded (two due to minor vascular malformations, and two due to movement artefact; see below), leaving 90 fMRI scans from 21 normal controls and 69 high-risk subjects. On the day of scanning all subjects underwent a structured psychiatric interview (the Present State Examination; Wing *et al.*, 1974). Twenty-seven high-risk subjects reported psychotic symptoms (usually isolated delusions or hallucinations); the remainder of the high-risk group and all of the controls had no such symptoms (Table 1). None of the subjects was on anti-psychotic medication or seeking treatment, or indeed saw themselves as unwell. They therefore could not be said to fulfil diagnostic criteria for any psychiatric disorder. All subjects provided written informed consent, and the study was approved by the Psychiatry and Clinical Psychology subcommittee of the Lothian research ethics committee.

Scanning procedure

Imaging was carried out at the Brain Imaging Research Centre for Scotland (Edinburgh, UK) on a GE 1.5 T Signa scanner (GE Medical, Milwaukee, WI, USA) equipped with 23 mT/m 'Echospeed' gradients having a rise time of 200 μs. After a localizer scan, subjects were imaged with a multislice T2-weighted fast spin-echo sequence [repetition time/echo time (TR/TE) = 6300/102 ms]. Twenty slices (5-mm thickness, 1.5-mm gap), aligned parallel to the anterior commisure–posterior commisure (AC–PC) line, covered the brain. A structural scan with 1-mm pixel size was next acquired using a 3D inversion recovery-prepared T1-weighted sequence [inversion time (TI) = 600 ms]. One hundred and twenty-four slices (thickness 1.7 mm) were aligned perpendicular to the AC–PC line. Finally, axial gradient-echo planar images (EPI) [TR/TE = 4000/40 ms; matrix 64 × 128; field of view (FOV) 220 × 440 mm] were acquired continually during the experimental paradigm. Thirty-eight contiguous 5-mm slices were acquired within each TR period. Each EPI acquisition was run for 204 volumes, of which the first four volumes were discarded. Visual stimuli were presented using a

Table 1 *Demographic details*

	Controls (n = 21)	High risk without symptoms (n = 42)	High risk with symptoms (n = 27)
Age (years) [mean (SD)]	26.8 (2.7)	26.8 (3.4)	25.1 (3.1)
Gender (male:female)	13:8	17:25	13:14
Mean NART IQ (SD)	97.95 (24.02)	99.56 (18.12)	97.86 (10.60)
Handedness (R:L:A)	19:2:0	39:2:1	21:4:2
Genetic liability (1st degree:2nd degree)*	N/A	32:10	16:11

*First or second degree relatives with schizophrenia. N/A = not applicable; R = right; L = left; A = ambidextrous.

screen (IFIS; MRI Devices, Waukesha, WI, USA) placed in the bore of the magnet; corrective lenses were used where necessary.

Experiment

The participants in the study performed the verbal initiation section of the Hayling sentence completion test (Burgess and Shallice, 1997) in the scanner. Subjects were shown sentences with the last word missing and were asked to silently think of an appropriate word to complete the sentence (i.e. without speaking the word), and press a button when they had done so. Sentences were selected from Bloom and Fischler's set of sentence completion norms (Bloom and Fischler, 1980). The task was adapted for fMRI to have four levels of difficulty, according to the range of suitable completion words suggested by the sentence context. Examples of each difficulty level are presented in Table 2. Sentences were presented in blocks of fixed difficulty; each block lasted 40 s and included eight sentences. Sentences were presented for a period of 3 s followed by a fixation cross for 2 s. Subjects were instructed to respond at any time (by button press) until the next sentence appeared. The rest condition consisted of viewing a screen of white circles on a black background for 40 s. The order of the blocks was pseudo-random, and each block was repeated four times (different sentences were used for each sentence block). This design allowed both a standard subtraction (sentence completion versus rest) and parametric analysis (examining areas of increasing activation with increasing task difficulty). Verbal instructions were given prior to scanning and were standardized across subjects. During the same scanning session a further functional imaging study was performed, the results of which are not discussed here.

Immediately after scanning, subjects were given the same sequence of sentences on paper and requested to complete each sentence with the word they first thought of in the scanner. Word appropriateness scores were determined from the word frequency list of sentence completion norms (Bloom and Fischler, 1980), which provides respective probabilities of possible responses. A score of 1 was given to the most frequently produced word in the word frequency list, a score of 2 for the next most frequently produced word, etc. Mean scores for both word appropriateness and reaction time were determined for each constraint level. For a number of subjects (six controls, eight high risk without and five high risk with individual psychotic symptoms), no reaction time measures were recorded in the scanner. Since these subjects indicated at debriefing that they had indeed performed the task in the scanner, they were included in the analysis.

Table 2 *Example sentences*

(1) High constraint	"He mailed the letter without a. . ."
(2) Medium high constraint	"The train was still on. . ."
(3) Medium low constraint	"Not even the cast liked the. . ."
(4) Low constraint	"Rushing out he forgot to take his. . ."

Numbers 1–4 represent increasing difficulty.

Scan processing

The EPI images were reconstructed offline in ANALYZE format (Mayo Foundation, Rochester, MN, USA). Scan analysis was performed using SPM99 (Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) running in Matlab (MathWorks, Natick, MA, USA), and was performed blind to the group status of the individual. For each subject, EPI volumes were realigned to the first volume in the series using rigid body transformations. Details of within-scanner movement are presented in Table 3; two subjects presented significant motion artefacts and were excluded from further analysis. One subject was excluded on the basis of having moved >3 mm, peak-to-peak, over less than 20 images, and one subject presenting large correlations (>0.5) between movement parameters and task regressors was also excluded. The images were then normalized to the SPM99 EPI template using a linear affine transformation followed by non-linear deformations, and resampled using sinc interpolation to cubic voxels of size 8 mm^3. Normalized images were spatially smoothed with a $6 \times 6 \times 6$ mm^3 FWHM (full width half maximum) Gaussian filter to minimize residual inter-subject differences, and in order to meet assumptions for statistical analysis regarding the distribution of residuals.

Statistical analysis

Statistical analysis was performed using the general linear model approach as implemented in SPM99. At the individual subject level the data were modelled with five conditions (the four difficulty levels and the rest condition), each modelled by a boxcar convolved with a synthetic haemodynamic response function. The estimates of the subject's movement during the scan were also entered as 'covariate of no interest'. Before fitting the model, the subject's data were filtered in the time domain using both a low-pass [Gaussian kernel, 4 s (FWHM)] and a high-pass (400 s cut-off) filter. Contrasts were constructed to examine all four sentence completion conditions versus rest, and areas of increasing activation with increasing task difficulty (the parametric contrast).

For each contrast of interest (sentence completion versus rest, and parametric effects) one contrast image per subject was entered into a second level random effects analysis to examine areas of activation within each of the three groups (one sample *t*-test), and differences in activation between the groups (ANOVA). Differences in activation due to symptomatic 'state' effects were initially examined by comparing the non-symptomatic groups (controls plus high risk without symptoms) versus high-risk subjects with symptoms (and

Table 3 *Within-scanner movement*

	Controls (n = 21)	High risk without symptoms (n = 42)	High risk with symptoms (n = 27)
Max movement in x (mm) (SD)	0.80 (0.51)	1.10 (0.83)	1.45 (0.93)
Max movement in y (mm) (SD)	0.80 (0.47)	0.82 (0.38)	0.99 (0.46)
Max movement in z (mm) (SD)	1.11 (0.74)	1.06 (0.65)	1.25 (0.83)

Estimate of movement parameters determined from realignment stage of preprocessing.

Table 4 *Behavioural measures*

Group	Mean word appropriateness score (SD)				Mean reaction time (ms) (SD)			
Constraint levels	Low	Med low	Med high	High	Low	Med low	Med high	High
Controls	6.40 (1.25)	3.25 (0.53)	1.93 (0.32)	1.10 (0.08)	2643 (528)	2543 (605)	2413 (615)	2375 (630)
High risk without symptoms	6.30 (1.00)	3.17 (0.59)	1.97 (0.35)	1.12 (0.09)	2501 (547)	2366 (591)	2242 (587)	2237 (618)
High risk with symptoms	6.38 (0.79)	3.35 (0.59)	2.01 (0.40)	1.12 (0.08)	2530 (671)	2441 (671)	2246 (699)	2337 (703)

Constraint levels high to low represent increasing difficulty.

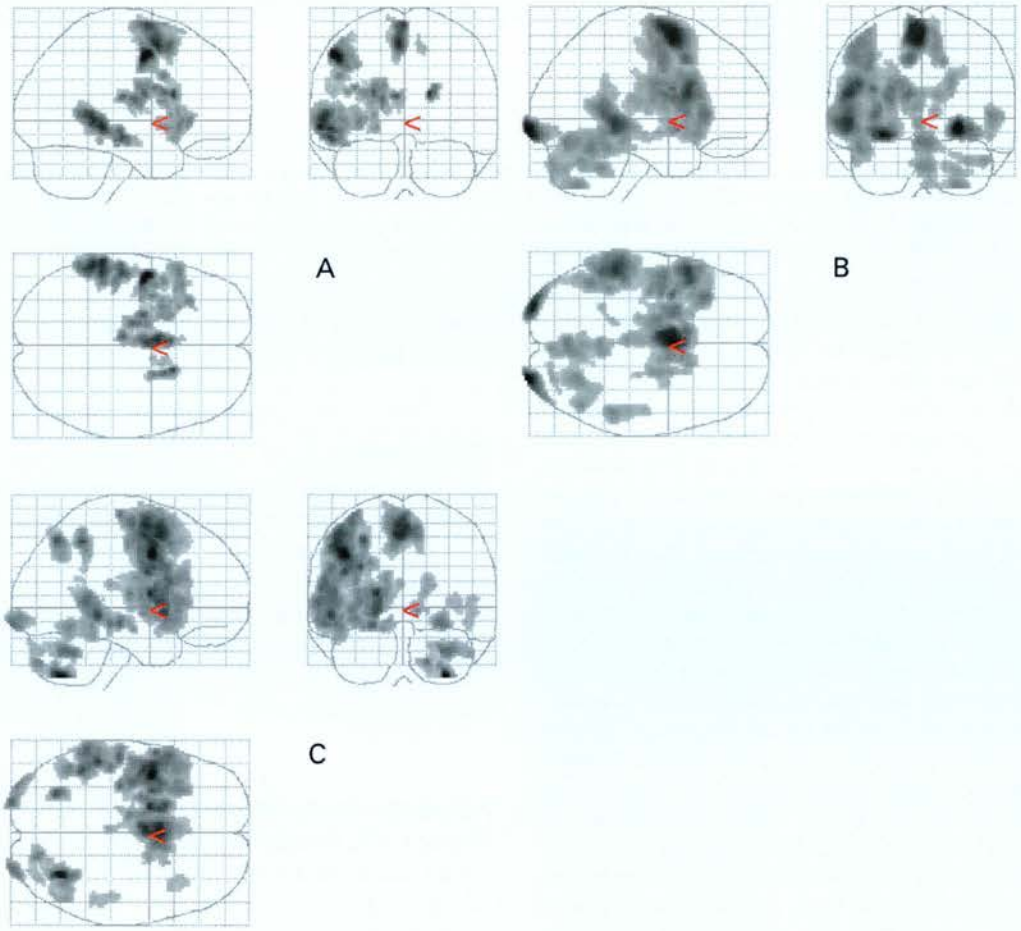

Fig. 1 Sentence completion versus rest: within-group analysis. (**A**) Controls (n = 21); (**B**) high risk without symptoms (n = 42); (**C**) high risk with symptoms (n = 27). Activations displayed on 3D 'glass brain', maps thresholded at $P < 0.0001$ uncorrected voxel level, extent threshold 50 voxels.

vice versa). Differences due to 'trait' effects were initially examined by comparing controls versus all high-risk subjects (and *vice versa*). This analysis structure was chosen to simplify the reporting of results and to minimize the number of group comparisons. As the groups were matched on demographic variables, and there were no significant differences in movement parameters, we did not include these factors as potential confounds in the model. However, to be confident that these factors were not having a significant effect, further examination of the influence of these variables was performed. Potential confounders [detailed in Tables 1 and 3: age, gender, handedness and within-scanner movement in x (mm)] were examined by entering them as covariates in the second-level random effects analysis. Even at lenient thresholds (regions considered significant at voxel-level P corrected <0.10, for maps thresholded at 0.01, F-test), none was found to have a significant effect.

In order to further clarify any state/trait related findings, *post hoc* group comparisons were conducted. In this model groups were also split in order that we could also examine differences according to degree of genetic risk, i.e. those with any first degree, or only second degree, relatives with the disorder. The criteria we followed for differences to be identified as potential state-specific effects were that similar differences should be found between 'high risk with symptoms versus controls', and between 'high risk with versus high risk without symptoms', but not between 'high risk without

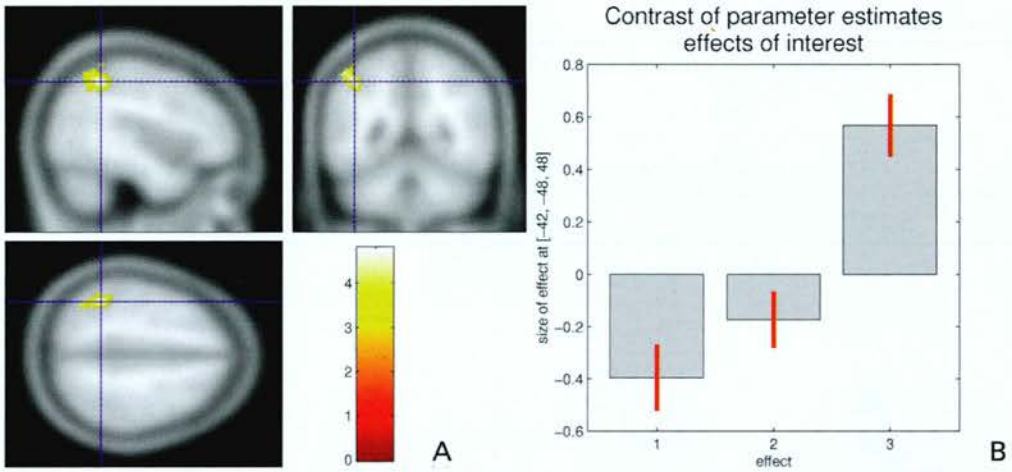

Fig. 2 Sentence completion versus rest: between-group differences. (**A**) Group comparison showing relatively greater activation in left inferior parietal lobule in high-risk subjects with psychotic symptoms versus controls and high risk without symptoms. Maps thresholded at $P < 0.001$ uncorrected voxel level, extent threshold 50 voxels. Colour bar represents Z score. (**B**) Effect size at peak co-ordinate (1 = controls; 2 = high risk without symptoms; 3 = high risk with symptoms).

symptoms versus controls'. Criteria for any potential trait-specific effects were that similar differences should be found between 'controls versus high risk with symptoms' and between 'controls versus high risk without symptoms', but not between 'high risk with versus high risk without symptoms'. In addition to these more detailed group comparisons, we also performed a masking procedure in SPM to examine regions fulfilling the above criteria for state and trait effects. In order to derive regions signifying potential state effects, an exclusive mask was generated between 'high risk with versus high risk without symptoms' and 'high risk without symptoms versus controls', at an uncorrected mask threshold of 0.05. This mask was then converted into binary format to define an image to be used to examine areas of overlap between this exclusive mask and the contrast 'high risk with symptoms versus controls'. Along similar lines, in order to derive regions signifying potential trait effects, an exclusive mask was generated between 'controls versus high risk without symptoms' and 'high risk with versus high risk without symptoms', which was then used to examine areas of overlap in the contrast 'controls versus high risk with symptoms'. Finally, interactions between state and trait effects were examined by looking at areas where there were linear effects across the groups, for example controls greater than high risk without symptoms, greater than high risk with symptoms, and *vice versa*.

Statistical maps were thresholded at a level of $P = 0.001$ uncorrected, and regions were considered significant at $P < 0.05$ cluster level corrected for multiple comparisons, unless otherwise stated. All P-values quoted in the text are at the corrected cluster level. Co-ordinates were converted from MNI (Montreal Neurological Institute) to Talairach co-ordinates using a non-linear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging).

Results

Demographic details

There were no statistically significant differences in age, gender, handedness or IQ between the subject groups. There were also no significant differences between the high-risk groups (with and without psychotic symptoms) in terms of genetic liability (Table 1).

Behaviour

All three groups showed the expected pattern of quicker reaction time and higher word appropriateness scores with greater contextual constraint (Table 4). This pattern of reaction time confirms that the subjects were performing the task appropriately during scanning. There were no significant differences between the groups in terms of their performances on either word appropriateness scores or reaction time measures.

Sentence completion versus rest

Figure 1A–C displays the results for the sentence completion versus rest contrast (all levels of difficulty versus baseline) within each of the groups. This demonstrated activation in regions commonly activated with this task, including the left precentral gyrus, inferior frontal gyrus, medial/superior frontal gyrus, middle/superior temporal gyrus, right posterior lobe of the cerebellum and the occipital lobes bilaterally. Visual inspection of these maps indicated an additional region of activation in the high-risk subjects with psychotic symptoms in the left parietal lobe, which was not seen in either of the other two groups.

No significant between-group differences were found between the controls and all the high-risk subjects for the sentence completion versus rest contrast, but, as suggested by the within-group maps described above, there was significantly greater activation in the high-risk subjects with psychotic symptoms versus the non-symptomatic groups in the left parietal lobe ($x = -42$, $y = -48$, $z = 48$; $P = 0.001$; Fig. 2A, B; Table 5). This activation was located in the

Table 5 *Random effects analysis: main group differences*

P-value	Extent	*Z*	Peak height (*x*, *y*, *z*)	Region
Sentence completion versus rest				
Symptom 'state' contrast: controls, high risk without symptoms (n = 63) < high risk with symptoms (n = 27)				
0.001	353	4.50	–42, –48, 48	Left parietal lobe: inferior parietal lobule BA 40
			–48, –49, 54	Left parietal lobe: inferior parietal lobule BA 40
			–30, –46, 36	Left parietal lobe
Parametric				
Inherited 'trait' contrast: controls (n = 21) > all high risk (n = 69)				
0.033	145	4.01	14, 47, –1	Right frontal lobe: medial frontal gyrus BA10/32
			2, 53, 5	Right frontal lobe: medial frontal gyrus BA10/32
0.004	223	3.97	–2, –78, –11	Left cerebellum
			–14, –78, –15	Left cerebellum: posterior lobe, declive
			–22, –73, –17	Left cerebellum: posterior lobe, declive
0.001	279	3.89	8, –13, 6	Right cerebrum: thalamus
			–6, –15, 12	Left cerebrum: thalamus
			18, –11, 10	Right cerebrum: thalamus

Table 6 *Random effects analysis: further examination of potential 'state'-related effects*

P-value	Extent	*Z*	Peak height (*x*, *y*, *z*)	Region
Sentence completion versus rest				
Controls (n = 21) < high risk with symptoms (n = 27)				
0.001	334	4.17	–42, –50, 48	Left parietal lobe: inferior parietal lobule BA 40
			–48, –48, 55	Left parietal lobe: inferior parietal lobule BA 40
			–31, –36, 56	Left parietal lobe: inferior parietal lobule/postcentral gyrus
Controls (n = 21) < high risk without symptoms (n = 42): n/s				
High risk without symptoms (n = 42) < high risk with symptoms (n = 27)				
0.007	235	4.40	–42, –48, 48	Left parietal lobe: inferior parietal lobule BA 40
			–40, –62, 54	Left parietal lobe: inferior parietal lobule BA 40
			–32, –46, 34	Left parietal lobe
Analysis with respect to degree of risk				
High risk with first degree relatives (n = 48) > high risk with second degree relatives (n = 21)				
0.003	274	4.23	–6, –99, 4	Left occipital lobe: cuneus
			10, –93, 14	Right occipital lobe: cuneus
			7, –72, 2	Right occipital lobe: lingual gyrus BA18
High risk with second degree relatives (n = 21) > high risk with first degree relatives (n = 48): n/s				

n/s = not significant.

intraparietal sulcus, contained mainly within the inferior parietal lobule [Brodmann area (BA) 40], and to a lesser extent involved the superior parietal lobule (BA 7). No regions were shown to be relatively less active in the symptomatic group versus the non-symptomatic groups for this contrast.

A more detailed analysis between groups of this parietal lobe difference (using the model with the high-risk groups also split into those with first and second degree relatives) is presented in Table 6. These group comparisons indicated that there was significantly greater activation in the left inferior parietal lobule in the high risk with symptoms versus controls (x = –42, y = –50, z = 48; P = 0.001), and versus the high risk without symptoms (x = –42, y = –48, z = 48; P = 0.007), but not between the controls and high-risk subjects without symptoms (nearest cluster at x = –34, y = –44, z = 46; T score = 1.74). The masking procedure confirmed these findings, where regions of overlap were seen in the left inferior parietal

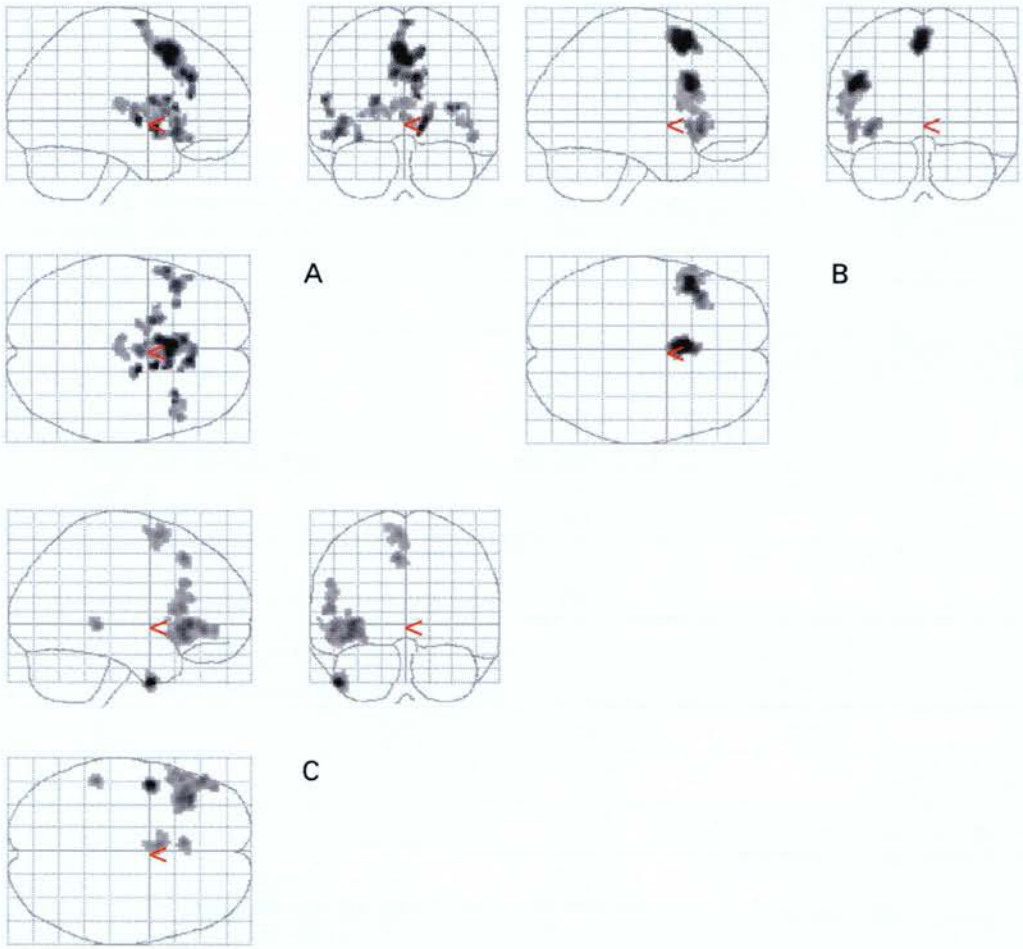

Fig. 3 Parametric: within-groups analysis. (**A**) Controls (n = 21); (**B**) high risk without symptoms (n = 42); (**C**) high risk with symptoms (n = 27). Activations displayed on 3D 'glass brain', maps thresholded at $P < 0.001$ uncorrected voxel level, extent threshold 50 voxels.

lobule in high risk with symptoms versus controls, and versus the high risk without symptoms, but not between controls and high risk without symptoms (x = –42, y = –50, z = 48; $P <$ 0.001).

The analysis with respect to degree of genetic risk indicated no significant differences in the parietal lobe (see Table 6). The analysis examining linear trends across the three groups, however, suggested that parietal lobe activity was lowest in the controls, followed by the high risk without symptoms, and greatest in high risk with symptoms (x = –42, y = –50, z = 48; P = 0.019). There were no significant findings for the reverse contrast.

Parametric analysis

For the parametric contrast, each group presented two main regions of increasing activation with increasing task difficulty: the left superior/medial frontal gyrus (BA 6) and the left inferior frontal gyrus (BA 47) (see Fig. 3A–C).

Analysis of group differences for the parametric contrast revealed significantly greater increases in activation with increasing task difficulty in the controls compared with the high-risk group as a whole in several regions: one involving the right medial frontal gyrus and to a lesser extent the anterior cingulate gyrus (x = 14, y = 47, z = –1; P = 0.033), another in the left posterior lobe of the cerebellum (x = –2, y = –78, z = –11; P = 0.004) and finally in the thalamic nuclei (x = 8, y = –13, z = 6; P = 0.001) (Fig. 4A, B; Table 5). No other group differences were observed.

Further examination of these activation differences (using the model with the high-risk groups also split into those with first and second degree relatives) are presented in Table 7. The largest number of differences were seen between the controls and high-risk subjects without symptoms in several regions, including: the right medial frontal gyrus (x = 6, y = 53, z = 12; $P <$ 0.001) and left posterior lobe of the cerebellum (x = –14, y = –79, z = –22; P = 0.001). While we did not find thalamic differences at the standard threshold of P = 0.001 uncorrected, at a lower threshold of P = 0.005 uncorrected, differences also emerged between the controls and high-risk subjects without symptoms in this region (x = 8, y = –13, z = 10; $P <$ 0.001). Also at this lower threshold,

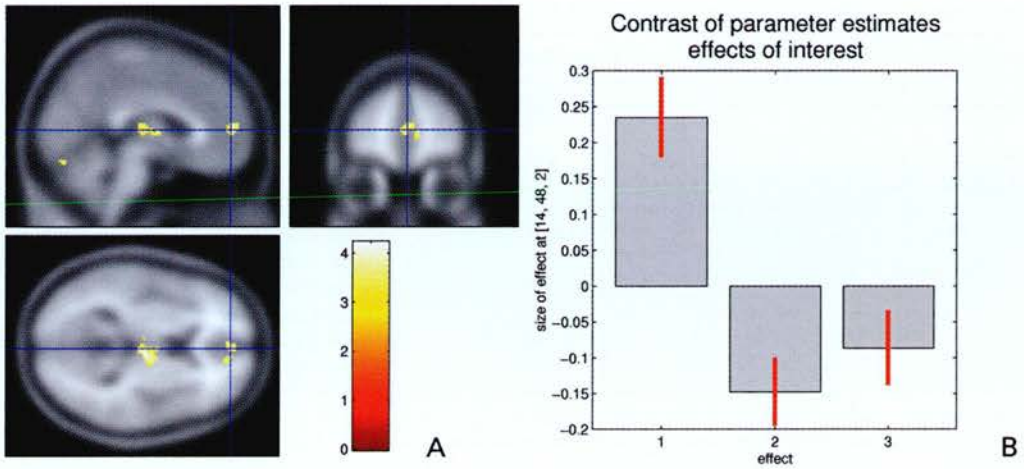

Fig. 4 Parametric: between group differences. (**A**) Group comparison showing greater increases in activation with task difficulty in controls versus all high-risk subjects in medial frontal, thalamic and cerebellar regions. Maps thresholded at $P < 0.001$ uncorrected voxel level, extent threshold 100 voxels. Colour bar represents Z score. (**B**) Effect size at peak co-ordinate for medial frontal region (1 = controls; 2 = high risk without symptoms; 3 = high risk with symptoms).

Table 7 *Random effects analysis: further examination of potential 'trait' related effects*

P-value	Extent	*Z*	Peak height (*x*, *y*, *z*)	Region
Parametric				
Controls (n = 21) > high risk without symptoms (n = 42)				
<0.001	819	4.95	6, 53, 12	Right frontal lobe: medial frontal gyrus BA10
			12, 49, 2	Right frontal lobe: medial frontal gyrus BA10/32
			–2, 42, 9	Left frontal lobe: anterior cingulate gyrus BA32
<0.001	675	4.79	–2, –42, 30	Left limbic lobe: cingulate gyrus BA31
			4, –53, 33	Right parietal lobe: precuneus BA31
			5, –39, 39	Right limbic lobe: cingulate gyrus BA31
0.001	308	4.08	–14, –79, –22	Left cerebellum: posterior lobe, declive
			–2, –83, –20	Left cerebellum
			–5, –63, –7	Left cerebellum: anterior lobe
0.008	211	4.02	0, 18, 40	Cingulate gyrus BA32
			0 –5, 48	Cingulate gyrus/ medial frontal gyrus BA6/24
0.005	236	4.01	38, –69, 20	Right temporal lobe: middle temporal gyrus
			45, –71, 35	Right parietal lobe: precuneus/angular gyrus
			37, –55, 39	Right parietal lobe: inferior parietal lobule
Controls (n = 21) > high risk with symptoms (n = 27)				
0.120	109	3.82	6, –14, 4	Right thalamus
High risk without symptoms (n = 42) > high risk with symptoms (n = 27): n/s				

n/s = not significant.

significant differences between controls and high risk with symptoms were seen in the thalamus ($x = 6$, $y = -14$, $z = 4$; $P < 0.001$) and left posterior lobe of the cerebellum ($x = -4$, $y = -81$, $z = -14$; $P = 0.033$). No differences were seen between the two high-risk groups at either statistical threshold. The masking procedure produced consistent findings, whereby regions of overlap were seen in controls versus high risk with symptoms and high risk without symptoms, but not between high risk with and without symptoms in the region of the cerebellum ($x = -4$, $y = -80$, $z = -8$; $P = 0.049$), and at a trend level, in the thalamus ($x = 8$, $y = -14$, $z = 4$; $P = 0.075$).

Table 8 presents analysis of the potential 'trait'-related effects with respect to degree of genetic risk. These results indicated greater increases in activation with increasing difficulty in those with first degree relatives versus those with second degree in the right medial frontal gyrus ($x = 16$, $y = 32$, $z = 40$; $P = 0.021$). No significant differences were observed for the reverse contrast. The analysis examining linear trends

Table 8 *Random effects analysis: further examination of 'trait' effects, analysis with respect to degree of risk*

P-value	Extent	*Z*	Peak height (*x*, *y*, *z*)	Region
Parametric				
High risk with first degree relatives (n = 48) > high risk with second degree relatives (n = 21)				
0.021	173	4.47	16, 32, 40	Right frontal lobe: medial/superior frontal gyrus BA8
			24, 32, 35	Right frontal lobe: middle frontal gyrus BA8/9
			21, 19, 30	Right frontal lobe
0.063	132	4.40	6, –42, 52	Right parietal lobe: paracentral lobule BA5/7
			5 –50, 60	Right parietal lobe: precuneus BA7
			16, –34, 43	Right parietal lobe: paracentral lobule
High risk with second degree relatives (n = 21) > high risk with first degree relatives (n = 48): n/s				

n/s = not significant.

across the three groups suggested thalamic increases in activity were greatest in the controls, followed by the high risk without symptoms, and lowest in high risk with symptoms (x = 6, y = –18, z = 6; P = 0.003). There were no significant findings for the reverse contrast.

Discussion

We used fMRI in combination with a sentence completion test to examine trait and state effects of brain activation in subjects at high risk of schizophrenia. This report presents the results as part of a study of these individuals over the time period at which they are at the greatest risk of becoming ill. Importantly, none of these subjects was considered ill at the time of testing; those referred to as high risk with psychotic symptoms had reported isolated or transient psychotic symptoms in the setting of unimpaired function. They did not meet diagnostic criteria for any psychiatric disorder, and all subjects were antipsychotic naïve at the time of investigation. We found that the high-risk subjects with isolated psychotic symptoms demonstrated significantly increased activation in the left inferior parietal lobule compared with controls and non-symptomatic high-risk subjects. The controls showed significantly greater task-related increases in activity in medial prefrontal, thalamic and cerebellar regions compared with the high-risk group as a whole.

The differences in brain activation patterns occurred against a background of closely similar task performance. This has important implications in relation to interpretation of the data. Many functional imaging studies examining patient populations (often medicated) reveal activation differences alongside performance differences. Thus it is difficult to determine whether activation differences are due to a core neuronal abnormality, or whether they are a secondary effect of poor performance (and/or medication). Functional imaging studies have often reported hypofrontality (in dorsolateral regions) in schizophrenic subjects, particularly with executive tasks in which the patients perform worse than controls. Studies that have controlled for task performance, however, suggest that hypofrontality may only become evident when performance fails (Frith *et al.*, 1995; Fletcher *et al.*, 1998). This is in agreement with the current findings, where we did not find evidence for decreased dorsolateral prefrontal activity in the presence of matched task performance. Others have suggested a more complex relationship between performance and either decreased or increased prefrontal cortex activation, particularly with reference to increasing working memory load (see Manoach, 2003).

An alternative explanation is that abnormal dorsolateral prefrontal activation may be specifically associated with deficits relating to the established illness. Results from other functional imaging studies of high-risk subjects are at present inconsistent. One study of clinically unaffected relatives reported increased activation of the right dorsolateral prefrontal cortex associated with increased risk in the absence of performance deficits during a working memory paradigm (Callicott *et al.*, 2003). This was reported to involve BA 9/10/46, and so would appear to be more lateral than the prefrontal region reported to be positively associated with increased familial risk in this study (high-risk subjects with first degree relatives versus second degree relatives; Table 8; BA 8). In contrast, other studies of unaffected relatives report no differences in prefrontal cortex activation during the Wisconsin Card Sorting Test (Berman *et al.*, 1992), reductions in anterior cingulate and left inferior prefrontal perfusion at rest (Blackwood *et al.*, 1999), or no differences in prefrontal activation during a verbal fluency test (Spence *et al.*, 2000). It is, however, important to consider, since schizophrenia is a disorder with a restricted age of onset, that the subjects in the four studies mentioned above were generally outside the period of maximum risk (mean ages 34, 32, 39 and 55 years, respectively, and including a number of individuals >40 years old), and were therefore unlikely to develop the disorder. Our younger high-risk subjects will therefore presumably be in different risk strata than these older

relatives, which may account for differences between these four studies and the current findings.

A matter that requires consideration is where our symptomatic high-risk subjects lie in relation to well, asymptomatic individuals and individuals with schizophrenia. These subjects did not have schizophrenia according to any diagnostic criteria. Their psychotic symptoms were fleeting and non-disabling, and their functioning good, but they do of course have an enhanced liability to the disorder. It has recently been reported that fleeting psychotic symptoms like these occur in ~10% of the normal population at some time (Verdoux and van Os, 2002). In this high-risk sample the occurrence lies nearer 40% (reported over the previous 18 months). Some of those 40% may still go on to develop schizophrenia, as it has been reported that the cases who do have often, but not always, had transient or partial psychotic symptoms prior to becoming ill (Johnstone *et al.*, 2000). We conclude that in this sample the presence of these transient or partial psychotic symptoms is indicative of a state of genetically induced vulnerability to schizophrenia that, in some cases, will translate into psychosis.

Although we did not find lateral prefrontal differences, medial prefrontal-thalamic-cerebellar regions of reduced activation were seen in our high-risk group as a whole compared with control subjects. More detailed group comparisons also indicated differences between controls and both high-risk groups (but not between the two high-risk groups) in thalamic and cerebellar regions (although some only became evident at lower thresholds). Medial prefrontal differences were, however, only observed between controls and high-risk subjects without symptoms. Similar results were also reflected in the masked analysis. It is interesting and unexpected that more differences were observed between the high risk without symptoms versus controls than between the high risk with symptoms versus controls. This may be due to the larger number of subjects in this group, giving greater statistical power, particularly in a contrast examining incremental differences associated with increasing task difficulty rather than in the simpler contrast of sentence completion versus rest. Nevertheless, as a whole, these results are consistent with findings that this group of regions may be dysfunctional in schizophrenia (Andreasen *et al.*, 1996), and in part with another study of unaffected relatives (Callicott *et al.*, 2003) that also reported decreased activation in medial frontal, thalamus and cerebellar regions in unaffected relatives. Our favoured interpretation of these findings is that as task difficulty increases, healthy controls are able to increase activation in a network of areas involving medial prefrontal-thalamic and cerebellar regions, but those at genetically enhanced risk of schizophrenia were less able to do so. We suggest that the presence of the schizophrenia genotype (i.e. the high-risk trait) is associated with a restricted ability to activate this network, but this may only have behavioural consequences on more demanding tasks where the deficit can not be compensated. Although we did not find increasing or decreasing degree of risk to be associated with activity in these specific regions within the high-risk group using the categorical measure of inheritance described, greater familial risk was found to be associated with increased activity in a region with the maxima located in the right medial/superior prefrontal region (BA 8). This region was slightly more superior and posterior to the medial prefrontal region referred to above (BA 10/32). It should also be considered that, although the finding of increasing activation with increasing task difficulty in the thalamus met criteria for a genetically mediated effect, the analysis examining linear differences across the groups suggested that this increased activation was greatest in the controls, followed by the high risk without symptoms, followed by high risk with symptoms. This indicates this effect may not be purely trait related, and that there may be some state modulation of trait effects with regards this finding.

Other studies of the Hayling sentence completion test in normal subjects have reported areas of activation similar to those reported here (Nathaniel-James *et al.*, 1997; Lawrie *et al.*, 2002). The study by Lawrie *et al.* (2002) compared schizophrenics ($n = 8$) and controls ($n = 10$), but did not report any functional localization differences between groups. However, Lawrie *et al.* (2002) examined medicated subjects with established schizophrenia who were not specifically selected to be at enhanced genetic risk of the disorder. Alternatively, the present, much larger, study was perhaps able to distinguish groups, because of increased statistical power.

We used the verbal initiation section of the Hayling test because it had previously been shown to demonstrate differences between schizophrenic and control subjects (in terms of functional connectivity; Lawrie *et al.*, 2002), and is considered to be a refinement of the verbal fluency test, which is commonly found to elicit functional imaging abnormalities in schizophrenia (Frith *et al.*, 1995; Yurgelun-Todd *et al.*, 1996; Curtis *et al.*, 1998; Spence *et al.*, 2000). PET studies of verbal fluency report a relative failure to deactivate the left superior temporal gyrus in schizophrenic subjects compared with controls (Frith *et al.*, 1995), while fMRI studies report decreased left dorsolateral prefrontal activation (Yurgelun-Todd *et al.*, 1996; Curtis *et al.*, 1998). Differences in scanning methodologies, particularly with respect to the requirement for covert responses in fMRI, may account for inconsistencies in temporal lobe activations (Curtis *et al.*, 1998). fMRI paradigms that involve overt speech in response to a stimulus can be problematical, since the associated movement can cause large image distortions. Indeed, one limitation of the current study is that, although we collected within-scanner measures of performance by way of reaction time, on-line task performance in terms of word appropriateness involved covert word generation. At the time the study began, novel paradigms that allow overt verbal responses cued in the gap between image acquisitions (see Henson *et al.*, 2002) were not readily available, or indeed widely used.

In the current study we did not find any activation differences in either the left dorsolateral prefrontal cortex or

left superior temporal cortical region, which is consistent with another study of relatives of schizophrenic subjects (Spence *et al.*, 2000). Rather, we found parietal lobe over-activation in symptomatic high-risk subjects. Further examination of this over-activation suggested that this was a state-related effect. Differences were found between controls and high-risk subjects with psychotic symptoms, and between the two high-risk groups, but not between controls and asymptomatic high-risk subjects, even at more lenient thresholds. These results were also confirmed using the masked analysis. However, although the parietal lobe hyperactivity fulfilled criteria for state-specificity, the analysis regarding linear differences across the three groups suggested that the parietal activation was greatest in the high risk with symptoms, followed by high risk without symptoms, followed by controls. Hence there may also be an interaction between with genetically mediated and symptom-related effects in this region.

The majority of imaging studies in schizophrenia have focused on prefrontal and temporal brain abnormalities, but there are a number that report abnormalities of parietal lobe regions. In an early study investigating the relationship between hippocampal pathology and prefrontal hypofunction in monozygotic twins discordant for schizophrenia, there was an inverse relationship between hippocampal volume and activation in the parietal cortex in the affected twin group (Weinberger *et al.*, 1992). In a more recent study the earliest structural deficit (associated with early onset schizophrenia) was reported to occur in the parietal lobe, which only later progressed to involve frontal and temporal lobes (Thompson *et al.*, 2001). Several other functional imaging studies have reported increased activation in parietal lobe regions in schizophrenic subjects compared with controls during a variety of paradigms, including memory tasks (Crespo-Facorro *et al.*, 1999) and verbal fluency (Curtis *et al.*, 1998), decision making (Paulus *et al.*, 2002), and in a study of voluntary movements in patients with symptoms specifically attributed to self-monitoring failures (Spence *et al.*, 1997).

One interpretation of the relative over-activation of the parietal lobe in our symptomatic high-risk subjects is that this region may be recruited to enable them to perform the task at a similar level to the other groups. Since we did not find that activation in the left intraparietal area was linearly related to task difficulty, we suggest that the relative over-activation in this region represents a general dysfunction in those at high risk with psychotic symptoms. In other words, the presence of the early symptomatic state is associated with a compensatory over-activation of the parietal lobe at all levels of task engagement. The intraparietal area is considered to be involved in attentional maintenance (Corbetta *et al.*, 2000; Hopfinger *et al.*, 2000), response preparation (Snyder *et al.*, 1997) and response monitoring (Garavan *et al.*, 1999; Menon *et al.*, 2001), functions that have been shown to be abnormal in schizophrenia (Frith, 1992; Schatz, 1998). A compensatory additional activation in the parietal lobe in the high-risk subjects with symptoms may therefore be related to attentional aspects of the task and the preparation and monitoring of suitable responses. This interpretation is consistent with reports that subjects in the prodrome to schizophrenia commonly report difficulties focusing attention and a reduced sense of control of behaviour (McGhie and Chapman, 1961; Klosterkötter *et al.*, 1997). Alternatively, regions of the posterior parietal cortex have been implicated in the role of distinguishing between self and others (Meltzoff and Decety, 2003). Hyperactivity in this region in our symptomatic high-risk subjects may therefore suggest deficits of this neural system. Misattribution of internally generated actions as being externally generated are considered to be a potential neurophysiological basis of positive symptomatology. Indeed, findings of hyperactivity in parietal areas in subjects with the established illness experiencing passivity phenomena are consistent with this hypothesis (Spence *et al.*, 1997). Regardless of the origin of this abnormality, a relative over-activation of the parietal lobe could represent one of the earliest pathological changes by which the trait of high genetic risk of schizophrenia switches to the state of incipient psychosis.

It is conceivable that the differences in male:female ratios across the groups could contribute to the reported findings, especially since there is evidence to suggest gender differences in brain activation during language-based tasks (Shaywitz *et al.*, 1995). However, there were no statistically significant differences in gender between the groups; furthermore, we were unable to demonstrate differences in activation due to gender at the second level.

Overall, these results indicate that there are state and trait features of schizophrenia that can be demonstrated with functional imaging. Group differences of apparent genetic cause were found in medial prefrontal-thalamic-cerebellar regions, and differences were found in subjects at high risk with isolated psychotic symptoms in the parietal lobe. These patterns of activation reveal information about the pathophysiology of the state of vulnerability to schizophrenia, and about the mechanisms involved in the development of schizophrenic symptoms.

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