AN *IN VIVO* INVESTIGATION OF NEURONAL FUNCTION
IN FIRST EPISODE SCHIZOPHRENIA:
THE EFFECTS OF RISPERIDONE
ON PATTERNS OF CEREBRAL METABOLISM AND SYMPTOM PROFILES.

By

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ABSTRACT

Risperidone is a novel-atypical antipsychotic agent, effective in treating the symptoms of schizophrenia with fewer side effects than typical antipsychotics. Despite the wide-spread clinical use of this drug, studies examining the brain areas associated with risperidone treatment in human subjects are limited. Positron emission tomography (PET) employing the tracer fluorodeoxyglucose (FDG) labeled with the isotope ^{18}F, is an *in-vivo* imaging technique that can be used to observe changes in the patterns of cerebral metabolism following administration of typical and atypical antipsychotics. We completed a series of studies in first-episode unmedicated schizophrenic subjects before and after an initial 2mg dose of risperidone and again following 6 weeks of treatment to study the effects of treatment on patterns of cerebral metabolism. We also examined the correlations between changes in regional metabolism and changes in symptom severity. This is the first reported study of such relationships in first episode patients. We found that reductions in temporal metabolism were correlated with alleviation of delusions and hallucinations, while there was a strong trend for reduction in medial frontal metabolism to be correlated with reduction in disorganization symptoms. Correlations between successful treatment (indicated by reduced symptoms) and patterns of neuronal activity, provided a biochemical measurement of symptom improvement.

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LIST OF ABBREVIATIONS

Alpha 1 (α_1)   The rate constant representing the rate of loss of radioactive tracer from the metabolic compartment.

DA   Dopamine

D_2 Dopamine 2 receptor

DSM-IV   Diagnostic and statistical manual (version 4)

DSM III-R   Diagnostic and statistic manual (version 3 revised)

DLPFC   Dorso-lateral prefrontal cortex

FDG   Fluorine deoxyglucose

FOV   Field of view

GAS   Global assessment scale

Gpe   Globus palidus externa

Gpi   Globus pallidis interna

FWHM   Full width at half maximum

MRI   Magnetic resonance imaging

PET   Positron emission tomography

rCBF   Regional cerebral blood flow

SPECT   Single photon emission computerized tomography

SPM96   Statistical parametric mapping 1996

SAPS   Scale for assessment of negative symptoms

SANS   Scale for assessment of positive symptoms

SCID   Structures clinical instrument of the DSM-IIIR

s.d.   Standard deviation

SPGR   Spoiled gradient MRI pulse sequence

ν Degrees of freedom

VTA   Ventral tegmental area

z   Standardized score with a mean of zero and a standard deviation of 1

z_t Threshold value for z

^{18}F Radioactive fluorine 18 a positron emitter

$5HT_2$ Serotonin 2 receptor

ACKNOWLEDGEMENTS

PREFACE

This thesis presented by Ms CMJ Lane, entitled 'An in vivo investigation of neuronal function in first episode schizophrenia: the effects of risperidone on patterns of cerebral metabolism and symptom profiles,' contains material from five jointly authored manuscripts that report the findings of a series of studies for which the senior investigator was Dr Peter F Liddle. In accord with the requirement of the regulations of the Faculty of Graduate Studies for the preparation of theses, this preface provides a summary of the main contributions of each of the authors of these manuscripts.

Chapter 2. Immediate effects of risperidone on cortico-striato-thalamic loops and the hippocampus. Manuscript by Liddle PF, Lane CMJ, Ngan ETC.

Liddle PF: Development of the theory that is tested in this study; supervision of study design, data collection and analysis; writing of manuscript.

Lane CMJ: Participation in design of PET scanning procedure; collaboration with ETN in recruitment of patients, principal person responsible for PET scanning sessions; execution of data analysis; re-writing the manuscript under the supervision of PFL, to make it compatible in style with remainder of thesis.

Ngan ETC: Participation in design of PET scanning procedure; collaboration with CMJL in recruitment of patients; assessment of symptoms; clinician responsible for patients throughout the study.

Chapter 3. Immediate and delayed effects of risperidone on cerebral metabolism: correlations with symptom change. Manuscript by Ngan ETC, Lane CMJ, Ruth TJ and Liddle PF.

Ngan ETC: Participation in overall design of study and in design of PET scanning procedure; collaboration with CMJL in recruitment of patients (same subjects as for Ch 2); assessment of symptoms; clinician responsible for patients throughout the study; development of some of the data analysis procedures; writing manuscript for publication.

Lane CMJ: Participation in design of PET scanning procedure; collaboration with ETN in recruitment of patients (same subjects as for Ch 2), principal person responsible for PET scanning sessions; execution of data analysis; re-writing the manuscript under the supervision of PFL to make it compatible in style with remainder of thesis.

Ruth TJ: Overall supervision of PET procedures.

Liddle PF: Overall design of the study in collaboration with TC Ngan; supervision of data collection, analysis, and preparation of manuscript.

Chapter 4: Effects of risperidone on cerebral activity in healthy subjects. Manuscript by Lane CMJ, Ngan ETC, Yatham L, Ruth TJ, Liddle PF,

Lane CMJ: Participation in design of PET scanning procedure; recruitment and assessment of subjects, principal person responsible for PET scanning sessions; execution of data analysis; writing of manuscript for publication and re-writing under the supervision of PFL to make style compatible with remainder of thesis.

Ngan ETC: Physician responsible for subjects during scanning sessions.

Yatham L: Design of study (in collaboration with PFL),

Ruth TJ: Overall supervision of PET procedures.

Liddle PF: Design of study (in collaboration with LY), supervision of data collection, analysis, and preparation of manuscript.

Appendix 1: The two scan procedure: allowing for residual radioactivity. Manuscript by: Liddle PF, Lane CMJ, Ngan ETC.

Liddle PF: Design of the study and mathematical modeling; supervision of data collection and analysis; writing of manuscript.

Lane CMJ: Participation in design of PET scanning procedure; recruitment and assessment of subjects (same subjects as chapters 2, 3 and 4), principal person responsible for PET scanning sessions; execution of data analysis.

Ngan ETC: Physician responsible for subjects during scanning sessions; development of spread sheet for calculation of residual radioactivity based on mathematical model developed by PFL.

Appendix 2: Evaluation of spatial normalization. Manuscript by Lane CMJ, Atkins S, and Liddle PF. Accepted for presentation at the World Brain Imaging & Registration Workshop, Bled, Slovenia August 1999. Accepted for publication in "Proceeding of the WBIR Workshop".

Lane CMJ: Participation in design of PET scanning procedure; recruitment and assessment of subjects (same subjects as chapters 2 and 3), principal person responsible for PET scanning sessions; execution of data analysis; writing of manuscript for publication and re-writing to make style compatible with remainder of thesis.

Atkins S: Discussion and partial supervision of writing of manuscript.

Liddle PF: Development of the concept of voxel concordance as a measure of adequacy of spatial normalization; design of the study and writing of software for computing voxel concordance; supervision of data collection, analysis and preparation of manuscript.

Peter F Liddle
Jack Bell Professor of Psychiatry
June 19,1999

I.   INTRODUCTION

Antipsychotic agents influence various neurotransmitter systems including dopamine. Despite an understanding of how these agents block specific receptors and the time scale upon which this occurs, the mechanism by which these drugs produce changes in cerebral function, and how this leads to an eventual alleviation of schizophrenic symptoms, remains unknown. In this thesis we examine the changes in brain function brought about by treatment with the antipsychotic risperidone. In particular we explore the time course of changes and the relationship between the observed changes in particular regions of the brain and the changes in severity of specific groups of symptoms.

The symptoms of schizophrenia

The diverse symptoms of schizophrenia include disorganized thought, delusions, hallucinations, poverty of speech, flattened affect, apathy and social withdrawal. Despite 100 years of study, the classification of schizophrenic symptoms is still a subject of debate. Many investigators have proposed that symptoms should be divided into positive and negative symptoms. Perhaps the first discussion of this distinction was by Reynolds (review by Berrios 1985) who defined positive symptoms as those that entailed the presence of abnormal activity and negative symptoms as those that reflected the absence or diminution of normal mental activity. This dichotomy provided a basis for Crow (1980) who proposed that two pathological processes occur in schizophrenia, each leading to a distinct group of symptoms. The type 1 process is expressed as positive symptoms, such as delusions, hallucinations and formal thought disorder, which are usually transient. Crow proposed that these symptoms arise from dopamine imbalance. The type 2 process generates negative symptoms, such as poverty of speech, blunted affect and apathy. These symptoms tend to be chronic. Crow proposed that these symptoms arise from structural brain damage.

The relationships between symptoms however, are more complex than can be described by a simple dichotomy. On the basis of factor analysis, many investigators have concluded that there are at least three distinguishable clusters of symptoms (Liddle 1984, 1987; Bilder et al., 1985; Liddle & Barnes 1990; Mortimer et al., 1990; Peralta et al., 1992; Arndt et al., 1991). In a study of 40 chronic schizophrenic subjects, Liddle (1984,1987) found the symptoms of schizophrenia to segregate into three distinct groups: psychomotor poverty (flat affect, poverty of speech, decreased spontaneous movement), disorganization (formal thought disorder, distractibility, inappropriate affect), and reality distortion (delusions and hallucinations). Other studies in both chronic and acute patients have also found these symptoms to cluster, although discrepancies concerning which symptoms factor into a given syndrome remains under debate. For example Bilder et al., (1985) found poverty of speech to be more closely related to the disorganization syndrome than to the psychomotor poverty syndrome as identified by Liddle (1984, 1987).

Mechanism of antipsychotic action

According to the dopamine hypothesis, schizophrenic symptoms arise from overactivity of the dopamine system (Matthysse 1973). This hypothesis arose from speculations by Carlsson and Lindqvist (1963) that neuroleptics from various classes were blocking the then yet unconfirmed

dopamine receptors. Once the Dopamine D2 receptor had been identified, a high correlation between the concentration of a drug needed to block dopamine D2 receptors in vitro and the daily dose necessary to control psychosis was established (Seeman 1992). Further evidence in support of the dopamine hypothesis came from the observation that stimulants such as amphetamine evoked schizophrenia-like psychotic symptoms in healthy subjects (Connell 1958, Bell 1965), and aggravated psychotic symptoms in schizophrenic subjects (Angrist 1987). Amphetamine increases extracellular catecholamines including dopamine (Snyder 1972). Indeed all antipsychotics proven in clinical double blind trials to be effective in the treatment of schizophrenia block dopamine D2 receptors.

Despite proven efficacy, D2 blockers are characterized by numerous side effects and research into atypical antipsychotics such as clozapine with a high 5HT2 and low D2 binding potential demonstrated their clinical efficacy and lower propensity for inducing side effects. Unfortunately, clozapine is prone to agranulocytosis and requires weekly blood testing which is not feasible for many schizophrenic patients. Therefore, research into other therapeutic agents based on other neurotransmitters, such as serotonin, is continuing, to determine therapeutic agents with reduced side effects.

Studies of dopamine metabolites, receptors and endogenous dopamine, have been inconclusive. Higher brain levels of dopamine receptors and or metabolites have been found in some post mortem studies of schizophrenic subjects. However, these results are inconsistent (Davis et al., 1991), and are difficult to interpret given that antipsychotic medications up-regulate dopamine receptors, at least in animals (Seeman 1976). In-vivo receptor studies in neuroleptic naïve schizophrenic subjects using both PET and SPECT have also been inconclusive showing either increased or unchanged receptor density, though differences in ligand may be responsible for the reported differences (Farde et al., 1990, Wong et al., 1986, Seeman 1987).

SPECT studies indicate that there might be an increased release of endogenous dopamine in response to amphetamine challenge in schizophrenic subjects. In these studies (Laruelle et al., 1996) the amount of endogenous dopamine released is inferred from the displacement of the ligand ^{123}I-IBZM from the D2 dopamine receptor. There is still uncertainty regarding the interpretation of the displacement of the ligand because the magnitude of the effects are less than would be predicted on the basis of microdialysis studies of endogenous dopamine release in animal models (Moghaddam 1990). Not withstanding this uncertainty, the magnitude of the displacement of ^{123}I-IBZM following amphetamine challenge is strongly correlated with the severity of transient induced psychotic symptoms (Laruelle et al., 1996).

Despite the relative success of D2 blockers as antipsychotics, there are two main caveats to the dopamine hypothesis. First, although dopamine receptor blockade occurs immediately (Seeman 1992), in treatment trials, the alleviation of symptoms is not statistically significant until several weeks after the beginning of treatment (Hirsh & Barnes 1995). This observation suggests that a slowly developing still unknown homeostatic change in the brain is the mechanism of action for a drugs antipsychotic effect. An example of such a delayed effect is that the dopamine neurons decrease their firing rate after long term, but not short term, administration of D2 antagonists, an effect referred to as depolarization blockade (Aghajanian & Bunney 1974). A second limitation of the dopamine hypothesis is that schizophrenic symptoms can persist despite a high level of D2 Blockade (Wolkin et al., 1989). This suggests a mechanism other than dopamine

transmission may be involved in the generation of schizophrenic symptoms. Indeed other neurotransmitters have been implicated in the generation of psychotic symptoms.

In addition to changes in neurochemistry, schizophrenia is characterized by a number of subtle structural and functional brain differences occurring in both cortical and subcortical regions. At the gross anatomical level, schizophrenic brains show abnormalities involving shrinking of the cortical gyri, and an enlargement of the third and fourth ventricles (Shelton and Weinberger 1987; Ross and Pearlson, 1996). Microscopic abnormalities are also found in cortical and subcortical areas in various brain areas (Falkai & Bogerts 1994). For example microscopic decreases in dendritic spines and interneurons are found in the frontal cortex of schizophrenic post mortum tissue (Benes 1992). Some, but not all, functional imaging studies have found decreased frontal blood flow or metabolism in schizophrenia (Andreasen et al., in review 1992) Other functional imaging studies have reported correlations between severity of schizophrenic symptoms and abnormal regional blood flow in a variety of different cerebral areas (e.g. Liddle et al., 1992; Ebmeier et al., 1993; Yuasa et al., 1995; Silbersweig et al., 1995).

These various changes in neurochemistry as well as in brain structure and function, have lead some researchers to propose that schizophrenia results not from a single, localized abnormality, but by the dysfunction of broadly distributed circuitry which may result from a dysfunction of cortical regulation of subcortical dopamine neurotransmission (Weinberger 1987; Robbins 1990; Davis et al., 1991; Grace 1991).

Figure 1 shows the neuronal circuitry connecting the basal ganglia with other brain regions. Carlsson and Carlsson (1990) emphasized the inhibitory influence of the striatum on the thalamus and thus interpreted the cortico-striato-thalamocortico pathways as feed back loops. There are two pathways in the cortico-striato-thalamocortico circuit, the direct and indirect. These pathways are modulated by dopamine glutamate, GABA and serotonin.

1) Dopamine effects on the direct and indirect pathway

Dopamine exhibits both an excitatory and inhibitory influence on the direct and indirect pathways. This allows dopamine to serve as a regulator of the balance of negative and positive feedback in the striatum. A low dopaminergic tone would favor negative feedback and a high tone, positive feedback (Carlsson 1995).

2) Dopamine/Glutamate interaction

Glutamate is excitatory on both pathways, and removal of excitatory glutamatergic tone, acting on striatal cells, causes behavioral stimulation. Glutamate and dopamine can antagonize each other in the striatum (Carlsson 1995).

Glutamate plays a major role in transmission between cortex and basal ganglia and action of glutamate receptor (NMDA) antagonists such as phencyclidine (PCP) and ketamine can mimic schizophrenic symptoms such as auditory hallucinations and disorganization (Lahti et al., 1995). Both PCP and ketamine bind to the non competitive binding site on the NMDA receptor. This might suggest that enhanced glutamate function would be therapeutic in the treatment of schizophrenia; however, in practice such treatment is potentially problematic due to the cytotoxic and convulsant effects of excitatory neurotransmitters such as glutamate.

Figure 1: Neurocircuitry of the cortico-striato-thalamic tracts
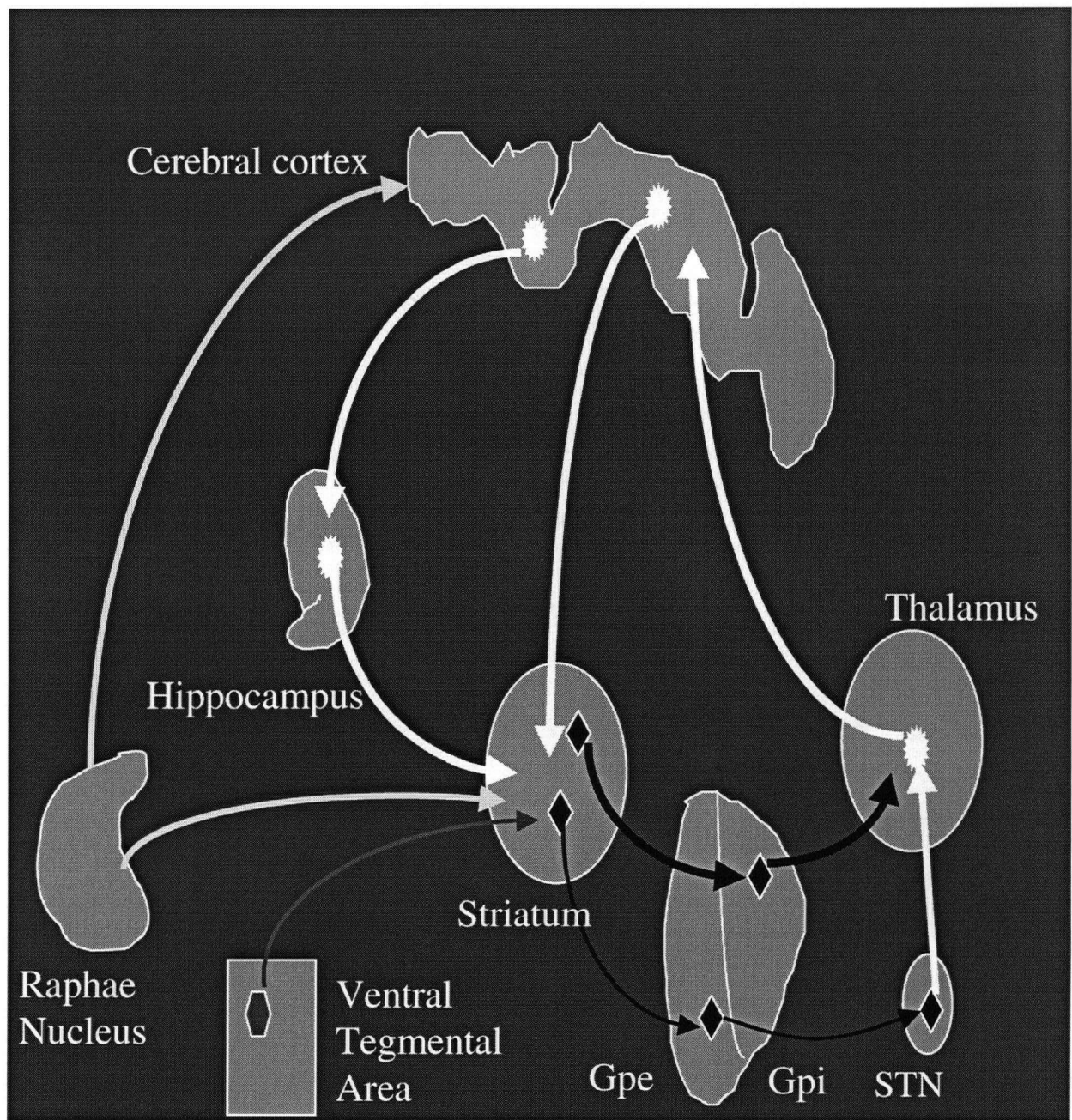
Glutamine fibers (excitatory) - white; GABA fibers (inhibitory) - black, thick lines represent the direct while thin lines represent the indirect projections; Dopamine fibers (excitatory and inhibitory) - red; Serotonergic fibers (inhibitory) - yellow. STN subthalamic nucleus.

3) GABA

GABA plays an inhibitory role in both the direct and indirect circuits. The effects of GABAergic agent would depend on which pathway was dominant in a particular situation. Furthermore, GABA plays a role in regulation of dopamine release (Santiago et al., 1993). It is possible that a deficiency in GABAergic function in schizophrenia could result in excessive dopaminergic activity (Dewey et al., 1997).

4) 5HT/DA interaction

There is convincing evidence that serotonergic projections inhibits dopamine function at two levels: at the level of the midbrain they inhibit the firing of the dopamine cells projecting from the substantia nigra, and in the striatum and cortex they inhibit the synaptic release of dopamine and probably the synthesis of dopamine. As a result the serotonergic agonists, serotonin precursors, and SSRI's enhance the inhibition of the dopamine system. Conversely, lesions of the raphae nuclei, 5HT1a agonists (through the action of autoreceptors) and 5HT2 antagonists disinhibit the dopamine system (Kapur & Remmington 1996 in review). This disinhibition of the dopamine system has been suggested to be one mechanism by which EPS are reduced in patients treated with 5HT2/D2 drugs such as risperidone despite a high dopamine D2 receptor blockade.

Serotonin dysfunction has also been implicated in the pathogenesis of schizophrenic symptoms. Wooley and Shaw (1954) were the first to hypothesize that schizophrenia might result from changes in serotonergic function in the CNS. This was based on the fact that 5HT2 receptor agonist such as lysergic acid diethylamide (LSD) induces psychosis. Though inconclusive, studies reporting serotonin (5HT2) receptor decreases in frontal cortex in schizophrenia also point to possible serotonergic abnormalities in schizophrenia (Mita et al., 1986; Laruelle et al., 1993).

The evidence that we have considered above indicates that characteristic positive schizophrenia-like symptoms may be evoked following exposure to various drugs which affect the dopamine, glutamate, and serotonin systems. Therefore, while striatal DA overactivity is strongly implicated in the generation of psychotic symptoms, it is probable that other transmitters that modulate cortical and basal ganglion function play a role.

The role of cortex and subcortical nuclei in the pathogenesis of schizophrenic symptoms might be elucidated in functional imaging studies of schizophrenic subjects following treatment with antipsychotics. Previous studies (Wolkin et al., 1985; De Lisi et al., 1985; Buchsbaum et al., 1987, 1992a, 1992b and 1992c; Bartlett et al., 1991; Potkin et al., 1994; see Liddle 1999 for review) of antipsychotic effects on neuronal activity have employed typical agents such as haloperidol in chronic schizophrenic subjects. The most commonly reported effect of sustained treatment of typical antipsychotic agents on patterns of neuronal activity in chronic schizophrenic subjects is a diffuse decrease in frontal activity and an increase in activity within the basal ganglia.

At least some of these changes are likely related to non-therapeutic effects. Typical antipsychotics (e.g. haloperidol) induce a variety of extrapyramidal side effects (EPS) including

parkinsonism, akinesia and akathisia. The observation that the dorsal striatum is heavily involved in motor activity, while ventral striatum is connected to prefrontal cortex and limbic cortex, which are more strongly implicated in cognitive and emotional processes (Alexander & Delong 1986), suggests the dorsal D2 blockade might mainly lead to EPS, while ventral D2 blockade might be more directly related to antipsychotic effect. The observation that typical antipsychotics induce immediate early genes such as C-fos in dorsal and ventral striatum, while atypical antipsychotics induce C-fos in ventral striatum and prefrontal cortex (Robertson et al., 1994) suggests that one of the cardinal sites involved in antipsychotic action is the ventral striatum.

This is consistent with the observation by Liddle et al., (1992), and supported by Silbersweig et al., (1995), that the expression of delusions and hallucinations is associated with increased cerebral blood flow in ventral striatum. Liddle et al., and Silbersweig et al., also observed that the expression of hallucinations is associated with increased cerebral blood flow in medial temporal lobe and various other cortical sites. This suggests that the mechanism by which dopamine blockers might exert an antipsychotic effect involves modulation of transmission in the cortico-striato-thalamic circuits that regulate cortical function.

However, several lines of evidence indicate that the action of antipsychotics is unlikely to merely be a matter of altering the gain in the feedback in the cortico-striato-thalamic feedback circuit. First, it is relatively common for delusions and/or hallucinations to persist even after the acute overarousal associated with an acute psychotic episode has responded to treatment with dopamine blockers. Furthermore, in patients with psychotic symptoms that persist despite treatment with high doses of antipsychotics, PET studies confirm that dopamine D2 receptors in the striatum are virtually fully blocked (Wolkin et al., 1989). Thus, it is likely that some process in addition to dopamine blockade in the ventral striatum is required for the alleviation of delusions and hallucinations.

In a theory outlined in chapter 2, Liddle et al., have proposed that there are two principle pathological processes that contribute to the generation of delusions and hallucinations. Over-activity in the cortico-striatal-thalamic circuits creates a predisposition to the full gamut of acute psychotic symptoms (including delusions and hallucinations), while the cardinal event in the generation of delusion and hallucinations is aberrant firing of the hippocampus. According to this theory, treatment with antipsychotic medication should produce an immediate reduction in activity in the cortico-striatal-thalamic circuit in acutely psychotic patients. Furthermore, there will be a reduction in hippocampal activity that might vary between patients, and the degree of this reduction will predict the subsequent degree of resolution of delusions and hallucinations.

However, actual expression of psychotic symptoms involves over-activity at multiple cerebral sites, as discussed above. The areas implicated in delusions and hallucination include not only ventral striatum and hippocampus, but also extensive areas of temporal and frontal cortex. For example, Liddle et al., (1992) found that reality distortion (delusions and hallucinations) is associated with over-activity of left parahippocampal gyrus and hippocampus as well as left temporal pole. In a SPECT study, Silbersweig et al., (1995) found that auditor hallucinations were associated with over-activity in hippocampus, parahippocampal gyrus and medial frontal cortex.

Several SPECT studies have examined the pattern rCBF (regional cerebral blood flow) associated with hallucinations (Musalek et al., 1989; McGuire et al., 1993). Musalek et al., found that patients with hallucinations had increased rCBF in medial temporal lobe, compared with that in control subjects. In a longitudinal study of patients who were scanned while suffering from hallucinations and again after the resolution of the hallucinations, McGuire et al., found the first principal component of the change in rCBF between the first and second scans loaded positively on a set of cerebral sites that included left medial temporal lobe, anterior cingulate and left lateral frontal cortex. This finding indicates that the experience of hallucinations is associated with overactivity in a distributed circuit that includes the left medial temporal cortex.

Furthermore, several studies have found that symptoms of disorganization (formal thought disorder; inappropriate affect) are associated with over-activity of right medial frontal cortex. Liddle et al., (1992) found that disorganization was associated with increased rCBF in right medial frontal cortex and thalamus, and also with decreased rCBF in right ventral striatum and contiguous insula. Using SPECT, Ebmeier et al., (1993) and also Yuasa et al., (1995) found that disorganization was correlated with increased rCBF in right medial frontal cortex.

Thus, it would be expected that successful antipsychotic treatment would result in a decrease in activity in temporal and frontal cortex. In particular, we would predict that the magnitude of decrease in activity in left medial temporal lobe and temporal pole occurring during treatment would be correlated with the degree of decrease in reality distortion symptoms while the magnitude of decrease in activity in right medial frontal lobe occurring during treatment would be correlated with the degree of decrease in disorganization symptoms.

In the event that the predicted changes in right medial frontal cortex and left temporal lobe were to be observed, this observation would raise the question of whether the change in activity preceded symptom resolution, as would be expected if the change in cerebral activity were an essential component mechanism of antipsychotic action, or alternatively, the changes in activity developed as a consequence of symptom resolution. This question might be addressed by measuring cerebral metabolism in the regions of interest immediately after the first dose of antipsychotic before any substantial reduction in symptoms would be expected, and again after an adequate course of treatment. If the cerebral changes were detectable immediately after the first dose, this would support the hypothesis that the changes in metabolism were an essential component of the mechanism of antipsychotic action, rather than being a consequence of the alleviation of symptoms.

Effects of risperidone on cerebral activity in schizophrenic subjects

This series of studies was designed to investigate the mechanism of action of antipsychotic drugs by studying the changes in regional cerebral metabolism produced by the atypical antipsychotic drug, risperidone, in first episode, previously un-medicated schizophrenic patients under single blind conditions.

To identify the immediate changes in cerebral activity that precedes symptom change, we scanned schizophrenic patients immediately before treatment and again 90 minutes after the first dose of risperidone. A 90 minute period was selected based on studies of risperidone plasma levels (Janssen-Ortho Inc, 1998) and studies of the temporal effects of antipsychotic agents on

dopamine D2 binding potential (Nyberg et al., 1993). To identify the additional changes that occur as symptoms resolve, we performed a third scan after 6 weeks of treatment.

The hypotheses that we tested were:
- That antipsychotic medication produces an immediate reduction in activity in the cortico-striatal-thalamic loops that play a role in the regulation of cortical activity.
- That antipsychotic medication produces an immediate decrease in hippocampal activity that predicts subsequent resolution of delusions and hallucinations.
- That after six weeks treatment, there will be decreases in metabolism in medial frontal cortex and in the left medial and anterior temporal lobe.
- The magnitude of change in left medial temporal lobe and temporal pole will be correlated with the change in reality distortion score.
- The magnitude of change in right medial frontal lobe will be correlated with the change in disorganization score.
- Significant reductions in metabolism in right medial temporal lobe and left anterior and medial temporal lobe, will be discernible immediately after the first dose of risperidone.

We also performed exploratory analyses to address the following questions:
- Is 6 weeks of treatment associated with decreased metabolism in cerebral areas other than those areas specified in our hypotheses (i.e. ventral striatum, thalamus, right medial frontal cortex and left lateral temporal lobe?)
- Do such changes correlate with changes in severity of symptoms?
- Are changes discernible immediately after the first dose of risperidone, in the vicinity of the changes detected after 6 weeks treatment.

Effects of risperidone on cerebral activity in healthy subjects

To clarify the interpretation of our findings in schizophrenic patients, we also performed a study of the effect of a single dose of risperidone on cerebral activity in healthy subjects. Healthy subjects were scanned twice, one week apart under double blind randomized conditions 90 minutes following either a placebo or a 2mg dose of risperidone.

In the study of healthy subjects we tested the following hypothesis:
- Changes in cerebral activity would be seen in those cerebral areas where the schizophrenic patients exhibited changes that were unrelated to symptomatic improvement.

We also performed an exploratory analysis to identify changes occurring in any other cerebral areas in healthy subjects. In particular, we performed a directed search in those regions where the patients exhibited changes associated with symptomatic improvement, in order to determine whether or not there was any evidence of cerebral change in healthy subjects in these areas. Such changes would support the hypothesis that the changes underlying the therapeutic effects arise from normal pharmacological actions of risperidone, whereas the absence of such changes in healthy subjects would suggest that the therapeutic action arises from pharmacological effects that occur only in patients.

Selection of patients

Selection of patients is a crucial issue because there are several factors that might lead to differences in the effects of treatment on brain activity at different phases of the illness. Patient selection is likely to be a significant contributor to the discrepancies (Andreasen et al., 1992) between earlier brain imaging research in schizophrenia. There are at least three factors related to patient selection that might account for discrepancies in the brain imaging literature:

1) Possible progression of illness

The issue of whether or not schizophrenia is a progressive disease, as implied by Kraepelin (1896) when he initially named the disorder Dementia Praecox, remains a subject of debate. One hypothesis is that schizophrenia is due to abnormal neuronal development which leads to a clinical manifestation of symptoms in early adult life. Some studies indicate that brain abnormalities are present at the onset of illness and might reflect abnormal early development (Weinberger 1993). Nonetheless, there is also evidence for progressive brain abnormality. For example, several studies have reported an increase in ventricular size as the illness progresses, and that ventricular enlargement is associated with poor response to treatment (De Lisi et al., 1994). Therefore, the nature of the cerebral response to treatment might change as the illness develops, and it is likely that the cerebral response to treatment will be more homogeneous in a group of patients who are all at a similar stage in the progression of their illness.

2) Selection artifact

Whether or not schizophrenia usually progresses, cases recruited at an advanced stage in the illness are likely to include an over-representation of treatment non-responsive cases. Poorly responsive cases are likely to require higher doses of medication, increasing the risk of side effects and the cerebral changes associated these side effects. Conversely, a sample of patients recruited at an early phase of illness is likely to include a greater proportion of treatment responsive cases. Keeping in mind that schizophrenia may be progressive in nature, older subjects with previous episodes and treatment are less likely to respond to medication than first episode cases (DeLisi et al., 1994; Weinberger et al., 1993). First episode cases therefore, would generally be more suitable for a study of cerebral changes associated with response to antipsychotic treatment.

3) Prior medication might produce confounding changes in brain activity.

Prior medication might result in adaptive changes such as up-regulation of neurotransmitter receptors, that might alter the pattern of response to subsequent medication (Seeman 1992). In addition, unless the washout period is very long, the presence of residual medication might contribute to variance between cases in the observed response to the study medication.

Therefore we elected to study first episode, unmedicated subjects in this investigation. Such patients are more likely to respond to treatment at relatively low doses, thereby minimizing confounding side effects. In addition, in previously un-medicated patients we would not anticipate potentially confounding effects due to changes in cerebral activity produced by prior treatment. However, there are two disadvantages of studying first episode cases: first, the diagnosis is often unclear in the early stages of the illness. A diagnosis of schizophrenia cannot

be made according to DSM-IV diagnostic criteria (American Psychiatric Association, 1994) until a mixture of characteristic signs and symptoms have been present for a significant portion of time during a one month period with some of the signs of the disorder having persisted for at least six months. After one month of illness, the diagnosis of schizophreniform illness can be made. Therefore, in this study we elected to include both schizophreniform and schizophrenic cases, and to follow all the cases for the minimum length of time necessary to establish a diagnosis of schizophrenia.

Secondly, negative symptoms in first episode cases might differ from the negative symptoms characteristic of the chronic phase of illness (Liddle 1999). In particular, secondary negative symptoms such as social withdrawal secondary to delusions or hallucinations might play a relatively greater role in acutely ill, first episode patients. On the other hand, there is evidence for a more marked association between negative symptoms and cognitive impairment in the chronic phase of the illness (Baxter & Liddle 1998). Therefore prediction based the patterns of cerebral activity associated with persistent negative symptoms (Liddle et al., 1992) might not be valid in first episode patients. Consequently, in this study we only test hypotheses regarding positive symptoms.

Selection of antipsychotic

For our study minimization of the potential cerebral changes associated with extra pyramidal side effects (EPS) is a high priority. Hence, the use of an atypical antipsychotic with low propensity to produce EPS is preferable. At the time this study was designed, the only atypical antipsychotics approved for use in Canada were clozapine and risperidone. In view of the risk of agranulocytosis associated with clozapine (Meltzer et al., 1995), we selected risperidone for this study.

Risperidone is effective in treating many of the symptoms of schizophrenia with a significant reduction in (EPS) compared to typical neuroleptics (Peuskens et al.,1995). In some multicenter trials 6mg of risperidone has been found to be more effective in reducing both positive and negative symptoms of schizophrenia with reduced EPS as compared to either 10 or 20mg of haloperidol (Chouinard et al. 1993, Marder et al., 1994). In the other major multicenter trial, risperidone was not found to be significantly more effective in treating the symptoms of schizophrenia, but nonetheless did produce fewer EPS than 10mg of haloperidol (Peuskens et al., 1995). Furthermore risperidone has been shown to be effective in treating the majority of first episode patients at doses no greater than 4 mg daily (Kopala et al., 1997).

The receptor profile of risperidone has a high affinity for the serotonin 5HT2 receptor (100 fold to that of the D2 receptor), while its affinity for the D2 receptor is comparable to that of the typical antipsychotic, haloperidol (Nyberg et al., 1993). The pharmacology of risperidone also includes limited but clinically observable affinity of both histaminergic and adrenergic receptors (Leyson et al., 1993) (Table 1).

Compared to other atypicals such as clozapine or olanzapine, risperidone has a receptor binding profile which is relatively simple. Hence, interpretation of findings in terms of receptor occupancy is likely to be more feasible than with other atypical agents.

Table 1: Rector binding profiles (affinity expressed as Ki in nmol/l for ligand indicated) for risperidone verses clozapine and haloperidol.

receptor	3H-ligand	risperidone	clozapine	haloperidol
5-HT2	ketanserin	0.14	16	48
alpha 1 adrenergic	WB 4101	0.81	7	8
D2	haloperidol	1.13	156	1
histamine 1	pyrilamine	2.23	4	4390
alpha 2 adrenergic	clonidine	7.54	>10000	>10000

Data from Leyson et al., 1994; Roth et al., 1992, 1994, 1995, 1998; Van Tol et al., 1991

^{18}F-FDG-PET measurements of cerebral metabolism as an index of regional neuronal activity.

Neuronal firing involves both electrical and chemical events occurring on a time scale of several milliseconds. While local measurement of electrical activity might in principle provide the most detailed information about neuronal activity, direct precisely localized measurements of electrical activity in the living human brain are not practical. Because glucose use and neuronal activity are closely coupled, regional rates of glucose metabolism provide a measurable index of regional neuronal activity.

Regional glucose metabolic activity can be measured using PET with the tracer fluorodeoxyglucose labeled with the positron emitting isotope, ^{18}F. PET is a technique that provides three-dimensional images of the distribution of tracers labeled with positron emitting radioisotopes. The mode of positron decay is particularly advantageous for the detection and quantification by external measurement. The positron is slowed by loss of energy to surrounding matter along its path and ultimately combines with an electron. Positron annihilation ensues, with the simultaneous emission of energy equivalent to their combined mass of 1.022 MeV. The emitted energy is in the form of two gamma rays (photons with energies of 511 keV) that travel in opposite directions (Phelps et al., 1979). An energy 511 keV is sufficient to ensure that a substantial proportion (approximately 10%) of gamma rays traverse the brain and are available for detection by a ring of external detectors.

Coincidence detection algorithms are employed to record the occurrence of simultaneous detection of photons at two detectors. The simultaneous detection of a pair of photons travelling in opposite directions indicates that a positron annihilation event has occurred along the line between the two detectors and thereby provides information about the location of that event. The mathematical technique of back-projection allows reconstruction of an image of the spatial distribution of the positron-emitting isotope. Coincidence detection gives a more precise determination of the direction of travel of the photons than would be feasible using collimators alone. Consequently, PET images have a high signal to noise ratio.

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The limit on spatial resolution is determined by the distance that the emitted positron is likely to travel before annihilation. Because the positron emitted by ^{18}F has a relatively low kinetic energy (compared with that emitted by other isotopes such as ^{15}O) the distance traveled before annihilation is relatively short (typically a few millimeters). After taking account of the loss of resolution attributable to geometrical factors such as the finite aperture width of the detectors, the spatial resolution achieved with current PET cameras is approximately 5mm (CTI, Knoxville TN).

Such a spatial resolution will lead to some loss in signal intensity from small cerebral structures such as the ventral striatum (which is a globular mass approximately 8mm in diameter). This is because the signal from a gray matter structure this small will be mixed with the lower signal from adjacent white matter, which is expected to have a lower metabolic rate. Hence it is a priority to maximize the sensitivity of measurement by ensuring that the as much of the emitted radiation is detected as is possible. In particular, it is advantageous to employ 3D image reconstruction based on recording not only those pairs of gamma rays that travel in a plane orthogonal to the long axis of the PET camera, but also on recording of pairs of gamma rays that travel obliquely (Sossi et al., 1998).

FDG is an analog of glucose that crosses the blood brain barrier and undergoes the initial step of glycolytic metabolism. Unlike glucose, ^{18}F-FDG-6 phosphate is not a substrate for the next enzyme in the glycolytic pathway and thus becomes trapped. Thus the rate of accumulation of ^{18}F-FDG is an index of to the rate of glucose metabolism.

The Sokoloff-Phelps three-compartment model provides a quantitative expression for the relationship between accumulation of the radio-labeled deoxyglucose and glucose metabolism (Phelps et al., 1979). This model is based on the assumption that the total concentration of tracer in tissue is the sum of contributions from tracer in the blood, free tracer in tissue and tracer that has been phosphorylated to ^{18}F-FDG-6-phosphate. The time course of the tracer in each of these three compartments is illustrated in Figure 2.

Because the majority of the uptake of radioactivity occurs within a period of approximately 30-40 minutes after administration of the tracer, the PET FDG technique is suitable for quantifying changes in cerebral activity that occur on a time scale of this uptake period. However, it should be noted that for periods longer than an hour it is necessary to include a term that allows for de-phoshorylation of the FDG-6-phosphate in the equations describing the accumulation of radioactivity in tissue (Phelps et al., 1979). The available evidence indicates that the onset of the pharmacological effects of antipsychotic medication occurs over a period of approximately one hour (Tamminga 1999). Thus, PET with ^{18}F-FDG is potentially a suitable technique for our purpose.

Figure 2. Typical temporal distribution of ^{18}F activity in grey matter between free FDG and FDG-6-PO$_4$ in human subjects (from Sokoloff et al., 1987).
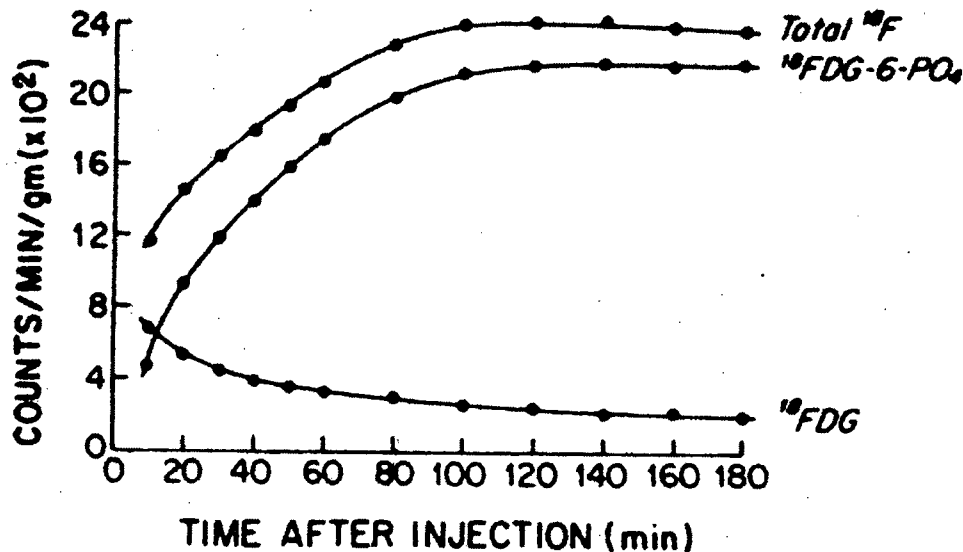
Data analysis and statistical issues

There are currently two main methods currently in use for the analysis of brain imaging data, namely region of interest analysis (ROI) and voxel based analysis. One problem associated with region of interest analysis is that the location of functionally homogeneous cerebral areas rarely coincides with a region that can be delineated precisely using macroscopic structural landmarks. Therefore, it is often preferable to employ a voxel based analysis rather than an analysis based on the average metabolic change in regions defined in relation to structural landmarks (Fox et al., 1985). In a voxel-based analysis, the significance of change is evaluated in every voxel in the entire field of view.

However, voxel-based analysis presents two major challenges. First, it is necessary to transform the images that are to be compared so that corresponding loci from all images are located in the vicinity of the same voxel. This is called spatial normalization. The second challenge is the need to correct for multiple comparisons allowing for the large number of voxels in the region that is examined.

Spatial normalization to a standard space

Fox et al., (1985) proposed translating, rotating and re-sizing each image to match the brain represented in the Talairach atlas of the human brain (Talairach & Tournoux 1988) to ensure that corresponding loci from different images are located in the same voxel. The Talairach brain

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is located in a coordinate frame that has its origin at the mid-point of the anterior commissure, with the x axis directed from left to right, the y axis passing from back to front through the posterior and anterior commissures, and z axis directed from the base of the brain towards the vertex. The location of any feature in the brain can be specified by giving the distances in x, y and z directions from the origin. However, the linear transformation of images advocated by Fox et al., does not account for the fact that brains of different individuals differ not only in size but also in shape. The next major step towards satisfactory spatial normalization was the development by Friston et al., (1991a) of non-linear deformation ('warping') of the brain to match the shape of a standard brain template. However the optimum method for achieving this remains a subject of debate (Zuk & Atkins 1996). In part this is because hitherto there has been no accepted method for quantifying the goodness of the match achieved by different methods. For the purpose of choosing the most appropriate method for use in this study, we have developed a measure of the adequacy of the match between image and template and have applied it to the comparison of several methods of spatial normalization. This comparison is presented in appendix 2.

Correction for multiple comparisons in testing statistical significance

Since a functional image typically includes over 150,000 voxels, the correction for multiple comparisons when a statistical test is applied to all voxels, is extremely stringent. However, the appropriate correction is not as severe as a simple Bonferroni correction (Miller 1966) for the number of voxels examined. Because the image intensity in adjacent voxels in an image is usually correlated, the number of independent tests is somewhat less than the number of voxels. The first practical approach to making the appropriate allowance for the correlations between nearby voxels was developed by Friston et al., (1991b). This method was further refined by Worsley et al., (1992). Nonetheless, even after allowing for the correlation between image intensity in adjacent voxels, the required correction is still very stringent if the entire brain volume is examined. When testing a specific hypothesis about the location of a predicted change in brain activity, the statistical power can be increased by confining the search to a restricted region of the brain. There are two accepted approaches to testing significance of focal changes when statistical tests are applied to all voxels within a restricted search volume:

1) The method of Worsley et al., (1996) based on the theory of random Gaussian fields of finite extent.
2) The method of Friston (1997) based on the extent of a cluster of supra-threshold voxels in the vicinity of the predicted effect.

Worsley's method effectively determines how many independent comparisons are performed when examining all the voxels in a designated region of finite extent, taking into account the consequences of 'edge effects' while allowing for the fact that changes in adjacent voxels are correlated. However, statistical power is reduced when the ratio of surface area to volume of the region of interest is large. Statistical power may be increased by reducing the volume examined from the entire cerebral volume to a specified small globular region. Thus, Worsley's method is appropriate when testing anatomically precise hypotheses, such as our predicted changes in ventral striatum, thalamus and hippocampus (see chapter 2).

However, Worsley's method does not provide much power when testing for relatively diffuse changes of moderate magnitude in cerebral cortex. Under such circumstances, Friston's method

is likely to offer greater power. Friston's method is based on the identification of a cluster of voxels that exceed some threshold for significance in the vicinity of the predicted effect, and determining the probability that a cluster size might occur by chance. We apply Friston's method for testing the significance of changes produced by risperidone in cortical regions in schizophrenic patients (see chapter 3).

Testing for significance of non-focal brain changes

When changes in brain activity in an extensive area of the brain are anticipated, it is more relevant to test for a change in image intensity throughout the predicted area, than to test for the significance of change at a particular locus. Worsley et al., (1995) developed a sensitive test for the significance of non-focal change in brain images based on measurement of the sum of squares (SS) of the z values for all voxels in a specified volume of interest. If the image intensity in different voxels were to be uncorrelated, SS would be distributed as chi squared with the degrees of freedom equal to the number of voxels in the volume of interest (N), under the null hypothesis of no systematic change between scans. However, in PET images, image intensity in adjacent voxels is correlated, thereby reducing the number of degrees of freedom. Worsley et al., demonstrated that the effective number of degrees of freedom is determined by the number of resolution elements in the volume of interest, where a resolution element is the volume enclosed by the contour at half maximum of the point response function of the PET camera. Worsely et al., 1995 has shown that the number of degrees of freedom is given by the expression, n= 0.829.RESEL, where RESELS is the number of resolution elements in volume of interest. Under the null hypothesis, the quantity n.SS/N is distributed as chi squared with n degrees of freedom. Therefore, the probability of chance occurrence of a value of SS as large as the observed value can readily be determined. If the observed value of SS is higher than is likely to have arisen by chance, it can be concluded that there has been a significant distributed change in image intensity in the volume of interest, but it is not possible to conclude on the basis of this test that the change at a specific location within the volume is statistically significant.

Correction for residual radioactivity

Because one of our goals is to measure the effects of risperidone on cerebral activity in schizophrenic patients before there has been an appreciable change in severity of symptoms, it is imperative that the first and second PET scans are performed within a few hours on the same day. An initial scan was followed three hours later with a second scan to determine the immediate effects of 2mg of risperidone on patterns of cerebral metabolism relative to a baseline placebo condition. When using the PET tracer ^{18}F-FDG, which has a half-life for radio-active decay of 110 minutes, and performing two scans a few hours apart, a substantial amount of radioactivity taken up into tissue after the first injection of ^{18}F-FDG will still be present during the second scan. Hence, it is necessary to correct for this residual radioactivity when determining the amount of ^{18}F-FDG taken up after the second injection.

Brooks et al., (1987) have shown that in brain tissue, it is possible to make a satisfactory allowance for residual radioactivity using a prediction for the tissue concentration of ^{18}F-FDG based on the Sokoloff-Phelps (Phelps et al., 1979) three compartment model for the distribution of radioactivity in cerebral tissue, when the interval between the two scans is one hour. After ^{18}F-FDG is taken into cells, it is phophorylated to ^{18}F-FDG-6 phosphate, which is not a suitable substrate for the next enzyme in the glycolytic pathway, and hence becomes trapped (Sokoloff

et al., 1977). The major physiological process that accounts for change in the distribution of tissue radioactivity at times greater than one hour after the injection of ^{18}F-FDG is the slow conversion of ^{18}F-FDG-2 phosphate back to ^{18}F-FDG, which can escape from the brain. This de-phosphorylation is catalyzed by the enzyme glucose-6-phosphatase. In the Brook's method, the transfer coefficient, k4, that reflects the rate of de-phophorylation is not measured directly, but is assigned a nominal value.

However, in our study, it is necessary that the interval between the first and second scans should be approximately three hours to allow an adequate period of time for the risperidone administered after the first scan to reach the brain.   Although the amount of residual activity is expected to be less when the inter-scan interval is three hours compared to the one hour interval employed by Brooks et al., (1987), the amount of residual radioactivity is nonetheless appreciable. It can readily be shown, using the equations describing the Sokoloff-Phelps model, that the uncertainties arising from using a nominal value of the transfer coefficient, k4, are substantial when the interval between scans is several hours, and might lead to an appreciable error in the estimation of residual radioactivity. We have therefore developed a technique that employs a measurement of tissue radioactivity measured in a scan performed immediately prior to the second injection of ^{18}F-FDG to provide information necessary to estimate the rate of de-phosphorylation in each individual subject, and then utilizes this estimate in the calculation of the residual activity present during the second scan. In appendix 1 we report a study that validates this method.

Overview

The hypotheses regarding the predicted changes in the cortico-striato-thalamic circuit and hippocampus in schizophrenic patients are addressed in chapter 2. The hypotheses regarding changes in the right medial frontal lobe and left temporal lobe are addressed in chapter 3. In addition, chapter 3 includes an exploratory analysis to identify any changes in other cerebral areas. The effects of risperidone on cerebral metabolism in healthy subjects is reported in chapter 4. General conclusions are presented in chapter 5.   In appendix 1 we present a study to validate our method for correcting for residual activity. Appendix 2 compares various methods of spatial normalization.

II. IMMEDIATE EFFECTS OF RISPERIDONE ON CORTICO-STRIATO-THALAMIC LOOPS AND THE HIPPOCAMPUS.

Manuscript by Liddle PF, Lane CMJ, Ngan ETC. To be submitted to the British Journal of Psychiatry.

Introduction

Post mortem studies of neuropathology and structural imaging studies have provided strong evidence for abnormality of the temporal lobes, especially the left temporal lobe, in schizophrenia (Falkai & Bogerts 1994). In particular, several of these studies have revealed evidence that the hippocampus has a reduced volume (Bogerts et al., 1990). Functional imaging studies (reviewed by Liddle, 1999) reveal abnormal function of the temporal lobes in schizophrenia. The majority of the evidence indicates that the experience of delusions and hallucinations is associated with increased rCBF in the temporal lobe, especially in its ventro-medial aspect (Musalek et al., 1989; Liddle et al., 1992; McGuire et al., 1993; Silbersweig et al., 1995). Most of these studies were longitudinal studies that compared brain activity during the experience of symptoms with that in the absence of symptoms, in the same subject, and therefore indicate cerebral activity associated with the experience of symptoms rather than the predisposition to symptoms. In contrast, several studies have reported an association between under-activity of left lateral temporal lobe and delusions and/or hallucinations (Liddle et al., 1992; Ebmeier et al., 1993; McGuire et al., 1995). All of these studies were cross-sectional rather than longitudinal, and hence might have been expected to reveal features associated with the predisposition to delusions and/or hallucinations, in addition to features associated with the experience of these symptoms.

The observation that for typical antipsychotic drugs the level of dopamine D2 receptor occupancy in the basal ganglia is predictive of therapeutic effect (Nordstrom et al., 1993) suggests that the basal ganglia might also be implicated in delusions and hallucinations. Several functional imaging studies have reported that delusions and /or hallucinations are associated with over-activity in the ventral striatum (Liddle et al., 1992; Silbersweig et al., 1995).
In summary, there is substantial evidence that both the ventral striatum and also temporal lobe structures such as the hippocampus are implicated in the production of delusions and hallucinations. The striatum is a major relay station in the cortico-striato-thalamic- cortical feedback loops that modulate frontal cortical activity (Alexander et al., 1986). There is growing evidence that the hippocampus plays a cardinal role in gating transmission in these feed-back loops (Grace 1998). In particular, Grace and colleagues have shown that striatal spiny neurons only generate an action potential in response to input from frontal cortex when there is concurrent input from the hippocampus. This lead Grace to propose that hippocampal under-activity plays a central role in the pathophysiology of schizophrenia.

While the finding of reduced hippocampal volume suggests a deficiency of hippocampal function in schizophrenia, the balance of evidence from functional imaging studies indicates that the actual experience of delusions and hallucinations is more likely to be associated with hippocampal over-activity than under-activity. In this paper, we propose a pathophysiological mechanism for delusions and hallucinations, and present findings of a Positron Emission

Tomography (PET) study of the effects of antipsychotic treatment on regional cerebral glucose metabolism that tests this proposal.

Proposed Mechanism of Delusions and Hallucinations

The cognitive processes underlying Reality distortion

Many studies (reviewed by Liddle, 1999) that have examined the relationships between the various symptoms of schizophrenia have found that delusions and hallucinations tend to be associated with each other more strongly, than either is associated with other symptoms characteristic of schizophrenia. This suggests that these two types of phenomena share features of their pathophysiology that are not shared by other symptoms. Both delusions and hallucinations entail a disorder of the mental mechanism for evaluation of reality. Liddle (1984,1987) designated this group of symptoms the reality distortion syndrome.

The nature of the putative defect in evaluation of reality might be defined more precisely by examining the relationships between reality distortion and performance in cognitive tasks. Such studies indicate that, in contrast to other groups of schizophrenic symptoms, reality distortion is not associated with widespread cognitive impairment (Liddle, 1987, Baxter and Liddle, 1998). In particular, there is no evidence that the essential problem is a lack of competence in formal tests of logic. However, several investigators have found that individual symptoms from the reality distortion syndrome are associated with impaired performance in tasks in which the common feature is an ability to place mental events in context from which they arose (Heilbrun, 1980; Morrison & Haddock, 1997). In particular, Frith has emphasized the relationship between delusions of control and impaired ability to monitor the source of self-generated mental activity (Frith & Done, 1989). These findings are consistent with the hypothesis that the cognitive deficit underling reality distortion is a failure of the mechanism for evaluating and monitoring the context of mental activity.

The relevance of context for evaluation of ideas and perceptions

Memories for facts ('declarative memories') are either episodic or semantic. Episodic memories are memories of specific events experienced by the subject. Their credibility is usually determined by contextual information. Semantic memories are memories for facts whose validity transcends the context within which they were learned. For example, an individual might know that Paris is the capital city of France without remembering the specific circumstances in which they first learned this fact. Novel ideas are initially evaluated in relation to the context in which they arose. Eventually an idea might be sufficiently consolidated (perhaps by cross-reference to other accepted information) that it is accepted without reference the context of its origin. At that point it becomes an accepted item in semantic memory. This implies that the cerebral representation of a novel idea is normally linked to the representation of the context of its origin.

The role of the hippocampus in the processing of context

The observation that bilateral damage to the hippocampus abolishes episodic memory (Milner 1972) without damaging semantic memory suggests that the hippocampus might play a role in the linking of an idea to its origin. The hippocampus receives direct projections from widespread areas of the cerebral cortex, and its neural architecture suggests that it is likely to play a role in integrating information from diverse sources. For example, Rolls (1989) has

proposed a model according to which pyramidal cells in the CA1 region of the hippocampus fire whenever a specific pattern of distributed cortical activity occurs. Hippocampal neurons project via glutamatergic fibers to the corpus striatum where they synapse on gaba-ergic spiny inter-neurons (Figure 1). As demonstrated by Grace et al., (1998) the afferents from hippocampus to striatum gate the feedback via cortico-striato-thalamic-cortical loops. Thus, the connections and architecture of the hippocampus provide it with the capacity to identify and reinforce specific patterns of cerebral activity. When the pattern of cerebral activity associated with a particular mental event coincides with a pattern of cerebral activity that had previously been associated with that event (such as the representation of the context in which the mental event was initially experienced) hippocampal firing might reinforce that pattern of mental activity. This evidence leads to the first hypothesis.

Hypothesis 1: <u>Under normal circumstances, hippocampal firing acts to reinforce mental events that are contextually appropriate</u>.

Such a mechanism would facilitate the retrieval of episodic memory by reinforcing the neuronal representation of an event when the representation of a contextual clue is activated. However, if aberrant firing of the hippocampal neurons unrelated to contextual information were to occur when the representation of a mental event is active, the cortical activity representing that event might be reinforced irrespective of context. Thus, an idea might receive reinforcement irrespective of context. In effect, it would escape evaluation in the light of context. Similarly, an internal verbalization might be mis-perceived as a real verbal stimulus. These considerations lead to a second hypothesis:

Hypothesis 2: <u>Aberrant firing of hippocampal neurons might lead to reality distortion, taking the form of either delusions or hallucinations.</u>

Once the pattern of cerebral activity associated with a mental event has received inappropriate reinforcement, that pattern of activity might itself continue to promote self-reinforcing hippocampal neural activity by normal cortico-hippocampal neurotransmission, thereby sustaining itself. Hence, delusional ideas might tend to persist even after the event during which they were generated, though in the absence of active reinforcement, such beliefs would be amenable to normal modification and might eventually fade.

Dopaminergic modulation of cortico-striato-thalamo-cortical feedback

Gaba-ergic neurons in the striatum are modulated by dopaminergic projections form the ventral tegmental area in the mid-brain. Although some controversy exists regarding the mechanism of this modulation, it appears that dopamine enhances positive feedback mediated by the direct pathways involving two inhibitory neurons, while it inhibits negative feedback via indirect pathways that involve three inhibitory neurons (Alexander and Crutcher, 1990). Thus dopamine release will tend to enhance concurrent cortical activity, by promoting positive feedback and inhibiting negative feedback. It would be anticipated that in an individual prone to aberrant hippocampal firing, dopaminergic over-activity would promote reality distortion. Thus, in schizophrenia, delusions and hallucination would generally be expected to occur at times of dopaminergic overactivity (such as might be induced by stress). However, individuals with a strong tendency to hippocampal over-activity might experience reality distortion in the absence of dopaminergic over-activity. Furthermore, dopaminergic hyperactivity would not only promote reality distortion, but also other patterns of cortical activity that are not related to

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aberrant hippocampal firing. In accord with experimental observation (Fibiger 1991), dopaminergic over-activity would promote psychomotor excitation and also the disorganization of mental activity that arises when the normal inhibition of inappropriate activity fails (Liddle, 1999).

Hypothesis 3: <u>Acute psychotic episodes are associated with dopaminergic over-activity that promotes psychomotor excitation and disorganization. In individuals, prone to aberrant hippocampal firing, it also promotes reality distortion.</u>

In view of the evidence that dorsal striatum is principally involved in feedback to premotor cortex, while ventral striatum is more strongly involved in feedback to prefrontal cortex which is involved in higher mental functions (Alexander et al., 1986) it would be expected that dopaminergic modulation of ventral striatum rather than dorsal striatum would be relevant relief of psychotic symptoms.

The action of antipsychotic drugs

Antipsychotic drugs diminish dopaminergic over-activity, and hence would be expected to alleviate all of the symptoms of an acute psychotic episode. However, hypothesis 2 implies that full remission of reality distortion will occur only if the decrease in widespread frontal cortical over-activity results in suppression of aberrant hippocampal firing (possibly mediated via decrease in activity in the abundant glutamatergic projections from frontal cortex to hippocampus). The patterns of cerebral activity representing mental events that had previously been inappropriately reinforced might still be maintained via normal hippocampal activity, but would be expected to fade with time.

Hypotheses 2 and 3 lead to the following hypotheses regarding the action of antipsychotic medication:

Hypothesis 4: <u>Administration of an antipsychotic during a psychotic episode will lead to immediate reduction of activity in ventral striatum, thalamus and frontal cortex. This effect will lead to alleviation of the acute psychotic episode, but the magnitude of the changes will not correlate with change in severity of any specific sub-group of symptoms.</u>

Hypothesis 5: <u>In cases where administration of the antipsychotic drug leads to reduction in aberrant hippocampal activity, there will be eventual reduction in severity of reality distortion.</u>

Hypothesis 4 can be tested directly by measuring regional cerebral activity in ventral striatum, thalamus and frontal cortex response to administration of antipsychotic medication. Hypothesis 5 can be tested by examining the correlations the reduction in hippocampal activity induced by acute administration of antipsychotic medication and the subsequent reduction in severity of reality distortion after several weeks treatment.

We have used PET to measure the changes in metabolism in ventral striatum, thalamus, hippocampus and frontal cortex, following a single dose of the antipsychotic drug, risperidone in a group of drug-naïve first episode schizophrenic patients, and examined the relationship between any changes observed and symptom change after 6 weeks of continuous treatment. Risperidone blocks dopamine D2 receptors and also serotonin 5HT2 receptors. We elected to use risperidone in first episode patients, because using this drug in such a patient group,

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symptom resolution can be expected at low doses in the majority of cases (Kopala et al., 1997) thus psychosis can be relieved with minimal extra-pyramidal side effects, thereby minimizing the likelihood that any changes observed in the basal ganglia are associated with extrapyramidal side effects.

Experimental procedures

Patients

Eight patients satisfying DSM-IV criteria for schizophrenia or schizophreniform psychosis (American Psychiatric Association, 1994) were recruited and scanned during their first episode of psychotic illness. Apart from one individual who had previously received two doses of risperidone (1mg) during the preceding week, subjects had not taken antipsychotic medication at any time prior to the first PET scan (Table 2).

Table 2: Characteristics of schizophrenic subjects

Demographic Variables	Mean	SD	
Age (years)	26.5	5.6	
Social class	2.8	1.0	
GAS at admission	31.9	6.5	
Lifetime hospitalization (months)	0.20	0.16	
Gender (female:male)	6:2		
Handedness (right:left)	7:1		

Experimental design

On the first scanning day, a placebo capsule was administered under single blind conditions 90 minutes prior to the injection of tracer for a baseline PET scan. At the completion of the baseline PET scan, 2mg risperidone was administered (single blind). 80 minutes later, a 10 minute duration pre-injection emission scan was performed to measure residual radioactivity remaining from the first injection of tracer. Ninety minutes after administration of risperidone, tracer was injected and a second ('first-dose') PET scan was performed. On the second day, patients received 2 mg of risperidone in divided doses. On the third day the dosage was then increased to 4mg per day (in divided doses) if clinically indicated, and subsequently increased to a maximum of 6 mg daily (in two divided doses) if clinically indicated. Dosages were decreased as necessary to minimize extrapyramidal side effects. The mean dosage after 6 weeks treatment was 3.75 mg/day (range 2-6mg/day). After 6 weeks, a third ('post-treatment') PET scan was performed after the morning dose of risperidone.

Symptoms were assessed using the Scale for Assessment of Negative Symptoms (SANS) and the Scale for Assessment of Positive Symptoms (SANS) (Andreasen 1987) on the day of the first PET scan, and subsequently at 2, 4 and 6 weeks. A score for Realty Distortion was derived by adding the global delusions and global hallucinations scores. Overall severity of illness was assessed using the Global Assessment Scale (GAS) (Endicott et al., 1976). Social status of patients was assessed on the basis of parental occupation. The experimental procedure was

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approved by the University of British Columbia and Vancouver Hospital's ethical review boards and all subjects gave written, informed consent.

Imaging procedure

Images of regional glucose metabolism were obtained with a CTI 953B PET camera (CTI Knoxville TN) using 18F-fluorodeoxyglucose (^{18}F-FDG), prepared by the procedure of (Hamacher et al., 1986). Between-plane collimating septa were retractable to permit oblique photon paths allowing reconstruction of images in three dimensions (Sossi et al.,1998). Data were collected in 31 contiguous axial slices covering an axial field of view of 10.8cm. For each scan, 2 millicuries of tracer was administered by slow injection over 1 minutes, via a forearm cannula. To permit estimation of the input of tracer to the brain, the concentration of ^{18}F-FDG in the plasma of arterialized venous blood was measured in 15 samples collected over a period of 120 minutes following injection. To ensure a standard mental state, the subjects were engaged in a continuous performance task during the period of uptake of ^{18}F-FDG. Digits were presented one at a time on a visual display unit, and the subject was instructed to press a bar whenever two identical digits were presented consecutively. Three-dimensional image data was collected in four 5 minute frames commencing 60 minutes after tracer injection. Correction for absorption of radiation was made using data from a transmission scan obtained employing a ^{68}Ge Germanium rod source.

Data analysis

Image analysis was performed using Statistical Parametric Mapping software (SPM 96; Wellcome Department of Cognitive Neurology, London). The four 5 minute frames in each scan were aligned and averaged. The 'first –dose' and 'post-treatment' images were then aligned to the baseline image. The residual activity from that injected from the 'baseline 'scan remaining during the 'first-dose' scan was computed using the procedure of Liddle et al., (appendix 1). This procedure utilizes the pre-injection emission scan to derive an estimate of the rate coefficient for the loss of tracer from the metabolic pool due to dephosphorylation, and then employs this rate coefficient to compute the rate of decrease of residual activity with time. Residual activity was subtracted from the observed image intensity during in the 'first-dose' scan to provide an estimate of tracer concentration attributable to the second injection of ^{18}F-FDG. We will refer to this image as the 'corrected first-dose scan'.

For each subject, a mean image was derived by averaging the baseline, 'corrected first-dose' and 'post-treatment' images, and this mean image was spatially normalized to match the PET image template in SPM96. This template is located in a coordinate frame that has its origin at the mid-point of the anterior commissure, y axis passing from back to front through the posterior and anterior commissures, and x axis directed from left to right. The parameters for the transformation were then applied to each of the three images for each subject. After spatial normalization, images were smoothed with a 10 mm full-width at half maximum Gaussian filter.

As a preliminary step, the effect of risperidone treatment on global metabolism was examined. The Phelps-Sokoloff model was employed to calculate metabolism in each voxel, using Metab Tool software (CTI, Knoxville, TN). Mean global metabolism was taken to be the mean value for all intra-cerebral voxels in the middle 10 slices of the image. We demonstrated that

risperidone produced no significant change in mean global metabolism. Therefore, in the subsequent analysis of regional metabolism, variation between scans in mean global metabolism was removed by proportional scaling, thereby removing noise due to errors of measurement of the radioactivity in blood.

For each voxel, the General Linear Model was used to estimate the mean change in scaled image intensity between the baseline scan and 'corrected first-dose' image, and also between the baseline scan and the 'post-treatment' image. For the purpose of testing the specified hypotheses regarding ventral striatum and thalamus, only those voxels within the pre-specified volumes of interest in ventral striatum and thalamus were examined. The results of a non-directed search of the entire image volume are reported separately (Ngan et al., chapter 3). The significance of the change for each voxel was determined applying the method developed by Worsley et al., (1996) based on the theory of Gaussian fields, as applied to finite search volumes. In effect, Worsley's method determines the number of independent measurements within the brain volume examined, taking account of the fact that the image intensity in adjacent voxels is correlated, and applies the appropriate Bonferroni-type correction.

To test the hypothesis that there would be a reduction in prefrontal cortex metabolism (defined as all areas of cortex anterior to y=20mm in the Talairach coordinate frame) after the first dose, and also after 6 weeks treatment, we employed the test for distributed non-focal activations proposed by Worsley et al., 1995. This procedure evaluates the significance of the observed sum of squares of z values for all voxels in the specified volume, taking account of the fact that the number of degrees of freedom is less than the number of voxels, due to correlations between adjacent voxels. It is an appropriate procedure for identifying changes that are expected to be extensive but relatively small in magnitude.

A separate analysis was performed to identify voxels in which there was a significant relationship between change in image intensity between baseline image and 'corrected first dose' image and change in reality distortion severity at six weeks. The first step was to create difference images representing the change in globally normalized image intensity between the baseline image and the 'corrected first dose' image. The General Linear Model was employed to determine the regression of difference in image intensity on change in symptom score at six weeks. Significance was determined employing the Worsley (1996) correction. A similar analysis was also performed to identify voxels in which there was a significant relationship between change in image intensity between baseline and 'post-treatment' scans and change in Reality distortion severity after 6 weeks treatment.

Results

After 6 weeks treatment, there was a substantial improvement in delusions and hallucinations. The mean reduction in reality distortion symptom score was 3.9 (s.d. 2.2).

In accordance with prediction, there were statistically significant decreases in regional metabolism between the baseline scan and the scan performed after the first doses in the right ventral striatum, right thalamus and frontal cortex (see Tables 3 and 4, and Figure 3). The frontal lobe voxels exhibiting a decrease in metabolism after the first dose of risperidone formed clusters in dorsomedial frontal cortex bilaterally, ventromedial frontal cortex bilaterally and left

lateral frontal cortex. The proportion of all cerebral voxels anterior to y=20mm that exhibited a decrease satisfying the criterion p<0.05 (uncorrected) was 20.3%.

Furthermore, in comparison with baseline, there were statistically significant reductions in metabolism in right ventral striatum and frontal cortex after six weeks of treatment (Tables 2 and 3). After 6 weeks treatment, the clusters of suprathreshold voxels in which there was a decrease in metabolism, had increased in size, and the proportion of all cerebral voxels anterior to y=20mm that satisfied the criterion p<0.05 (uncorrected) was 40%. Risperidone had no effect on global metabolism.

The analysis of relationships of metabolism change after the first dose with change in Reality distortion after six weeks revealed a statistically significant relationship between reduced left hippocampal metabolism and reduced severity of reality distortion (Table 5 and Figure 4). Furthermore, change in metabolism in left hippocampus after 6 weeks treatment was also significantly correlated with reduction in severity of reality distortion after 6 weeks There were no statistically significant relationships between change in reality distortion and changes in metabolism in ventral striatum either after the first dose of risperidone or after 6 weeks treatment.

Table 3: Decreases in regional metabolism in the right ventral striatum and right thalamus after the first dose of risperidone and after 6 weeks treatment.

Region	Talairach coordinate xyz	Z at peak voxel	Uncorrected p	Corrected p [1]	Search volume
After 1st dose					
Right ventral striatum	10 10 –6	2.78	.003	0.05	512 mm³
Right thalamus	16 -24 0	3.55	.0002	0.04	3375 mm³
After 6 weeks treatment					
Right ventral striatum	6 8 -8	3.22	.0006	0.02	512 mm³
Right thalamus	22 –34 4	2.21	0.014	n.s. [2]	3375 mm³

(1) Corrected for multiple comparisons using the Worsley et al., (1996) finite volume correction
(2) n.s. denotes not significant.

Table 4: Significance of changes in metabolism in prefrontal cortex after the first dose of risperidone and after 6 weeks treatment, determined using the Worsley et al., (1995) criterion for non-focal change.

	Chi squared	Degrees of freedom	p
First dose	205.8	112	<0.005
After 6 weeks	251.5	93	<0.001

Table 5: Loci in left hippocampus where the reduction in metabolism after the first dose, and after 6 weeks treatment, is associated with reduction in severity of reality distortion symptoms after 6 weeks treatment.

	Talairach coordinates of peak voxel	z at peak voxel	Uncorrected p	Corrected p	Search volume
After 1 st dose	-24 –14 –18	3.23	001	0.04	3375 mm^3
After 6 weeks treatment	-32 – 6 -22	3.25	.001	0.04	3375 mm^3

Figure 3: Right para-sagittal slices at x=8 and 14mm showing decreases in metabolism in ventral striatum, thalamus, and medial prefrontal cortex produced by 2mg of risperidone. The colour scale indicates z values.

Ventral striatum

Medial frontal cortex

Thalamus   Orbital cortex

Caudate
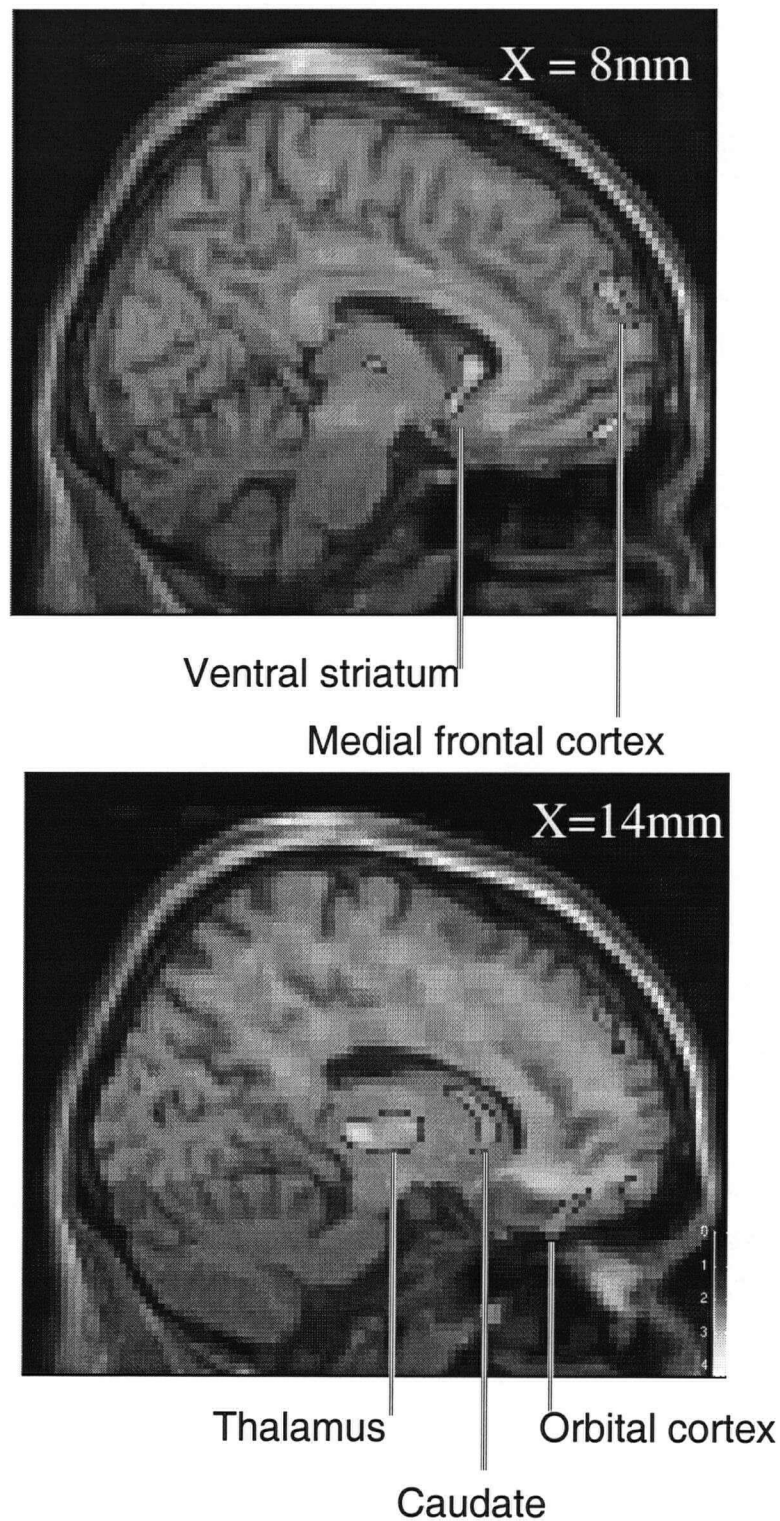
Figure 4: Axial slices showing voxels in the left hippocampus in which there was a significant relationship between reduction in metabolism after the first dose of risperidone and the subsequent reduction in reality distortion after 6 weeks treatment. The colour scale indicates z values.
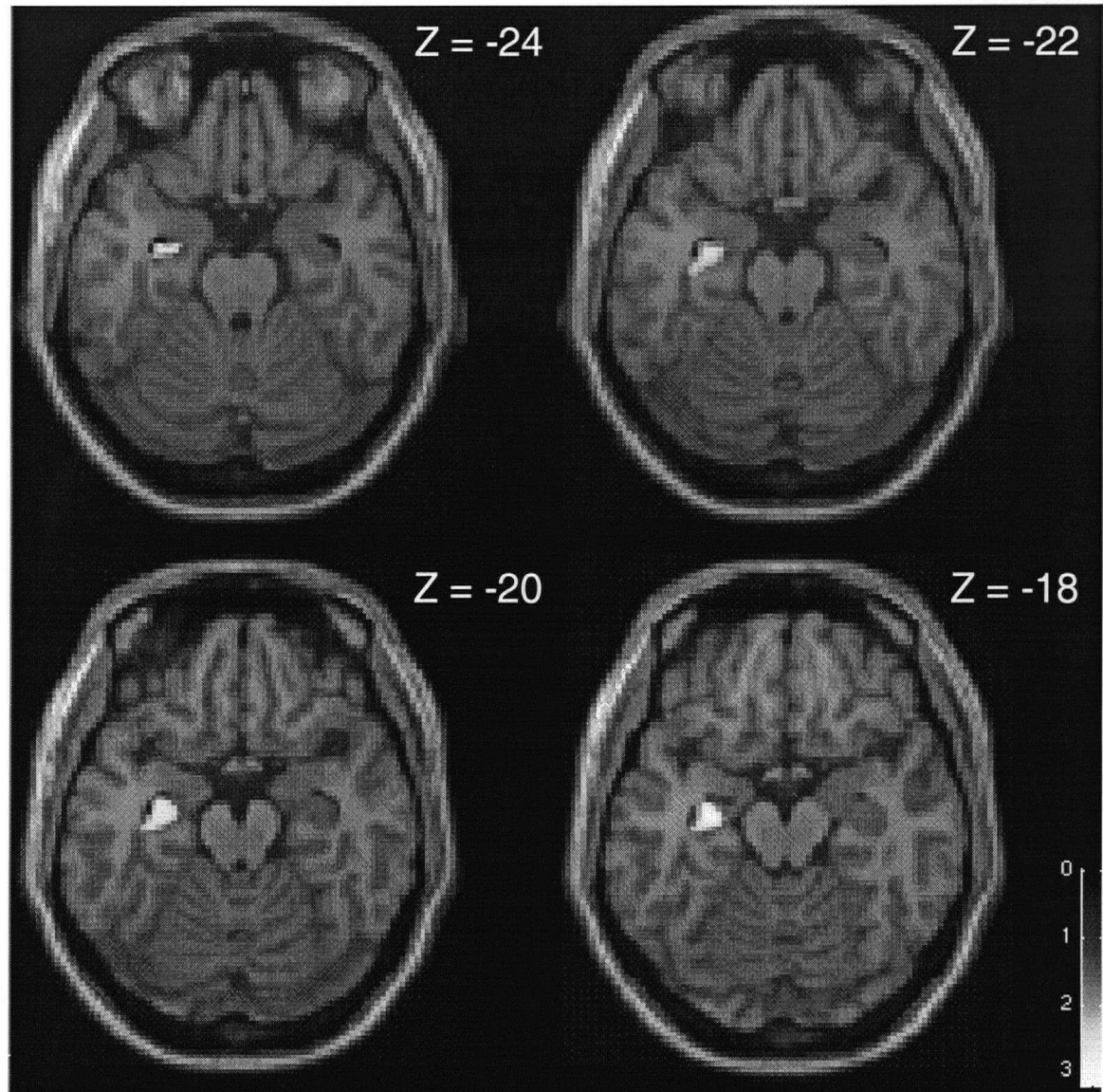
Discussion

In accordance with the predictions of the proposed mechanism of reality distortion, a single dose of risperidone produced significant reduction of metabolism (relative to mean global metabolism) in the ventral striatum, thalamus and prefrontal cortex. The changes in striatal and thalamic metabolism were only significant in the right hemisphere. This lateralization had not been predicted, though it is of interest to note that in their study of cerebral activity associate with hallucinations, Silbersweig et al., (1995) reported over-activity in right striatum and thalamus, together with over-activity in left hippocampus, a pattern consistent with the regional pattern of reductions in metabolism after administration of risperidone, observed in our study.

In addition, the magnitude of reduction in metabolism in symptom score in left hippocampus was strongly predictive of reduction in severity of reality distortion after six weeks treatment. This is consistent with the hypothesis that reduction of aberrant hippocampal firing is a pre-requisite for subsequent resolution of reality distortion.

At six weeks, statistically significant reductions relative to baseline in metabolism in ventral striatum and prefrontal cortex persisted. Furthermore, reduction in hippocampal activity after 6 weeks treatment was also significantly associated with reduction in symptoms at that stage, indicating that the reduction in hippocampal firing was sustained for six weeks.

It should be noted that to test our hypotheses, we elected to test for significant changes only at the cerebral sites specified in the hypothesis, because statistical power is markedly reduced when the entire cerebral volume is examined due to the much more severe Bonferroni correction that is required. This reduction in power would reduce the likelihood of detecting changes in small structures such as the ventral striatum, where signal is reduced by partial volume effects. A non-directed search of the entire cerebral volume for changes in metabolism (reported by Ngan et al., in preparation) revealed more extensive reductions in metabolism at 6 weeks compared with baseline, especially marked in medial frontal cortex, left lateral frontal cortex and left temporal lobe. A discussion of these changes and their relationships with changes in symptoms is beyond the scope of this paper.

This study provides strong support for the hypothesis that aberrant hippocampal activity is a cardinal feature of the pathophysiology of reality distortion. In addition it supports the hypothesis that over-activity in the ventral striatum is associated with acute psychosis, irrespective of symptom profile.

III. IMMEDIATE AND DELAYED EFFECTS OF RISPERIDONE ON CEREBRAL METABOLISM: CORRELATIONS WITH SYMPTOM CHANGE.

Manuscript by Ngan ETC, Lane CMJ, Ruth TJ and Liddle PF. To be submitted to American Journal of Psychiatry.

Introduction

Schizophrenia is a heterogeneous disorder with diverse symptoms and clinical course. This diversity is likely to represent a variety of physiological disturbances in different brain regions. The symptoms of schizophrenia have been divided into three distinct symptom clusters consisting of reality distortion, disorganization and psychomotor poverty (Liddle, 1984; Bilder et al., 1985; Peralta et al., 1992; Malla et al., 1993; Miller et al., 1993; Johnstone and Frith, 1996). Each syndrome has been associated with a distinct pattern of aberrant cerebral activity. Reality distortion symptoms (delusions and hallucinations) are associated with increase activity in the left temporal lobe (Musalek et al., 1989; Liddle et al., 1992; Silbersweig et al., 1995) whereas disorganization symptoms (formal thought disorder and inappropriate affect) are associated with increased activity in the right medial frontal regions (Liddle et al., 1992, Ebmeier et al., 1993, Yuasa et al., 1995). Chronic psychomotor poverty (poverty of speech, blunted affect, decreased spontaneous movement) is associated with underactivity of left lateral frontal cortex (Liddle et al., 1992).

Clinical improvement following treatment with antipsychotics is presumably the result of alterations in cerebral activity which represent an amelioration of aberrant activity underlying clinical symptoms. Previous studies of antipsychotic effects on neuronal activity have employed typical agents such as haloperidol in chronic schizophrenic subjects. The results of these studies have reported a variety of results. The most commonly reported effect of sustained treatment of typical antipsychotic agents on patterns of neuronal activity in chronic schizophrenic subjects is a diffuse decrease in frontal activity and an increase in activity within the basal ganglia (Wolkin et al., 1985; De Lisi et al., 1985; Buchsbaum et al., 1987, 1992a, 1992b and 1992c; Bartlett et al., 1991; Potkin et al., 1994; see Liddle 1999 for review). The majority of the studies which have addressed the association between change in cortical activity and symptom change have used anatomically determined regions of interest and global improvement in clinical symptoms as the variables of interest. Differences in the pattern of cerebral activity abnormalities associated with distinct symptom profiles suggest that the association between clinical improvement and changes in cerebral activity may be specific to the individual syndromes. Evaluation of changes in cerebral activity in relationship to specific symptom dimensions with known anatomical correlates may help further elucidate the physiological changes associated with clinical improvement following treatment with antipsychotics.

In this study we investigated the immediate and delayed effects of risperidone on cerebral metabolism in 8 unmedicated, first-episode schizophrenic patients. We tested the hypothesis that six weeks of treatment with risperidone would result in a decrease in cerebral activity in the left temporal region and the right medial frontal cortex. Furthermore we predicted that the decrease in the left temporal region would be correlated with improvement in reality distortion and that the decrease in the right medial frontal cortex would be associated with improvement in

disorganization. Because psychomotor poverty score during an acute episode of illness is confounded by the presence of secondary negative symptoms (e.g. social withdrawal due to hallucinations or paranoia; underactivity due to depression) we did not make a prediction regarding the changes in metabolism associated with changes in psychomotor poverty.

To assess whether observed changes were a direct effect of medication that preceded the changes in the clinical state, or, alternatively, a consequence of the alleviation of symptoms, we measured the changes in cerebral metabolism following the first dose of risperidone in addition to the changes after six weeks of treatment.

Experimental Procedures

Patients

Eight unmedicated subjects meeting DSM-IV criteria for schizophrenia were recruited into this study. The patient demographics are shown in Table 2 (see Chapter 2). Each patient underwent a semi-structured clinical interview conducted by ETCN. Symptom severity was rated using the scale for assessment of positive symptoms (SAPS) and scale for assessment of negative symptoms (SANS) (Andreasen 1997). Movement side effects were rated using the Simpson-Angus Scale (Simpson & Angus 1970), Automatic Involuntary Movement Scale (Guy 1976), and the Barnes Akathisia Scale (Barnes 1989). Patients were assessed prior to treatment and every two weeks for six weeks. Reality distortion was defined as the sum of the global delusion and global hallucinations scores. Disorganization was defined as the sum of positive formal thought disorder, inappropriate affect and poverty of content. In addition a score for psychomotor poverty was determined by adding the scores for global scores for poverty of speech, blunted affect and avolition. All subjects gave written ethical approval before the beginning of the study.

Imaging procedure and treatment protocol

Each subject underwent positron emission tomography (PET) scans using ^{18}F-fluorodeoxyglucose prior to initiation of risperidone, after the first dose, and after 6 weeks treatment. On the first study day, placebo was administered orally under single blind conditions 90 minutes before the injection of 18-FDG for the first scan. The second scan was performed 3 hours after the first. A 2mg dose of risperidone was administered orally 90 minutes before the injection of 18-FDG for the second scan.

On the second day, patients received 2 mg of risperidone in divided doses. On the third day the dosage was then increased to 4mg per day (in divided doses) if clinically indicated, and subsequently increased to a maximum of 6 mg daily (in two divided doses) if clinically indicated. Dosages were decreased as necessary to minimize extrapyramidal side effects. The mean dosage after 6 weeks treatment was 3.75 mg/day (range 2-6mg/day). After six weeks treatment, a third scan was performed 90 minutes after a 2mg dose of risperidone.

PET images were acquired using a CTI 953B scanner (CTI Knoxville TN) using a three dimensional acquisition protocol (Sossi et al., 1998). Subjects were injected with 2 millicuries of ^{18}F-FDG prior to each scan. In order to standardize mental state during glucose uptake, all subjects were engaged in a computerized continuous performance task prior to and for twenty

minutes after the injection of the radio labeled ^{18}F-FDG. Images were acquired in 4x5 minute frames beginning 60 minutes after injection.

Data Analysis

Image analysis was performed using Statistical Parametric Mapping software (SPM96, Wellcome Department of Cognitive Neurology, London UK). The images were aligned, spatially normalized, smoothed and corrected for variance in mean global image intensity as described in chapter 2. The effects of risperidone administration were modeled using the General Linear model and contrasts were performed to determine the change between baseline and first dose, and between baseline and 6 weeks treatment.

Effects of risperidone on cerebral metabolism

We tested for the effects of the first dose and of 6 weeks of treatment in regions specified on the basis of previous functional imaging studies of the relationship between schizophrenic symptoms and regional cerebral activity. A rectangular volume was defined in left anterior and medial temporal lobe (from x = -50 to –20, y=-20 to 20, z= -2 to –32) to include the area in which rCBF was found to be associated with the reality distortion syndrome (Musalek et al., 1989; Liddle et al., 1992; Silbersweig et al., 1995). A rectangular volume was defined in right medial frontal lobe (from x=0 to x=20mm, y=20mm to y=76mm, z=15 to z=35mm) to include the area in which rCBF was found to be associated with the disorganization syndrome (Liddle et al., 1992, Ebmeier et al., 1993, Yuasa et al., 1995)

To assess the main effects of treatment on the specified regions of interest, we employed Friston's criteria for assessment of significant changes in a priori regions (Friston, 1997). The method entails determining the probability, p(k), of chance occurrence of the observed number of contiguous voxels for which the z value exceeds a specified threshold, z_t. The optimum threshold depends on the type of effects predicted. When small amplitude diffuse effects are predicted, the optimum threshold is low, whereas when larger effects are predicted the optimum threshold is higher. Therefore, for testing hypotheses regarding the effects of a single dose of risperidone, we set the threshold at $z_t = 1.96$, and for testing the hypothesized effects of 6 weeks treatment, we set the threshold at $z_t = 2.33$.

In addition to the examination of the specified regions, we performed an exploratory analysis of the effects of 6 weeks treatment in the entire field of view, using the SPM96 correction for multiple comparisons appropriate for the entire field of view. In the regions of significant change, we then tested for changes after the first dose, using the Friston criterion for significance.

Correlation of changes in metabolism with symptom change

For the disorganization and reality distortion syndromes, we determined the correlation between syndrome score and change in metabolism after six weeks treatment in the right medial frontal and left anterior and medial temporal regions specified in our hypothesis. We calculated the Pearson correlation between syndrome change and average change in metabolism in those voxels with $z_t > 2.33$ in the most significant cluster in each of the two specified regions.

In addition we calculated the correlation between changes in reality distortion, disorganization and negative scores and changes in metabolism in those regions where significant change after 6 weeks treatment had been identified in the exploratory analysis. Bonferonni correction for multiple tests was used in testing the significance of all correlations for which we did not have a priori predictions.

Results

There were significant reductions in severity of reality distortion (3.9; s.d. 2.2) and disorganization (4.0; s.d. 2.2) after 6 weeks of treatment. Parkinsonian symptoms were mild in two subjects (Simpson & Angus score: 2) and moderate in one subject (Simpson and Angus score: 4). This subject also displayed mild akathisia (Barnes akathisia scale score: 1). One subject showed mild involuntary movement (Abnormal Involuntary Movement Scale Score: 1).

<u>Main effects of treatment on cerebral metabolism</u>

There were statistically significant changes in the right medial frontal cortex and the left temporal lobe after six weeks treatment with risperidone (Table 6). Following the first dose, there was a significant decrease in metabolism in the right medial frontal region, while there was a trend towards a significant decrease in the left anterior temporal search area [p(k) <0.09] (see Table 6, Figures 5 and 6). Risperidone had no effect on global metabolism.

Table 6: Decreases in metabolism after six weeks treatment with risperidone, and after a single dose, in right medial frontal cortex and in left temporal lobe.

	Talairach coordinates (x,y,z)	Z-score	uncorrected p value for voxel	Cluster Size (No. of voxels)	p(k) value for cluster size
Six weeks treatment					
Right medial frontal lobe	(0,56,28)	3.79	.00007	2456*	<.001
Left anterior-temporal lobe	(-30,10,-30)	3.45	.00025	5400*	<.001
Single Dose					
Right medial frontal lobe	(4,60,22)	2.7	.0035	1400	.001
Left anterior temporal lobe	(-50,-16,-8)	3.32	.00045	249	.09

* The clusters extended beyond the specified search areas. However, in both areas, the number of voxels within the search area exceeded the cluster size required to satisfy Friston's criteria for significant change at the level p<0.05.

Figure 5: Decreases in metabolism produced by 6 weeks treatment with risperidone in schizophrenic patients in:
a) left temporal lobe and ventral frontal cortex (plane: z = -16);
b) right medial frontal cortex (sagittal section: x=24)

Left temporal
lobe

a

Right medial
frontal cortex

b
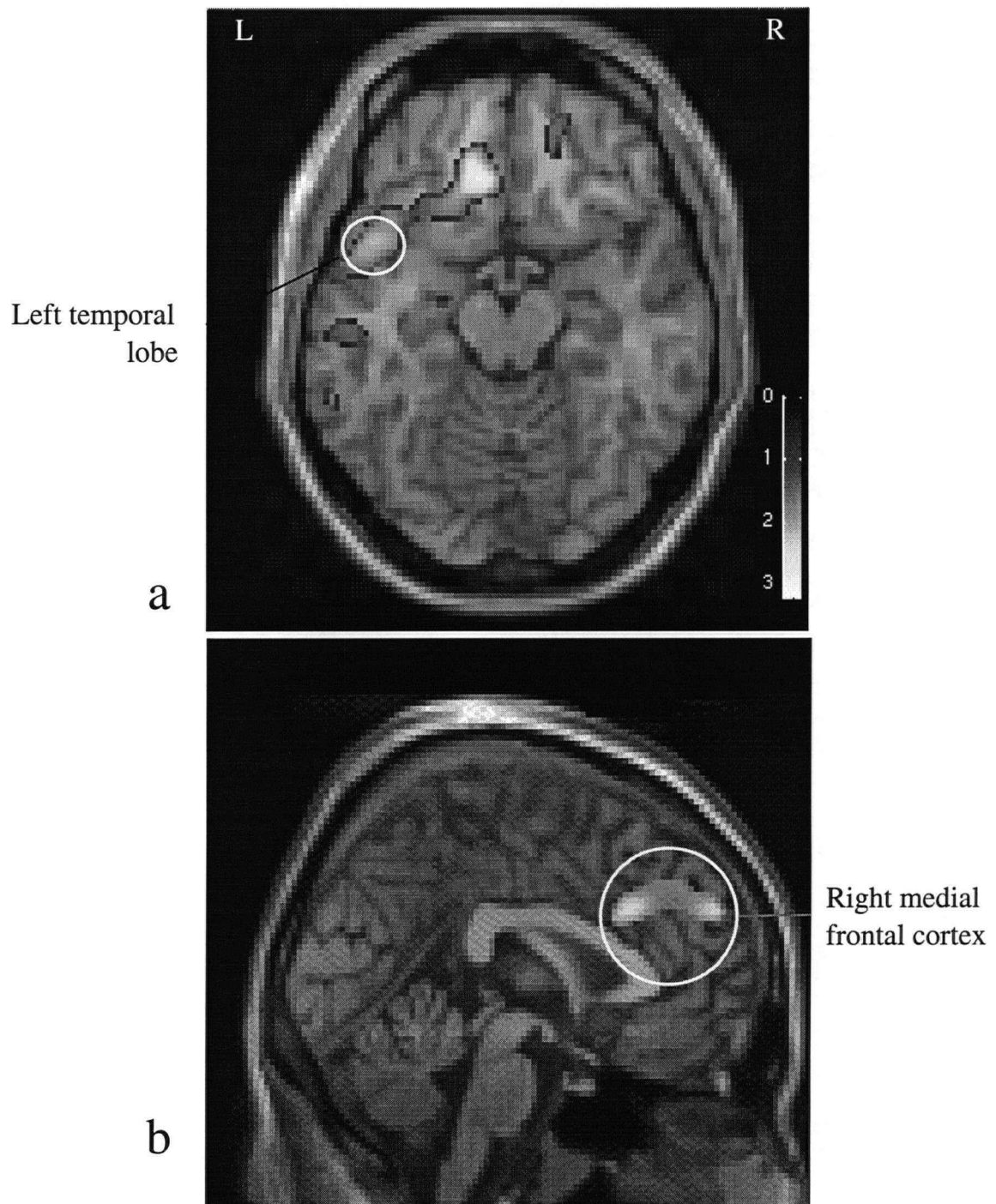
Figure 6: **Decreases in metabolism produced by 2mg risperidone in schizophrenic subjects, in:**
a) right medial frontal cortex (plane z=32);
b) left temporal lobe (plane z=8)
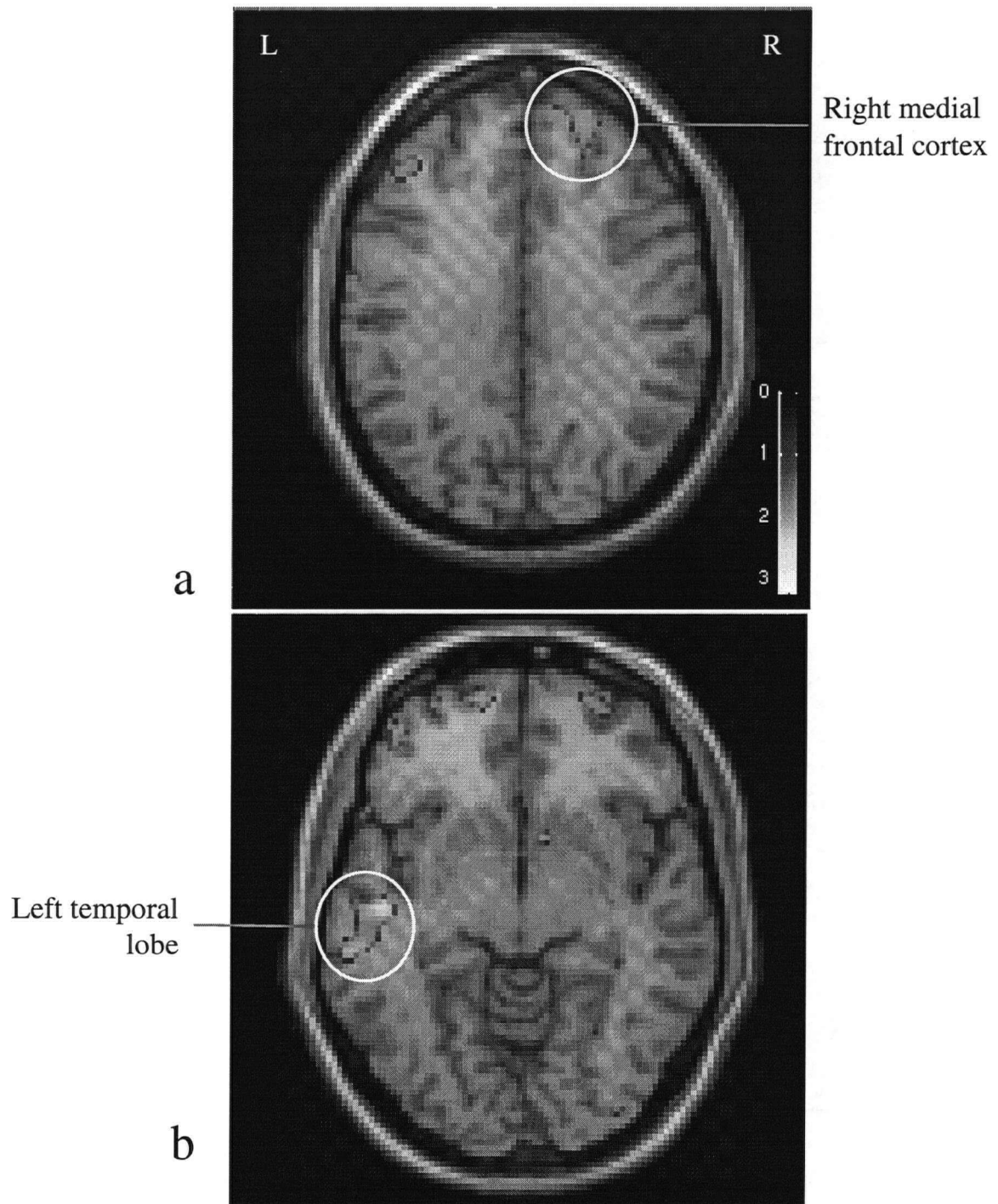
The exploratory analysis of the entire field of view found three areas of significant decrease in metabolism with local maxima in the left ventral-medial frontal, left dorsal-lateral frontal and left medial frontal regions (Table 7). A search of these areas in the single dose vs. baseline contrast found a significant decrease in metabolism in the left lateral frontal cortex (Table 8).

Table 7: Exploratory analysis of effects of risperidone on cerebral metabolism following six weeks treatment. The regional maximum z values indicating significant reduction in metabolism, that satisfy voxel-level criterion for significance after stringent correction for multiple comparisons, are presented.

	Talairach coordinates (x,y,z)	Z-score	corrected p value for voxel
Left ventro-medial frontal cortex	(-10, 34, -18)	4.94	0.008
Left dorso-lateral frontal cortex	(-30,10,-30)	4.6	0.034
Left dorso-medial frontal cortex	(-4, 24, 32)	4.56	0.04

Table 8: Exploratory analysis of effects of treatment following a single dose of risperidone in areas where significant change had been identified in a prior analysis of the effects of six weeks of treatment.

	Talairach coordinates (x,y,z)	Z-score	uncorrected p value for voxel	Cluster Size (No. of voxels)	p(k) value for cluster size
Left ventro medial frontal	(-22, 32, 29)	3.17	0.0008	1096	0.25
Left dorso-lateral frontal	(-36, 56, 8)	3.25	0.0006	2466	0.001
Left dorso-medial frontal	(-6, 39, 49)	3.64	0.00014	220	0.11

Correlations between metabolism change and symptom change

In accordance with a priori predictions the decreased activity in the left temporal region correlated with improvement in reality distortion (r=.712, p=.023). There was a strong trend in the predicted direction for the correlation between decrease activity in the right medial frontal cortex and improvement in disorganization symptoms (r=.573, p=.068).

There were no significant correlations between any of the three syndromes and metabolism change in the three left frontal regions identified in exploratory analysis. All of these correlations were less than 0.2 apart from that between change in disorganization and change in left dorsal medial frontal metabolism. The magnitude of this correlation (r=0.589) was similar

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to that between disorganization and metabolism in the adjacent right medial frontal cortex (r=0.573). The changes in metabolism in the right and left dorsal medial frontal cortices were highly correlated with each other (r=.894).

Discussion

In accordance with predictions, six weeks treatment with risperidone produced a significant decrease in activity in the left anterior and medial temporal lobe and in the right medial temporal lobe. The decrease in left temporal lobe metabolism correlated with reduction in reality distortion. There was a trend towards a significant correlation between decrease in metabolism in the right medial frontal cortex and decrease in severity of disorganization.

Ninety minutes following a single dose of risperidone, the decrease in metabolism in these two regions was present but less in magnitude and extent. These results suggest that the symptom improvement following treatment is due to the ability of risperidone to ameliorate the abnormally elevated activity in these brain regions. Furthermore, the significant changes detectable following a single dose of risperidone in these areas suggest that the changes in cerebral activity are the direct effect of the medication and not secondary to symptom resolution. In contrast, the decrease in activity in the left ventral-medial frontal lobe and left dorsal lateral frontal regions did not show significant correlations with symptom improvement.

IV.   EFFECTS OF RISPERIDONE ON CEREBRAL ACTIVITY IN HEALTHY SUBJECTS

Introduction

The site of action of antipsychotic drugs remains a subject of debate, although the fact that all established antipsychotic drugs block dopamine D2 receptors which are most abundant in the basal ganglia, has directed attention towards the basal ganglia. However, functional imaging studies indicate that the symptoms of schizophrenia are associated with aberrant cerebral activity at a diverse array of cerebral sites, including frontal and temporal cortex in addition to subcortical gray matter (Liddle et al., 1992, Ebmeier et al., McGuire et al., 1993, Silbersweig et al., 1995, Yuasa et al., 1995). Therefore, an understanding of the way in which antipsychotic drugs produce changes in function in diverse cerebral areas is essential for a full understanding of antipsychotic action.

There is an emerging body of evidence regarding the effects of antipsychotic drugs on regional cerebral metabolism in schizophrenic subjects (Bartlett at al., 1994, 1996; Tamminga 1999). One step in delineating the nature of the pharmacological processes that lead to these changes is the identification of which of these changes reflect the effects of antipsychotic medication on the human brain irrespective of clinical status, and which effects arise through an interaction of the pharmacological effects of the drug with the pathophysiology of schizophrenia. To achieve this step, it is necessary to compare the effects seen in schizophrenic subjects with those seen in healthy subjects.

Studies of the effects of typical antipsychotics in schizophrenic subjects have produced relatively consistent results. The principal features are reduction in frontal metabolism or regional blood flow (rCBF) and increases in metabolism or rCBF in the basal ganglia (Buchsbaum et al., 1987, 1992a, 1992b, 1992c; Wolkin et al., 1985; De Lisi et al., 1985; Bartlett et al., 1991; Potkin et al., 1994). These studies have examined the effects of sustained treatment, rather than a single dose. In a small study using SPECT, Berman et al., (1996) reported 6 weeks treatment with the atypical antipsychotic, risperidone, in elderly schizophrenic subjects produced reductions in frontal and temporal cortex blood flow.

Antipsychotic medications do not achieve their full effect for 4-6 weeks despite immediate receptor occupancy following initial administration (Seeman 1992). This raises the possibility that the cerebral changes produced by antipsychotics might develop over a period of several weeks. Therefore, it is important to compare the immediate effects of a single dose with the effects of sustained treatment. In a preliminary study of the time course of the effects of a single 10 mg dose of haloperdidol in schizophrenic subjects, Tamminga et al., (1999) found decreases in rCBF midline frontal cortex (including anterior cingulate) and left hippocampus, and increases in caudate/putamen and left thalamus. This study suggests that the reductions in cortical metabolism previously reported after sustained antipsychotic treatment are discernable even 2 hours after the first dose.

There have been no studies of the effects of sustained antipsychotic treatment on cerebral metabolism or blood flow in healthy subjects, but two published studies have reported the effects of a single dose of the typical antipsychotic, haloperidol, on regional metabolism in healthy subjects. Bartlett et al., (1994) reported that 12 hours after treatment with 5 mg of haloperidol there were widespread decreases in cortical, putamen and cerebellum metabolism. In contrast, Bartlett et al., (1996) reported no significant changes in regional metabolism 2 hours after administration of 5mg of haloperidol

In this paper we compare the effects of a single dose of risperidone on regional metabolism in healthy subjects with those regional changes produced by a single dose of risperidone in schizophrenic subjects, reported by Ngan et al., (chapter 3 of this thesis). In both studies, metabolism was measured in the interval for 90 to 140 minutes after oral administration of 2 mg of risperidone. Whereas in patients the need to proceed with treatment dictated that the first two scans in each subject be performed under single blind conditions, in fixed order, within a single day, these constraints did not apply in the study of healthy subjects. In healthy subjects it is feasible to compare cerebral activity following either placebo and or risperidone, administered under double blind conditions on two occasions that are a week or more apart. Such an experimental design is potentially more sensitive to small changes (because there is no need to correct for residual activity during the second scan) and also avoids possible bias due to a fixed order of scans, we elected to use this design in the healthy subjects.

In schizophrenic subjects, we found a significant decrease in metabolism in right medial frontal cortex and left dorsolateral frontal cortex, following a single 2mg dose of risperidone. There was also a trend towards a significant decrease in left anterior and medial temporal lobe. We found that after 6 weeks treatment there were significant decreases in all three of these areas. Furthermore, in accord with prior hypotheses based on the patterns of regional cerebral activity associated with schizophrenic symptoms, we found that the decrease in left anterior and medial temporal metabolism was significantly correlated with reduction in severity of reality distortion symptoms (delusions and hallucinations), while there was a trend towards a significant correlation between the decrease in right medial frontal metabolism and severity of disorganization symptoms (formal thought disorder and inappropriate affect). In contrast, the reduction in left lateral frontal metabolism was not related to changes in symptom severity.

On the basis of the observation that changes in left lateral frontal cortex were not related to symptom change in schizophrenic subjects, we hypothesized that similar changes would be seen in this region in healthy subjects. The expectation for changes in medial frontal lobe and left temporal lobe is less clear. It is possible that the therapeutic effects in patients might arise from a pharmacological process that also occurs in healthy subjects. Alternatively, the therapeutic effects might arise from an effect that involves the pathophysiology of psychosis. We therefore performed an exploratory examination in healthy subjects of changes in the areas where changes were related to symptom resolution in schizophrenic subjects.

Experimental procedures

Experimental design

Healthy subjects were administered either a placebo or 2mg of risperidone orally under double blind randomized conditions on two occasions, separated by one week. On both occasions, the subjects underwent a PET scan. Injection of ^{18}F- FDG was administered 90 minutes after the administration of the placebo or risperidone and scanning commenced 60 minutes later. The University of British Columbia and Vancouver Hospital ethical review boards approved the experimental procedure and all subjects gave written informed consent.

Subjects

Eleven healthy subjects were recruited for the study. One subject withdrew from the study following the first scan, another was excluded due to poor positioning in the PET camera. A total of 9 subjects (5 male, 4 female) were entered into analysis. These subjects were of similar age to the schizophrenic subjects studied by Ngan et al., (Chapter 3). The age range for schizophrenic subjects was 18 to 36 years (mean 26.5). For healthy subjects the range was 18 to 42 (mean 28).

Eight of the nine subjects were right handed according to the Annett handedness scale (Annett, 1970). Subjects were recruited using an advertisement and screened for a current or past history of psychiatric illness using the SCID structured clinical instrument of the DSM IIIR (Spitzer et al., 1990). A family history was also taken for each subject using collateral information when available.

The inclusion criteria for healthy controls were as follows:
- No lifetime history of psychotic illness, and no current axis 1 diagnosis, according to DSM-IIIR criteria.
- No history of schizophrenia, delusional disorder or bipolar disorder in a first degree relatives.
- Must be free of any psychotropic medication or drugs for the preceding 6 weeks.
- No current medical illnesses.

Imaging procedure

Images of regional glucose metabolism were obtained with a CTI 953B PET camera (CTI Knoxville TN) using ^{18}F-fluorodeoxyglucose (^{18}F-FDG), prepared by a modified procedure of Hamacher et al., (1986). Between-plane collimating septa were retracted to permit oblique photon paths allowing reconstruction of images in three dimensions (Sossi et al., 1998). Data were reconstructed into 31 contiguous axial slices covering an axial field of view of 10.8cm. For each scan, 2 millicuries of tracer was administered by slow injection over 1 minute, via a forearm cannula. To permit estimation of the input of tracer to the brain, the concentration of ^{18}F-FDG in the plasma of arterialized venous blood was measured in 15 samples collected over a period of 120 minutes following injection. To ensure a standard mental state during the period of uptake of ^{18}F-FDG, the subjects were engaged in a repeated stimulus continuous performance task. Digits were presented one at a time on a visual display unit, and the subject was instructed to press a bar whenever two identical digits were presented consecutively. Three-dimensional

image data was collected in three 5-minute frames commencing 60 minutes after tracer injection. Correction for absorption of radiation was made using data from a transmission scan obtained employing a ^{68}Ge germanium rod source.

Data analysis

Image analysis was performed using Statistical Parametric Mapping software (SPM 96; Wellcome Department of Cognitive Neurology, London) prepared as follows:
1) For each scan, the three 5 minute frames were aligned and averaged.
2) The 2mg risperidone images were then aligned to the placebo image.
3) Images were spatially normalized to match the PET image template in SPM96. To minimize the possibility that spatial normalization would remove differences between images due to risperidone, spatial normalization was performed on a mean image derived by averaging the placebo, and 2mg risperidone images. The normalization parameters were then applied to each image.
4) Images were smoothed using 10mm isotropic Gaussian filter to improve signal to noise.
5) Variation between scans in mean global image intensity was removed by proportional scaling, using SPM96.
6) For each voxel, the General Linear Model was used to estimate the mean change in scaled image intensity between the placebo scan and 2mg-risperidone image.
7) In addition, we identified voxels in which there was a conjunction between the effects of a single 2mg dose in healthy subjects and the effects of a single 2mg dose in schizophrenic subjects. This is a procedure for identifying voxels where the joint probability of metabolism decreases in both healthy subjects and schizophrenic subjects would be unlikely to be accounted for by chance.

In steps 6 and 7, we tested for changes in rectangular volumes of interest specified in the Talairach coordinate frame (Talairach &Tournoux 1988) in the cerebral areas that had been specified in the study of schizophrenic patients (Ngan et al., chapter 3). The volume in right medial frontal cortex had been specified on the basis of previous studies of the site of overactivity associated with the disorganization syndrome (Liddle et al., 1992; Ebmeier et al., 1993; Yuasa et al., 1995). That in the anterior and medial temporal lobe had been defined on the basis of previous studies of over-activity associated with reality distortion syndrome (Musalek et al., 1989; Liddle et al., 1992; Silbersweig et al., 1995). The volume in left lateral temporal lobe was defined to include the region in which metabolism was significantly decreased in schizophrenic patients after 6 weeks treatment in the study by Ngan et al., (chapter 3). These regions were:
•   Right medial frontal: x = 0 to 20 mm, y = 20 to 76 mm, z = 15 to 35 mm.
•   Left anterior and medial temporal lobe: x = -50 to –20, y = -20 to 20, z = –32 to –2.
•   Left lateral frontal cortex: x= -30 to -50 y= 10 to 50, z = 0 to 30 mm.
We applied the Worsley (1996) correction for multiple comparisons in a finite search volume, for each of these regions.

Results

In healthy subjects, risperidone produced extensive decreases in metabolism in left lateral frontal cortex and right medial frontal cortex (Figure 7). There was no evidence of changes in

basal ganglia or in the left temporal lobe even if a lenient criterion of p<0.05 (one-tailed, uncorrected) were to be applied. Risperidone had no effect on global metabolism.

Figure 7: Areas of decreased activity rendered onto right medial surface and left lateral surface in healthy subjects following 2mg risperidone, and in schizophrenic subjects following 2mg of risperidone and after 6 weeks treatment.
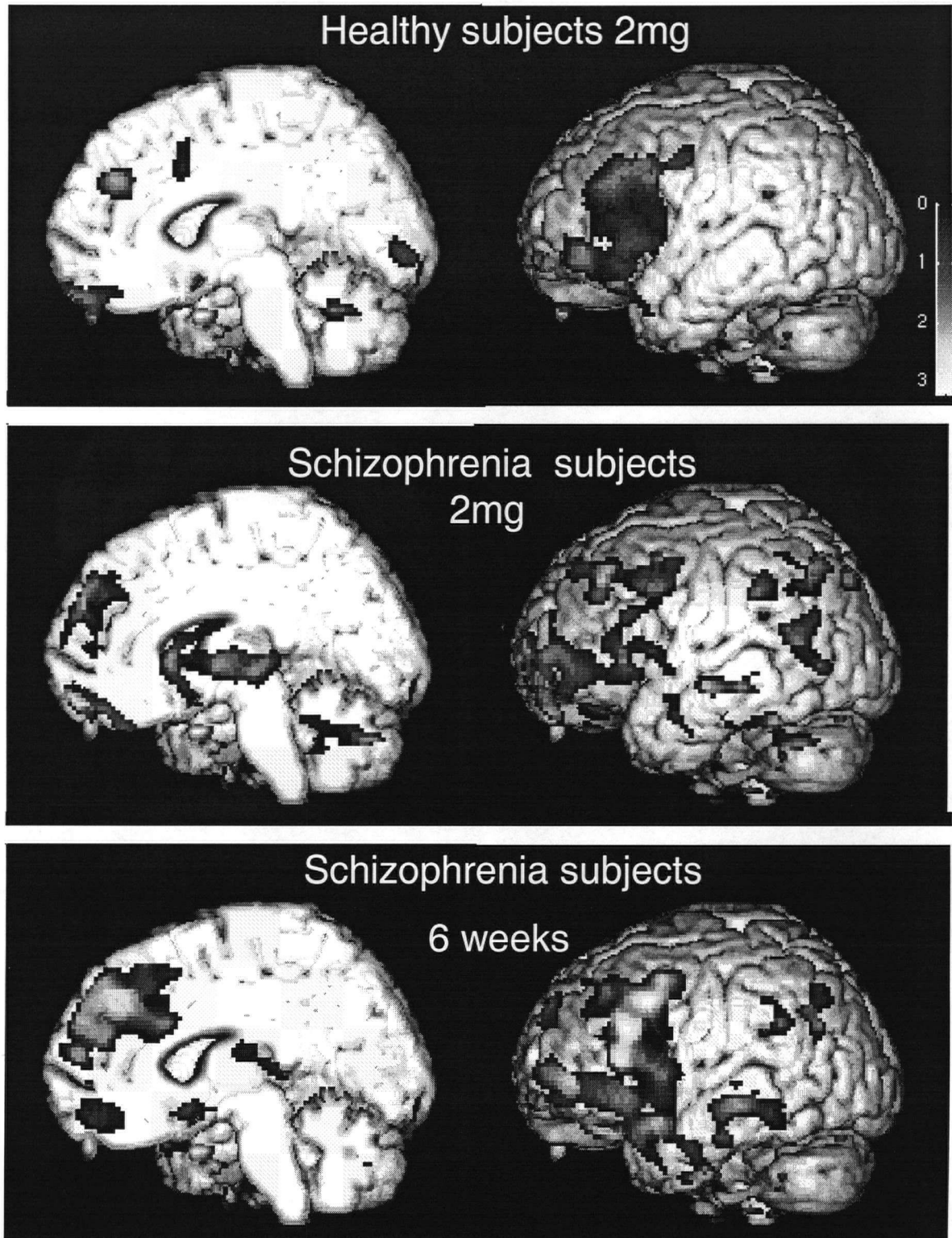
The reduction in metabolism in the specified region of the left lateral frontal cortex was statistically significant (Table 9). There was also a significant conjunction between the decrease in healthy subjects and in patients following the 2 mg dose of risperidone in this area (Figure 8, Table 10). The decrease in right medial frontal cortex was not significant after correction for multiple comparisons in patients (Ngan et al., chapter 3) following the 2 mg dose of risperidone in this area (Table 10). Nonetheless the proximity of this cluster to that observed in schizophrenic subjects, as illustrated in Figure 7, might suggests that this cluster is unlikely to be a chance occurrence. A deactivation in cerebral metabolism in the right medial frontal cortex of healthy subjects was confirmed by the observation of a significant conjunction between the decrease in healthy subjects and in patients in the right medial frontal cortex (Table 10).

Figure 8: Areas of conjunction between decreased metabolism observed in both schizophrenic and healthy subjects following 2mg risperidone, in the right medial and left lateral frontal cortex.
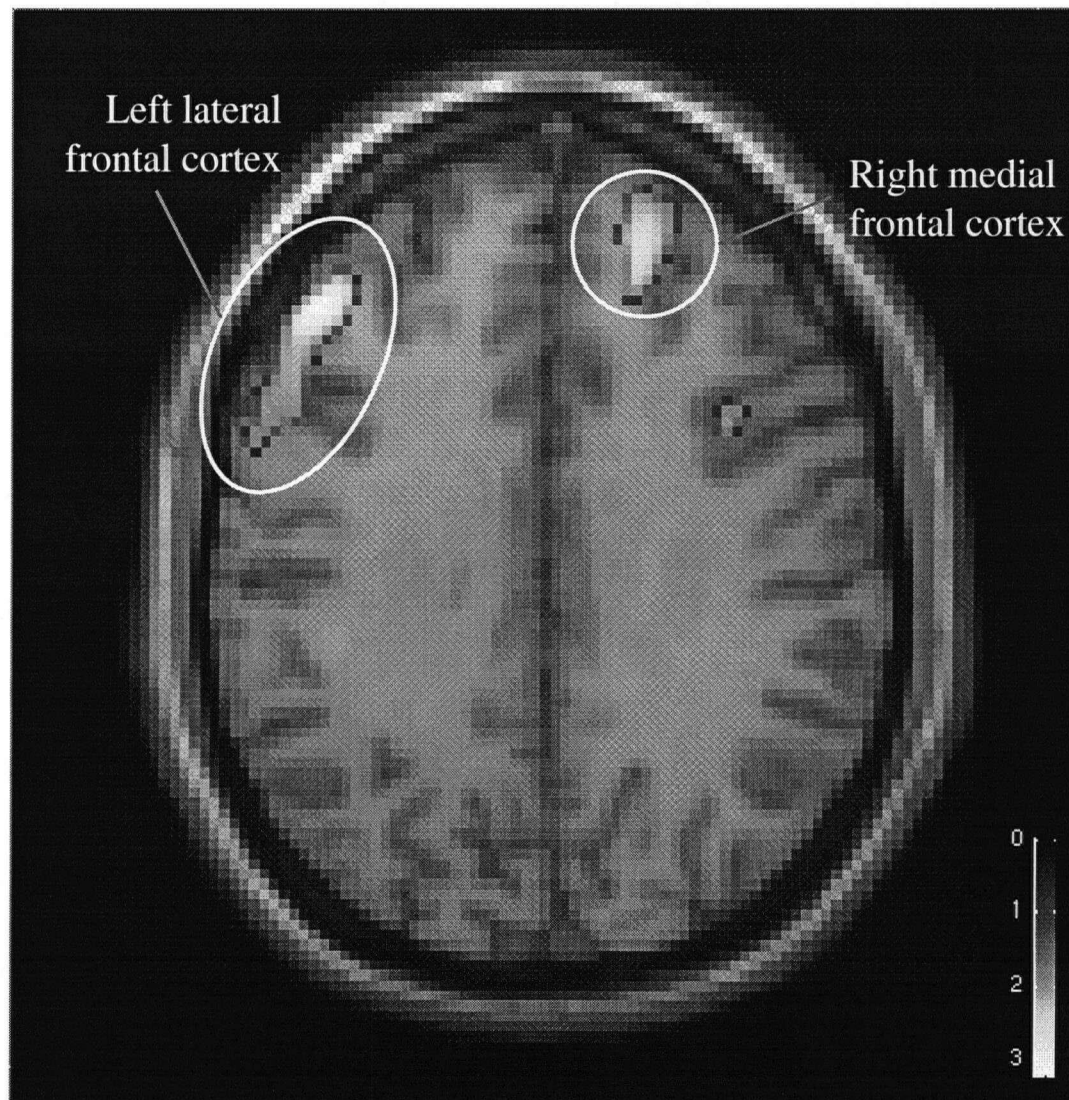
Table 9: Decreases in metabolism following 2mg risperidone in specified frontal lobe search volumes in healthy subjects

	Talairach coordinates of peak change	Peak z	uncorrected probability of peak z	Corrected probability of the peak z	Cluster size (k voxels)	Cluster probability p(k)
Left lateral frontal cortex	-48, 38, 28	3.88	0.0005	0.039	338	0.038
Right medial frontal cortex	14, 42, 32	3.57	0.0002	0.11	125	0.215

Table 10: Conjunction analysis demonstrating sites at which both schizophrenic and healthy subjects exhibit a reduction in metabolism following 2mg risperidone.

	Talairach coordinates of peak conjoint change	Peak z	uncorrected probability of peak z	Corrected probability of the peak z	Cluster size (k voxels)	Cluster probability p(k)
Left Lateral Frontal Cortex	-46, 44, 2	4.54	<0.0001	0.0022	2599	0.031
Right medial frontal cortex	18, 44, 32	3.75	0.00008	0.039	415	0.433

Discussion

As observed in schizophrenic patients, a single 2mg dose of risperidone produced a decrease in metabolism in right medial frontal cortex and in left dorsolateral frontal cortex. The finding of frontal cortical decreases is consistent with the observation by Bartlett et al., (1994) that 5mg of haloperidol produces extensive cortical decreases after a period of 12 hours. The greater extent of the reductions seen by Bartlett et al., possibly reflects the greater duration of time between administration of the medication and scanning or the differences arising from the difference receptor blocking profiles of haloperidol and risperidone.

The therapeutic effects of risperidone

The conjunction analysis demonstrated that risperidone produces a decrease in metabolism in healthy subjects at an identical location in right medial frontal cortex to the reductions produced in schizophrenic subjects. These reductions were in the region previously shown to be overactive in patients with the disorganization syndrome (Liddle et al., 1992, Ebmeier et al., 1993, Yuasa et al., 1995), and hence are likely to be related to the therapeutic effects of risperidone. This suggests that the therapeutic effect in this area arises from a pharmacological effect that occurs in healthy subjects.

In contrast, there was no evidence for a reduction in metabolism in the left anterior temporal lobe in healthy subjects, despite the fact that risperidone was observed to produce a reduction in metabolism in this area in schizophrenic patients that was correlated with reduction in reality distortion symptoms (Ngan et al., chapter 3). While the possibility that our study did not have sufficient power to detect a change in this area cannot be excluded, our finding nonetheless suggests that the therapeutic effect in this area arises from a pharmacological effect of risperidone that does not occur in healthy subjects. Liddle et al., (chapter 2) had demonstrated that the alleviation of reality distortion was predicted by the reduction of hippocampal activity produced by a single dose of risperidone. This is consistent with the hypothesis that the therapeutic effect depends on reduction of aberrant hippocampal firing that does not occur in healthy subjects.

The effects of risperidone on the left lateral frontal cortex

The observation that risperidone produces a significant reduction in metabolism in left lateral frontal cortex in both healthy subjects and schizophrenic patients indicates that it is an effect that is independent of illness status. In schizophrenic subjects this decrease became more marked after 6 weeks treatment (Ngan et al., chapter 3). However, unlike the changes observed in right medial prefrontal cortex or in left anterior and medial temporal lobe, there was no evidence that the changes in lateral frontal cortex are associated with therapeutic effects in patients. This raises the question of what are the functional implications of the decrease in left lateral frontal cortex induced by risperidone.

The left dorso lateral frontal cortex has been implicated in executive function, working memory and depression. Lesions of dorso-lateral prefrontal cortex in monkeys leads to deficits in working memory and to underactivity resembling the negative symptoms of schizophrenia (Goldman-Rakic, 1991).

In humans, lateral frontal lesions produce a dysexecutive syndrome (Chow and Cummings, 1999) and a syndrome of pseudo-depression characterized by apathy and poverty of speech (Blumer and Benson, 1975). Functional imaging studies demonstrate left lateral frontal under-activity in several conditions that are characterized by hypokinesia. For example, Liddle et al., (1992) demonstrated that left frontal under-activity is correlated with symptoms of psychomotor poverty in schizophrenia. Dolan et al., (1993) found that left frontal underactivity was associated with psychomotor retardation in depression. Several studies have demonstrated left frontal under-activity in progressive supranuclear palsy, a disorder characterized by akinesia and abnormal movements (Asanuma et al., 1993, Defebvre et al., 1995).

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Some studies of primary depression found significant hypometabolism involving the frontal dorsolateral cortex (primarily on the left hemisphere) and the caudate nuclei (Baxter et al., 1989). More recently, Mayberg et al., (1997) in a fluoxetine treatment trial in a group of patients with major depression found that a positive clinical response was associated with a significant metabolic increase (as measured with FDG-PET) in the dorso frontal cortex, and this increase was a normalization of the pretreatment hypometabolic pattern (Mayberg et al., 1995). While depression is also associated with underactivity of medial frontal cortex and anterior cingulate, it is possible that the lateral frontal underactivity in depression is predominantly associated with the executive abnormalities that produce symptoms such as poverty of speech (Dolan et al., 1993).

Depression is a frequent finding among patients with traumatic brain injury (Federoff et al., 1993). The severity of the depression is related to the distance of the lesion from the frontal pole on the left but not on the right hemisphere, an effect also seen in frontal stroke patients (Robinson and Szetela 1981).

Thus, there is substantial support for Blumer and Benson's observation that left lateral prefrontal cortex lesions can lead to a pseudodepression syndrome. It should be noted that medial frontal lesions especially lesions of the anterior cingulate cortex can also result in akinesia (Chow & Cummings 1999). It might be that the hypokinesia arising from medial frontal lesions reflects damage to the limbic neural systems engaged in motivation, whereas the hypokinesia associated with lateral prefrontal underactivity is part of a dysexecutive syndrome that includes impaired ability to plan and initiate activity. Thus, it is possible that the hypokinesia associated with lateral prefrontal cortex underactivity may be part of a dysexecutive syndrome (Goldman-Rakic, 1991) that includes impaired ability to plan and initiate activity.

It is therefore paradoxical that risperidone has been reported to be effective in treating negative symptoms (Marder et al., 1994) and working memory deficits (Green et al., 1997) in schizophrenia. However, it should be noted that the studies that have demonstrated these effects have compared risperidone with haloperidol. For example, in the study by Marder et al., (1994), various doses of risperidone were compared with haloperidol 20 mg per day. At such a dose, haloperidol produces marked akinesia (Van Putten et al., 1990). Thus it is possible that despite being beneficial compared with haloperidol, risperidone can nonetheless produce sub-clinical hypokinesia even at small doses. This possibility is consistent with the observation that the most common psychological adverse effect of risperidone in long term treatment is asthenia/lassitude/increased fatigability (Janssen-Ortho Inc, 1998). If this speculation is correct, perhaps the ideal antipsychotic would be one that produced decreased activity in medial frontal cortex and in temporal lobe, but not in lateral frontal cortex.

V. GENERAL DISCUSSION

We have demonstrated that risperidone produces an immediate reduction in metabolism in the cortico-striato-thalamic circuit, and also an immediate reduction in hippocampal metabolism that correlates with subsequent reduction in reality distortion symptoms. These findings are consistent with recent animal studies in rats, which show decreases in local cerebral glucose utilization of the hippocampus and mediodorsal nucleus of the thalamus following acute administration of risperidone (Huang et al., 1999).

It is likely that the reduction of activity in the cortico-striato-thalamic circuit reduces the propensity to all of the symptoms characteristic of an acute schizophrenic episode including not only reality distortion but also disorganization and psychomotor excitation. The correlation between reduction in hippocampal metabolism and subsequent symptom resolution supports the hypothesis that aberrant hippocampal activity plays a cardinal role in the pathophysiology of reality distortion. Although this is consistent with the accumulating body of evidence indicating that reality distortion syndrome is associated with left medial temporal lobe overactivity it is at first sight inconsistent with that evidence that removal of the hippocampus evokes psychotic symptoms (Falconer and Taylor 1968). This suggests that underactivity of the hippocampus may be responsible for psychotic symptoms. On the other hand, it is possible that following resection of the hippocampus, spurious firing of the post-synaptic neurons downstream from the hippocampus results in denervation supersensitivity. This spurious post synaptic firing would achieve the same effect of hippocampal firing thus leading to a possible psychosis.

However, the expression of reality distortion is associated with more widespread cortical over-activity (Liddle et al., 1992, Silbersweig et al., 1995). Our finding of reduction in metabolism in left temporal lobe after 6 weeks treatment that correlates with reduction in severity of reality distortion is consistent with the hypothesis that the immediate reduction in aberrant hippocampal firing and in cortico-striato-thalamic feedback to the cortex leads to subsequent resolution of the cortical over-activity associated with reality distortion. Furthermore, the evidence suggesting that these changes are already discernible 90 minutes after the first dose, (before significant resolution of symptoms) indicates that these changes are a direct reflection of the therapeutic process, rather than a consequence of symptom resolution.

Overall, our findings provide support for the conclusion that the reduction in left temporal metabolism produced by risperidone is a manifestation of its antipsychotic action. Furthermore, it should be noted that there was no evidence that risperidone produced changes in metabolism in the temporal lobe in healthy subjects. This suggests that the putative therapeutic effect is not a manifestation of the normal pharmacological action of risperidone, but rather, arises from an interaction between the pharmacological action of riperidone and the pathophysiology of psychosis.

The right medial frontal cortex metabolism was significantly decreased following an initial dose of risperidone and following 6 weeks treatment. The magnitude and extent of this effect was significantly increased at the end of 6 weeks treatment. There was a strong trend towards a significant correlation between the decrease in metabolism in the right medial frontal cortex and a decrease in the severity of disorganization. This suggests that the symptom improvement following treatment is due to the ability of risperidone to ameliorate the abnormally elevated activity in these brain regions. It should be noted that we also observed a significant

conjunction between the sites of reduction in right medial frontal cortex metabolism after a single dose of risperidone in patients and in healthy subjects. This indicates that if indeed the reduction in metabolism in this cerebral region is an aspect of the therapeutic effect of risperidone, it reflects a process that also occurs in the healthy brain.

Overall, our conclusions regarding the therapeutic relevance of the effects of risperidone on the right medial temporal lobe must remain tentative. Despite the observed trend towards the predicted correlation between reduction in metabolism and reduction in severity of disorganization symptoms, it should be borne in mind that this effect did not reach statistical significance, and any interpretation should be made cautiously. Our inability to draw a definite conclusion for this brain area is a probably a consequence of the limited sample size in our study.

We found a similar reduction in left lateral frontal lobe metabolism after a single dose of risperidone in schizophrenic patients and in healthy subjects. In the patients, this effect became more pronounced after 6 weeks of treatment. However, the reduction in metabolism in this cerebral area was not related to change in symptom severity. These findings indicate that the observed reduction in lateral frontal metabolism is a part of the normal pharmacological effect of risperidone, but it is not directly related to its therapeutic action. In light of the evidence (discussed in chapter 5) that left lateral frontal hypometabolism is related to akinesia in a variety of different circumstances, it is possible that the observed reduction in left lateral frontal metabolism is a reflection of a tendency for risperidone to cause a degree of hypokinesia.

Time course of effects of antipsychotics on cerebral activity

We found that changes in cerebral metabolism following treatment are discernable after a 2mg dose of risperidone and are strengthened following 6 weeks of treatment. In particular we found decreases in metabolism in ventral striatum, right medial frontal cortex and left lateral cortex that were discernable within two hours after a 2 mg dose of risperidone.

There have been few previous studies of the immediate effects of antipsychotics. In a study by Bartlett et al., (1996) no immediate effects on cerebral glucose metabolism were observed 2 hours following oral administration of 5 mg of haloperidol in a study of healthy subjects. However, in an earlier study these authors had reported wide spread cortical decreases 12 hours following administration of a 5 mg dose of haloperidol (Bartlett et al., 1994). Furthermore, after 12 hours there were significant increases in metabolism in the caudate and putamen.

Using PET with ^{15}O, Tamminga et al., (1999) found decreased rCBF in frontal and cingulate cortex during the first four hours following an oral administration of 10mg of haloperidol. These studies also demonstrated increased rCBF in the caudate that lasted through the first 8 hours of haloperidol administration. The finding of Tamminga et al., and Bartlett et al., regarding the effects of typical antipsychotics are consistent, insofar as both groups of investigators reported reduction in cortical activity and increase in basal ganglion activity, although when using a larger dose of haloperidol Tamminga et al., (1999) observed changes that were detectable after two hours whereas Bartlett et al., (1996) did not.

Of particular interest is that these studies of the effects of the typical antipsychotic haloperidol found increases in dorsal striatum, whereas we did not. This effect may be related to antipsychotic induced EPS associated more commonly with haloperidol than with risperidone (Peuskens et al., 1995).

Our finding of immediate effects in those regions where changes in cerebral metabolism were correlated with subsequent symptom alleviation is consistent with the time course of action of risperidone reported by Chouinard et al., (1993) (Figure 9). They found that the greatest rate of symptom reduction during treatment with risperidone (6mg/day), was during the first week. This observation of a discernable decrease in symptoms in the first week of

Figure 9. The clinical effects of risperidone compared to haloperidol in patients with schizophrenia (Chouinard et al., 1993).
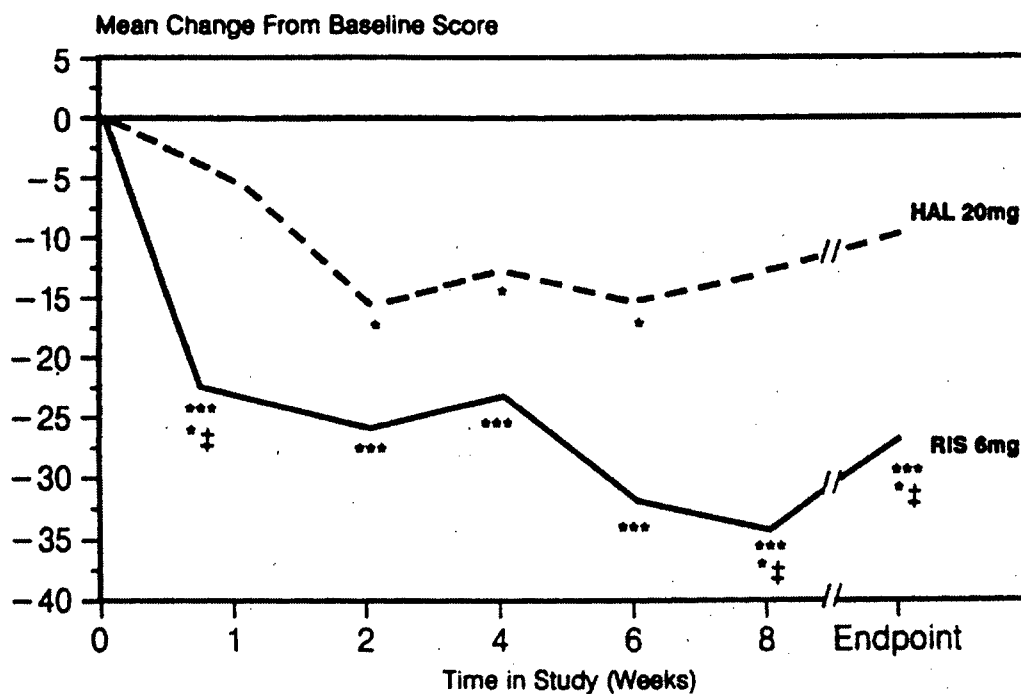
Figure 10. The clinical effects of α and β flupenthixol compared to placebo in recently admitted patients with schizophrenia (from Johnstone et al., 1978).
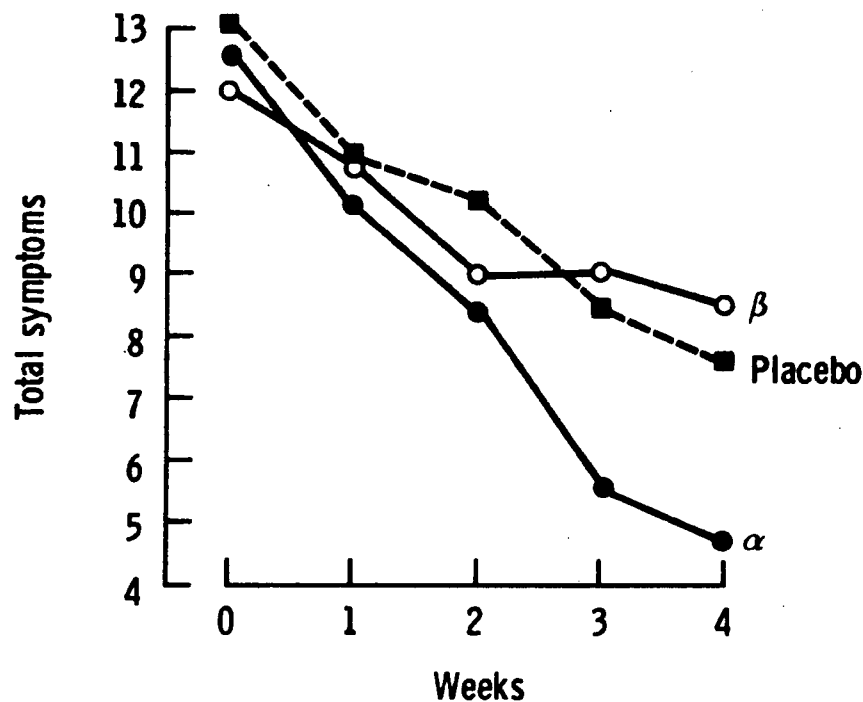
treatment is consistent with the observations from other treatment trials with other antipsychotics. For example in a trial of alpha flupenthixol, Johnstone et al. (1978), the rate of reduction in symptoms during the first week was similar to that during subsequent weeks of the trial.

Statistical considerations when observing the immediate effects of antipsychotics.

We have shown that the magnitude and the extent of the increase of metabolism is small following the first dose and becomes more extensive and greater in magnitude over the 6 weeks of treatment with risperidone. Due to the repeated measures design of our data we were able to observe changes following an initial 2mg dose of risperidone in those areas which showed significant reductions following 6 weeks of treatment. In addition we were able to observe if areas where change in cerebral metabolism was correlated with changes in schizophrenic symptoms were observable immediately following a 2mg dose.

The sensitivity to detect small changes whose location cannot be defined precisely a priori is low when using a region of interest (ROI) method as employed by Barlett et al., (1994, 1996), because it is unlikely that the specified borders of the ROI will coincide exactly with the region of metabolic change. The ability to see immediate changes in both the study of Tamminga et al., (1999) and our own work may be due to the use the voxel based method incorporated in SPM. When used with a test for statistical significance that allows for the multiple comparisons involved in searching a restricted search volume, voxel based analysis is potentially very sensitive.

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In chapter 2 we report schizophrenic subjects to show a correlation between decreased metabolism in the hippocampus following a single 2mg dose of risperidone and a change in reality distortion syndrome 6 weeks later. Assuming there is no systematic trend for metabolism to increase in this area in any subgroup of patients this observation would lead to the expectation that the group would exhibit a decrease in metabolism in the hippocampus. However, we did not observe a significant decrease in hipocampal metabolism following the single 2mg dose of risperidone. Nonetheless, we observed a trend (p <.09) for decreased metabolism. It is probable that our failure to observe a significant change is due to the limited statistical power of this study.

Future implications for pharmacotherapy

It would be a significant advancement in pharmacotherapeutics, if it were to be possible to provide a direct measurement of expected therapeutic effects after the administration of a single dose of an antipsychotic. Our observation that the changes in cerebral function ultimately associated with symptom resolution can be discerned after a single dose of risperidone opens the door to this possibility. In the future drugs might be developed with the object of achieving the cerebral changes that we have shown are related to therapeutic effects.

In addition to assisting in development of new drugs, an understanding of the cerebral changes that lead to the alleviation of symptoms might, at least in principle, facilitate the efficient selection and adjustment of medication in individual patients. At present, the adjustment of medication on the basis of clinical response is slow and liable to be confounded by a multiplicity of extraneous factors that can influence the observable clinical state. However, testing the response of individual subjects to schizophrenic treatment would not be feasible using PET due to the high costs and limited accessibility of this technique. Other forms of functional imaging (ERP, fMRI) are emerging to provide economical tests of neuronal changes following drug administration and may provide a novel method to test drug specificity and efficacy in individual subjects without waiting a period of weeks. These techniques may be used in schizophrenic subjects in hospital during the initial phase of their treatment.

Although the majority of our hypotheses were confirmed and most of our findings reached statistical significance, we must be cautious of interpretation and generalization to the entire first episode medication free schizophrenic population because the sample size was small. Replication of this study with a larger sample size is warranted.

APPENDIX 1: THE TWO SCAN PROCEDURE: ALLOWING FOR RESIDUAL RADIOACTIVITY.

Introduction

Serial measurements of glucose metabolism using the FDG PET method offer the possibility of measuring the effects of pharmacological agents on regional cerebral activity. Because the time scale over which glucose metabolism is measured is much longer than the time scale of interest of the variation in cerebral activity associated with cognitive tasks, the FDG PET technique has been superseded by regional cerebral blood flow (rCBF) techniques for the purpose of measuring regional cerebral activity associated with cognitive tasks. However, for the measurement of changes in cerebral activity associated with pharmacological effects, which typically occur on a time scale of several hours, the FDG PET technique offers potential advantages over rCBF techniques. By virtue of the relatively long half-life of the isotope ^{18}F, radioactive decay events can be counted over a relatively long time scale and the ratio of signal to noise in FDG PET images is usually higher than that in rCBF images. Furthermore, the short path length of positrons emitted by ^{18}F ensures better spatial resolution than is possible with the isotope ^{15}O used in techniques for imaging rCBF. This enhanced spatial resolution is valuable when measuring cerebral activity in relatively small structures such as the ventral striatum, which is of special interest in the pharmacological treatment of schizophrenia.

For the study of disorders such as acute schizophrenia, in which clinical state might change within a time scale of several days, it is preferable to be able to measure the changes in regional cerebral activity produced by a pharmacological agent within a single scanning session. However, because the half-life for radioactive decay of ^{18}F is 110 minutes, a substantial amount of radioactivity from the first (pre-treatment) scan will remain in the brain at the time of the second (post-treatment) scan.

Brooks et al., (1987) have demonstrated that it is possible to make a satisfactory correction for the residual radioactivity when FDG scans are performed 60 minutes apart. In the method used by Brooks, the residual radioactivity was predicted using the Sokoloff model, as modified by Phelps et al., (1979) to include the effects of the phosphatase activity. The Sokoloff-Phelps model employs four transfer coefficients, k_1, k_2, k_3 and k_4, to model the transfer of FDG between three pools: FDG in blood; FDG in the extra-vascular precursor pool; and FDG-6-phosphate in the metabolic pool. K_1 is the transfer coefficient for transfer from blood to precursor pool, k_2 is the coefficient for transfer from precursor pool to blood, k_3 is the bimolecular rate constant for phosphorylation of FDG, and k_4 is the unimolecular rate constant for de-phosphorylation of FDG-6 phosphate. Because FDG-6-phosphate is a poor substrate for the enzyme glucose-6-phosphatase, k_4 is small and phosphatase activity has an effect that is not clearly detectable until about 120 minutes after injection of 18-FDG.

In principle, estimation of glucose metabolic rate requires the derivation of the four transfer coefficients from the observed time course of accumulation of radioactivity in brain tissue. This is not practical on a routine basis in patients, so the usual practice is to perform a single

phosphate trapped in the metabolic pool and a much smaller contribution from FDG in the precursor pool. The amount trapped in the metabolic pool is approximately proportional to $k_1k_3/(k_2+k_3)$, which is in turn proportional to the metabolic rate for glucose. The exact expression for accumulated radioactivity contains a major term that is proportional to $k_1k_3/(k_2+k_3)$, modified by relatively small correction terms that can regarded as representing the radioactivity in the precursor pool; the lag in equilibration of tracer between blood to the metabolic pool; and the action of glucose-6 -phosphatase. These small correction terms depend on values of the rate constants, k_2, k_3 and k_4. A single measurement of tissue radioactivity at about 40 minutes can be used to estimate the quantity $k_1k_2/(k_2+k_3)$ for that subject, provided that nominal values for the individual constants k_2, k_3 and k_4 are employed to estimate the relatively small correction terms. The nominal values of the rate constants used in estimating the correction term are usually based on measurement of the full time course in a reference group of subjects.

The method used by Brooks et al., (1987) for accounting for residual radioactivity from the first scan contributing to radioactivity measured during the second scan in a repeated scan procedure, in effect, employs the radioactivity measured during the first scan to estimate the quantity $k_1k_3/(k_2+k_3)$ for that subject, and then uses this value together with nominal values for the individual rate constants, k_2, k_3 and k_4 in the small correction terms, to predict residual tracer in the metabolic pool during the second scan. Under circumstances where the second injection of radioactivity was given immediately after completion of the first scan (resulting in an inter-scan interval of 60 minutes) Brooks et al., (1987) achieved a satisfactory test re-test variation in estimated glucose metabolism ranging from 2% to 9%, in four subjects. However, for the purpose of studying the effects of pharmacological agents on cerebral activity, an inter-scan interval greater than 60 minutes is required because the pharmacological agent cannot be administered until after completion of the first scan, and time must be allowed for the pharmacological effect of the drug to be achieved. For example, assuming that tissue radioactivity is to be measured over a period of 20 minutes, starting 40 minutes after injection of radioactivity, and furthermore, that we wish to measure the pharmacological effect of the drug in the period 90-120 minutes after drug administration, the required inter-scan interval is 150 minutes, and the time from first injection to the mid-point of the second scan is 200 minutes.

With a longer inter-scan interval, the amount of residual radioactivity is expected to be less than with a shorter inter-scan interval, reducing the importance of accurate estimation of residual activity. However, the magnitude of the phosphatase effect will be greater, increasing the expected error due to use of a nominal value for k_4. The uncertainty due to inaccurate estimation of the phosphatase effect might in principle be reduced by performing a pre-injection scan shortly before the second injection of radioactivity, thereby providing two widely spaced measurements in the time course of accumulation of radioactivity from the first injection. This would permit estimates of two parameters, one proportional to $k_1k_3/(k_2+k_3)$ and the other related to k_4, for each individual subject. This would in turn allow more accurate estimation of the residual radioactivity in the metabolic pool.

The objects of the present report are two-fold:
1) To measure the variation in k_4 between schizophrenic subjects, and to compare the predicted residual tracer concentration at time of a second scan performed three hours later, derived using the subject-specific value of k_4, with the predicted value derived employing a nominal value of k_4. (The subjects in this study were participants in a double-FDG scan protocol, in

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which they received a second injection of FDG approximately 3 hours after the first injection, and hence, in this study it was not possible to actually measure the residual activity.)

2) To test the accuracy of using a measured value of k_4 to predict residual radioactivity 3 hours after the first scan, in a group of subjects who did not receive a second FDG injection, but were nonetheless scanned again approximately three hours after the first scan.

It should be noted that it is only possible to determine total residual activity rather than residual FDG-6-phosphate in the metabolic pool. Residual FDG-6-phosphate is of greater relevance for correcting for residual radio-activity in a subsequent post-treatment scan, because residual FDG in the precursor pool after treatment will be converted to FDG-6-phosphate at the post-treatment metabolic rate. However, during a pre-injection scan at approximately 180 minutes the amount of FDG in the pre-cursor pool is very small, so comparing measured residual total radioactivity with predicted total radioactivity is an adequate test of the accuracy of the prediction of residual FDG-6-phosphate in the metabolic pool.

Predicting Residual Concentration in Tissue:

The expression predicting total tissue radioactivity at T_2 from that at time T_1 can be derived from the expression for the tissue concentration of radioactive tracer. Using the nomenclature employed by Brooks et al., (1987):

$$c_i(t) = A \ I(0,t,\alpha_1) + B \ I(0,t,\alpha_2) \qquad \qquad ......(1)$$

$$\text{where } I(t_a,t_b, \alpha) = \int_{t_a}^{t_b} \exp{-\alpha(t_b-t')}c_p(t') \ dt'$$

$c_p(t')$ is the plasma concentration of tracer at time t'. A,B, α_1 and α_2 are combinations of the rate constants, k_1, k_2, k_3 and k_4 defined by:

$$A = k_1 \ (k_3+k_4-\alpha_1) \ / \ (\alpha_2-\alpha_1)$$
$$B = k_1 \ (\alpha_2-k_3-k_4) \ / \ (\alpha_2-\alpha_1)$$

$$\alpha_1=0.5(k_2+k_3+k_4 - \sqrt{(k_2+k_3+k_4)^2-4k_2k_4}\ )$$

$$\alpha_2=0.5(k_2+k_3+k_4 + \sqrt{(k_2+k_3+k_4)^2-4k_2k_4}\ )$$

In equation 1 and all subsequent equations, radioactivity concentrations have been corrected for the physical decay of ^{18}F to a standard reference time.

If the phosphatase correction is ignored (i.e. k_4 is taken to be zero), the asymptotic value of $c_i(t)$ as t approaches infinity, contains a factor $k_1.k_3/(k_2+k_3)$ which is proportional to glucose metabolic rate. Hence, it is convenient to re-formulate the expression for $c_i(t)$ in the form of a factor $[k_1.k_2/(k_2+k_3)]$ multiplied by a second factor that contains terms involving the individual rate constants, (reflecting corrections for FDG in the precursor pool, lag in equilibration between plasma and tissue, and phosphatase activity). If this second factor is evaluated using nominal values of the rate constants, the resulting approximate expression for $c_i(t)$ would be expected to be valid at times which are sufficiently long that the amount of FDG in the precursor pool is small and the effects of lag in equilibration are negligible, yet not so long that

phosphatase activity has produced appreciable dephosphorylation of FDG. Re-writing equation (1) in such a form yields:

$$c_i(t) = (k_1k_3/(k_2+k_3)) [\beta_1. I(0,t,\alpha_1) + \beta_2(k_2/k_3) I(0,t,\alpha_2)] \qquad ....(2)$$

where
$$\beta_1 = (k_3+k_4-\alpha_1)(k_2+k_3) / k_3(\alpha_2 - \alpha_1)$$
$$\beta_2 = (\alpha_2-k_3-k_4)(k_2+k_3) / k_2(\alpha_2 - \alpha_1)$$

It can readily be shown that provided k_4 is much less than k_2 and k_3, β_1 and β_2 are near to unity. The factor in square brackets in equation (2) can be evaluated using nominal values of the rate constant k_2, k_3 and k_4. The ratio of tissue concentration of tracer at time T_2 to that at time T_1 is given by:

$$R = [\beta_1 I(0,T_2,\alpha_1) + \beta_2(k_2/k_3)I(0,T_2,\alpha_2)] / [\beta_1 I(0,T_1,\alpha_1) + \beta_2(k_2/k_3)I(0,T_1,\alpha_2)] ....(3)$$

From the computational point of view, it should be noted that R depends only on k_2, k_3 and k_4, and the time course of the concentration of tracer in plasma. Hence, if nominal values of the rate constants (for gray matter) are used to evaluate this expression for R, the estimated value of the ratio is constant across all gray matter regions. Furthermore, k_1 does not appear explicitly in the expression. The brain responds to varying metabolic demand by adjusting capillary vasodilation, thereby leading to changes in k_1, and k_1 tends to be the most variable of the rate constants.

Phelps et al., (1979) report that plasma concentration of tracer can be fitted in a satisfactory manner by four exponentials. If the concentration of tracer in plasma decreases exponentially, the integrals in equation (3) can readily be evaluated exactly. For those subjects for whom the plasma time course can not be fitted satisfactorily by such an exponential time course, the integrals can be evaluated using a piece wise fitting procedure for the plasma time course.

Study 1: Measurement of the variation in phosphatase activity (k_4) within a group of schizophrenic subjects.

Subjects

6 schizophrenic patients undergoing a study of the effects of the anti-psychotic drug, risperidone, on cerebral activity.

Experimental Procedures

Scanning Procedure

In the period 40-60 minutes (denoted by T_1) after injection of 2mCi of ^{18}F-FDG, tissue radioactivity was measured using a CTI B PET scanner (CTI Knoxville TN) in 2D acquisition mode. At approximately 100 minutes after the injection of radioactivity, a 2 mg dose of risperidone was administered. Approximately 180 minutes after injection (denoted by T_2) a 10 minute duration scan was performed prior to the injection of a second dose of 2mCi of ^{18}F-FDG. (A subsequent post-treatment scan was performed 40-60 minutes after the second injection. The

findings regarding the effects of treatment on regional metabolism are reported separately. The present study addresses the issue of the accuracy of predicting residual activity at time T_2). Venous blood, arterialized by heating the hand to approximately 44^0C (Phelps et al., 1979), was collected for estimation of plasma radioactivity at approximately 1 minute intervals in the first 3 minutes, at 5 minutes intervals in the subsequent 25 minutes and then at approximately 45, 60 and 190 minutes after the first injection of ^{18}F-FDG.

Data Analysis

Tissue radioactivity at 50 minutes and 190 minutes after injection of 18-FDG was determined and averaged in three slices in each subject, using MPITool image analysis software (Multi Purpose Imaging Tool MPITool, Advanced Tomovision). All measured radioactivity values were corrected for physical decay. For each subject, the mean ratio of corrected tissue radioactivity, $R(T_2:T_1)$, at 190 minutes to that at 50 minutes was determined. The value of α_1 for that subject was determined from the measured value of $R(T_2:T_1)$ using equation (3). This equation gives the value of R corresponding to a specified value of α_1. The value of α_1 corresponding to the measured value of R was determined by serial adjustment of α_1 starting from the value 0.00455. The measured time course of blood radioactivity in blood was fitted by a four exponential curve employing the procedure of Whittal et al., (Whittal & MacKay, 1989). When the blood radioactivity curve consists of a sum of exponentials, the integrals in equation (3) can be evaluated exactly, for any specified values of α_1 and α_2. (α_2 varies by a negligible amount as k4 varies within the naturally occurring range). The coefficient α_1 is approximately equal to k4, and can be regarded as an indirect measure of phosphatase activity.

For each subject, the residual activity three hours after the first scan (i.e. 240 minutes after injection) predicted using the estimated value of α_1 for that subject was compared with that predicted using a nominal value of α_1. The nominal value of α_1 (0.00455) was calculated from the nominal value of the transfer coefficients employed in Metab Tool software (CTI Knoxville TN).

Study 2: Testing the accuracy of prediction of residual tracer based on estimation of phosphatase activity for each individual subject.

Subjects

Three healthy control subjects and one schizophrenic subject participated in study 2. In the healthy control subjects, the procedure was performed twice, two weeks apart: once after receiving placebo and once after receiving risperidone, in random order. The schizophrenic subject was one of the patients who had been scanned for study 1. He was scanned again on a single occasion after 6 weeks of treatment with risperidone.

Experimental Procedure

Scanning procedure

A 2 millicurie dose of ^{18}F-FDG was injected 90 minutes after the administration of either risperidone or placebo. Scanning in 2D-acquisition mode was performed at 50 minutes and 180 minutes after ^{18}F-FDG injection, for the purpose of estimating α_1. A dummy injection

containing no radioactivity was administered at 190 minutes and tissue radioactivity again determined 240 minutes after the initial injection of radioactivity, for the purpose of comparing observed tissue radioactivity with that predicted on the basis of the estimated value of α_1. Arterialized venous blood was collected as in study 1, and the time course fitted by a four exponential decay curve.

Data analysis

Tissue radioactivity at 50 minutes, 190 minutes and 240 minutes after injection of 18-FDG was determined and averaged in 6 regions in each subject, using MPI tool, and corrected for physical decay. For each subject, the mean ratio of corrected tissue radioactivity, R(T2:T1), at 190 minutes to that at 50 minutes was determined. The value of α_1 for that subject was determined from the measured value of R(T2:T1) using equation (3), as in study 1. Using this value of α_1, the predicted value of the ratio of tissue radioactivity, R(T3:T2), at 190 minutes to that at 50 minutes was determined, and compared with the measured value.

Results

Study 1: Estimation of the variability in α_1 between subjects.

The range of values of α_1 vary between subjects from 0.0039 min^{-1} to 0.0066 min^{-1} (see table 11). The discrepancies between the predicted values using the measured and nominal values of α_1 range from −10% to +25%.

Table 11: Comparison between the predicted tissue radio-activity concentration at the time of the second scan (relative to that at the first scan) determined using the measured value of the rate constant, α_1 , for each schizophrenic subject, with that predicted using a nominal value of α_1.

Subject No.	α_1	R(T3:T1) predicted using measured α_1	R(T3:T1) predicted using nominal α_1	% discrepancy
1	0.0066	0.950	1.188	+25.0%
2	0.0059	1.400	1.604	+14.6%
3	0.0059	0.928	1.090	+17.5%
4	0.0057	1.130	1.284	+13.6%
5	0.0039	1.214	1.139	-6%
6	0.0046	1.055	1.067	+1%

T1 is time of the first scan,
T3 is the scheduled time of the second scan,
R(T3:T1) is the ratio of tissue radioactivity at time T3 to that at time T1,
α_1 is the rate constant representing the rate of loss of radio-labeled tracer from the metabolic pool.

Study 2: Testing the accuracy of prediction of residual tracer.

The scan for healthy subject 3 after administration of placebo was excluded on account of a technical flaw in the data. A total of six scanning sessions in four subjects were subjected to analysis. As shown in table 12, the range of values of α_1 estimated from the scans at 50 minutes and 180 minutes after injection, was similar to that seen within the schizophrenic subjects in study 1. In the healthy subjects, there was a non-significant trend for α_1 to be greater after administration of risperidone. However, in the schizophrenic patient the value was virtually identical in this study, after 6 weeks treatment with risperidone, as it had been at baseline, at which time the patient had never previously received anti-psychotic treatment.

The mean magnitude of the discrepancy between observed radioactivity 3 hours after the first scan and that predicted using the estimated value of α_1 for each subject, was 4.6% (S.D. = 2.6). In contrast, the mean magnitude of the discrepancy between observed radioactivity and that predicted using a nominal value of α_1 was 19.6% (S.D. = 9.2). Thus, the estimate of residual radioactivity is significantly more accurate (t=3.1, p<0.05, degrees of freedom=11) using the measured value of α_1, rather than the nominal value.

Table 12: Discrepancies between the observed residual tissue radioactivity at the time of the second scan and the predicted values, in subjects who received a sham second injection. The discrepancies when the prediction was based on the measured value of the rate constant α_1 , are compared with the discrepancies when the predicted value was determined using a nominal value of α_1

Subject	Medication	α_1	Observed R(T3:T1)	R(T3:T1) predicted using measured α_1	discrepancy using measured α_1	R(T3:T1) predicted using nominal α_1	discrepancy using nominal α_1
HC 1	Placebo	0.0026	0.927	0.950	2.4%	0.739	-20.3%
HC 1	Risperidone	0.0058	1.007	0.956	-5.1%	1.07	6.3%
HC 2	Placebo	0.0052	0.944	0.970	2.8%	1.038	10.0%
HC 2	Risperidone	0.0082	0.856	0.786	-8.1%	1.104	29.1%
HC 3	Risperidone	0.0078	0.931	0.858	-7.9%	1.23	31.8%
Sz 4	Risperidone	0.0058	0.800	0.812	1.5%	0.958	19.8%

T1 is time of the first scan,
T3 is the scheduled time of the second scan,
R(T3:T1) is the ratio of tissue radioactivity at time T3 to that at time T1
α_1 is the rate constant representing the rate of loss of radio-labeled tracer from the metabolic pool.
HC denotes healthy control, Sz denotes schizophrenic subject.

Discussion

Study 1 reveals that there is substantial variation between subjects (in a schizophrenic patient population) in the α_1 coefficient that reflects phosphatase activity. Furthermore, use of a

nominal value for the phosphatase rate constant would lead to discrepancies of approximately 14% in predicting residual radioactivity compared with the prediction based on the measured value of α_1. Study 2 indicates that healthy subjects exhibit as much variability of α_1 as was observed in the schizophrenic subjects in study 1. Furthermore that the prediction of residual radioactivity during a scan three hours after the first scan is significantly more accurate when a measured value of α_1 is employed than when a nominal value of α_1 is employed.

The mean magnitude of the discrepancy between observed radioactivity and that predicted using the measured value of α_1 was less than 5%. In view of the fact that after three hours tissue radioactivity will have decayed to approximately 30% of its value during the first scan, the anticipated error in estimation of residual radioactivity is less than 1.5% of the tissue radioactivity arising from the second injection (assuming the second dose of FDG is equal to the first). This error is acceptably small, since it is less than the test-retest variability of glucose metabolism measurements (Goldman et al., 1995). It should be noted that in double FDG studies in which the interval between injections of FDG is three hours or more, it is not more efficient to divide the doses unequally such that a substantially larger dose is administered in the second injection in the manner recommended (Brooks et al., 1987) when the interval between injections is shorter.

This study did not have adequate power to exclude the possibility that risperidone treatment might modify the value of α_1, but provides no substantial evidence for such an effect. However, the possibility of effects of pharmacological agents on phosphatase activity should be taken into account in the design of pharmacological challenge studies.

In conclusion, we have demonstrated that when performing repeated FDG PET studies separated by an interval appropriate for pharmacological challenge studies in patient populations, residual radioactivity can be predicted with acceptable accuracy using an estimate of phosphatase activity derived from an additional scan performed shortly before the second injection of FDG.

APPENDIX 2: EVALUATION OF SPATIAL NORMALIZATION.

Introduction

Statistical parametric mapping (SPM 96; Wellcome Department of Cognitive Neurology, London) is used to characterize physiology in terms of regionally specific responses in brain image volumes. SPM achieves this characterization by treating each brain voxel separately (i.e. it is a univariate approach), and by performing voxel-wise statistical analyses in parallel creates an image of a statistic or 'significance'. For this voxel-based analysis to work, data from different subjects must derive from homologous ("co-registered") parts of the brain. Spatial transformations may therefore applied that move and 'warp' the images such that they all conform (approximately) to some idealized or standard brain. This normalization facilitates inter-subject averaging and the reporting of results in a conventional way. The transformation of an image into a standard anatomical space (usually that described in the atlas of Talairach and Tournoux (1988) as proposed by Fox et al., (1985) is called "spatial normalization".

There are currently two general methods for translating positron emission tomography (PET) data to Talairach space:

1. A subject's PET scan is first registered to the same subject's MRI scan of a complete volume of the head. This MRI (together with its registered PET scan) is then spatially normalized to a MRI template in Talairach space such as the T1-weighted *T1.img* template from Montreal used as a standard in the SPM 96 package.

2. A subject's PET scan may be directly spatially normalized to a PET template, such as the blood flow image template *PET.img* also contained as a standard in the SPM 96 package.

There may be problems associated with each of these methods including:

1. MRI data has distortions of up to 2mm for our scanner, an amount that could have serious repercussions during coregistration.

2. The majority of PET centers have scanners with a limited field of view (FOV). This "incomplete brain" leads to errors associated with transformations (especially with nonlinear transformations) to a complete PET template such as the *PET.img*. By using estimates of the start points before registration these effects may be reduced.

This study examines some of the different methods for image registration using algorithms available in the SPM image analysis software package. To aid comparisons between the methods, a new objective measure of voxel concordance was developed. This measure helps the researcher to decide which image registration method is most accurate for particular image normalizations.

In this paper we describe this new objective evaluation measure, called the voxel concordance measure, and show its use in a particular case study of schizophrenic subjects. In this study, 8 schizophrenic subjects each had an MRI scan and 3 ^{18}F-FDG PET scans. We used 5 different methods to spatially normalize the PET images to the *PET.img* blood flow template, and evaluated the accuracy of the registrations using our new voxel concordance measure, to decide which was the most suitable method for our subsequent analyses.

Experimental procedures

The FDG PET scans have dimensions 128 x 128 x 31 and were acquired on a CTI scanner ECAT953B (CTI, Knoxville, TN) having a 108 mm FOV in the z direction resulting in a truncation of the brain in the z direction. The voxel size = 2.608 mm^2 with a slice thickness of 3.375 mm.

The MRI scans have dimensions 256 x 256 x 124 and were acquired on a GE 1.5 Tesla scanner, using a SPGR sequence with FOV 260mm (hence voxel size = 260/256 = 1.0156 mm^2) and slice thickness of 1.5 mm.

Both the PET template *PET.img* (a blood flow image) and the MRI template *T1.img* (a T1-weighted template) have dimensions of 91 x 109 x 91 voxels, where each voxel is cubic, 2 x 2 x 2mm. Hence the FOV of the template is 182mm, which holds the complete brain.

A total of five methods of spatial normalization were compared as outlined below. After each method, the normalized PET image was smoothed using a 10mm Gaussian filter, so the resulting PET image matched the template for smoothness.

Methods of spatial normalization

Method 1. MRI2PET linear

i.   subject's MRI coregistered to subject's PET using a 6 parameter rigid body transformation (parameters defined as translation, rotation about the x, y, z axes)
ii.   subject's MRI spatially normalized to the T1 template first using the starting parameters of the co-registration above, followed by a 12 parameter affine transformation to the T1 template (parameters defined as rotation, translation, shearing and zoom about the x, y, and z axes)
iii.   subject's PET spatially normalized using normalization parameters from the subject's MRI to the T1 image.

Method 2. MRI2PET non linear

i.   subject's MRI coregistered to subject's PET using a 6 parameter rigid body transformation
ii.   subject's MRI spatially normalized to the T1 template using the starting parameters of the co-registration and a 12 parameter affine transformation with a 4x5x4 non linear component
iii.   subject's PET spatially normalized using normalization parameters from the subject's MRI to the T1 image.

Method 3. PET2PET linear

i.　　subject's PET spatially normalized to PET template using a 12 parameter affine transformation.

Method 4. PET2PET non linear

i.　　subject's PET spatially normalized to PET template using a 12 parameter affine transformation and a 4x5x4 non linear component

Method 5. PET2PET non linear masked template

i.　　Identical to method 4 except using a template masked in the z dimension to match the subject's specific z dimension as determined by the PET FOV during their scan. The template mask was created to include only those voxels lying within the FOV of each subject's PET image. Each binary image was then multiplied by the PET template to create a new 'masked template' specific with regard to the FOV for each subject. This new 'masked template' was then used to spatially normalize each subject's PET data using the non linear transform.

Best fit using concordant voxel analysis

The match between each normalized PET scan and the PET template *PET.img* for each method was evaluated both visually and by the new objective measure, the voxel concordance. The new objective voxel concordance measure is based on the notion that in a good match for two brain images, the overlap, or concordant voxels, is highest when normalization is the "best fit". The method entails classifying voxels as either gray or non-gray voxels, and determining the proportion of voxels for which there is concordance in this assignment.

To calculate the concordance we created a binary mask of both the PET template and the subject's coregistered image, by setting voxels above a given percentage of the image mean to one and others to zero. The similarity of these two binary images is computed as the sum of the overlapping masked voxels (the so-called gray concordant voxels) and the sum of the overlapping zero voxels (the so-called non-gray concordant voxels such as white matter and cerebral spinal fluid), all divided by the total number of voxels in the image.

An example of the binary masks used for the PET template *PET.img* and a subject's PET image linearly transformed using method 3 above is shown in Figure 11, rows 1 and 2 respectively. Figure 8 row 3 shows the matching so-called "gray concordant" voxels in white, the matching non-gray voxels in black, and the non-concordant voxels in gray.

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Figure 11: Transformation of template images.
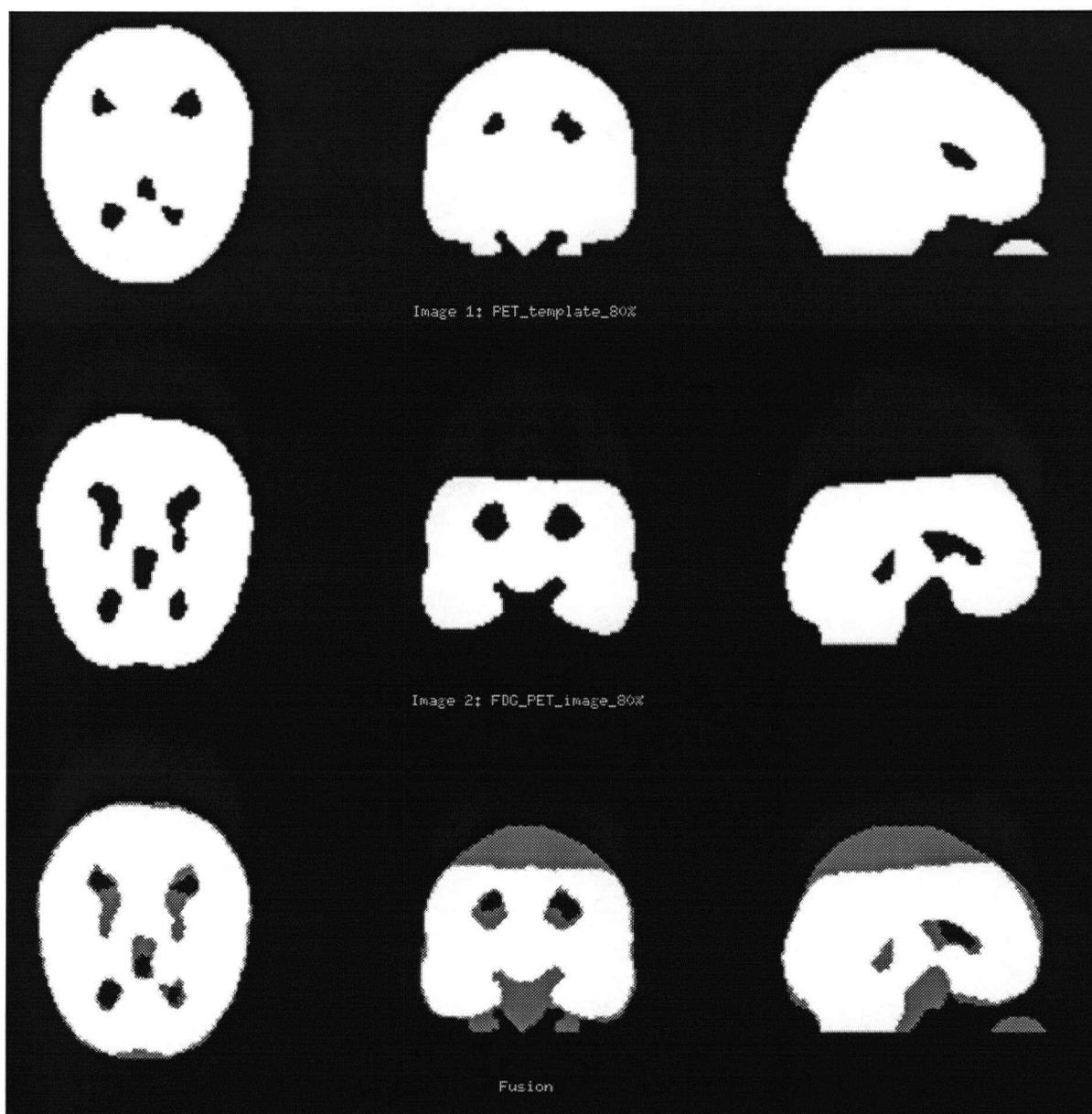
Row 1: Binary mask of the PET template image thresholded for voxels >80% of mean,
Row 2: Binary mask of the subject image co-registered to the template using Method 3, PET2PET linear, thresholded for voxels >80% of mean. Row 3: Fusion of the Template and subject image in row 1 and 2. Gray voxels are discordant. Voxel concordance of these images is 92.78% (see table 17).

Images were prepared and transformations applied using Statistical Parametric Mapping (SPM 96; Wellcome Department of Cognitive Neurology, London). Visual displays were prepared using the Multi-Purpose Imaging Tool, MPITool (Multi Purpose Imaging Tool MPITool, Advanced Tomovision). The measure of voxel concordance was obtained from the images using a MATLAB routine (Matrix Laboratory) developed by the authors.

Sensitivity of the concordance measure to system parameters

The concordant voxel method to determine goodness of fit was validated by performing a series of manipulations that produced a mis-placement of the images. This amount of mis-placement was known. Having performed validation experiments on the concordance measure, we applied the voxel concordance method to a case study of 8 Schizophrenic subjects with multiple PET and MRI scans.

Results

The new normalization evaluation method, voxel concordance, was evaluated both visually and by simulation methods employing deliberate mis-matches. The evaluation process is illustrated in Figure 12. The first row shows three views (axial, coronal and sagittal) of the *PET.img* template thresholded at 80% of the mean of the intensity of brain voxels. The second row shows the same image displaced by 10mm in the x-direction. The third row shows the fusion of these two images, where the discordant voxels are gray. From Table 13, the concordance of these images is 85.88%. Tables 12 and 13 show that the concordance changes almost linearly with respect to the displacement in both x and y directions, which is a desirable trait for any performance measure.

Figure 12: Example of the voxel concordance method
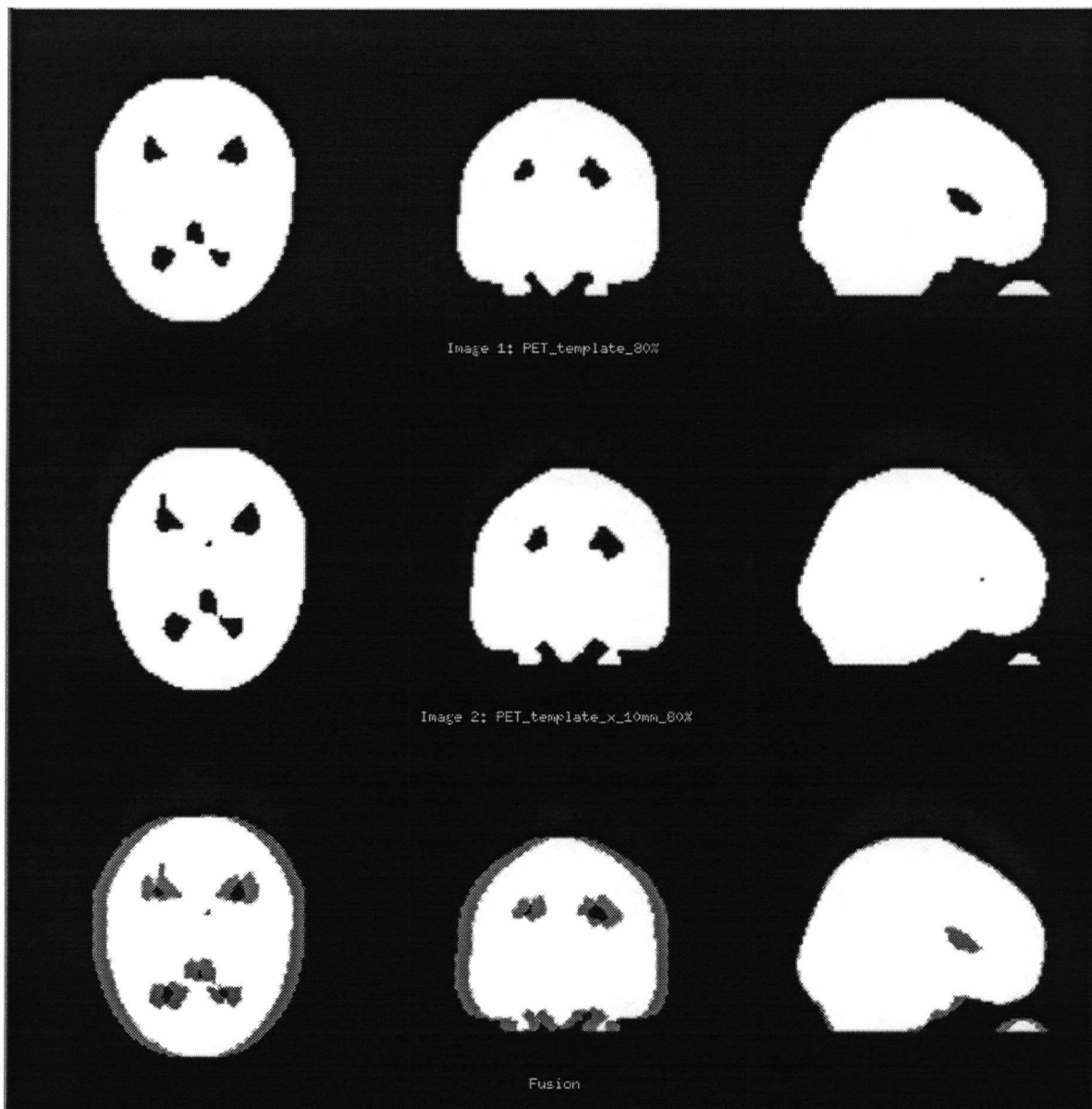
Row 1: Binary mask of the PET template image thresholded for voxels >80% of mean.
Row 2: Binary mask of the PET template displaced by 10mm in the x direction, thresholded for voxels
>80% of mean. Row 3: Fusion of the Template and the displaced template image in row 1 and 2. Gray
voxels are discordant. Voxel concordance of these images is 85.88% (see table 13).

Displacement in x or y directions

Concordance is most sensitive to displacement in x (almost 1.5% reduction in concordance per mm displaced; see table 13) whereas a displacement in y by 1 mm reduces concordance by approximately 0.7% (see table 14). The greater sensitivity to displacement in the x direction can be accounted for by the elliptical shape of the brain.

Table 13: The decrement in concordance resulting from the displacement of a PET blood flow image from its original position, towards the right (i.e. in the x direction).

Displacement in x in mm	percent concordance with the pet template
0	100.00
5	92.62
10	85.88
15	80.22

Table 14: The decrement in concordance resulting from the displacement of a PET blood flow image from its original position, in the anterior direction (i.e. in the y direction).

displacement in y in mm	percent concordance with the pet template
0	100.00
5	96.42
10	93.05
15	89.96

Smoothing

Introducing a discrepancy in smoothing between the image and the template had relatively minor effects on the degree of concordance, as seen in Table 15. Discrepancies in smoothness between the images corresponding to a difference of 5mm in FWHM of smoothing filter produced a 3% reduction in concordance. Differences in smoothness greater than 5mm FWHM are readily perceived on inspection, and can be removed by appropriate filtering of the least smooth of the two images. Therefore, undetected differences between the smoothness of the image and the smoothness of the template are unlikely to diminish the measured concordance by more than 3%.

Table 15: Concordance of an FDG PET image smoothed with 4 different isotropic Gaussian spatial filters with FWHM of 5,10,15, and 20 mm, respectively, with the same image smoothed with an isotropic Gaussian filter with FWHM of 10mm.

smoothing in mm	percent concordance
5mm	97.49
10mm	100.00
15mm	96.92
20mm	93.32

Voxel threshold

The voxel concordance method was relatively insensitive to the gray matter threshold provided the thresholding was within a reasonable range approximating to the gray matter. Table 16 shows that a 20% change of threshold as percent of mean gives only 1.5% change in concordance.

Table 16: The effect of adjusting the specified gray-matter threshold for an FDG image on the calculated concordance between that image and a PET template.

gray matter threshold as a percent of mean brain voxel value[1]	percent concordance
50	90.97
60	91.44
80	90.42
100	87.68
120	85.94

(1) Gray matter threshold was specified as a percentage of mean global value for cerebral voxels in the middle 10 slices of the image.

Testing the best method of spatial normalization using FDG-PET data.

Methods 3, 4, and 5 using the subjects' image directly spatially normalized to the PET template were all superior to the methods using the MR image. Table 17 shows the concordance for each subject for each method. Figure 13 shows the concordance for patient 1 with the *PET.img* template using Method 3 (PET2PETlinear) with a gray-matter threshold of 80%. It can be seen that the subject's ventricles are larger than the *PET.img* template ventricles, yielding a concordance of 92.78%.

Table 17: Comparison of the percentage concordance obtained using 5 different methods of spatial normalization

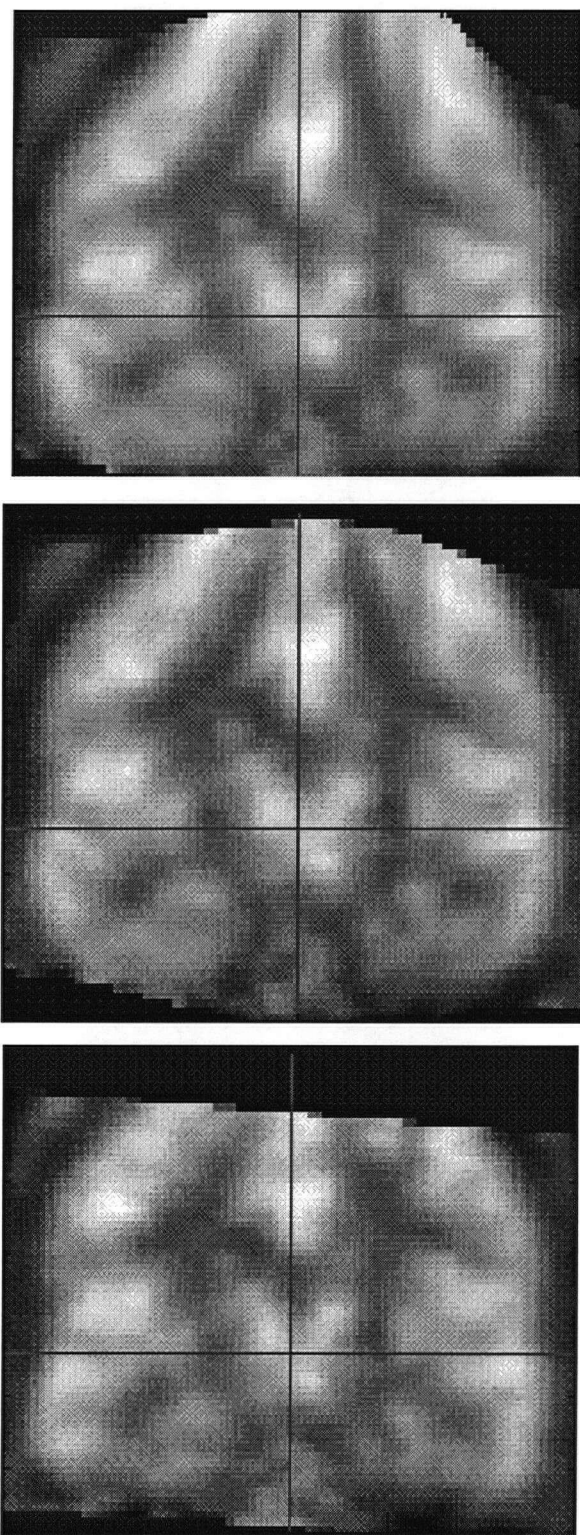| subject	Method 1: MRI2PET linear	Method 2: MRI2PET non-linear	Method 3: PET2PET linear	Method 4: PET2PET non-linear	Method 5: PET2PET nonlinear masked template
1	92.04	92.45	92.78	94.64	94.75
2	92.70	92.90	93.19	93.97	95.46
3	90.15	88.84	93.88	96.30	96.05
4	91.54	91.41	92.98	95.61	95.06
5	91.19	92.53	93.33	95.58	95.75
6	92.10	93.02	93.28	95.79	95.51
7	93.77	90.11	94.91	96.43	96.28
8	93.89	92.80	94.70	95.57	95.95
mean	92.17	91.56	93.63	95.48	95.60

Statistical analysis

A one-way, block design ANOVA revealed a significant difference between methods $F_{(4,39)}=23.4$, $p<.0001$. Post hoc tests revealed the PET2PET non linear method with masked template produced the highest degree of concordance and was significantly higher than PET2PET linear (post hoc Least Squares Difference , $p<.006$). There was no significant difference between the method using a masked vs. a total PET template. The MRI2PET non linear produced the least concordance and was significantly less than the PET2PET non linear and the PET2PET linear (post hoc Least Squares Difference, $p<.001$).

Image deformation by non-linear transformation

Despite the high concordance resulting from using an incomplete PET image registered to a complete or cropped template, care should be given when performing a warping to any data set with missing data. Edge effects (deformations) were noticeable (Figure 13) in two subjects when a non-linear transformation was used regardless of the template (MRI, PET, PET masked). For this reason a PET2PET linear transformation was selected as the best method to spatially realign FDG-PET data into standard space.

Figure 13: Image deformation following non-linear transformation (coronal sections).

From top to bottom:
PET2PET nonlinear (Method 4),
PET2PET non linear with masked template (Method 5)
and PET2PET linear (Method 3).

Discussion

The best method overall was the PET2PET non-linear with the masked template (Method 5), though it did not differ significantly from the PET2PET non-linear (Method 4). However both these methods are limited in their use due to the truncated FOV in the PET data resulting in pulling of brain data towards the edges for some subjects, as shown in Figure 13.

Therefore we chose PET2PET linear (Method 3) as the method for spatial normalization of subject PET images to standard space, as it resulted in the highest degree of concordance with the least aberration to the data. This finding is in accordance with others who have pointed out the difficulties in performing non-rigid body co-registrations (Zuk et al., 1996).

Poor voxel concordance using the MRI template may be due to distortions in the MR images or due to limitations of SPM's registration algorithms. The MRI has significant distortions especially using the SPGR and other sequences up to 2-3 mm. From our results we know an increasing displacement in x or in y leads to a relative decrease in concordance. A 2mm distortion in the x-direction could explain the 3-4% discrepancy between methods using a MRI vs. PET template.

In conclusion, the new voxel concordant measure was helpful in determining which spatial normalization method to use in our case study. However, visual evaluation of each method was still required to rule out aberrant transformations in incomplete data sets.

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