Biological and Clinical Determinants of Treatment Resistant Schizophrenia

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BIOLOGICAL AND CLINICAL DETERMINANTS

OF

TREATMENT RESISTANT SCHIZOPHRENIA

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Abstract

Up to one third of patients with schizophrenia show only limited response to dopamine blocking antipsychotic medication. This could be due to distinct neurobiological abnormalities in this subgroup of patients. While there is robust evidence to suggest that the neurobiology of schizophrenia involves increased presynaptic striatal dopaminergic elevation, little is known as to whether this abnormality is present in treatment resistance, and consequently the relationship between this dopamine abnormality and the lack of response to treatment remains unknown. Furthermore, it remains unclear whether treatment resistance manifests at the outset of illness, and perhaps has a neurodevelopmental origin, or whether it evolves over time, possibly as a result of a neurodegenerative process.

The first study in this thesis investigated striatal presynaptic dopamine synthesis in twelve treatment resistant schizophrenic patients, twelve patients with schizophrenia who had responded to antipsychotics, and twelve healthy volunteers, using [^{18}F]-DOPA Positron Emission Tomography (PET). Thus, it was possible to test the hypothesis that the response to treatment is determined by differences in presynaptic dopamine function. The results demonstrated that there were no significant differences in striatal dopamine synthesis capacity between treatment resistant patients and healthy volunteers, whilst dopamine synthesis capacity was significantly increased in responders relative to treatment resistant patients. The difference was most marked in the associative and the limbic striatal subdivisions.

A second, large follow-up study of first episode psychosis (FEP) patients, examined the course of treatment resistance over the 10 year follow up. It was found that over 80% of treatment resistant patients were persistently resistant from the initiation of antipsychotic treatment. My PET study, due to its cross sectional design, could not determine whether the normal dopamine levels predate the antipsychotic exposure in treatment resistant patients. However, by demonstrating that a great majority of treatment resistant patients are resistant to dopamine blocking antipsychotics at first ever initiation of treatment, my second study raises the possibility that these patients may have had normal dopamine levels even at the outset of their psychotic illness. In the same FEP cohort it was possible to investigate neurodevelopmental predictors of treatment resistance. The finding that the negative symptom dimension and younger age of onset were significant predictors of treatment resistance is compatible with the view that TRS may be of neurodevelopmental origin.

Overall, my observations in this thesis indicate that TRS may be a distinct and enduring subtype of schizophrenic illness of a possible neurodevelopmental origin whose pathophysiology is not marked by alterations in dopamine synthesis capacity. Findings emerging from this thesis provide a platform for future studies, which may lead to the discovery of much needed new treatments for this disabling and intractable condition.

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Abbreviations

5-HT	5-hydroxytryptamine
AAADC	Aromatic Amino Acid Decarboxylase
AESOP	Aetiology and Ethnicity in Schizophrenia and Other Psychoses
ARSAC	Administration of Radioactive Substances Advisory Committee
AST	Associative striatum
BPRS	Brief Psychiatric Rating Scale
DA	Dopamine
DSM	Diagnostic and Statistical Manual of Mental Disorders
DUP	Duration of Untreated Psychosis
$[^{18}F]$-DOPA	Fluorine-18-L-dihydroxyphenylalanine
FEP	First Episode Psychosis
GABA	Gamma-Aminobutyric Acid
GAF	Global Assessment of Functioning
IBZM	Iodobenzamide
ICD	International Classification of Diseases
IGC	Item Group Checklist
K_i^{cer}	$[^{18}F]$-Dopa influx rate constants relative to uptake in cerebellum
LCS	Life Chart Schedule
LSD	Lysergic Acid Diethylamid
LST	Limbic Striatum
MNI	Montreal Neurological Institute
MPA	Minor Physical Anomalies
MRI	Magnetic Resonance Imaging
NICE	National Institute for Clinical Excellence
NSS	Neurological Soft Signs
NMDA	N-Methyl-D-Aspartate

PANSS	Positive and Negative Syndrome Scale
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
pHVA	Plasma Homovanillic Acid
ROI	Region-of-Interest
SPM	Statistical parametric mapping
SCAN	Schedules for Clinical Assessment in Neuropsychiatry
SMST	Sensorimotor Striatum
SPECT	Single Photon Emission Computed Tomography
TRS	Treatment Resistant Schizophrenia
VTA	Ventral Tegmental Area

Acknowledgements

Personal contribution to the investigations

First study (Chapter 3)

I have contributed to the study design and methodology, development of the thesis hypotheses and have completed the study protocol. In addition, I have designed and planned the recruitment strategies and contact procedures. I have also been responsible for completion and submission of applications for both Ethics and Administration of Radioactive Substances Advisory Committee (ARSAC) approval. I was solely responsible for all the recruitment, assessments and PET scanning of all subjects and, in addition statistical and image analyses.

Second study (Chapter 4)

I participated in the follow-up data collection of the Aetiology and Ethnicity in Schizophrenia and Other Psychoses (AESOP) study. Additionally, for the baseline data, I calculated the IGC algorithm, (part of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN)) symptom scores for 484 patients and further, using factor analysis categorized these symptoms in five psychopathological dimensions that were used in thesis investigation. At the follow up, I was involved in collection of NSS, MPA and DNA data. The medication and life chart data collection was completed by my AESOP colleagues. I have been responsible for identifying treatment resistant and responder patients, and was responsible for the design, hypotheses and relevant data entry, as well as analysis and interpretation of results.

Chapter 1 Introduction

1.1 Clinical Subtypes of Schizophrenia

Since its conception, schizophrenia has been described in terms of distinct clinical subtypes. However, the adequacy of this categorical approach, which till recently formed the basis of modern descriptive systems of psychosis, such as those in the ICD-10 and the DSM-IV, has been criticized for its failure to adequately capture the rich and heterogeneous psychopathology of the disorder (Demjaha et al., 2009). Following the DSM-V revision, the categorical subtypes of schizophrenia (paranoid, disorganized, catatonic, and undifferentiated) were removed, and instead the use of psychopathological dimensions that provide the more useful description of psychotic patients is encouraged in an appendix (Tandon et al., 2013).

Nevertheless, the modern diagnostic systems continue to ignore the important subtyping based on treatment response, despite the knowledge that the former diagnostic subtypes were not a good predictor of future response to antipsychotics (Bromet et al., 2011). Although not formally operationalized, the clinically highly relevant sub-entity of schizophrenia: "Treatment Resistance," is clearly delineated, both in terms of criteria and specific treatment. However, the important question of whether treatment resistant schizophrenia (TRS) is aetiologically and neurobiologically distinct subtype of schizophrenic illness remains unanswered.

1.2 **Treatment resistant schizophrenia**

Schizophrenia is a severe and often incapacitating mental disorder with an average life-time prevalence of about 0.5 to 1.0 percent of the population worldwide, and contributes considerably to the global burden of disease (Murray and Lopez, 1997; Rossler et al., 2005) with a 2- to 3-fold increase in mortality risk compared with the general population (Saha et al., 2007). The fortuitous discovery of Chlorpromazine, more than a half a century ago (Delay *et al.,* 1952), brought new treatment hopes and prognostic improvement (Creese et al., 1976). However, despite considerable scientific and therapeutic progress, up to one third of patients (Lindenmayer, 2000) continue to experience distressing delusions, hallucinations, thought disturbances and functional impairment. Treatment resistance in this condition is one of the greatest therapeutic challenges to psychiatry, significantly affecting patients, their families and society in general. Improved understanding of the neurobiology of schizophrenia is prerequisite to successful treatment; therefore identification of a precise molecular abnormality, particularly in patients who fail to respond to available treatment is of utmost clinical importance. Whilst there is sufficient evidence to suggest that the neurobiology of schizophrenia involves presynaptic striatal dopaminergic elevation (Fusar-Poli and Meyer-Lindenberg, 2012; Howes et al., 2012), little is known as to whether this abnormality is present in patients resistant to antipsychotic treatment. Consequently, the reasons for this non-response remain elusive, which poses a considerable constraint on developing novel and much needed neurobiologically determined treatments. It is clear, however, that treatment resistance to dopamine blocking antipsychotics remains a major neurochemical challenge to the dopamine

hypothesis in that the theory fails to explain the refractory nature of some cases of schizophrenia. This failure is highlighted by the fact that clozapine, the only antipsychotic to date that is effective in majority of those who fail to respond to conventional antipsychotics (Taylor and Duncan-McConnell, 2000), is only a weak D2 blocker, and some think, it exerts its therapeutic effect via mechanisms involving other neurotransmitters such as glutamate or serotonin (Gellman and Aghajanian, 1994; Javitt, 2004; Tanahashi et al., 2012).

1.3 Neurobiology of schizophrenia

1.3.1 Dopamine Hypothesis

The DA hypothesis heuristically survives as one of the most eminent theories of schizophrenia, reputed to play a principal role in explaining the neurobiological mechanisms of this complex disorder. Its principles lie in Arvid Carlsson's seminal work which showed that the administration of chlorpromazine and haloperidol stimulated the turnover of DA in the brains of mice (Carlsson and Lindqvist, 1963). The hypothesis claims that subcortical hyperdopaminergia contributes to the positive psychotic symptoms, which may explain the success of dopaminergic blocking drugs in alleviating these symptoms. And indeed, the DA hypothesis received support from pharmacological evidence indicating that the efficacy of antipsychotic drugs is related to DA D2 receptor blockade, beginning with observations that the relative clinical potencies of antipsychotic drugs closely parallels their affinity to bind and block the DA D2 receptor subtype (Creese et al., 1976b; Seeman et al., 1976). Additional support for a role of DA came from observations that psychotogenic drugs, such as

amphetamine, can lead to psychosis as well as that many patients with schizophrenia experience worsening of symptoms following acute exposure to amphetamines (Connell, 1958).

Later, the original DA hypothesis was revised to account for the neurobiology of negative and cognitive symptoms (Davis et al.,1991), suggesting that these symptoms are related to a deficit in cortical DA transmission. Therefore, the revised version incorporates both the subcortical hyperdopaminergia and frontal hypodopaminergia, and their co-existence in schizophrenic patients has been subsequently supported by several PET and Single Photon Emission Computed Tomography (SPECT) studies (Abi-Dargham et al., 2002; Abi-Dargham, 2004). Evidence from animal studies offers a molecular explanation for the mechanism, suggesting that frontal hypodopaminergia results in increased levels of DA in the striatum. However, there is also evidence to suggest that prefrontal cortical function can be impaired by increased dopamine D2 receptor expression in the striatum (Kellendonk *et al.*, 2006). More recent modifications of the DA hypothesis took into account DA's important roles in learning and motivation to further explain positive symptoms of schizophrenia. In line with this, Gray et al., (1991) suggested that coincident events are regarded as highly salient in states of hyperactive DA signalling. These ideas have evolved further to propose that elevated dopaminergic neurotransmission leads to increased salience being assigned to insignificant events or normal external and internal stimuli, and this in turn is responsible for genesis of psychosis (Kapur, 2003; Gray et al, 2004; Kapur et al., 2005).

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1.4 **Imaging the DA system**

In the last two decades, by using neurochemical imaging techniques such as PET and SPECT, it has been possible to obtain direct evidence of enhanced dopamine transmission in a living human brain. PET and SPECT studies indicated that multiple aspects of dopaminergic transmission, specifically increased DA D2 receptor occupancy, increased DA release and elevated DA synthesis, may cause dopaminergic overactivity that is responsible for symptom formation in schizophrenia. The emphasis of the research in this field was mainly on the striatum, given that it is highly populated by D2 receptors and has rich dopaminergic projections and connections with other brain areas, particularly with the thalamus, ventral tegmental area (VTA), and the septo-hippocampal system. Striatum is not uniform histologically, and can be functionally divided, on the basis of differential striatal-cortical connectivity, into limbic (LST), associative (AST), and sensorimotor (SMST) subdivisions, along the dorsolateral to ventromedial gradient (Martinez et al., 2003; Joel et al., 2000). These subdivisions are equivalent to anatomical substructures and functionally relate to emotion, reward mechanisms, motor and cognitive processes (Huber *et al.*, 1975; Martinez *et al.*, 2003). Thus, LST is involved in reward, motivation and emotional regulation, AST processes cognitive information, and SMST is concerned with sensorimotor processing (Figure1.1). Anatomical equivalents, projections and related functions are presented in Table 1. 1.

Figure1.1 Functional striatal subdivisions (from Haber *et al.* 1999)

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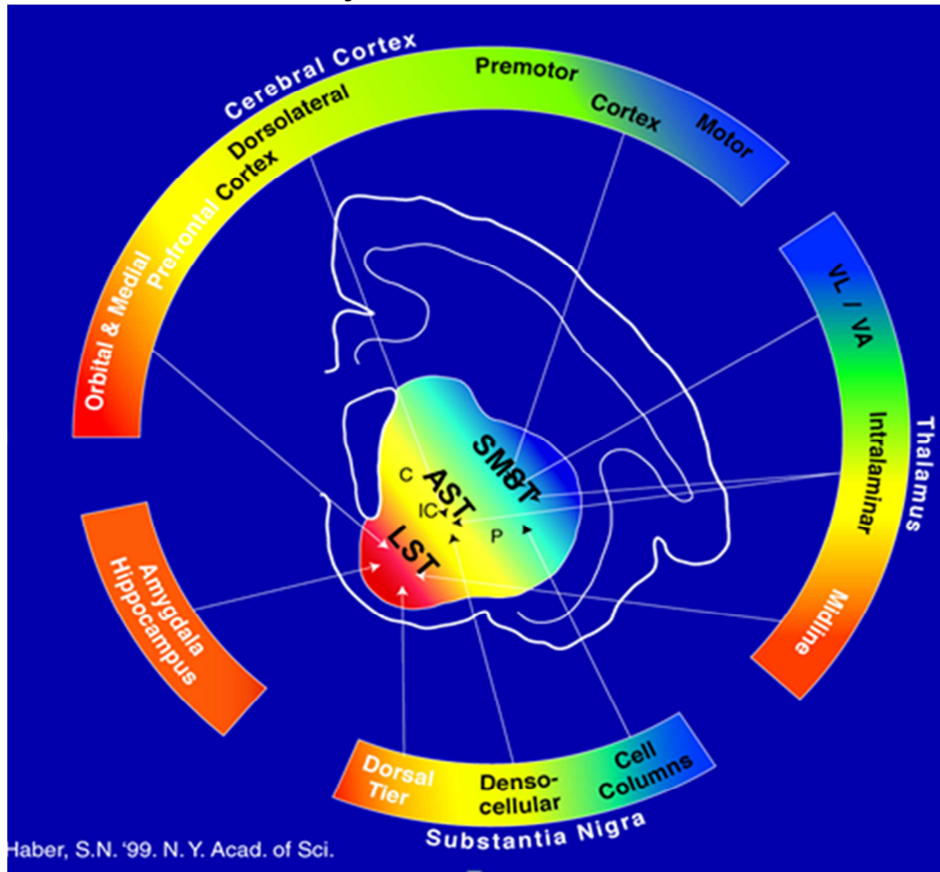
Summary diagram illustrating striatal functional subdivisions (LST, AST, and SMST) based on inputs from specific cortical, thalamic, and midbrain regions. LST receives input from limbic structures, AST from associative cortical regions and the dorsolateral prefrontal cortex, and SMST from motor and premotor areas

Table1.1 Striatal anatomical substructures and their functional equivalents

Anatomic striatal subdivisions	Functional striatal subdivisions	Projections from:	Function
Ventral striatum (VST)	LST	Hippocampus, Amygdala, Nucleus Accumbens	Emotional responses Mechanisms of Reward
Precommissural dorsal putamen Precommissural dorsal caudate Postcommissural caudate	AST	Associative cortical regions The dorsolateral prefrontal cortex	Cognition
Postcommissural putamen	SMST	Motor cortex Premotor cortex Supplementary motor cortex	Locomotion

1.4.1 DA D2 receptors occupancy in schizophrenia

Striatal DA D2 receptors have received much attention and have been investigated extensively in recent decades in schizophrenia studies, which is not surprising given the fact that they are a principle target of antipsychotic medication. DA D2 were the first neuroreceptors that were imaged by PET and SPECT scanning that permitted their visualization in vivo (Wagner *et al.*, 1983), using a variety of radiotracers, most commonly 11C Raclopride, 11C N-methylspiperone and [123I]IBZM.

Molecular imaging studies observed alterations in DA D2/D3 receptors, however the results were conflicting (Farde et al.,1995), which was largely attributable to the effects that chronic exposure to antipsychotics has on DA receptor up-regulation. Meta-analytic work of molecular imaging studies (Laruelle, 1998; Zakzanis and Hansen, 1998) revealed much smaller increase in DA D2 receptors than that observed in early post-mortem studies, confirming the confounding effect of exposure to antipsychotic medication, that was not possible to account for in post-mortem status. However, in a subsequent meta-analysis (Weinberger and Laruelle, 2002), a modest (12 percent), yet significant increase in DA D2 receptors was confirmed in drug naive patients with schizophrenia compared to healthy controls.

Contrary to this, the most recent meta-analysis showed no increase in DA D2/3 receptor availability in drug-naive patients, although an elevation of small effect size (Cohen $d = 0.26$) in previously medicated patients was observed (Howes et al., 2012). Studies using DA depletion paradigms, where depletion is achieved

by using α-methyl para-tyrosine (AMPT), which blocks tyrosine hydroxylase and thus prevents new DA synthesis, have demonstrated increased DA D2 receptor occupancy in schizophrenic patients compared to controls (Abi-Dargham et al., 2000). Intriguingly, one study demonstrated that DA D2 receptor levels are elevated in healthy monozygotic co-twins compared to dizygotic co-twins of twins with schizophrenia, which may suggest that DA D2 receptor up-regulation confers a genetic risk (Hirvonen, 2005).

Most imaging studies of DA D1 receptors have observed no abnormalities of striatal DA D1 receptors in schizophrenic patients (Abi-Dargham et al., 2000; Karlsson et al., 2002 et al; Okubo et al., 1997). However, in the pre-frontal cortex of drug-naïve patients one study reported no change (Karlsson et al., 2002), one study reported decreased levels (Okubo et al., 1997), and two others reported increases (Abi-Dargham et al., 2012; Abi-Dargham et al., 2002).

On the whole, despite extensive research, although it is possible that a small DA D2 receptor elevation is present in brains of drug-naïve schizophrenic patients, the evidence remains equivocal and mostly unconvincing, which, as examined by Weinberger and Laruelle, (2002), is a consequence of underpowered studies, imaging protocol, or sampling effects that may have contributed to results in occasional positive studies.

1.4.2 Increased DA release

In contrast to DA receptor studies, the evidence of increased dopamine release in schizophrenia has been more consistent and robust. The principle underlying an excessive amphetamine-induced dopamine release technique is based on endogenous competition between the released dopamine and the radiotracers for binding to DA D2 receptors, which reduces radiotracer binding; this then provides an indirect index of released dopamine. In these studies, brain scans are performed twice, before and after amphetamine challenge, and the difference between these two scans reflects the amount of DA release following the challenge.

Both PET and SPECT studies that have used this paradigm have shown similar results, demonstrating an excessive amphetamine-induced dopamine release by reporting approximately doubled radiotracer displacement in patients with schizophrenia compared with controls (Abi-Dargham et al., 1998; Breier et al., 1997; Kestler et al., 2001; Laruelle *et al.*, 1996; Laruelle and Abi-Dargham, 1999). Furthermore, enhanced DA release was associated with increased positive symptoms (Laruelle et al., 1996). However, although evident even at the onset of illness, or during acute relapses or exacerbation, excessive release has not been associated with remission status (Laruelle et al., 1999).

1.4.3 Increased presynaptic DA synthesis capacity

The most widely used tracer for measuring presynaptic DA synthesis is [^{18}F]-DOPA, which is decarboxylated to [^{18}F] - Fluorodopamine and stored in DA

vesicles in areas rich in Aromatic Amino Acid Decarboxylase (AAADC), such as the striatum. This accumulation reflects DOPA uptake and conversion to DA, quantified as the influx constant Ki, that parallels decarboxylase activity, giving an indication of the DA synthesis capacity in the presynaptic terminals of striatal dopaminergic neurons (Moore, 2003). The biochemistry of DA synthesis is presented in a schematic diagram (Figure 1.2).

PET derived evidence indicates that a dysfunction of the presynaptic striatal DA system may be a critical factor in developing schizophrenia. At present this neurochemical abnormality constitutes the most replicated, robust finding in dopaminergic studies in schizophrenia (Howes and Kapur, 2009), which is confirmed in two recent meta-analyses (Fusar-Poli & Meyer-Lindenberg 2012; Howes et al., 2012). Howes and colleagues (2012) examined all aspects of presynaptic DA transmission (DA synthesis capacity, synaptic DA levels, and DA release) and reported a highly significant increase in presynaptic function with a large effect size of 0.79. Fusar-Poli & Meyer-Lindenberg (2012) focused solely on studies that examined DA synthesis capacity. The meta-analysis of 11 PET studies comprising collectively 113 subjects and 131 healthy volunteers (Table 1.2), revealed 14 % elevation in dopamine synthesis capacity in patients with schizophrenia.

All the studies that reported increased DA synthesis involved acutely psychotic patients. The three remaining studies, of which one reported decreased [^{18}F] - DOPA synthesis (Elkashef et al., 2000) and 2 others observed no significant change (Dao-Castellana et al., 1997; Shotbolt et al., 2011), were conducted on

patients who were relatively symptom free at the time of scanning. Interestingly, studies involving medication free/naïve subjects have reported elevated DA synthesis suggesting that medication cannot explain the elevation seen in these patients. Three positive studies included first-episode psychosis patients (Hietala et al., 1995; Hietala et al., 1999; Lindstrom et al., 1999). Furthermore, increased DA synthesis capacity has been demonstrated even in individuals who are at high risk of developing schizophrenia thus indicating that this dopaminergic abnormality predates the onset of frank psychosis (Howes et al., 2009), and progressively increases in those who go on to develop psychotic disorder (Howes et al., 2011).

Figure 1.2 Schematic presentation of presynaptic DA regulation
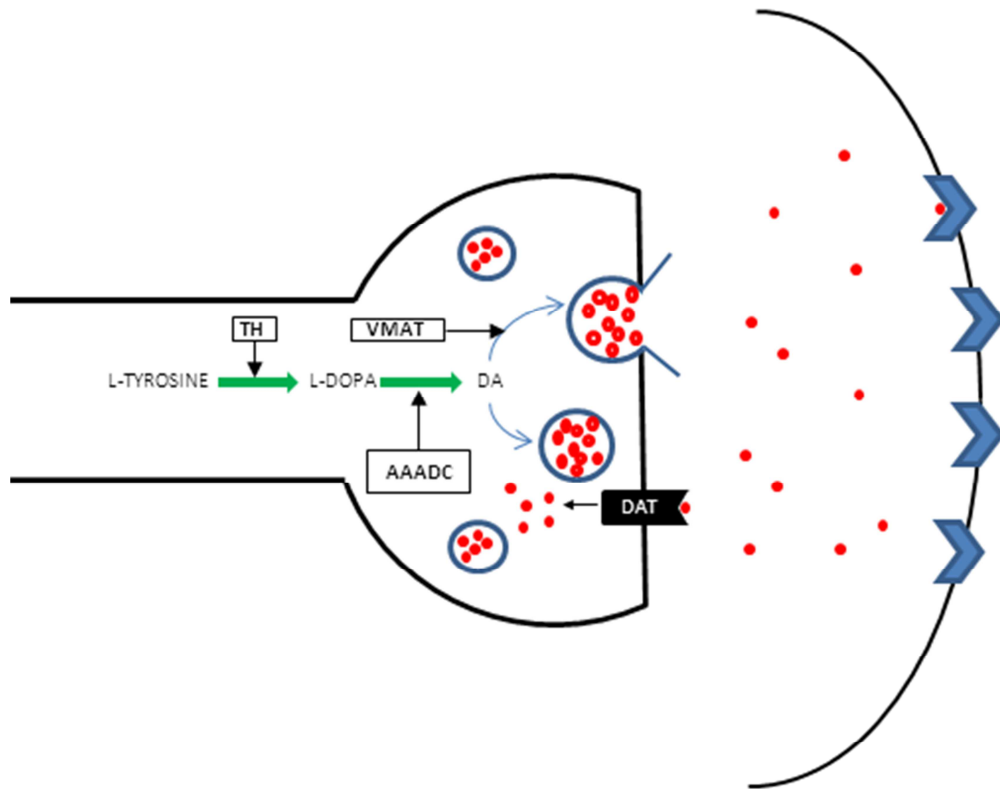
Conversion of L-tyrosine (4-hydroxyphenylalanin) to L-3, 4 dihydroxyphenylalanine [L-DOPA] constitutes the first step in a complex pathway of dopamine synthesis. L-tyrosine is derived mainly from dietary sources, although a small quantity originates from L-Phenylalanine converted to L-tyrosine by phenylalanine hydroxylase (PHA). L-tyrosine is converted to L-DOPA by tyrosine hydroxylase (TH). AAADC then acts on L-DOPA to convert it to DA. The DA uptake transporter (DAT) plays an additional role in increasing cytoplasmic DA levels via the reuptake of extracellular DA and thus maintains extracellular dopamine homeostasis. From the cytoplasm the majority of DA is stored in specialized synaptic vesicles by the vesicular monoamine transporter (VMAT) and is ready for release upon arrival of the action potential.

Table 1.2 PET studies of DA synthesis capacity in schizophrenia

Study	Patients (N)	Controls (N)	Age	Illness Stage	Radiotracer	Medication Status	Effect size
Reith et al., 1994	5	13	38	Chronic	$[^{18}F]$-DOPA	4 MN 1 MF	1.38
Hietala et al., 1995	7	8	26	FEP	$[^{18}F]$-DOPA	MF	0.63
Dao-Castellana et al., 1997	6	7	26	Chronic	$[^{18}F]$-DOPA	2 MN 4 MF	0.27
Hietala et al., 1999	10	13	30	FEP	$[^{18}F]$-DOPA	MF	0.68
Lindstrom et al., 1999	12	10	31	FEP/Chronic	$[^{11}C]$-DOPA	10 MN 2 MF	0.88
Elkashef et al., 2000	19	13	36	Chronic	$[^{18}F]$-DOPA	9 MF 10 M	-0.20
Meyer-Lindenberg et al., 2002	6	6	35	Chronic	$[^{18}F]$-DOPA	MF	1.89
McGowan et al., 2004	16	12	38	Chronic	$[^{18}F]$-DOPA	M	1.57
Kumakura et al., 2007	8	15	37	Chronic	[18F]-DOPA	3 MN 5 MF	0.53
Nozaki et al., 2009	18	20	36	FEP/Chronic	$[^{11}C]$-DOPA	14 MN 4 MF	0.69
Howes et al., 2009	7	12	36	FEP	$[^{18}F]$-DOPA	3 MN 4 MF	1.2
Shotbolt et al., 2011	7	10	43	Chronic	$[^{18}F]$-DOPA	M	0.68

FEP, First Episode Psychosis, MN, Medication Naïve, MF, Medication Free, M, on Medication

1.5 The role of other neurotransmitters in neurobiology of schizophrenia

Although increased presynaptic dopamine synthesis has been documented in most PET studies, and as such, it constitutes the most replicated and robust finding in dopaminergic studies in schizophrenia, there is an overlap in dopamine synthesis capacity between patients and healthy subjects (Hietala et al., 1999; McGowan et al., 2004), where values in some patients are observed to be within the normal range. This observation suggests that dopamine dysfunction may not be the only abnormality in all patients with schizophrenia, and that the pathophysiology of such a complex disorder may involve aberrations in other neurotransmitters (Carlsson et al., 2001).

Among other neurotransmitters, glutamate has received particular attention in recent decades (Coyle et al., 2003; Kim et al., 1980; Tamminga, 1998) due to the psychogenic effects of phencyclidine ("angel dust") or ketamine, that are powerful non-competitive antagonists of the N-methyl-d-aspartate (NMDA) glutamate receptor (Lodge and Johnson, 1990), and can precipitate relapse in remitted patients (Cohen et al., 1962). Furthermore, glutamate may impact differentially to DA on schizophrenic symptomatology and be more accountable for negative symptoms (Stone et al., 2007), which is of clinical significance, specifically in terms of new drug developments.

DA, glutamate and Gamma-Aminobutyric Acid (GABA) interactions in complex neurocircuits are hypothesized to be of particular importance for psychosis formation (Carlsson and Carlsson, 1990; Goff and Coyle, 2001). It has been

postulated that the dysfunction in cortical systems involving glutamate and GABA neurotransmission may be responsible for the pathophysiology of schizophrenia (Grace, 2000; Lewis et al., 1999). Carlsson and colleagues hypothesized that cortical hypoglutamatergia, either acting directly as an "accelerator" or via GABA interneurons projections, as a "brake," modulates the firing of dopaminergic neurons that can in turn lead to either decrease or increase in dopaminergic activity (Figure 1.3). Thus, for instance, the reduced glutamate activity enhances DA release in dopaminergic pathways, which then via negative feedback, mediated, at least in part, via the striatum and the thalamus, regulates glutamate release that would then act as "a brake" on cortical DA production (Carlsson et al., 2000; Carlsson et al., 2001; Stone et al., 2007).

Figure 1.3 Hypothetical cortical glutamate and GABA mediated regulation of DA activity in schizophrenia (modified from Carlsson *et al.*, 2001)

Cortex

Glu   Glu

Brake   Accelerator

GABA

DA,

Cortical hypoglutamatergia acting directly as an "accelerator" or via GABA interneurons projections, as a "brake," to modulates the firing of dopaminergic neurons '
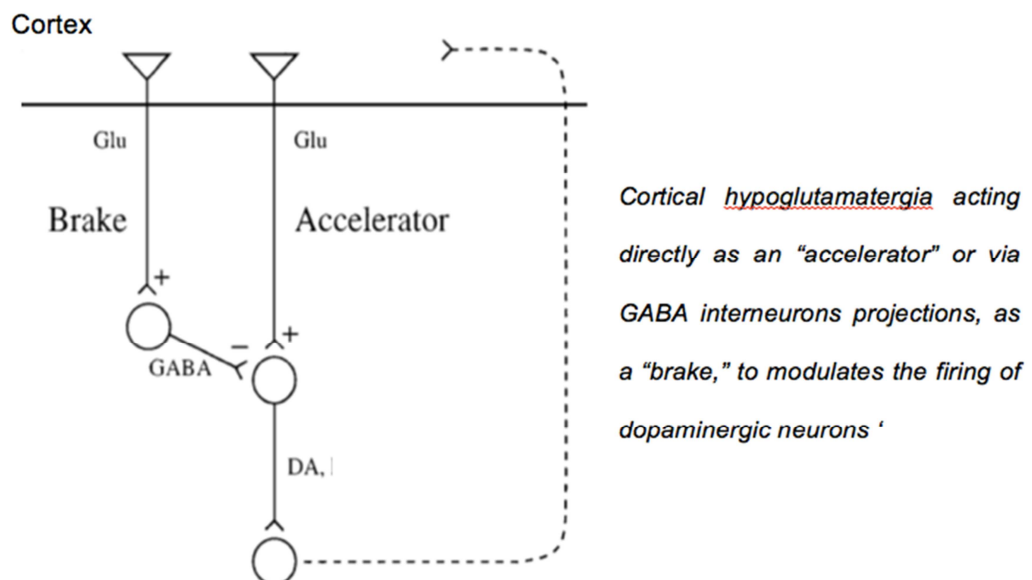
Abnormalities in both GABA and serotonin (5-HT) transmission in schizophrenia have also been observed. GABA is widely distributed and the principal inhibitory neurotransmiter in the brain. The deficiency of GABAergic interneurons in the prefrontal cortex and hippocampus has been consistently reported in both preclinical and in vivo studies (Bussato et al., 1997; Lewis et al., 1999; Perry et al., 1997; Reynolds et al., 2001). However, contrary to these findings and subsequent expectations, GABA agonists have not proved to be effective in treatment of schizophrenia or have shown relatively low effect, which suggests that perhaps the inhibitory deficiency is not directly related to GABA in patients with schizophrenia (Taylor D., 2006).

Findings regarding serotonergic abnormalities are less consistent. The support for serotonin involvement in schizophrenia was derived from the observations that LSD induces a psychotic state characterized by visual hallucinations. LSD, similarly to newer atypical antipsychotics, has a specific affinity for serotonin receptors, particularly 5-HT$_2$A subtype (Glennon et al., 1983, Taylor D; 2006). The fact that clozapine exerts antagonistic effect at the 5-HT2 receptor (Lieberman et al., 1998), and is effective where higher affinity DA blockers fail, gave additional support to serotonin model and DA-Serotonin hypothesis of schizophrenia (Meltzer et al., 1989). Since clozapine has a much higher affinity for the DA D4 than for the DA D2 receptor, combined DA D4 and 5-HT2 receptor antagonism seemed of particular significance in the treatment of schizophrenia. However, fananserin, a potent antagonist at these two receptors, was demonstrated to be ineffective for schizophrenic symptoms (Truffinet et al., 1999). Another potential antipsychotic amperozide, which is a

selective 5-HT$_2$A antagonist, has been shown in some studies to have antipsychotic efficacy, although the evidence was inconclusive and it has not been approved for the use in treatment of schizophrenia (Meltzer and Nash 1991). The majority of post-mortem studies reported increase in 5-HT$_2$A receptor density in the prefrontal cortex (PFC) (Abi-Dargham, 2007), however in vivo studies were less convincing. Thus, Lewis et al., (1999) in their PET study failed to confirm the reduction in 5-HT$_2$ receptors found in postmortem studies of schizophrenia and concluded that serotonergic abnormality in schizophrenia, if at all present, is unlikely to be associated with the 5-HT$_2$ receptors.

Overall, these neurotransmitters are both anatomically and functionally interconnected, and the emerging evidence suggests that they all contribute to the pathogenesis of schizophrenia, although knowledge of precise mechanisms of their interplay in schizophrenia is currently lacking. It has been suggested that the schizophrenic pathophysiology may not even involve these neurotransmitters directly, but could be related instead to general connectivity defects that may originate from the disrupted neurodevelopment (Carlsson et al., 2001, Weinberger, 1995; Weinberger, 1987).

1.6 Neurobiology of treatment resistance

Little is known about the neurobiology of treatment resistance in schizophrenia. There is some evidence from magnetic resonance imaging (MRI) studies that patients who show limited response to treatment have increased cortical atrophy in comparison with responders (Bilder et al.,1994; Stern et al.,1993).

These findings are confirmed in a recent MRI study conducted in 25 responders and 14 non-responders experiencing first episode psychosis. The authors reported increased cortical thickness in responders, which was also associated with more rapid response (Szeszko et al., 2012). However, these studies may have been confounded by the reported effects of antipsychotics on cortical volume (Moncrieff, 2011). Dorph-Petersen and colleagues (2005) observed an 8–11% reduction in mean fresh brain weights of macaque monkeys that were chronically exposed to olanzapine and haloperidol. Subsequently Ho et al., (2011) confirmed in their large FEP longitudinal study that antipsychotic treatment had a significant influence on brain volumes of these patients. More recently, Zipursky and Murray (2013) reported that some of the decreases in brain tissue volumes are explainable by the effects of antipsychotic medication.

PET studies have demonstrated a significant association between striatal DA D2 occupancy and prediction of short-term clinical response to antipsychotic treatment (Kapur et al., 2000), and have indicated that at least 60% of DA D2 receptor occupancy is necessary to achieve clinical response to antipsychotic treatment, which has been shown with both typical antipsychotics (Kapur et al., 2000; Nordstrom et al.,1993) and atypical antipsychotics (Abi-Dargham & Laruelle, 2005; Zipursky et al., 2005), excluding clozapine that shows lower DA D2 occupancy (Nordstrom et al., 1995). In line with this, Wolkin et al., (1989) using PET examined DA D2 receptor occupancy in 10 treatment resistant schizophrenics, hypothesizing that the lack of response in these patients may result from inadequate DA D2 receptor blockade. However, almost identical striatal DA D2 receptor occupancies in both responders and non-responders

were observed (Wolkin *et al.,* 1989). Correspondingly, a [^{123}I] IBZM Single Photon Emission Tomography (SPET) study conducted on 10 responders and 8 non-responders reported a similar degree of DA D2 occupancy in both groups (Pilowsky et al., 1993). Moreover, in a third study that examined DA D2 occupancy in six medicated patients with chronic schizophrenia using PET radiotracer, [11C]-methylspiperone, more than 95% DA D2 occupancy was demonstrated (Coppens *et al.*, 1991). Intriguingly, Kane et al., (1988) in their seminal trial included the most severely ill and treatment resistant patients with schizophrenia who failed a prospective trial of haloperidol at doses of at least 60mg/day, which indirectly suggests that DA receptor occupancy was sufficiently achieved.

These findings imply that whilst adequate DA D2 blockade may be necessary, it is not sufficient in all cases and does not guarantee a response. Notably, differential blockade does not seem to explain the mechanisms of treatment resistance to dopaminergic blocking antipsychotics.

There is, however, some evidence to suggest that presynaptic dopaminergic elevation instead may be linked to treatment response. Abi-Dargham and colleagues found that higher synaptic DA levels, as measured using DA depletion paradigms, were associated with a better response to antipsychotic treatment (Abi-Dargham et al., 2000). Furthermore, studies that examined dopamine metabolites in relation to antipsychotic response have reported higher pre-treatment plasma homovanillic acid (pHVA) in responders compared to treatment resistant patients (Chang et al., 1990; Duncan et al., 1993), and conversely treatment resistant patients had lower DA metabolite levels in their

cerebrospinal fluid (Van Kammen and Schooler, 1990). Thus the available evidence raises an important question of whether in treatment resistance: a) dopaminergic dysfunction is not a primary abnormality b) or, is such that antipsychotics fail to adequately normalize its activity. However, to date no study has examined DA synthesis capacity in vivo in specifically treatment resistant patients and the relevant question whether this abnormality is present in this sub-group of schizophrenics remains unanswered.

1.7 **Clinical course and predictors of TRS**

1.7.1 The course of TRS

Another question that remains unanswered is whether TRS manifests right at the onset of psychosis or evolves over time as a result of perhaps neurodegeneration or neurochemical sensitization. At the beginning of the last century, Kraepelin, referring in his textbook to Albrecht's observations that one third of his cases with hebephrenia reached terminal state within a year of onset, concluded: "Often enough the unmistakable symptoms of dementia appear already within the first year" (Kraepelin, 1919), citing, in addition, several other similar findings by experienced psychiatrists at the time. Thus, even prior to modern psychopharmacology, there were reports to suggest that bad outcome may identify a distinct and enduring subtype of schizophrenic illness, one that is perhaps more severe and of neurodevelopmental origin (Lieberman et al., 1997).

Considering the other possibility, that a proportion of patients become less responsive to pharmacological treatment as the illness progresses, Kolakowska and colleagues (Kolakowska et al., 1985) examined retrospective accounts of medicated patients 2 - 20 years after their first presentation. However, the authors found that majority of 40 poor responders in their sample were unresponsive throughout the illness, and thus concluded that treatment response is related to the type and not the stage of illness. The main methodological limitation of this study is the retrospective examination of clinical records that could have introduced information bias, i.e. the retrospective

examination of medical records could have lead to a measurement error (Rothman et al., 1987). Still, as the authors rightly state, the bias would be towards "over-estimation" of remission particularly if there was no association with gross behavioural disturbances. In addition, previous longitudinal studies have similarly observed that a type of illness course is apparent in early stages of illness, thus supporting this notion (Bleuler M, 1978; Huber et al., 1975).

However, Wyatt (1991) reviewed the evidence derived from twenty two studies of predominantly FEP patients, that examined the effect of medication on the natural course of schizophrenia, suggested that early psychopharmacological intervention improves the outcome and prognosis of illness. They proposed that a neurodegenerative process may be inherent to psychosis and thus unfavourably affect the clinical course in those who are non-compliant and subjected to multiple relapses. Numerous MRI studies have subsequently addressed this question, but no clear support for the hypothesis has been found (Zipursky et al., 2012).

On the other hand, there is evidence from animal studies that chronic treatment with DA blocking antipsychotics induces DA D2 receptor up-regulation, which in turn can lead to breakthrough dopamine supersensitivity and consequently reduce the efficacy of antipsychotic treatment despite clinically relevant levels of DA D2 occupancy (Ginovart et al., 2009; Samaha et al., 2007). This may imply that dopamine supersensitivity may predispose some patients to becoming resistant following repeated and long-term exposure to antipsychotic treatment. And, indeed it has been shown that in a proportion of patients not only the time

to remission is longer in subsequent episodes, but less, if at all, achievable (Lieberman, 1993; Sheitman et al., 1997).

Nonetheless, still up to 20 percent of FEP patients appear to be resistant to medication at a very early stage of their illness, and at the time of initiation of treatment (Agid et al., 2011; Lieberman et al., 1996; MacMillan et al., 1986; Robinson et al., 1999), which cannot be explained by the effects of medication, neurochemical sensitization or neurodegeneration.

Taken together, evidence to date raises the question of whether there may exist a distinct subgroup of schizophrenic patients whose pathophysiology is not marked by DA alterations and hence they do not derive therapeutic benefits from dopamine blocking antipsychotics. However, there may be another form of treatment resistance that indeed does develop at various stages of illness. On the other hand, some treatment resistant patients may achieve spontaneous remission or start responding to treatment later in life (Meltzer, 1997), which is in line with previous observations that older schizophrenic patients require much less intensive maintenance antipsychotic treatment than their younger counterparts (Fenton WS and McGlashan MD, 1987), and could perhaps be explained by the fact that DA system is age dependent, with significant reductions in dopaminergic transmission in older subjects being observed (Dreher et al., 2008; Reeves et al., 2002).

1.7.2 Neurodevelopmental predictors of treatment resistance in schizophrenia

The neurodevelopmental aetiology of schizophrenia has been considered in some of the earliest psychiatric descriptions (Kraepelin E, 1919). The neurodevelopmental theory of schizophrenia proposes that early risk factors, present prior to the onset of psychosis, especially during the gestation period, interfere with normal neurodevelopment resulting in pathological aberrations that increase the likelihood of psychosis in early adolescence or young adulthood (Weinberger, 1987; Murray and Lewis 1987).

More than a century ago, Clouston referred to the disorder as "developmental insanity" proposing that schizophrenia may be of neurodevelopmental origin (Clouston TS, 1891). The idea that patients with potential neurodevelopmental aetiology are more likely to show poor treatment outcome has long been considered (Murray et al.,1992; Murray, 1994). Murray and colleagues (1992) hypothesized that brain aberrations in the foetal and neonatal period may give rise to psychotic symptoms in early adolescence, which is particularly associated with poor outcome. The authors suggested that "congenital schizophrenia" is distinguished from other forms of illness by several features such as obstetric complications, lower level of premorbid functioning, presence of a deficit state, male gender, younger onset of illness, and neurological alterations, which have all been linked to poor treatment response. Accordingly, it has been shown that a history of perinatal insults was highly prevalent in patients with poor prognosis (Wilcox & Nasrallah, 1987).

This was subsequently confirmed in prospective first episode psychosis studies that found obstetric complications to be considerably more prevalent in a non-responder group (Alvir et al., 1999; Robinson et al., 1999). Furthermore, it has been shown that obstetric complications are associated with ventricular enlargement that tends to be present at the onset of the disorder (Weinberger et al.,1982) which supports a neurodevelopmental model of schizophrenia (Cannon et al., 2002).

Kolakowska and colleagues (1985) and Scottish FEP studies (Scottish Schizophrenia Research Group et al.,1989) reported that most of their non-responders were men and, in a later study, men with neurological impairment and prominent negative symptoms at the onset of illness were particularly at risk of treatment resistance. Other studies have also reported associations of negative symptoms with poor treatment response (Lindenmayer et al., 2000). Higher prevalence of negative symptoms and their association with premorbid developmental impairments has been reported in adolescent schizophrenia (Hollis C, 2000), and additionally, prodromal negative symptoms have been associated with subsequent transition to psychosis (Demjaha et al., 2012), which potentiates their neurodevelopmental origin. Furthermore, the deficit syndrome, introduced in the late eighties by Carpenter and colleagues (Carpenter et al., 1988) to describe primary enduring negative symptoms, is regarded as a distinct entity, shown to be marked by distinct neuroanatomical correlates (Cascella et al., 2010; Galderisi et al., 2008).

Younger age at onset has consistently been linked with poor outcome and poor response (Jomli et al., 2012; Mohamed, 2013), and specifically treatment resistance (Vanelle, 1995), which suggests an increased likelihood of early neurodevelopmental insults in TRS patients (McDonell and McClellan, 2007).

Family history has been reported to be associated with poor prognosis of illness, and in addition, with negative symptoms (Malaspina et al., 2000). Other neurodevelopmental factors such as severe cognitive impairment and poor premorbid functioning (Lindenmayer, 2000; Meltzer, 1997), as well as NSS (Smith et al., 1999) have been associated with treatment resistance. Finally, Sheitman and Lieberman (1998) suggested that the accumulation of a critical number of neurodevelopmental factors, rather than individual ones, may predispose the individual to treatment resistance.

1.8 **Aims and hypotheses**

Evidence to date raises the possibility that TRS may constitute a neurobiologically distinct subtype of illness, which is not marked by dopamine alterations. While there is sufficient evidence to suggest that the neurobiology of most schizophrenia, in general, involves increased presynaptic striatal dopaminergic elevation, little is known about whether this abnormality is present in patients resistant to antipsychotic treatment. The lack of this knowledge considerably limits the development of much needed novel and more rational treatments for this group.

The first study in my thesis has been specifically designed to address this important question using high resolution PET scan imaging to investigate DA synthesis in a cohort of treatment resistant patients. I tested the hypothesis that the neurobiological difference between treatment resistant patients and responders is determined by differences in presynaptic dopamine function. Either: a) no alterations in presynaptic striatal dopaminergic function will be observed in treatment resistant patients or b) they will have an exceptionally hyperactive dopaminergic system that current antipsychotic treatments are unable to block.

The second question relates to the issue of whether treatment resistance manifests at the outset of illness, for example due to disruption in neurodevelopment, or it evolves over time as a result of either a neurodegenerative process or neurochemical sensitization. This was tested in a large 10-year follow-up study of first episode psychosis patients with a

hypothesis that TRS tends to manifest at the outset and thus is related to a distinct subtype rather than a stage of illness.

Finally, using the same cohort, it was possible to investigate whether and which neurodevelopmental factors may predict treatment resistance, and thus test whether TRS is neurodevelopmentally distinct subtype of schizophrenic illness.

Chapter 2 Methodology

In this chapter the core methodological principles and issues pertinent to both treatment resistance and PET studies are described. First, the important issue of defining treatment resistance and remission in schizophrenia is considered. This is followed by a description of general principles underlying PET scanning and basic procedures of PET image analysis. The details of recruitment, sample characteristics, methodology and analyses performed in each study will be described in subsequent separate chapters.

2.1 Defining treatment response in schizophrenia

2.1.1 Defining 'treatment resistance'

The definition of treatment resistance in schizophrenia is an intricate process. It is generally accepted that TRS refers to a continuous course associated with substantial impairment in functioning, in which symptoms persist despite standard psychopharmacological treatment. However, the literature has been historically limited by inconsistencies in defining 'treatment resistance,' which could be attributed to difficulties related to disentangling illness severity from chronicity, or illness progression (Caspi et al., 2004).

In the absence of a universally accepted definition, studies have opted for different operational criteria, largely dependent on study aims and population used, which has resulted in disparity in response rates. Thus, studies recruiting patients for novel antipsychotic drug trials may use more stringent criteria than those testing psychological or behavioural interventions (Brenner et al.,1990). It

is clear, however, that any definition of treatment resistance should indicate that the patient has received an adequate trial of antipsychotic medication in terms of both dosage and duration. It is therefore essential to carefully assess for non-compliance or inadequate dose given prior to categorising a patient as unresponsive to medication.

Among the most renowned criteria for defining treatment resistance are those delineated by Kane and his collaborators for the Multicenter Clozapine Trial (Kane et al.,1988). Kane and colleagues were first to demonstrate clozapine effectiveness in schizophrenia patients who failed to respond to conventional antipsychotics.

According to these, prior to patients being deemed to be treatment resistant they are required to have:

1. A trial of at least three conventional antipsychotics from two different chemical classes at doses of ≥1000 mg chlorpromazine equivalents per day, lasting at least 6 weeks each.
2. No period of good functioning in the last five years.
3. Persistent moderate or severe psychopathology according to the Brief Psychiatric Rating Scale and Clinical Global Impressions criteria.

These criteria are considered highly stringent and permit the inclusion of the most severely ill schizophrenia patients only, who in addition, failed a prospective trial of haloperidol at doses of at least 60mg per day. However, in clinical setting patients show a varying degree of treatment response and cannot be always dichotomized into those who achieved full remission versus

those who are refractory to treatment. Evidently, there is a proportion of patients who fail to meet such treatment-resistance criteria, but still display moderate to severe negative or affective symptoms associated with poor social functioning. Thus, it may be more beneficial both in the research and clinical setting to assess treatment response as a continuum rather than use the strict criteria that categorize patients in one or the other subgroup (Meltzer 1997).

Due to the stringency of Kane's criteria, all of the three aspects of the first criterion specifically, the number of medication trials, the dosage and the duration, have been modified since.   It has been demonstrated that patients who do not respond to two medication trials have less than a 7% chance of responding to a third antipsychotic (Kinon et al., 1993). It is now generally accepted that two prospective drug trials are sufficient when defining treatment resistance (Barnes and McEvedy, 1996; Conley and Buchanan, 1997). Similarly, following important findings that chlorpromazine doses $\leqq$ 400 mg per day block 80 to 90% of dopamine receptors (Farde et al.,1994) and that higher doses, even in those who are considered resistant, are unlikely to produce direct therapeutic benefit (Wolkin et al.,1989), chlorpromazine equivalent of 400–600 mg antipsychotics is a current standard dose for an adequate treatment trial.   Thus, treatment resistance began to be defined as treatment failure to at least two different classes of antipsychotics administered for longer than 4 weeks in appropriate doses. Thus, for instance, Bondolfi and colleagues in their risperidone versus clozapine trial defined resistance as treatment failure to at least two different classes of antipsychotics administered for longer than 4 weeks in appropriate doses, although they do not specify what constitutes an

"appropriate dose" (Bondolfi et al., 1998), nor do they specify what proportion of their treatment resistant patients are treatment-intolerant. Furthermore, according to the UK National Institute for Clinical Excellence (NICE) criteria, a patient is considered treatment resistant if there is no satisfactory clinical improvement despite the consecutive use of two antipsychotics, one of which should be an atypical at recommended doses for 6 to 8 weeks duration (NICE, 2002). Finally, a 4- to 6-week duration of antipsychotic trial is now considered adequate (reviewed in Conley and Kelly 2001). However, Agid and colleagues have shown that whilst a great majority of FEP patients respond to initial antipsychotic treatment, a considerably lower response rate of less than 20 percent was observed with a second antipsychotic trial. Thus it has been advocated that clozapine should be considered early in the treatment of FEP patients, who do not respond to other second-generation antipsychotics within the first month of treatment (Agid et al., 2007; Agid et al., 2011).

Considering all the factors described above, in particular that the original Kane criteria are too stringent, the modified Kane's criteria have been used in the present work (Chapters 3 and 4) to identify treatment resistant patients. According to these, treatment resistance is identified if, despite a trial of at least two sequential antipsychotic for the 4-6 week duration, and at a dose of 400–600mg chlorpromazine equivalents/day, patients continued to experience persistent psychotic symptoms of at least moderate severity on one or more items on the positive symptom sub-scale of the PANSS (Kay et al., 1987), a total PANSS score of 75 and above (Leucht and Kane, 2006) and poor global functioning defined as a score of <59 on the Global Assessment of Functioning

(GAF), corresponding to at least moderate functional impairment as indicated in Chapter 3.

2.1.2 Defining symptomatic remission in schizophrenia

Similarly to defining treatment resistance, deciding what constitutes symptomatic treatment remission in schizophrenia has not been an easy process. First, for remission criteria to be applied patients have to have a diagnosis reached using recognized criteria; the second consideration relates to fact that remission does not equate to recovery, and finally patients can achieve remission spontaneously, that is without psychopharmacological treatment (Van Os et al., 2006). The issue of standardizing treatment remission has been considered by two working groups in recent years (Andreasen et al., 2005; Van Os et al., 2006). The US working group led by Andreasen, reached a consensus definition on remission in schizophrenia, and subsequently developed specific standardized operational criteria for its assessment. These criteria take into consideration two factors (for details see Chapter 3):

- Low symptomatic scores on the Positive and Negative Syndrome Scale (PANSS), but the symptom severity can also be determined by using the BPRS.
- A minimum of 6 months duration of symptomatic remission

2.2 General principles of PET imaging and analysis

PET is based on radioactive positron emission that was first discovered in 1933 (Joiot F, 1933; Thibaud J, 1933) following Anderson's discovery of the positrons in cosmic radiation (Anderson, 1932). It provides a unique and exciting opportunity for quantifying specific neurotransmitter alterations in a living human brain, at a very high sensitivity, which is unparalleled by other brain imaging modalities. Over the last 20 years, it has been extensively applied in psychiatric research, and permitted the investigation of the pathophysiology of schizophrenia and mechanisms of actions of antipsychotic drugs, providing important findings in both domains with significant subsequent theoretical and clinical implications. PET scanning requires administration of a short-lived positron emitting radiotracer (the radioisotope) into the blood circulation and subsequent tomographic imaging of the radiotracer distribution.

2.2.1 Radiochemistry and properties of radiotracer [^{18}F]-DOPA

Production of radiotracers is a highly specialized, time consuming and expensive procedure that has to be approved by national and international regulatory agencies. Radiotracers used in brain imaging must be able to cross the blood-brain barrier, have limited plasma and non-specific binding, peripheral metabolism and have appropriate kinetics for the study duration. Strict quality and pharmaceutical control requires tracers to be free of contamination by other radionuclides and toxic organic solvents and must guarantee sterility, stability, apyrogenicity and optimal pH and osmolality. The radiotracers are unstable with a short half-life and therefore most have to be manufactured at the

scanning centre and used virtually immediately after their synthesis. Therefore an onsite cyclotron, which is a compact subatomic particle accelerator, is useful, and often necessary, for short-half live radiotracers (Miller et al., 2008). The PET facility centre at the Hammersmith Hospital where the present work was conducted has an on-site cyclotron and radiosynthetic laboratory, in close proximity with PET scanners that permitted efficient manufacturing and transport of [^{18}F]-DOPA to the scanning room. The cyclotron, using powerful magnetic and electric fields that are positioned at a right angle within a vacuum chamber, energizes a beam of charged particles that then bombards stable target elements to induce a series of nuclear reactions, which gives rise to unstable positron emitting isotopes. In the following stage, the isotope is incorporated into the organic compound of interest. This process does not significantly affect the biochemical properties of the original compound (Miller et al., 2008).

One of the most widely used radiotracers for imaging the dopaminergic system is [^{18}F]-DOPA that was first applied for clinical use in 1983 (Garnett et al., 1983). The positron emitter, ^{18}F used here to label DOPA, is produced by bombarding ^{18}O with protons, which results in the nucleus losing a neutron and gaining a proton. It has a relatively long half-life of 110 minutes, which is considerably longer than that of many other radioisotopes and hence does not always necessitate on site production. Once injected, [^{18}F]-DOPA is transported across the Blood Brain Barrier (BBB), then decarboxylated to [^{18}F] - Dopamine by AAAD, and stored in DA vesicles. Similar to DA, it is further metabolized by monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT), but DA

metabolites exit the brain in a slow manner, therefore during the measurements, the activity usually accumulates during the measurement times that typically last up to 90 minutes (Cumming et al., 2001). In healthy subjects, F-DOPA images taken for 90 minutes following the peripheral injection of this radiotracer show high affinity for the brain areas richest in AAAD such as the striatum (caudate and putamen) and the mesencephalon, but significantly lower for the cerebral cortex or cerebellum (Herholz K et al., 2004).

2.2.2 Exposure to Radiation

There are associated risks of radiation exposure, in terms of DNA cellular damage, to both participants and the personnel involved in PET scanning procedures. The use of radiotracers, therefore, is rigorously regulated by ARSAC (UK) whose permission is required prior to the commencement of molecular imaging studies. The effective dose of 5-10 mSv is considered to be safe and in the range within which the risk is so small that it could be administered safely even in healthy volunteers.

2.2.3 PET data acquisition

Whilst undergoing decay, radiotracers emit positrons, the antiparticles of electrons, from radioactive nuclei, that travel a short distance of approximately 1-3 mm through a tissue, and when decelerated collide with surrounding slow moving electrons generating simultaneously a pair of annihilation gamma rays (or photons). The paired gamma rays are then detected as a 'coincident event' by PET scanners (or cameras) via scintillation crystal detectors, configured as circular rings. The 2 gamma rays are emitted at an angle of almost 180 to each

other, which makes the identification of the source of origin fairly accurate, i.e. they are likely to have occurred from a single annihilation event, somewhere at the point along the straight line between the two detectors. These lines are known also as a "lines of coincidence" or "lines of response" (LOR). The annihilation event will be recorded only if both detectors are activated simultaneously. During scanning time millions of coincidence events are registered forming a great number of LORs that are recorded and saved by PET scan computers as 2 dimensional matrices known as sinograms. During this process a few of the photons are absorbed by the surrounding tissue through which they first need to pass in order to reach the detectors, a process referred to as 'attenuation'. This results in a loss of detection of true coincidence events, which in turn leads to increases in noise, image artefacts, and image distortion. Therefore, prior to reconstruction of three-dimensional images, the sinogram data must be first corrected for attenuation (a process known as attenuation correction) and in addition for scatter, which occurs when photons are deflected by a collision with an electron, thus changing direction. Scatter correction is one of the most complex correction procedures. The three-dimensional images of corrected sinograms in the brain are reconstructed by specific computer analysis using filtered back-projection algorithms. Thus the PET image quantitatively reflects the dynamic radiotracer distribution in the specific region of brain over time. These reconstructed images are then formatted as a series acquired during adjacent time periods, which are known as frames (Figure 2.1).

Figure 2.1 Simplified diagram of PET data acquisition. (Partly adapted from Miller et al. 2008).
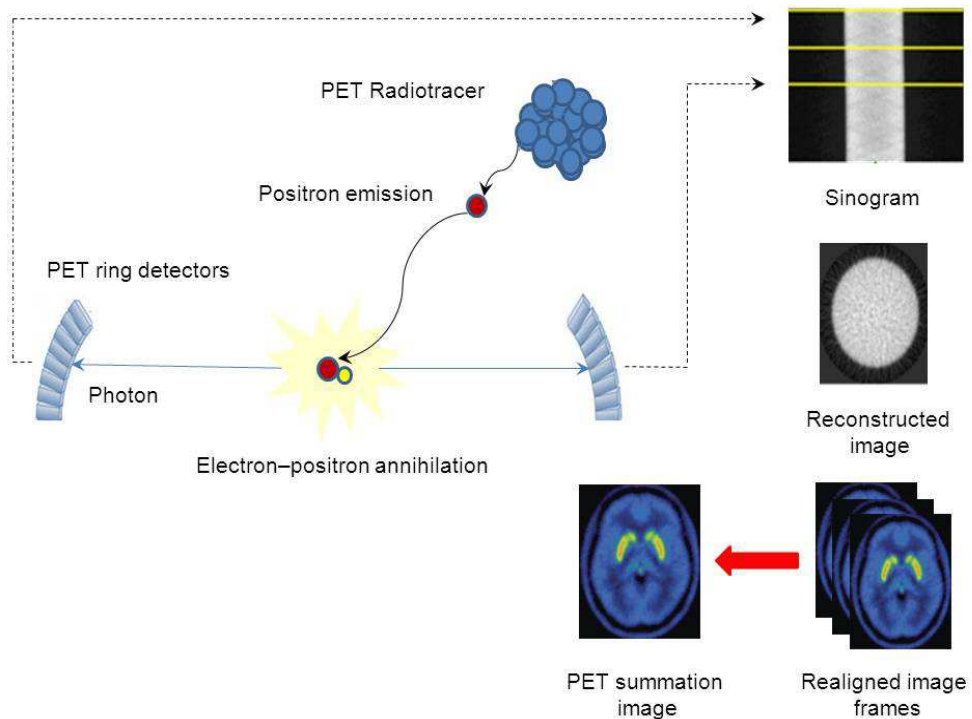
Radiotracers emit positrons from radioactive nuclei, that travel a short distance through a tissue, and when decelerated collide with surrounding electrons generating simultaneously a pair of annihilation gamma rays, which are then detected as a 'coincident event' by PET scanners. The three-dimensional images of corrected sonograms (2 dimensional matrices) in the brain are reconstructed by specific computer analysis using filtered back-projection algorithms. These images are then formatted as a series acquired during adjacent time periods, which are known as frames. Once realigned the time frames are integrated in a single summation dynamic image.

2.3 General principles of PET image analysis

2.3.1 Head movement detection and correction

Head movements, that may be expected even in the most co-operative subjects, during longer acquisition times can be a source of artefacts and image degradation, and as such present one of the major caveats in PET, but also other techniques of brain imaging. Significant head movements can potentially distort the data by either decreasing or increasing the signal, which is dependent on radiotracer concentration in tissues that surround regions of interest.

Numerous physical methods have been used in an attempt to minimize head movement. For example head restraints, such as individually moulded thermoplastic masks clamped to the scanner table, were used in two healthy volunteer studies (Green et al.,1994; Ruttimann et al.,1995). Although it was demonstrated that the effects can be reduced significantly by using these masks they fail to reduce head movement fully, and some, particularly patients with complex psychiatric or neurological disorders, could find these restraints uncomfortable to a degree that this would potentially result in increased movement.

An alternative strategy is to use an optical tracking system which enables head position measurements during the scan and subsequent movement correction conducted prior to image reconstruction (Goldstein et al.,1997; Montgomery et al.,2006).

The effects of head movement on image quality during data acquisition can be corrected for and therefore be minimized by performing post-hoc frame-by-frame (FBF) realignment of the data (Mawlawi et al., 2001). This is performed by realigning frames either to an initial frame or to a frame chosen for its high signal to noise ratio. In this thesis, the FBF realignment to a single frame acquired 7 minutes after [^{18}F]-DOPA injection was employed (Chapter 3). This method may have several potential limitations: it can give rise to artefacts between frame movements due to differences in radiotracer distribution between early and late frames, fails to correct for movement that occurs within frames, and finally it may lead to an incorrect attenuation correction as it is unable to account for movement that occurs between the transmission and emission scans.

Nevertheless, Montgomery and colleagues directly compared FBF realignment with a motion tracking system using a series of 11C-raclopride scans to examine their effect on both test-retest reliability (potential differences at 2 time points may be attributable to head movement), and noise in the data. They found that both methods of realignment were superior to the raw data, and although motion-tracking system performed better than FBF in some indices, FBF still dramatically improved the accuracy of data. For example, using FBF realignment of PET data, the variability of the ventral striatal signal was reduced from 13.5 percent, observed with row data, to 7.3 percent. Once realigned the time frames are integrated in a single summation dynamic image, known also as an ADD image (Figure 2.1).

2.3.2 General methodology of spatial normalization

Prior to entering statistical analyses, the dynamic images need to be normalized to a template first, which is essential when analysing data across different individuals. Normalization involves manipulation of individual brain images to match an atlas template. In other words, it registers images from different participants into approximately the same co-ordinate system which is achieved by using coregistration algorithms. The presence of considerable inter-individual anatomical variability in respect to a standard co-ordinate system necessitates atlases to be based on multiple subjects.

One of the most popular co-ordinate systems was developed by Talairach and Tournoux (Talairach J and Tournoux P, 1988), however, it is based on the transaxial planes that are demarcated by white matter structures, therefore not suitable for PET images where white matter is not visible. A standard brain atlas that is close to the Talariach coordinate system was created from 305 normal subjects at the Montreal Neurological Institute (MNI) (Evans et al., 2006). It is internationally recognized as the MNI standard brain atlas template for spatially normalized brain PET scans. For each tracer separate templates are created. For instance, the $[^{18}F]$-DOPA template used in the present study was created by several groups at Hammersmith Hospital from previously acquired images, using the same imaging protocol.

The described method was used for voxel based analysis where the parametric image for each patient was normalized into standard space, using the patients' PET summation image and the $[^{18}F]$-DOPA template. The normalization method

used, however, in ROI analysis differs somewhat; here, the [^{18}F]-DOPA atlas template was normalized together with ROI map (see the following section) individually to each subject's dynamic summation PET image, which remained in native space. Using this method, observer bias that can occur when defining ROIs for each subject, is avoided. Moreover, this way the sampling volume is adjusted to the individual's striatal volume, mitigating to some extent against partial volume effects that may otherwise introduce biases in radiotracer uptake measurements (Chapter 3).

2.3.3 Regions - of - interest (ROI) approach

The striatum is rich in AAADC, and as such is a region of particular interest for measurements of [^{18}F]-DOPA uptake. As described in a previous chapter, the striatum can be topographically organized into functional subdivisions along the dorsolateral to ventromedial gradient. In earlier dopaminergic studies investigation of dopaminergic transmission was only possible for the striatum as a whole, which precluded more precise identification of dopamine abnormality in schizophrenia (Martinez et al., 2003). However rapid and powerful advances in PET imaging resolution permit [^{18}F]-DOPA uptake measurements even on this sub-divisional level. This is highly important, as recent literature has demonstrated that certain striatal structures may be associated more strongly with schizophrenia. From recent studies it appears that associative striatal subdivision is more important than other functional subdivisions for schizophrenic pathophysiology (Kegeles , 2010). Congruently, Nozaki and co-authors (2009) have reported that presynaptic dopamine synthesis capacity is

increased in the caudate nucleus that anatomically corresponds to the associative subdivision.

In this present work, the regions of interest map that were defined in MNI space, comprised the whole striatum and its functional subdivisions, and in addition the cerebellum that acted as a reference region. As described in the previous section, the ROI map was normalized together with the [^{18}F]-DOPA atlas template to each individual PET scan. Correspondingly [^{18}F]-DOPA influx rate constants (Ki values) were calculated, using graphical analysis (Patlak et al., 1983), in the whole striatum and its sensorimotor (SMST) associative (AST) and limbic (LST) functional subdivisions relative to uptake in reference region (see Chapter 3).

2.3.4 Statistical parametric mapping (SPM)

A voxel-based comparison using SPM permits identification of significant differences at a voxel level across scan datasets that allows both between-group and within-group analyses. Using this approach, all the individual parametric images are first smoothed to improve the signal to noise ratio and compensates for slight inter-individual anatomical differences, and then normalized into the same three-dimensional standardized space using an isotropic Gaussian kernel. This way, each voxel from the data set in question is in the same stereotactic location as a corresponding voxel from a comparison data set, so that subsequent analyses can be performed at a voxel level using the general linear model (Friston et al., 1995). For each contrast a t statistics is computed for each voxel to produce a SPM (t). The SPM (t) is subsequently transformed to SPM (z), to enable comparison between studies with different

degrees of freedom. Following this, voxel based Z scores are collected into a 3D image, which is termed as statistical parametric map or Z map. The SPM constitutes a large number of measures over the brain. Significant clusters of mean group differences can then be identified, using appropriate thresholding that accounts for the large number of comparisons. These are then projected onto a 3D glass brain or a single subject T1 MRI image (Figure 2.2).

Figure 2.2 Between-group comparison using SPM

Group 1   Group 2
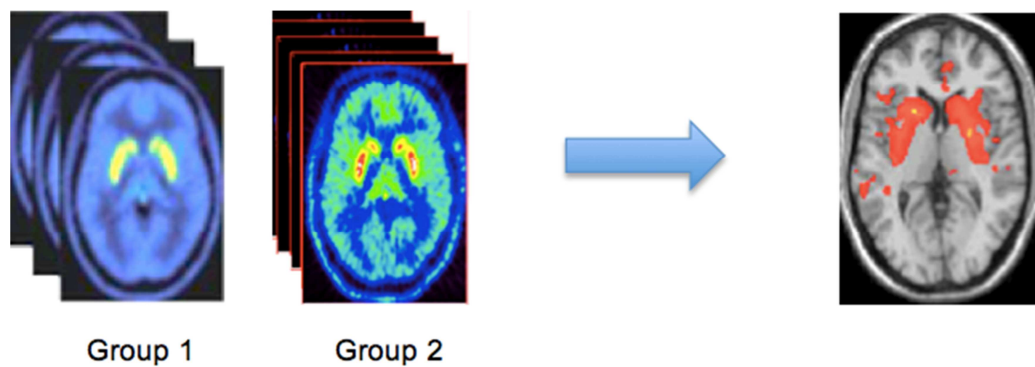
Statistical comparisons across scan datasets in corresponding voxel regions of the brain. Voxels with significant differences in mean tracer uptake are automatically transferred onto a standard T1 MRI.

2.3.5 Comparison between ROI and SPM methods

The ROI approach allows for the sampling of a tracer uptake in specific and preselected areas of the brain, identified as regions of interest, to address the specific research questions, based on an a priori hypothesis. It examines activity within a set of voxels that are functionally related. In this work, as previously described, the preselected regions of interest comprised the whole striatum and its functional subdivisions. However, the localized changes in the

striatum may not always be detected by a traditional ROI analysis. Statistical parametric mapping, on the other hand permits localisation of statistically significant changes on a voxel by voxel basis, without previous assumption of the distribution of differences between the images. It has been shown that SPM can be successfully applied to [^{18}F]-DOPA PET images (Ito et al.,1999). Furthermore, application of SPM permits examination of dopaminergic function in other brain regions outside the striatum, which could not be determined with the ROI approach. Therefore, both methods of image analysis, the automated ROI approach and SPM were used in the first thesis study (Chapter 3) to demonstrate that results are independent of the image analysis performed.

Chapter 3 PET study of dopaminergic activity in TRS

3.1 **Background**

As discussed earlier, a significant proportion, perhaps up to one third, of patients with schizophrenia show only a limited response to antipsychotic treatment (Lindenmayer et al., 2005). This 'treatment resistance' is a major clinical problem, impairing the lives of these patients. Over the last two decades, radiolabelled DOPA PET studies have directly demonstrated that elevated presynaptic striatal dopaminergic function is a robust feature of schizophrenic pathophysiology (Chapter 1). However, the relationship between this abnormality and the response to antipsychotic treatment remains unclear.

There is substantial evidence to suggest that the efficacy of antipsychotic drugs is related to dopamine D2 receptor blockade (Kapur et al., 2000). All currently licensed antipsychotic medications block dopamine receptors (Talbot et al., 2002), and their relative clinical potency closely parallels their affinity to bind and block the dopamine D2 receptor subtype (Creese et al.,1976). PET studies have indicated that at least 50% occupancy of D2 receptors is necessary to achieve clinical response (Abi-Dargham et al., 2005). Both PET and SPECT studies that have investigated D2 receptor occupancy specifically in treatment resistance in schizophrenia found almost identical levels of striatal dopamine receptor occupancy in responders and non-responders, suggesting that whilst adequate D2 blockade may be necessary for a therapeutic response, it does not necessarily guarantee it (Wolkin et al., 1989; Pilowsky et al., 1993; Coppens

et al., 1992). The limited data available suggests that patients who do not respond to current dopamine-blocking treatments may have a lower level of [18 F]-DOPA uptake than those who respond to dopaminergic blockers (Abi-Dargham et al., 2005; Duncan et al., 1993). However, it could also be argued that patients who do not respond have an exceptionally hyperactive dopaminergic system, which is not blocked by current antipsychotic treatments. This study was specifically designed to address this issue by using [18F]-DOPA PET to compare dopamine synthesis capacity in patients that had either shown a good or a poor response to treatment with antipsychotic drugs.

3.2 Methods

3.2.1 Participants

Two groups of patients were recruited, defined according to their response to antipsychotic treatment; all met DSM IV criteria for Schizophrenia, paranoid subtype determined by using Operational Criteria Checklist (OPCRIT) (McGuffin et al., 1991). The Treatment Resistant group (n=14) comprised patients who met modified Kane criteria for treatment resistance (see Chapter 2.1). According to these, all of these patients had received at least 2 prior sequential antipsychotic trials each of at least 4-6 weeks in duration at a dose of 400–600mg chlorpromazine equivalents/day, but had continued to have persistent psychotic symptoms defined as a rating of at least moderate severity on one or more items on the positive symptom sub-scale of the PANSS (Kay et al., 1987), a total PANSS score of 75 and above (Leucht and Kane, 2006) and poor global functioning defined as a score of <59 on the Global Assessment of Functioning

(GAF), corresponding to at least moderate functional impairment. The Responder group (n=12) comprised patients who met the Remission in Schizophrenia Working Group Criteria for treatment remission (Andreasen et al., 2005). These patients scored 3 or less on all items of the PANSS (corresponding to mild severity or no symptoms), and had not experienced a symptomatic relapse in the 6 months prior to the study.

All patients were recruited from the South London and Maudsley NHS Trust. A group of healthy controls (n=12), with no previous or current history of psychiatric illness as assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and the Structured Clinical Interview for DSM-IV Personality (SCID-II), and no family history of psychosis, were recruited through advertisement in the press. The groups were matched for age, sex, ethnicity, weight, and for smoking. Treatment Resistant patients and Responders were matched, in addition, for duration of illness and for antipsychotic dosage expressed in chlorpromazine equivalents. All antipsychotic doses were converted to chlorpromazine equivalents, according to the published equivalencies (Ho et al., 2011; Woods 2003; Taylor et al., 2012)

All patients were receiving antipsychotic medication other than clozapine at the time of scanning. Two patients (from the Treatment Resistant group) had been previously treated with clozapine, but it had been discontinued due to side effects, without reaching therapeutic blood levels. Compliance to medication was determined by measuring antipsychotic drug serum levels, and by

reviewing pharmacy and medical records. Subjects were excluded if, prior to the scan, there was evidence of non-compliance at any point in the six months prior to scanning, or where serum levels were not at adequate levels. Exclusion criteria for all groups were pregnancy (all women received a pregnancy test prior to scanning), contraindication to imaging, history of neurological or active medical illness or head injury, or alcohol or other drug abuse or dependency. All subjects received a urine drug screen prior to scanning and were excluded if this was positive for illicit substances.

One patient was excluded from the Treatment Resistant group because he did not complete the scanning, and another Treatment Resistant patient withdrew from the study. The data analysis was therefore restricted to 12 Treatment Resistant patients, along with 12 Responders and 12 healthy volunteers.

The protocol was approved by the Institute of Psychiatry, King's College London research ethics committee, and permission to administer radioactive substances was granted from the Administration of Radioactive Substances Advisory Committee, United Kingdom. All subjects, following a full description of the study, gave written informed consent to participate.

3.2.2 PET Protocol

The participants were instructed to fast and to refrain from caffeine, tobacco, and alcohol, for at least 12 hours before scanning. One hour prior to the start of each scan, all subjects received 150 mg of carbidopa, a peripheral AAADC inhibitor, and 400 mg of entacapone, a peripheral catechol-O-methyltransferase

inhibitor orally, to increase specific signal detection, as these compounds reduce the formation of radioactively labeled metabolites that may cross the blood-brain barrier and thus confound the measurements.

3.2.3 Data acquisition

PET imaging data were acquired on an ECAT EXACT HR+ 962 PET scanner in 3D mode, with an axial field of view of 15.5cm, which provides 63 transaxial planes. This camera has an axial resolution of 5.4 mm full width at half maximum (FWHM) and a transaxial resolution of 5.6 mm FWHM at 10 cm distance from the centre (Brix et al., 1997). Subjects were positioned in the scanner with the orbitomeatal line parallel to the transaxial plane of the tomograph. Head position was monitored via laser crosshairs, video camera and direct observation throughout the scanning period. To correct for attenuation, an initial 10-minute transmission scan was conducted prior to radiotracer administration. Approximately 180 MBq of the [^{18}F]-DOPA was injected as a bolus over 30 seconds into the antecubital vein, via a 22g cannula and three-way tap, simultaneously with the initiation of a 90-minute dynamic PET scan. Emission data were obtained as 26 frames of increasing duration over 90 minutes in list mode, starting with a background frame of 30 seconds, followed by injection of [^{18}F]-DOPA at the beginning of four 60-second frames, three 120-second frames, three 180-second frames, and, finally, fifteen 300-second frames (Figure 3.1).

Figure 3.1 PET protocol used in experiment
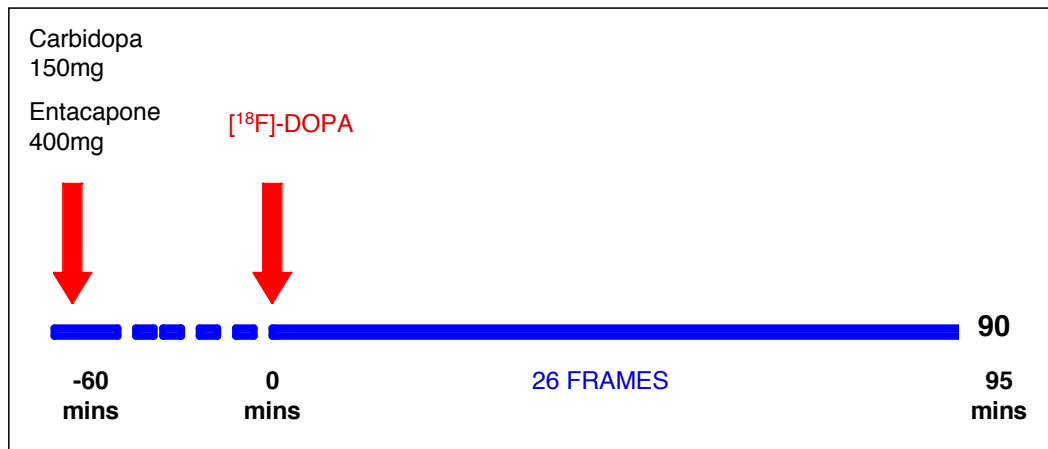
Subjects, in addition, underwent structural magnetic resonance imaging to exclude intracranial abnormalities. No gross abnormalities were detected in any subject following review by a neuroradiologist blind to the subject group.

3.2.4 Image analysis

Both automated ROI and voxel-based statistical image analyses, with the cerebellum as a reference region, were performed to examine striatal $[^{18}F]$-DOPA uptake. The ROI analysis included the whole striatum and its associative, limbic and sensorimotor sub-regions delineated as described in Chapter 2.

To correct for head movement, denoising of the non-attenuated dynamic image and frame-to-frame realignment to a single 'reference' frame acquired 7 minutes after $[^{18}F]$-DOPA injection, was conducted. Then, the transformation parameters were applied to the corresponding attenuation-corrected frames, and the realigned frames were summated to create a movement-corrected dynamic

image ready for the analyses. The ROI map was then normalized together with an [18F]-DOPA template to each individual PET summation image using SPM5 (www.fil.ion.ucl.ac.uk/spm), which allows ROIs to be placed automatically and without observer bias, on individual [18F]-DOPA PET dynamic images (Figure 3.2).

Figure 3.2 Striatal ROI map normalized together with an atlas template on PET summation image (Adapted from Shotbolt et al., 2011).
With kind permission from Psychological Medicine, Cambridge University Press
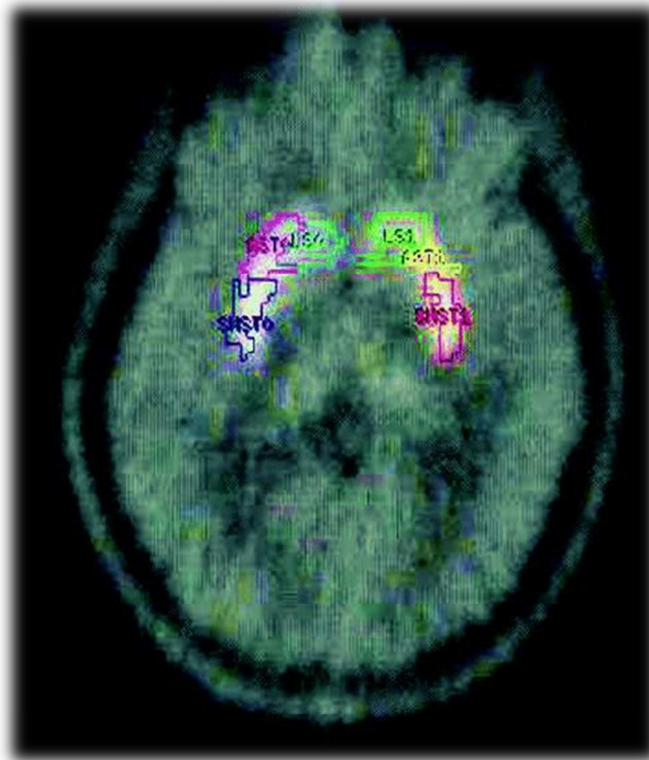
Green area represents LST, pink area AST, and purple area SMST

Patlak graphical analysis was used to compute striatal [18F]-DOPA influx rate constants (K_i^{cer} values) to index striatal DA synthesis capacity relative to uptake in the reference region (the cerebellum) for left and right sides combined. The

cerebellum is often used in [^{18}F]-DOPA PET studies as a reference region as it is known to have the lowest AAADC activity and consequently the lowest DA concentration (Brown et al., 1979; Moore et al., 2003).

3.2.5 SPM analysis

Voxel-based statistical image analysis was performed to independently confirm the results derived from ROI analysis and determine if there were sub-regional differences. Parametric maps of the influx rate constants for [^{18}F]-DOPA were constructed from movement-corrected images by using a wavelet-based kinetic modelling approach that increases the signal-to-noise ratio without affecting significantly resolution (see Howes et al., 2009). Following the normalization of parametric images, statistical analyses were performed using SPM5, restricted to the striatum using a mask, to examine differences between groups. The results presented were analysed corrected for multiple comparisons ($p < 0.05$, corrected at the Family Wise Error rate) and, in a further sensitivity analysis, without correction.

3.2.6 Statistical analysis

Preliminary tests were conducted to explore the homogeneity of variance, regression slopes, normality and reliable measurements of covariate. The Kolmogorov-Smirnov test confirmed that the data showed a normal distribution. To determine whether there was an effect of group on striatal K_i^{cer} values, and demographic, striatal volume, and clinical data, analysis of variance (ANOVA) and independent t tests were performed as appropriate. To examine differences

in K_i^{cer} values between the groups (Treatment Resistant, Responders and Healthy Volunteers), following a significant main effect, the posthoc pairwise comparisons based on t statistics between each groups were performed. To reduce the risk of a type I error due to multiple testing a Bonferroni correction of the p-value was applied.

To assess whether the effect was influenced by medication, an additional analysis of covariance (ANCOVA) with medication dose expressed as chlorpromazine equivalents added as co-variate, was performed. The independent variable was group whereas dependent variables comprised Ki^{cer} values for striatum and its three subdivisions. A two-tailed significance threshold of 0.05 was used throughout. In addition, Pearsons's correlations were performed to assess the relationship between the DA synthesis capacity and the total PANSS score or the negative and the positive subscales of the PANSS, in both treatment resistant and responder groups.

3.3 **Results**

Sociodemographic and clinical characteristics are presented in Table 3.1. No between group differences were observed for age, gender, ethnicity, weight, radiation dose received, cigarette smoking, duration of illness, or medication dose. In addition, there were no differences across the groups for the whole striatal volume or any of its subdivisions. The mean ages of treatment resistant patients, responders and volunteers were 45.7 [SD=9.8]; 44.0 [SD=11.9] and 44.2 [SD=8.9] respectively. The treatment resistant and healthy volunteer

groups comprised 5 males and 7 females each, whereas in a responder group there were 6males and 6 females. The number of smokers was equal in the treatment resistant and healthy volunteer groups (n=3), whilst there were 4 responders that reported smoking. As it is expected, treatment resistant patients received higher medication dose, by 110 mg/day approximately in chlorpromazine equivalents, then responders, and had significantly higher total PANSS score than responders. Types of antipsychotics that patients were receiving at the time of the scanning are presented in Table 3.2.

Figure 3.3 shows the mean dopamine synthesis capacity for the three groups. The analysis of variance, identified a statistically significant effect of group on K_i^{cer} values for the whole striatum ($F_{2,33}=5.4$, p=0.01), and for each of its associative ($F_{2,33}=6.7$,p=0.004), limbic ($F_{2,33}=4.0$, p=0.03) and sensorimotor subdivisions ($F_{2,33}=3.4$, p=0.05). For mean K_i^{cer} values and standard deviations, see Table 3.3.

Because one of the patients in the Responder group had particularly high K_i^{cer} values, we repeated the analysis after excluding this subject. The group effect remained significant for the whole striatum ($F_{2,32}=4.2$, p=0.02) and its associative subdivision ($F_{2,32}=6.4$, p=0.005), but not the other striatal sub-divisions. Individual K_i^{cer} values are presented on scatter plots before and after excluding the subject in question (Figures 3.4 and 3.5).

To assess the effect of antipsychotic medication, the analysis was repeated with the addition of medication dose, in chlorpromazine equivalents, at the time of

scanning as a covariate. The effects of group on K_i^{cer} values from the whole striatum ($F_{1,22}=7.2$, P =0.01) and the associative ($F_{1,22}=10.2$, P =0.005) and limbic ($F_{1,22}=6.4$, P =0.02) subdivisions remained significant, but there was no longer a significant difference in the sensorimotor subdivision.

Regarding the correlations between DA synthesis capacity and the symptom scores, in TRS there were no significant associations between the whole striatal Ki^{cer} values and the total PANSS, or its positive or negative subscale scores (r=0.11, r=-0.07, r=0.52 respectively). Whilst in Responder group, the significant relationship between DA synthesis capacity was observed between DA synthesis capacity and total PANSS and the negative subscale sores (r=0.60, p=0.039; r=-0.67, p=0.016 respectively), these associations no longer remained significant after removal of the subject with exceptionally high Ki^{cer} values (r=-0.36, p=0.27; r=-0.59, p=0.06), suggesting that significant correlations were affected by an extreme value.

Table 3.1 Demographic and clinical characteristics of the study participants

	Treatment Resistant (N=12)	Responders (N=12)	Controls(N=12)	P
Age	45.7 [9.8]	44.0 [11.9]	44.2[8.9]	ns
Gender	5M/7F	6M/6F	5M7F	ns
Smoking	3S/9N	4S/8N	3S/9N	ns
Ethnicity	5W/6B/1A	4W/7B/1A	6W/4B/2A	ns
Weight (Kg)	80.9 [19.2]	83.0 [20.7]	80.9 [23.3]	ns
Radioactivity injected (Mbq)	180.0 [5.5]	183.6 [4.3]	183.4 [5.6]	ns
Specific activity (GBq/Micromol)	0.025 [0.017]	0.033 [0.015]	0.025 [0.017]	ns
Whole Striatal Volume/mm3 Mean [SD]	15590.9 [1675.2]	16424.1 [1818.9]	16773.6 [1870.1]	ns
AST Striatal Volume/mm3 Mean [SD]	9644.0 [1165.5]	10185.0 [1164.1]	10005.0 [1768.2]	ns
LS Volume/mm3 Mean [SD]	1953.1 [262.3]	1965.5 [348.0]	2013.1 [269.3]	ns
SMST Volume/mm3 Mean [SD]	4260.2 [928.6]	4189.6 [513.5]	4394.4 [459.6]	ns
Duration of Illness (years) Mean [SD]	16.1 [8.6]	16.2 [10.1]	-----	
Chlorpromazine eqv mg/day Mean (SD)	396.1 [157.5]	283.9[159.14]	-----	
PANSS Total Mean [SD]	104.3 [10.6]	50.7 [5.8]	-----	
PANSS Positive Symptoms Mean [SD]	26.2 [3.8]	11.9 [2.4]	-----	
GAF score Mean [SD]	47.5 [3.9]	67.5 [4.5]	-----	

Table 3.2 Antipsychotic use in patients[a]

Antipsychotic	Treatment Resistant		Responders	
	N	%	N	%
Olanzapine	3	25.0	2	16.7
Quetiapine	2	16.7	1	8.3
Amisulpride	1	8.3	1	8.3
Risperidone	1	8.3	0	0.0
Risperidone depot	2	16.7	3	25.0
Aripiprazole	0	0.0	1	8.3
Chlorpromazine	1	8.3	0	0.0
Zuclopenthixol Decanoate	1	8.3	3	25.0
Flupenthixol Decanoate	1	8.3	1	8.3

[a]*There are no significant differences between groups*

Figure 3.3 Mean DA Synthesis Capacity for the whole striatum in three groups [a]
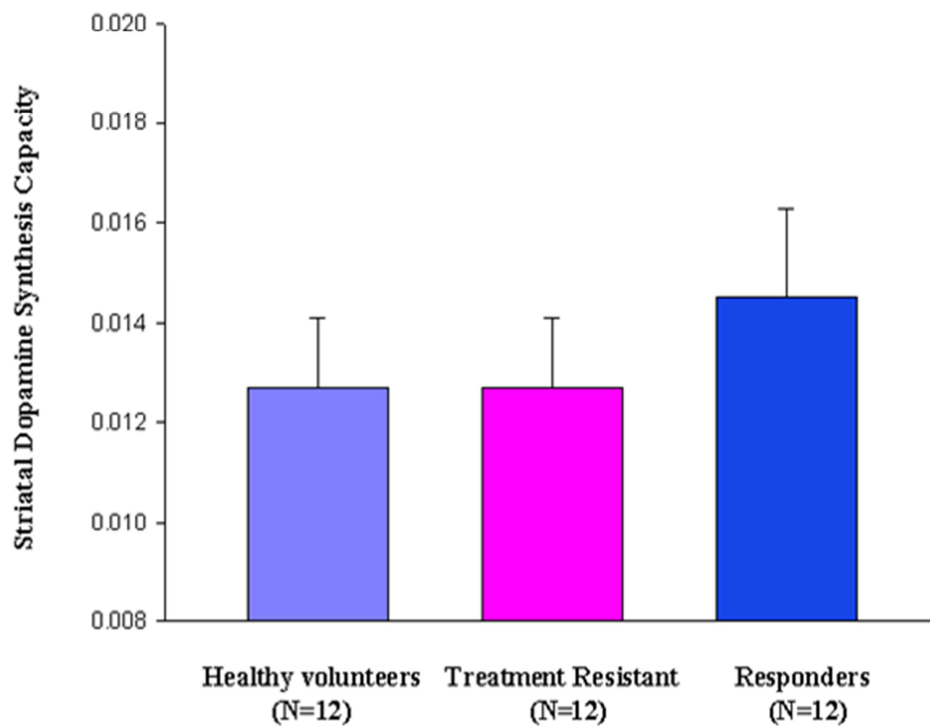
[a]*The treatment resistant group showed significantly lower dopamine synthesis capacity than the treatment responders (p= 0.02, corrected for multiple comparisons). There were no significant differences between treatment resistant patients and healthy volunteers. Error bars indicate standard deviation.*

Table 3.3 Comparison of mean presynaptic striatal K_i^{cer} values between treatment resistant patients, responders and healthy volunteers

	Treatment Resistant (N=12) Mean [SD]	Responders (N=12) Mean [SD]	Controls (N=12) Mean [SD]	F (df=2,33)	p
Whole Striatum	1.3×10^{-2} [0.14×10^{-2}]	1.4×10^{-2} [0.18×10^{-2}]	1.3×10^{-2} [0.14×10^{-2}]	5.38	0.01
Associative Striatum	1.2×10^{-2} [0.14×10^{-2}]	1.4×10^{-2} [0.15×10^{-2}]	1.2×10^{-2} [0.14×10^{-2}]	6.71	0.004
Limbic Striatum	1.3×10^{-2} [0.17×10^{-2}]	1.5×10^{-2} [0.23×10^{-2}]	1.3×10^{-2} [0.17×10^{-2}]	4.02	0.03
Sensorimotor Striatum	1.4×10^{-2} [0.20×10^{-2}]	1.6×10^{-2} [0.24×10^{-2}]	1.4×10^{-2} [0.15×10^{-2}]	3.38	0.05

Figure 3.4 Individual K$_i$^{cer} values for the whole striatum in three groups
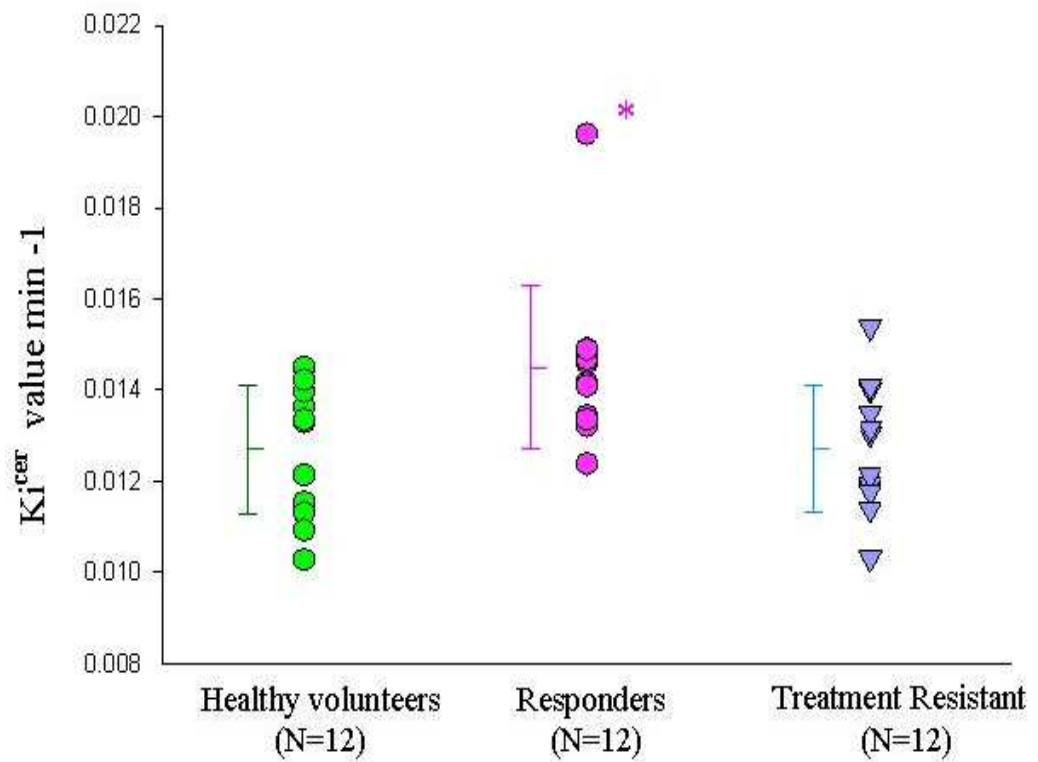
The treatment resistant group showed significantly lower dopamine synthesis capacity than the treatment responders (p= 0.02).   There were no significant differences between treatment resistant patients and healthy volunteers

Figure 3.5 Individual K_i^{cer} values for the whole striatum in three groups following the exclusion of a participant with very high K_i^{cer} value
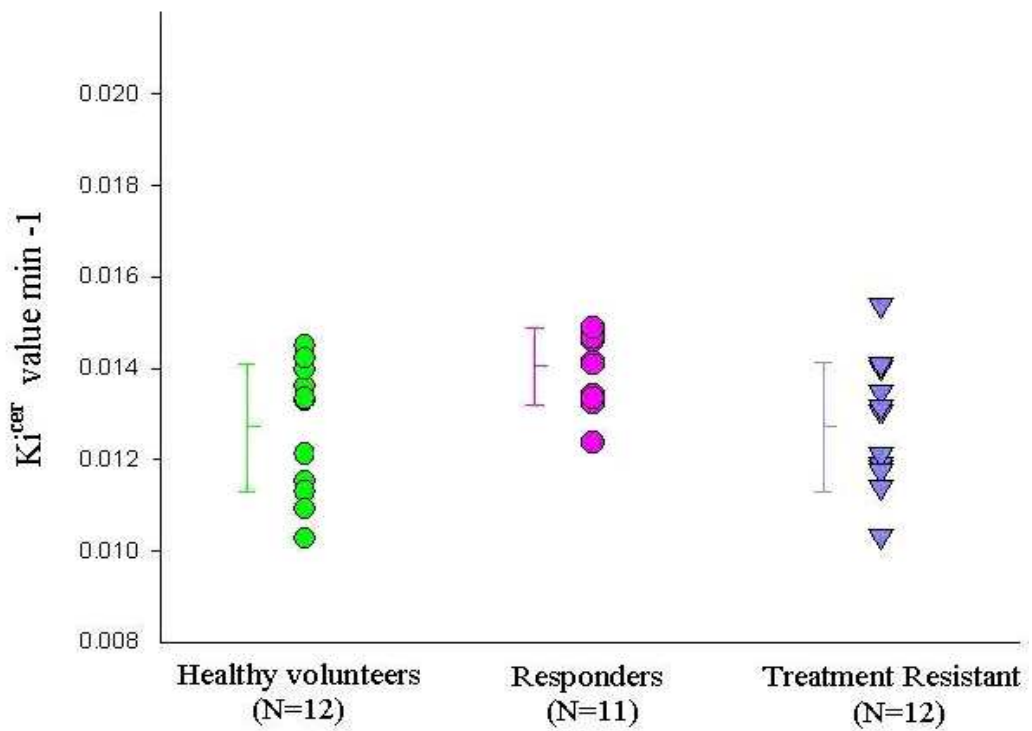
This result remained significant, after excluding the subject identified by the asterix in Figure 3.4. The treatment resistant group showed significantly lower dopamine synthesis capacity than the treatment responders (p= 0.01).

3.3.1 Between Group Comparisons

Treatment Resistant versus Responders

After adjustment for multiple comparisons, K_i^{cer} values were significantly greater in Responders than Treatment Resistant patients in the whole striatum (p=0.02, corrected; ES = 1.11), and the associative (p=0.008, corrected; ES= 1.31) and limbic subdivisions (p=0.03, corrected; ES=1.04). There was no significant difference in the sensorimotor subdivision (p=0.1, corrected).

Greater K_i^{cer} values in Responders than Treatment Resistant patients were also observed in the corresponding voxel-based analysis, with a peak in the head of caudate (p=0.039), which lies within the associative subdivision of the striatum (Figure 3.6). The difference was significant at p<0.05 corrected for multiple comparisons using the family–wise error rate. The Treatment Resistant group>Responder group contrast showed no significant differences, even at an uncorrected statistical threshold (p<0.05 uncorrected).

Responders versus healthy volunteers

K_i^{cer} values were significantly elevated in Responders compared to healthy volunteers, following multiple comparison adjustments, in the whole striatum (p=0.02, corrected; ES= 1.12) and the associative subdivision (p=0.01, corrected; ES=1.24), but not in the limbic (p=0.1, corrected), or sensorimotor subdivisions (p=0.06, corrected). Similarly, the voxel-based analysis revealed significantly greater K_i^{cer} values in the Responder group compared to healthy volunteers, with a peak in the caudate (p=0.037, corrected at the family-wise error rate). The voxel-based contrast of the healthy volunteers with Responders

showed no significant differences, even at an uncorrected threshold (p<0.05 uncorrected).

Treatment resistant versus healthy volunteers

There was no significant difference in mean striatal K_i^{cer} values between treatment resistant patients and healthy volunteers, in the whole striatum or its subdivisions. This was confirmed with the subsequent voxel based analysis for the contrast of the treatment resistant group> healthy volunteer group and for the contrast of the healthy volunteer group> treatment resistant group, even at an uncorrected threshold (p<0.05 uncorrected).

Figure 3.6 Increased Striatal Dopamine Synthesis Capacity in Responders relative to Treatment Resistant patients.[a]
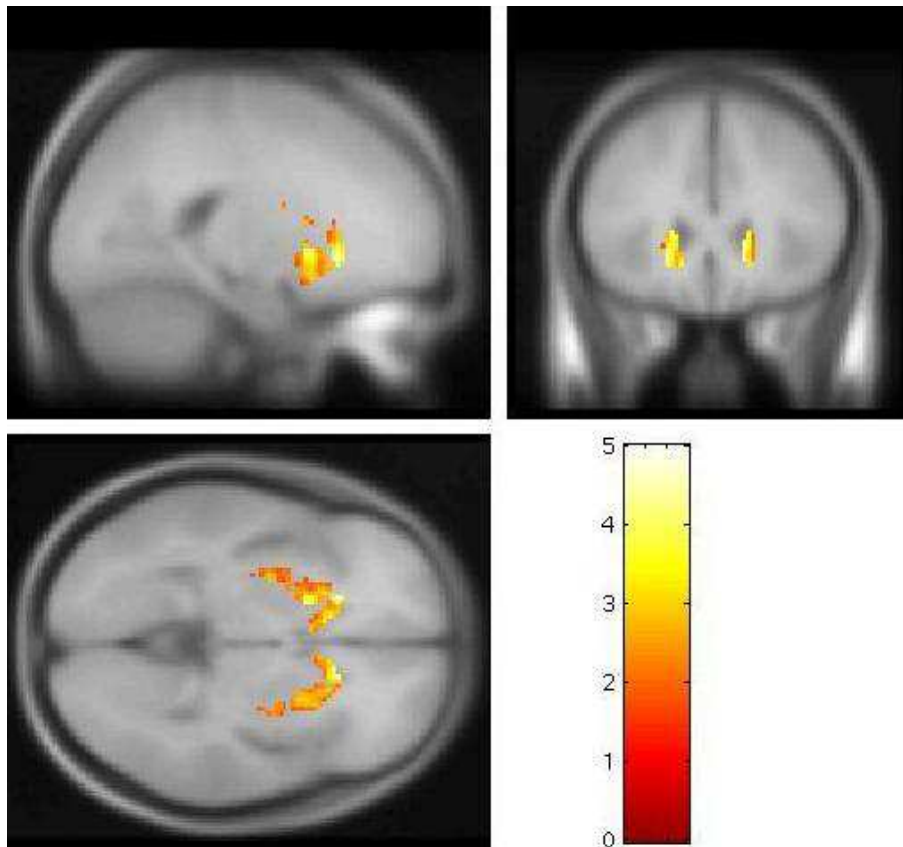
[a]*The images show increased dopamine synthesis capacity in Responders (N=12), relative to Treatment Resistant patients (N=12). The most significant increase was in the head of the caudate nucleus (p=0.039, corrected at the family-wise error rate).*

3.4 **Discussion**

This study provides direct evidence that dopamine synthesis capacity in schizophrenia is lower in patients that are treatment resistant than in those who show a good response to antipsychotic drugs. This suggests that treatments that involve the blockade of dopamine receptors may be effective in patients that have an elevation of dopamine synthesis capacity, but may be less useful in patients in whom dopamine synthesis capacity is relatively normal.

3.4.1 Limitations

The patients in this study were chronically medicated, which could have influenced pre-synaptic dopamine synthesis capacity (Carlsson et al.,1963) discussed in some detail in Section 3.4.3. The two patient groups, however, were matched for both the current dose of medication, and the total duration of treatment. The mean daily dose was higher in the Treatment Resistant group (reflecting their poor response to treatment), but the difference in dose was not statistically significant, and group differences in Ki^{cer} values remained significant after covarying for medication dose. Another potential limitation is that patients received various types of antipsychotic medication that could have differentially affected dopamine synthesis. Again, the groups were relatively well matched in terms of generation and type of antipsychotics (presented in Table 3.2). Two patients, one in the treatment resistant and the other in the responder group, were on amisulpride. At doses lower than 200mg daily, amisulpride may increase dopamine transmission via a preferential blockade of pre-synaptic D2-like autoreceptors. However, as these two patients received much higher doses, 800 mg and 600 mg daily respectively, than the level for a preferential

presynaptic action, and were in different groups, it is unlikely that this has affected the results. Only one patient (in the Responder group) received aripiprazole, an antipsychotic with the unique properties of being a partial dopamine receptor agonist (Miyamoto et al., 2004). Theoretically, partial agonists should reduce dopamine synthesis capacity, reflected in a lower Ki^{cer} value, although in practice little net effect has been observed during chronic aripiprazole administration in rats (Der-Ghazarian et al., 2010). As the aripiprazole treated individual was in the responder group, a reduction in Ki^{cer} value would reduce rather than account for the group differences we observed between responders and treatment resistant patients. Furthermore, as the Ki^{cer} values for this individual were similar to the mean Ki^{cer} value in this group, it is unlikely that including the aripiprazole treated individual has had a major effect on the overall results.

As the group sizes were relatively modest, the possibility that the absence of differences in dopamine synthesis capacity between the Treatment Resistant patients and controls could reflect limited statistical power should also be considered. However, data from previous studies in schizophrenia suggest that the effect size for the elevation in dopamine synthesis capacity measured using the same PET protocol is relatively large (>1)[4,25] and a formal power calculation indicated that a sample size of 12 per group had 80% power to detect an effect size of >0.7, using a two group two-sided t-test and p=0.05.

3.4.2 Presynaptic dopamine synthesis capacity in TRS

The lack of an elevation in presynaptic striatal dopamine synthesis capacity in patients who were treatment resistant could provide an explanation for the ineffectiveness of antipsychotic treatment in this subgroup. This finding is in agreement with studies of pHVA in patients prior to treatment with antipsychotics: levels were higher in responders than non-responders (Duncan et al., 1993). Furthermore, Abi-Dargham and colleagues (2000) found that higher synaptic dopamine levels, as indexed by D2 receptor occupancy, were associated with a better response to antipsychotic treatment. There is evidence from animal studies that chronic treatment with dopamine blocking antipsychotics induces D2 receptor upregulation, which can reduce the efficacy of antipsychotic treatment and may lead to breakthrough DA supersensitivity (Ginovart et al., 2008; Samaha et al., 2007). Whether these elevated D2 receptors may then affect dopamine synthesis in Treatment Resistant patients is not entirely clear. This study was cross-sectional, and therefore cannot determine whether presynaptic dopamine synthesis capacity was normal in Treatment Resistant patients at the onset of their illness, or whether it was initially abnormal, but then some change occurred so that the persistent psychotic symptoms were no longer related to a striatal dopamine excess. Kolakowska et al., (1985) observed that in most of their treatment resistant patients, the response to antipsychotic treatment had been insufficient throughout the illness, which led them to conclude that the treatment response was linked to the type, rather than stage of schizophrenia.

3.4.3 Higher presynaptic dopamine synthesis capacity in responders

Significantly higher presynaptic dopamine synthesis capacity was observed in patients who showed a good response to antipsychotics, with the strongest effect observed in the associative subdivision of the striatum, consistent with recent evidence (Howes et al., 2009, Howes et al., 2011, Kegeles et al., 2010). While the elevation in the responder group is consistent with some previous PET studies in chronic patients (McGowan et al., 2004; Reith et al., 1994), there are conflicting reports from other studies in chronic patients with schizophrenia, who were not acutely psychotic, at he time of scanning that found no significant DA elevation (Shotbolt et al., 2011; Elkashef et al., 2000).

Discrepant results may be due to small samples and hence were underpowered to detect the difference or different criteria used in identifying treatment response. The study by Shotbolt and colleagues (2011) had a relatively small sample size (n=6) that could have accounted for undetected between group differences. The authors also considered the possibility that, due to this modest sample size, they may have recruited an unusual subgroup of patients, which this could have limited the interpretation and generalizability of their findings. Dao-Castellana and colleagues (1997) observed a non-significant striatal DA elevation in patients with schizophrenia and, although Elkashef et al., (2000) reported decreased DA function in the ventral striatum, they found the opposite in posterior cingulate. Therefore, even in negative studies there is an indication of increased presynaptic DA synthesis capacity in schizophrenia (reviewed in Howes et al., 1997).

One tentative explanation for the paradoxically high dopamine synthesis capacity in the face of relative symptomatic remission could be that, in the context of chronic exposure to DA D2 receptor blockade, these patients do not have enhanced transmission due to the ambient post-synpatic DA D2 blockade. However, it is not clear whether antipsychotic drugs do normalise dopamine synthesis capacity. Acute treatment increased dopamine synthesis capacity in one study (Vernaleken et al., 2006), although no overall effect was observed in another study (Ito et al., 2009). On the other hand, it has been reported that longer-term antipsychotic treatment reduces presynaptic dopamine synthesis capacity (Grunder et al., 2003), and preclinical studies show that antipsychotics induce delayed depolarization block of presynaptic dopamine neurons which is more rapid in a rat schizophrenia model that shows increased dopamine neuron activity than in wild-type rats (Grace et al., 1997; Valenti et al., 2011). However, the study by Grunder and colleagues (2003) did not include healthy volunteers; thus, whilst antipsychotics reduced dopamine synthesis capacity, it remains unknown whether antipsychotic treatment normalised dopamine synthesis or not. This study, and some (Howes et al., 2009; McGowan et al., 2004) although not all (Elkashef et al., 2000) studies that involved antipsychotic treated patients, suggest that dopamine synthesis capacity is not completely normalised by antipsychotic treatment. Finally, the effect size seen in the treatment responder group approximates that observed in previous studies of dopamine synthesis capacity in schizophrenia, including those involving medication free/naïve subjects (Howes et al., 2009). Thus, overall, these findings suggest that medication does not explain the elevation seen in the responder group.

No associations were observed between symptom scores and Ki^{cer} values. The lack of the relationship between dopamine synthesis capacity and symptoms within treatment resistant group is not surprising. This further indicates that DA is not primarily or directly involved, but other neurotransmitters may be responsible for psychopathology in this subgroup of patients. Analogously, increased DA synthesis capacity did not correlate with PANSS scores in a Responder group. One explanation could be that these patients were stable and remitted for a considerable period of time, as reflected by the relatively low PANSS scores, suggesting that correlations may have been underpowered or affected by the low variance of symptom severity. This finding is in line with those reported by McGowen et al., (2004) who similarly observed that elevated Ki values did not correlate with symptom ratings in their study of stable patients with schizophrenia.

Chapter 4 Characterization of treatment resistance in a longitudinal first episode psychosis study: course and predictors

4.1 Background

There is an on-going debate as to whether TRS is an enduring subtype of schizophrenic illness, one that tends to manifest right at the outset, or whether it evolves over time as a result of neurodegeneration or neurochemical sensitization. In favour of the first notion is the evidence from a number of studies that have linked various underlying neurodevelopmental factors to treatment resistance (outlined in Chapter 1). Moreover as suggested by Sheitman and Lieberman (1993) these features tend to be most prominent in patients that are resistant at the onset, and in addition, they seem to have a cumulative effect in generating treatment resistance (Sheitman and Lieberman 1993). On the other hand, many authors have postulated that treatment resistance evolves, in the context of a long duration of untreated psychosis or multiple episodes of illness, and have therefore attributed chronicity to a change in responsiveness (Loebel et al., 1992; Wyatt, 1995). Whilst it is true that the majority of patients with schizophrenia show relatively good response in the initial phase of illness (Meltzer, 1997), first episode studies have documented that up to 20% of patients have persistent symptoms despite treatment, during the first episode of illness (Loebel et al., 1992). An earlier study conducted in chronic schizophrenics found that the majority of poor responders were unresponsive throughout their illness (Kolakowska et al., 1985). However, the studies to date are limited by small numbers, short periods of follow up, and imprecise definition of treatment resistance. The principal aim of the present

study was to determine whether treatment resistance manifests at the onset of psychosis by examining its course in a large first episode psychosis representative sample of patients who underwent a 10-year follow up. With respect to neurodevelopmental predictors of TRS, the literature to date has mostly focused on factors related to poor outcome or poor response to antipsychotic treatment, which, although related, are not synonymous with treatment resistance. Therefore, in this study, the associations of available putative neurodevelopmental features identified to be associated with either poor outcome, poor response, and in some studies with TRS (Chapter 1; Section1), were examined specifically in patients who were resistant at the initiation of antipsychotic treatment. Finally, the additive effect of putative neurodevelopmental features in predicting treatment resistance was examined. Thus, I set out to test the hypothesis that treatment resistance is a neurodevelopmentally pre-determined form of schizophrenic illness.

4.2 **Methods**

4.2.1 Design

AESOP study

The AESOP study is a 10 year, longitudinal follow-up, population based study of all incident cases of psychosis from defined catchment areas (Dazzan et al., 2008; Fearon et al., 2006). At a baseline, all patients aged 16–64 years who presented with a first episode of psychosis identified in the southeast London and Nottingham centres over a 2-year period were approached. Exclusion criteria were: (a) the presence of a disease of the central nervous system; (b) moderate or severe learning disabilities as defined by ICD-10; and (c) transient psychotic symptoms resulting from acute intoxication as defined by ICD-10.

At approximately 10 years after inclusion, it was attempted to trace, re-contact and re-assess all participants recruited at a baseline and, where possible, they were invited to participate in the follow up study. Ethical approval for both the baseline and follow up studies were obtained from the local research ethics committees. At baseline recruitment, patients provided detailed contact information for themselves, their GPs and relatives, and gave consent to be re-contacted for follow-up.

4.2.2 Baseline clinical assessment

Psychopathology was assessed as soon as possible after first contact was made with psychiatric services using the SCAN (SCAN; WHO, 1992). Ratings on the SCAN were based on clinical interview, case-note review and information from informants, when this was available (Fearon et al., 2006). Diagnoses were established according to ICD-10 Diagnostic Criteria for Research (WHO, 1992) during a series of consensus meetings with senior clinicians, including at least one principle investigator, blind to ethnicity, and based on all available information for each case, such as clinical vignettes prepared by the clinical assessor, and the SCAN interview. There was 80% agreement between raters on diagnostic category (kappa values ranged from 0.63 to 0.75, $p<0.001$). Further, using factor analysis, baseline symptoms were categorized in five psychopathological dimensions: manic, reality distortion, negative, depressive and disorganization symptom dimensions (Demjaha et al., 2009). DUP was defined as the period in weeks from the onset of psychosis to first contact with statutory mental health services. The end point for DUP was contact with secondary mental health services (for full details, see Morgan et al., 2006). Age of onset was established as the age at which first psychotic symptoms appeared. In line with previous studies (Harrison et al., 1996), mode of onset was rated, using the WHO Personal and Psychiatric History Schedule, according to two categories: acute (psychotic symptoms appeared incrementally within 1 month) and insidious (psychotic symptoms appeared incrementally over a period of more than 1 month).

Neurological soft signs were assessed as soon as possible after initial presentation using an expanded version of the Neurological Evaluation Scale (Buchanan and Heinrichs, 1989; Dazzan et al., 2004).

4.2.3 Follow-up clinical assessments

Using an extended version of the WHO Life Chart Schedule (LCS), (WHO 1992, 1992) extensive information was collected from medical case records, where possible, from follow-up interview, and treating clinicians. LCS includes, in addition to items that rate psychopathology and medication use, items that rate substance use and details of contact with mental health services. The WHO Life Chart, designed to collate information from multiple sources, including medical case records, has been successfully implemented in previous long-term follow-up studies. Presence of symptoms at follow-up was assessed using the SCAN (Version 2). Data from both the WHO Life Chart and SCAN were used to determine a lifetime diagnosis in consensus meetings with senior clinical investigators and other members of the research team. Data on antipsychotic medication prescribing and adherence to medication, throughout the follow-up period, were collated from the ward and community prescriptions, correspondence from the prescribing clinician, and from the patients' clinical records. Using all available information, case histories were reconstructed over the follow-up period to complete all sections of the Life Chart. This involved close examination of medication charts, medical records and clinical documentation that included reports of drug level testing where available and correspondence to general practitioners. Using a Medication History Timeline, the start and end date of all prescribed antipsychotic medication, dosage,

adherence to treatment; and the reason for change or termination was recorded. Data collection was conducted by, or under close supervision, of senior clinicians.

Figure 4.1 Flow chart documenting re-contacting and tracing original AESOP sample


```
┌─────────────────────────────────────────────────────────────┐
│                    10-years post baseline                     │
│                         target n 557                          │
└─────────────────────────────────────────────────────────────┘

        ┌──────────────────┐              ┌──────────────────┐
        │    ONS search    │              │     Excluded     │
        │                  │              │       n 8        │
        └──────────────────┘              └──────────────────┘

    ┌──────────┐   ┌──────────┐
    │   Dead   │   │  Abroad  │
    │   n 39   │   │   n 30   │
    └──────────┘   └──────────┘

        ┌─────────────────────────────────────────┐
        │         In contact with services         │
        └─────────────────────────────────────────┘

            ┌──────────┐
            │   Yes    │
            │  n 247   │
            └──────────┘

        ┌─────────────────────────────┐
        │     Contact via services     │
        └─────────────────────────────┘

    ┌──────────┐   ┌──────────┐        ┌──────────┐
    │   Yes    │   │    No    │        │    No    │
    │  n144    │   │  n 103   │        │  n 233   │
    └──────────┘   └──────────┘        └──────────┘

            ┌─────────────────────────────────────┐
            │      Write to last known address     │
            └─────────────────────────────────────┘

                ┌──────────┐        ┌──────────┐
                │ Contact  │        │No contact│
                │  n 169   │        │  n 167   │
                └──────────┘        └──────────┘

                        ┌─────────────────────────┐
                        │       Visit address      │
                        └─────────────────────────┘

                    ┌──────────┐        ┌──────────┐
                    │ Contact  │        │No contact│
                    │   N 98   │        │   n 69   │
                    └──────────┘        └──────────┘
```
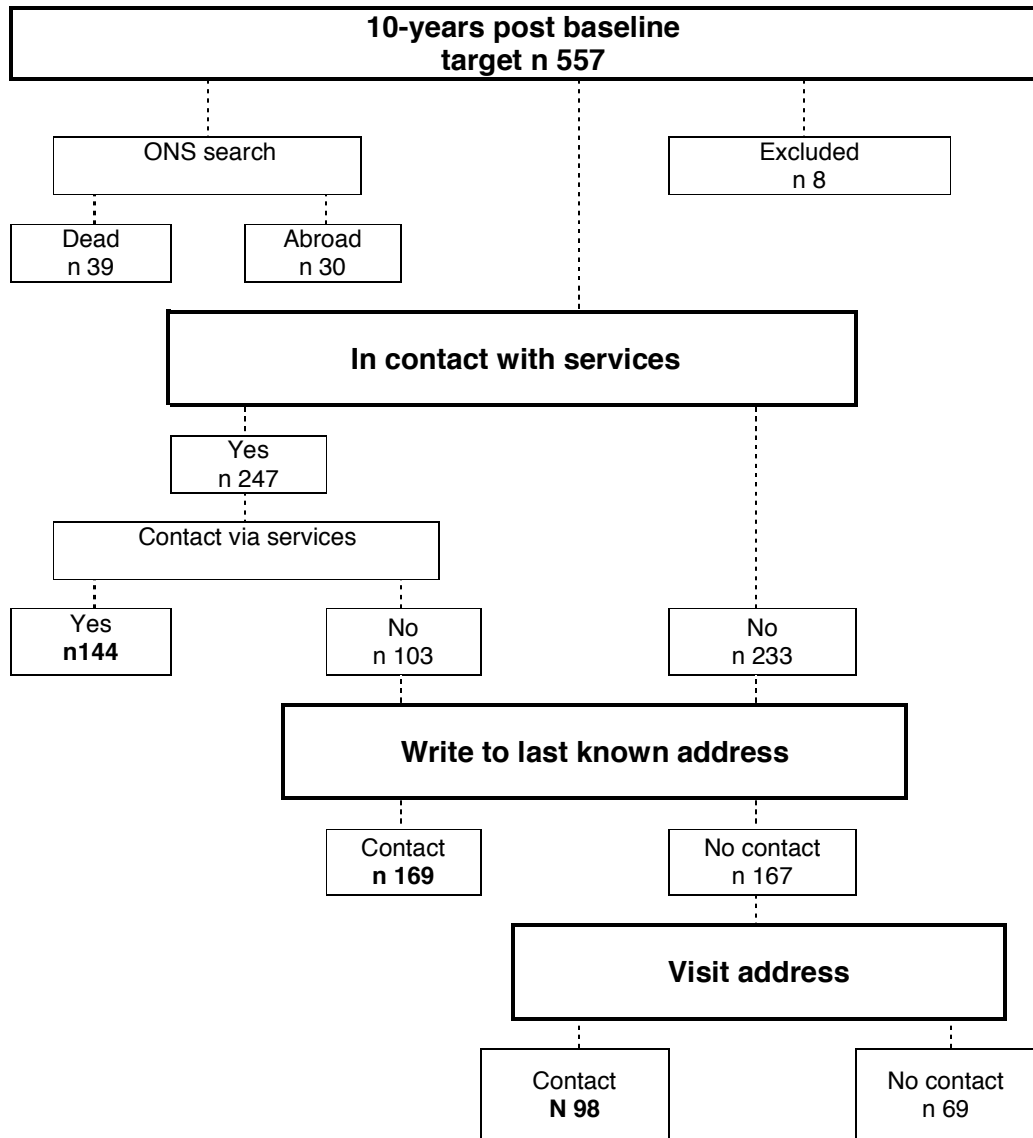
4.2.4 Defining treatment resistance and remission

Patients who were receiving clozapine, the only antipsychotic licensed for TRS in the UK (Taylor et al., 2007) during the follow up period were defined as treatment resistant. To receive clozapine the patients should meet NICE criteria, according to which they should have been treated with at least two sequential different antipsychotics (at least one from a second generation class), for an adequate duration (minimum 4 weeks), and at adequate dosage, with no adequate response to either antipsychotic as demonstrated by the presence of psychotic symptoms of at least moderate severity. The same criteria were applied to identify patients who had not yet received clozapine, but remained treatment resistant.

Treatment Response was defined as a state following a psychotic episode, of a duration of at least 6 months, in which none of the symptoms or disturbed behaviour required to define a psychotic episode were present as listed in a life chart schedule: hallucinations or pseudo-hallucinations in any modality, delusions, marked thought disorder, marked psychomotor disorder, bizarre or inappropriate behaviour, severe excitement or aggression, loss of interest, social withdrawal, states of overwhelming fear or severe anxiety, and gross and persistent self-neglect.

4.2.4.1 Defining treatment resistance at onset

Patients were classified as treatment resistant from the onset, if from the time of first ever initiation of antipsychotic treatment, they continued to exhibit psychotic symptoms of at least moderate severity despite receiving medication of adequate dosage (at least 400mg chlorpromazine equivalent) for at least 4

weeks and, which did not remit when treated sequentially with a different antipsychotic in adequate dosage for a minimum duration of 4 weeks.

4.2.4.2 Defining symptom severity

SCAN rating scale was used to assess the severity of psychotic symptoms. The ratings were based on duration, persistence, degree of interference with social functioning, effect on other people and frequent contact with services. Moderate severity (score 2) corresponds to symptoms being definitely present, on multiple occasions or for part of the time at a level sufficient to use in classification. Severe psychotic symptoms (score 3) was assigned to symptoms that have been continuously present throughout episode and in severe form, significantly interfering with functioning.

4.2.5 Statistical analysis

Analyses were performed using the SPSS (version 15.0; SPSS Inc., USA) computer software. To characterize the sample, a descriptive analysis was conducted, with the results expressed as frequencies and percentages or means ± standard deviations (SDs) as appropriate. Potential differences between the subjects with complete and incomplete clinical information in terms of demographic and clinical variables, were examined using Fisher's Exact or independent samples t-tests as appropriate

4.2.5.1 Logistic regression

Logistic regression model was performed to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs), and thus assess the effect of available neurodevelopmental factors (see Chapter1; Section 1.6.2) on treatment resistance. First, the relationship with individual variables: age of onset, gender, NSS and the negative symptom dimension was examined. The model consisted of the independent variables that showed statistically significant associations with treatment resistance: age of onset, gender, and the negative symptom dimension. Scores on treatment outcome (treatment resistance and treatment response) were entered as the dependent (outcome) variable. Second, to test the additive effect of neurodevelopmental factors on treatment resistance, a composite neurodevelopmental score was derived, by summing the scores on negative dimension (score 1 assigned to the presence of negative symptoms), age of onset (score 1 assigned if age of onset was below median) and NSS (score 1 assigned to scores above the median NSS score). This composite score was then entered as an independent variable and treatment response score as the dependent one. Although information was collected for MPA's, Family History and Premorbid IQ, this was only available for a very small number of the analytic sample and hence it was not possible to include these in the regression analyses.

4.3 Results

4.3.1 Characteristics of analytic sample

Of the 557 cases that were initially recruited, the follow up assessment was completed for 411 cases (see Figure 4.1). There were no significant differences in terms of gender, ethnicity, study centre, DUP or diagnosis between cases that the assessment was completed for, and those who were lost to follow up. However, the subjects who did not complete the study were significantly older than those who did (t=2.5; p=0.02). The information including medication, adherence to treatment and symptom ratings was complete and sufficient for 270 (48%) patients. These subjects did not differ from subjects with incomplete information (n=287) in terms of age, gender, ethnicity, study centre, DUP or diagnosis. Of these, 73 (27%) were identified as Treatment Resistant and 190 (70%) met criteria for treatment response. The remaining 7 (3%) couldn't be classified into either category, as they had never received an adequate trial of antipsychotic medication. Clinical and demographic characteristics of the Treatment Resistant Patients and Responders are presented in Table 4.1. About two thirds of treatment resistant patients were male, over 90% had diagnosis of schizophrenia, 40% were white British, a third had no qualifications, and the median DUP was 24 (interquartile range 4–101) weeks.

Responders had higher, by approximately 5 years, age of onset than treatment resistant patients, about half were males, 65% were diagnosed with schizophrenia, 43% were white British and the median DUP was 6 (interquartile range 2–24) weeks.

Table 4.1 Clinical and demographic characteristics of the analytic sample

	Treatment Resistant (N=73)	Responders (N=190)	Treatment Resistant from onset (N=61)*
Age of Onset in years Mean [SD]	25.2 [7.7]	30.5 [10.6]	24.7 [6.9]
Gender			
Male	53 [72.6]	99 [52.1]	44 [72.1]
Female	20 [27.4]	91 [47.9]	17 [27.9]
Ethnicity			
White British	30 [41.1]	86 [45.3]	25 [41.0]
Black Caribbean	21 [28.8]	51 [26.8]	18 [29.5]
Black African	13 [17.8]	24 [12.6]	11 [18.0]
White Other	5 [6.8]	13 [6.8]	4 [6.6]
Asian	3 [4.1]	11 [5.8]	3 [4.9]
Other	1 [1.4]	5 [2.6]	
Mode of Onset			
Acute	25 [34.2]	82 [43.4]	17 [27.9]
Insidious	35 [47.9]	81 [42.9]	32 [52.5]
Unknown	13 [17.9]	27 [13.7]	12 [21.6]
Diagnosis			
Schizophrenia	68 [93.2]	123 [64.7]	56 [91.8]
Manic	2 [2.7]	35 [18.5]	2 [3.3]
Depressive	2 [2.7]	32 [16.8]	2 [3.3]
Education			
Any qualification	44 [60.3]	133 [70.1]	35 [57.5]
No qualification	24 [32.9]	50 [26.3]	21 [34.4]
Unknown	5 [14.8]	07 [03.6]	5 [18.1]
DUP Median [Interquartile range] weeks	23.8 [4.4-101.1]	5.7 [2.0-24.5]	26.3 [4.4-88.4]
Length of follow-up period in years Mean [SD]	9.3 [2.21]	9.9 [2.05]	9.6 [2.32]

*The 61 patients that had been Treatment Resistant from the onset are a subgroup of the Treatment Resistant group, n=73. Of these, (n=73), 50 patients were receiving clozapine treatment

4.3.2 The course of treatment resistance

Rigorous examination of the relevant clinical information, using the life chart schedule that included information on presence, severity of symptoms, and medication data during the follow up period, revealed that a majority of patients, 61 (83%) of the treatment resistant sample, representing 15% of the follow up sample had not responded to antipsychotic treatment right from first presentation and initiation of treatment.

Twelve (17%) patients, who had initially responded to treatment, fulfilling criteria for remission outlined in Section 4.2.4, had become resistant to illness during the course of their illness. On qualitative exploration, patients who developed resistance to antipsychotics later in their illness were predominantly males (n=9), had a later age of onset by approximately 3 years than those resistant from beginning, 5 were white British, all had diagnosis of schizophrenia, and the majority (9) were commenced on clozapine. They developed treatment resistance 5 years, on average, after initial presentation and treatment, and had about 4 admissions prior to becoming resistant (between 1 and 18 each). Only one patient in this group had a history of substance misuse.

For 3 patients who were receiving clozapine there were not sufficient data on psychopathology, and therefore it wasn't possible to determine whether their resistance had been present from their first psychotic episode. However, the fact that one of these patients had received clozapine within the first year of presentation and that the other two were on high doses of antipsychotics right

from the initiation of psychopharmacological treatment increases the likelihood that these patients too may have been resistant to treatment from the onset.

4.3.3 Predictors of persistent treatment resistance

As presented in Table 4.2, treatment resistance was associated significantly with younger age of onset (OR=0.95, 95% CI 0.89-0.97), male gender (OR= 2.37, 95% CI 1.27-4.46), and higher scores on negative symptom dimension at baseline (OR=1.24, 95% CI 1.08-1.43). There were no significant associations with NSS. Following adjustment for potential confounders (DUP, diagnosis and for variables entered in the model), the associations with younger age of onset and the negative dimension remained statistically significant (OR=0.95, 95% CI 0.90-0.99) and (OR=1.17, 95% CI 1.01 -1.37) respectively. The association with gender was no longer significant (OR=1.047, 95% CI 0.49 -2.21).

4.3.4 Additive effect of neurodevelopmental factors on treatment resistance at onset.

There was an overall effect of the combined neurodevelopmental score on subsequent treatment resistance (OR=2.11; CI 1.29-3.47) as shown in Table 4.3. With respect to the additive effect of neurodevelopmental factors, the risk of developing treatment resistance increased in a linear fashion with each additional neurodevelopmental factor: patients with 1 factor vs. patients with 2 factors (OR=0.08, CI=0.01 – 0.73); patients with 2 factors vs. patients with 3 factors (OR=0.33, CI=0.09 – 1.25) patients with 1 factor vs. patients with 3 factors (OR=1.03, CI=0.34 – 3.09) (Figure 4.2).

4.3.5 The effect of gender on age of onset

Females had significantly higher age of onset by approximately 4 years compared to males (t =2.91; p = 0.004). Subsequent analyses stratified by response, revealed no significant differences in the age of onset between male and female treatment resistant patients (mean (sd) age; males = 24.3 (6.6); females= 25.9 (6.1); t =0.89; p = 0.38), whereas the age of onset was significantly greater in female responders compared to male responders (mean (sd) age; males = 28.4 (8.7); females = 32.8 (11.2); t =2.78; p = 0.006). Results are presented in Table 4.4. Taking into consideration the potential influence of diagnostic type on the relationship of age at onset to response to treatment and gender (Meltzer et al., 1997), the analysis was repeated for patients with diagnosis of schizophrenia only, and similar results were obtained: no significant differences in the age of onset between male and female treatment resistant patients (mean (sd) age; males = 24.3 (5.2); females= 24.9 (6.6); t =0.34; p = 0.73), with female responders showing significantly greater age of onset relative to male responders (mean (sd) age; males = 28.1 (9.1); females= 31.9 (10.3) (t =2.16; p = 0.03).

Table 4.2 The effect of neurodevelopmental factors on treatment resistance

Independent variable	Treatment Resistant N=61	Responders N=190	Odds Ratio (95% CI)	
			Unadjusted	Adjusted
Age of onset Mean [SD]	24.8 [6.4]	30.5 [12.2]	0.93 [0.89-0.97]**	0.94 [0.90-0.99]*
Gender N [%] Male Female	44 [72.1] 17 [27.9]	99 [52.1] 91 [47.9]	2.38 [1.27-4.46]**	0.96 [0.46-2.03]
Negative Symptom Dimension Mean [SD]	2.46 [2.4]	1.38 [1.9]	1.24 [1.08-1.43]**	1.17 [1.01 -1.37]*

*P<0.05; ** P<0.001.
Odds ratios are adjusted for variables in the table and in addition for DUP and diagnosis

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Table 4.3 The effect of combined neurodevelopmental score on treatment resistance at onset

Treatment resistance at onset

	Wald	df	p	Odds Ratio (95% CI)
Combined Neurodevelopmental Score*	8.994	1	0.003	2.1 [1.30 -3.446]

*Combined Neurodevelopmental Score was derived, by summing the scores on the negative dimension (score 1 asigned to presence of negative symptoms), age of onset (score 1 assigned if age of onset was below median) and NSS (score 1 assigned to scores above the median NSS score)

Figure 4.2 Additive effect of neurodevelopmental factors on treatment resistance
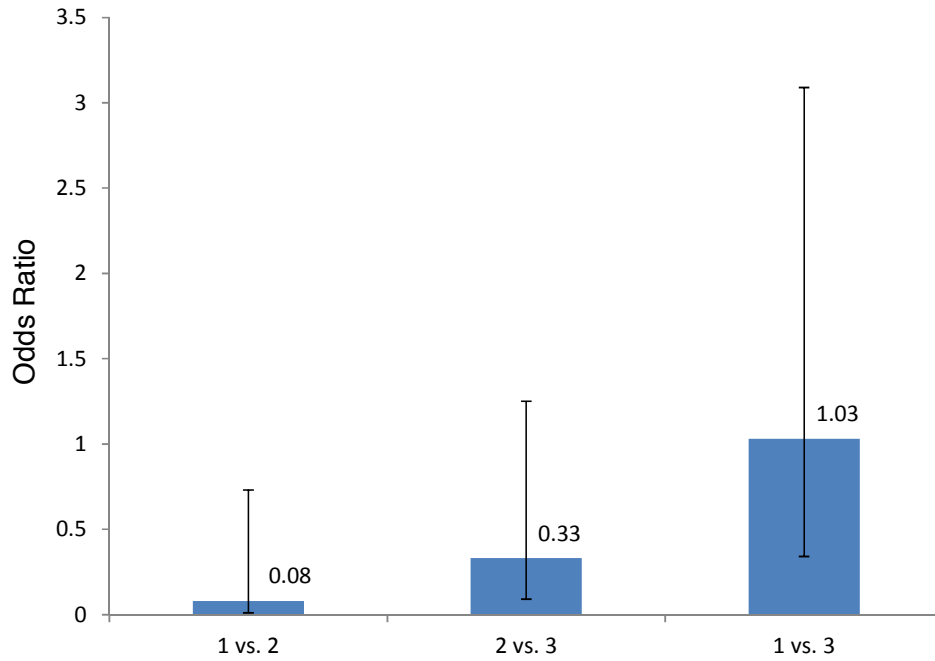
The risk of developing treatment resistance increased in an additive fashion with increasing number of factors present: patients with 3 factors present (earlier age of onset, higher NSS and negative symptoms) vs. patients with 1 factor were significantly more likely to develop treatment resistance. Error bars present 95% confidence intervals

Table 4.4 Comparison of age at onset in relation to gender in treatment resistant patients and responders

| Gender | TREATMENT RESISTANT | | | | | | RESPONDERS | | | | |
| | N | Mean | SD | t | p | N | Mean | SD | t | p |
				Age at onset							
Male	41	24.3	6.6	0.89	0.38	97	28.4	8.7	2.78	**0.006**
Female	17	25.9	6.1			88	32.8	12.1		

4.4 **Discussion**

The principal finding of this study is that treatment resistance to antipsychotics manifests at the outset of illness in the great majority of patients. Furthermore, to the best of my knowledge, this is the first study to investigate the predictive ability of putative neurodevelopmental markers specifically in patients who are persistently refractory to treatment right from the first presentation. Thus, the data demonstrated that the negative symptom dimension at a baseline and younger age of onset predispose to treatment resistance. Finally, the results suggest that the neurodevelopmental factors may have additive effect in increasing the risk of treatment resistance.

4.4.1 Treatment resistance from the onset

The finding that 61 patients, over 80% of treatment resistant patients, did not respond to antipsychotic medication from their first presentation indicates that in the majority, treatment resistance is an enduring trait of schizophrenic illness. Two first episode trials have similarly reported treatment resistance from the onset in a number of patients; Robinson et al., (1999) observed that 10 (8.5%) of their patients were unresponsive from their first episode despite intensive treatment that they received over one year follow up. A similar percentage of first episode patients with schizophrenia who did not respond to medication and were "never well enough to be discharged," during a 2 year follow-up, was reported in a previous first episode trial (MacMillan et al., 1986). Kolakowska and colleagues (1985) in their relevant study of chronic schizophrenic patients retrospectively observed that the majority of their poor responders had shown

inadequate response throughout their illness and concluded that response to antipsychotics is determined by type, and not the stage of illness as proposed earlier (Bleuler M, 1978; Ciompi, 1980; Huber et al., 1975). And, even some of the earliest psychiatric descriptions emphasize that the resistant type of illness may be already apparent in the initial phases of schizophrenia (Kraepelin, 1919).

However, a small number of patients (n=12; 17%) showed a good initial response to antipsychotics and developed resistance during the course of their illness, suggesting that there are two different types of treatment resistance; one form manifest at onset and the other that develops during the course of the illness, as previously advocated in the treatment resistance literature (Sheitman and Lieberman, 1998), although as evident in this work, the one that is always there and to which patients seem to be predestined, is more prevalent.

4.4.2 Putative neurodevelopmental origin of treatment resistance from the onset

Consistent with previous findings, younger age of onset and the negative symptom dimension were significant predictors of treatment resistance, even after the adjustment for diagnosis, DUP and gender. However, the association with gender, after the adjustment, fell short of statistical significance. Abundant evidence has linked both negative symptoms and age of onset to treatment resistance Eaton et al., 1992; Scottish Schizophrenia Research Group.,1987, Kolakowska et al., 1985; Robinson et al., 1999; Kinon et al., 1993; Loebel et al., 1992; Lindstrom, 1996; Meltzer et al., 2001). It has been suggested that age of

onset is one of the key factors that may provide an aetiological clue to schizophrenia (DeLisi, 1992). Studies in the late eighties documented that patients with a younger age of onset were more likely to have negative symptoms and cognitive impairments, (Johnstone et al.,1989) and additionally brain morphological defects (Crow et al., 1989) than those with later age of onset. These findings together with the observation that the higher the number of neurodevelopmental factors, the more likely it is that the patient will be resistant to treatment add weight to the idea that there is a neurodevelopmental aetiology to treatment resistance, in which disruptions during neurodevelopment have affected pathophysiology before the psychotic symptoms manifest. The findings here raise the possibility that it is TRS that is most compatible with the neurodevelopmental model proposed more than two decades ago (Meltzer et al.,2001; Murray et al.,1992; Murray and Lewis,1987; Murray et al.,1985; Weinberger,1987). Even Kraepelin, who advocated the neurodegenerative mechanism of illness, indicated that a in a proportion of 'dementia praecox' patients, who had low intellectual premorbid functioning, a neurodevelopmental origin was likely. It could be that these patients are most akin to contemporary treatment resistant patients.

4.4.3 Gender and age of first onset psychosis in relation to treatment response

It has long been evident that schizophrenia tends to manifest earlier in men. The majority of studies have reported that the age of onset between schizophrenic men and women differs approximately by 3 to 5 years (Angermeyer and Kuhnz, 1988; Beratis et al.,1994; Faraone et al.,1994;

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Goldstein et al.,1989; Hafner et al.,1992). Similarly, in my present work, men in general had earlier age of onset by 4 years; however, the gender-age of onset relationship differed in relation to treatment response. The age of onset in this sample is higher than that observed in other studies. This discrepancy is due to the fact that the AESOP study included all subjects who presented to the psychiatric services up to the age of 64, which is in contrast with most FEP studies that tended to use age 35 as an upper age limit. The gender difference in age of onset may suggest that the association between early age of onset and treatment resistance is biased by male gender. However, while the effect of gender on age at onset was obvious in responders, this effect disappears in TRS patients indicating that the age of onset is not gender-dependent and has direct influence on treatment resistance. These intriguing results replicate and confirm previous findings from the excellent study conducted by Meltzer and colleagues (2001) who were the first to address the issue of a gender effect on age at onset specifically in relation to treatment response, using stringent criteria for treatment resistance (Meltzer et al., 2001). The authors concluded that these two subgroups of patients are premorbidly distinct, and that women with the younger age of onset are particularly at risk of developing treatment resistance.

The later age of onset in female responders and more favorable course of illness could be explained, at least in part, by the antidopaminergic effect of natural oestrogen in women (Buckman and Peake, 1973). The protective effect of sex hormones specifically oestrogens, putatively mediated via reduction in dopaminergic transmission, was observed by Hafner and colleagues (1991),

who postulated that: "oestradiol might act as a protective modulator in schizophrenia by enhancing the vulnerability threshold for psychosis through the downward regulation of DA neurotransmission" (Hafner et al.,1991). Accordingly, with increasing age the oestrogen levels are reduced, which in turn will have less effect on reducing dopamine transmission and consequently at a later age, women may be at increased risk of developing a psychotic disorder brought on by modulation in dopamine receptor sensitivity (Angermeyer and Kuhnz, 1988; Seeman, 1981). This is of particular significance for the finding that in treatment resistance the age of onset is not gender dependent i.e. younger age of onset irrespective of gender is a critical factor on developing treatment resistance. As demonstrated in Chapter 4 (Demjaha et al., 2012), the pathophysiology of treatment resistance does not appear to involve dopamine alterations, therefore oestrogens' protective role in this condition is not relevant, or for as yet unknown reasons, dopamine-oestrogen interaction is impaired. Differential response to serotonergic challenge drugs among male and female patients with schizophrenia has been reported (Meltzer et al.,1993), and in addition, heterogeneity in central serotonergic sensitivity within the treatment resistant subgroup (Lindenmayer et al.,1997). In addition, these data demonstrate that it is the younger age of onset and not the gender that is instrumental in heralding treatment resistance, and reveal further that women of a younger age of onset are at particular risk of not responding to dopamine blockade.

4.4.4 Methodological considerations

Although this study is of a longitudinal design and patients were comprehensively assessed and examined at their first presentation to services, with a substantial proportion re-interviewed (41.8%) at follow up, the case histories had to be reconstructed over the follow-up period to complete sections of the Life Chart including data on medication, compliance and psychopathology, which could have introduced information or recall bias. However, it has been demonstrated that the WHO Life Chart can be used to obtain reliable ratings (Susser et al., 2000). In terms of symptomatology, however, as mentioned by Kolakowska et al., (1989), the tendency would be towards an overestimation of response, since patients who do not show significant behavioral disturbance, and particularly the ones with severe negative symptoms could often be rated by clinicians as "stable," even when they are still symptomatic. In all patients who were resistant to medication from the initiation of treatment rigorous examination of comprehensive medical records revealed that patients in this study have been persistently symptomatic. Another limitation is a small number of neurodevelopmental factors, specifically with regards to testing the additive value of these factors in generating treatment resistance. Nevertheless, this is one of the largest and longest follow up studies examining schizophrenic patients from their first psychotic episode that specifically addresses neurodevelopmental predictors of specifically treatment resistance that manifests at the time of initiation of antipsychotic treatment.

Chapter 5 General Discussion

5.1 Collective thesis findings

The principal finding of the thesis (Chapter 3) indicates that the pathophysiology of patients with schizophrenia, who are resistant to dopamine blocking antipsychotics, is not marked by abnormal pre-synaptic striatal dopamine synthesis capacity, raising the possibility that treatment resistance is a neurobiologically distinct subtype of schizophrenia. This is the first study in the field that, using the high resolution PET scanning, has measured in vivo presynaptic dopamine synthesis in specifically treatment resistant patients classified according to stringent criteria. Due to the study's cross-sectional design, it was not possible to determine whether normal dopamine levels predated the antipsychotic exposure. It could be that antipsychotic medication reduces the initially high presynaptic dopamine levels without achieving symptomatic remission as postulated by Grunder et al., (2003), however, as shown in this work treatment does not normalize dopamine levels in responders that were significantly increased compared to those in treatment resistant patients. Furthermore, the study in Chapter 4 revealed that over 80% of patients are persistently treatment resistant from the initiation of antipsychotic treatment. Moreover, these persistently resistant patients tended to have more severe negative symptoms at baseline and younger age of onset, and moreover, were more likely to develop resistance, if more than one of these factors were present. Finally, the finding that women whose schizophrenic illness manifests earlier are particularly at risk of becoming treatment resistant may add further support to the notion that dopamine may be normal at the

commencement of illness, and that other neurotransmitters may be instrumental or play a more prominent role in the neurobiology of treatment resistance, which precludes ooestrogen from exerting its protective effect as discussed.

Taken together, the work in this thesis suggests that TRS is a distinct and enduring subtype of schizophrenic illness, one which, is probably neurodevelopmentally mediated, and perhaps characterized by different molecular mechanisms that do not lead to increased dopamine transmission.

Integrating findings from this thesis with those derived from published literature (Chapter 1), a putative or preliminary model of predicting treatment resistance that integrates normal pre-synaptic dopamine levels and various indicators of neurodevelopmental pathology may be derived (Figure 5.1).

Figure 5.1 Putative model integrating factors that may predict treatment resistance in schizophrenia
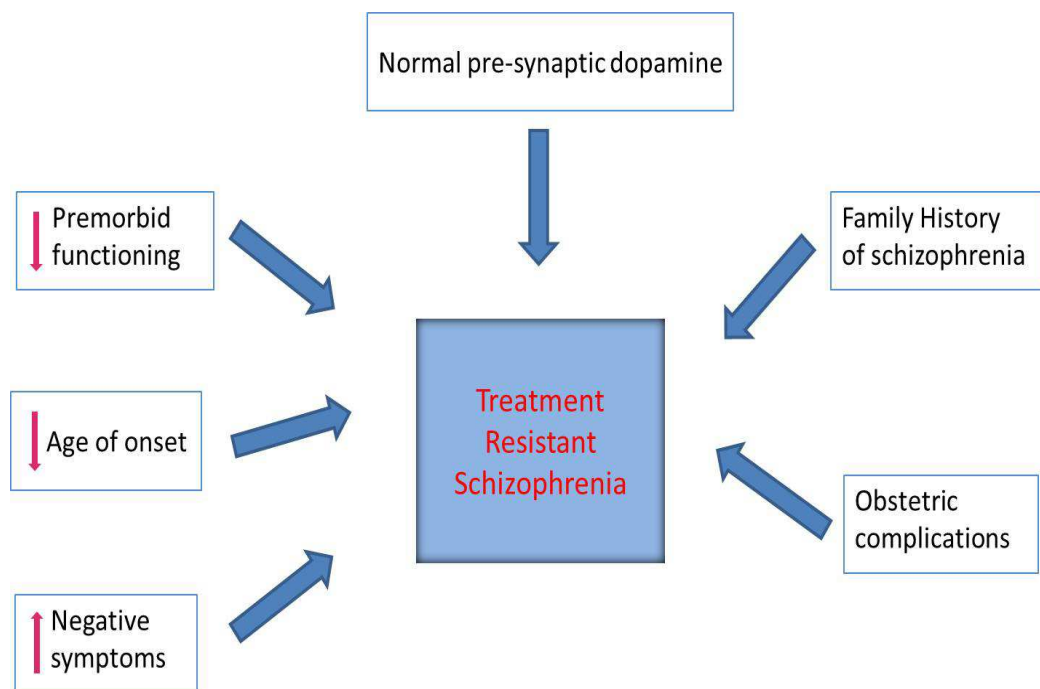
5.2 Scientific and clinical contribution of the thesis findings in the field of treatment resistance

One of the main impediments to finding novel and effective treatments for incapacitating psychotic symptoms that in a considerable proportion of patients persist despite treatment is the present lack of understanding of the neurobiology of TRS, notwithstanding over 50 years of scientific work in the field. Not only has the neurobiology of treatment resistance remained

mysterious, but it has not been easy to predict who will initially not respond to treatment. Work in this thesis illuminates understanding of the neurobiology of treatment resistance, which may subsequently inform new drug development. Furthermore, in combination with previous findings, it may alert clinicians to patients, early in their illness, who are more likely to be at risk of not responding to dopaminergic blockade, and encourage them to commence clozapine as early as possible, and thus prevent established and enduring treatment resistance in such a disabling condition as schizophrenia.

5.2.1 Implications for the DA hypothesis

Despite criticism and intense research efforts over the decades to deconstruct the complex pathophysiology of schizophrenia, DA hypothesis continues to remarkably thrive in a rapidly changing scientific milieu. This is not entirely surprising, taking into account the fact that: a) the DA blocking antipsychotics are effective in a proportion of patients with schizophrenia, and b) psychogenic agents increase DA levels. And indeed for the patients who are responsive to dopamine blockers, the hypothesis does hold true, or plays a role to an extent, but over the years failed to provide explanations for patients who remain treatment resistant despite guaranteed DA blockade. Direct confirmation in this thesis of the absence of hyperdopaminergia in patients with limited response to dopaminergic drugs, clarifies for the first time why since its genesis, the DA hypothesis could not account for the neurobiology of treatment resistance in schizophrenia. The findings provide support for other and distinct neurobiological mechanisms in this subgroup of patients that may involve

complex interactions of other neurotransmitters, as described in a following section.

5.2.2 New insights into neurobiology of treatment resistance and implications for novel treatment approaches

This thesis provides some of the first inklings into the pathophysiology of TRS, and may in part explain resistance to current dopamine blocking antipsychotics. Thus, the findings in Chapter 3 indicate that other molecular mechanisms, most probably involving glutamate and GABA, but possibly also other neurotransmitters, may be contributing to pathophysiology of treatment resistance. In particular, evidence that glutamate dysfunction may contribute to the pathophysiology of schizophrenia and the efficacy of clozapine in patients who have not responded to treatment with strong D2 antagonists suggests that changes in the glutamate system and its interaction with other neurotransmitters may be an important pathophysiological factor in this subgroup of patients. The importance of the glutamate system in treatment resistance has been substantiated by the effectiveness of lamotrigine, as an augmentation strategy in treating these patients, via inhibition of excessive glutamate transmission in the brain, (Tiihonen et al.,2003). Most recent data preliminary show that treatment resistant patients, in the face of relatively normal striatal dopamine synthesis capacity, have elevated anterior cingulate cortex glutamate levels (Demjaha et al.,2013) indicating that glutamate is a logical new therapeutic target. Recent meta-analytic work has demonstrated significant effects of glutamatergic agents, such as glycine/D-serine site antagonists, on negative symptoms (Tsai et al., 2010) that are generally resistant to dopamine blocking

antipsychotics. In a view of complex interplay of neurotransmitters governing schizophrenia and particularly treatment resistance, another promising therapeutic approach is stimulation of GABA receptors to overcome glutamatergic deficits, which is yet to be tested in clinical trials (Kantrowitz, J.T., and Javitt,.2010).

5.2.3 Contribution to the debate on neurodevelopmental versus neurodegenerative origin of treatment resistance

The findings in Chapter 4 add weight to the notion that treatment resistance may be a stable and neurodevelopmentally marked phenotype of schizophrenic illness. Whilst there is an on-going debate in the field as to whether schizophrenia is of a neurodevelopmental or neurodegenerative origin, from this work it appears that treatment resistance is the schizophrenic subtype most compatible with neurodevelopmental model proposed in the early eighties (Murray and Lewis,1987; Murray et al.,1985, Weinberger,1987). Even a century ago, although Kraepelin held that "dementia praecox," is of a neurodegenerative nature, he importantly recognized a subtype of illness arising from neurodevelopmental disruption affecting patients with low intellectual premorbid functioning that may be akin to contemporary treatment resistant patients. There is, however, a small proportion of patients identified in this work, who whilst they initially responded to medication, ultimately developed treatment resistance, which suggests that treatment resistance in schizophrenia is heterogeneous. Whether the development of treatment resistance in these patients is due to the effect of repeated exposure to

medication, dopamine sensitization, or the effect of neurodegeneration remains to be answered.

The finding that patients with more severe negative symptoms and younger age of onset are most likely to be resistant adds further weight to existing evidence, and the presence of more than one of the neurodevelopmental factors renders patient more likely not to respond to treatment.

5.3 **Methodological issues**

Even though this work has started to unravel the enigma of mechanisms of TRS, the results should be considered in the context of methodological limitations that in some detail were discussed in respective chapters.   The main issue is that the cross-sectional design of the study did not permit me to determine whether these patients initially have high dopamine levels similar to those observed in their counterparts who show good response to medication, and then some neurochemical change in response to chronicity and exposure to various dopamine-blocking drugs occurs that results in normal dopamine levels in a context of persistent symptoms. The fact that the majority of patients in the second thesis study have been resistant right from the onset of illness raises the possibility, albeit indirectly, that relatively normal dopamine levels predate exposure to antipsychotic treatment. All patients in the first study had a continuous course of illness with no recorded periods of remission and the clinical records in most of our cases indicated that treatment resistant patients had shown poor response to treatment from the outset of illness. However, since presynaptic dopamine levels have not been directly and prospectively

measured in the same sample, firm conclusions at this stage should not be drawn.

Another issue is related to the choice and number of neurodevelopmental factors used to examine associations with TRS in my second study. As discussed in Chapter 1, there is evidence that early age of onset, male gender and negative symptoms are good markers of poor outcome or TRS therefore, based on these findings, it was reasonable to hypothesize that these factors will be related to TRS. Due to limited and insufficient information on obstetric complications, family history, premorbid function and MPA, it has not been possible to test these important predictive factors in relation to TRS

5.4 **Future directions**

Establishing directly whether differences in the severity of dopamine dysfunction predate exposure to antipsychotics will require long-term prospective studies of therapeutic response in patients who are initially medication-naïve. Similar work investigating dopamine synthesis capacity in first episode psychosis prior to starting antipsychotic medication and how this determines treatment response is currently underway (Howes et al., 2013). Outstanding unanswered questions relate to other aspects of DA transmission such as DA release, or extra striatal DA transmission, in treatment resistance that would require more selective and specific tracers.

Future studies, in addition, need to determine the mediating role of other neurotransmitters in the pathophysiology of treatment resistance. As evident in

Carlsson's and Grace's work (Chapter 2.4 and Chapter 3), the interactions between different neurotransmitters are complex, and precise mechanisms of how and whether they affect the dopamine system needs to be clarified in new studies. This will require the development of new radiotracers to investigate non-dopaminergic systems that would hopefully illuminate further the understanding of neurobiology of TRS.

Further characterization of the important role of negative symptoms in predicting treatment resistance is also needed, which could have implications for both criteria for defining treatment resistance and treatment of these incapacitating symptoms. Most treatment resistance definitions have largely focused on persistent positive symptoms. In view of the negative symptom significance for treatment resistance, consideration should be given to their inclusion, in addition to positive psychotic symptoms, in criteria for treatment resistance. Refractoriness of these symptoms represents a critical unmet treatment need, which has been highlighted by the National Institute of Mental Health (NIMH) initiative (Kirkpatrick et al., 2006).

Overall, recognizing which patients are destined to show poor response to treatment (Figure 5.1) would prevent their unnecessary exposure to unwanted side effects of ineffective antipsychotics, and commencement of clozapine in the earliest stages of illness, which may halt the devastating effect of intractable and persistent psychopathology on patients' lives. This is of particular importance for women with a younger age of schizophrenia onset, who are especially sensitive to antipsychotic induced hyperprolactinaemia, which may

lead to long-term endocrine disturbances (Smith et al., 2002). Therefore, further research is needed to elucidate mechanisms governing treatment resistance specifically in young women and clarify the status of ooestrogens and its effect on dopamine synthesis in these patients. Finally, studies, examining all relevant neurodevelopmental factors documented in the published literature to date are required to contribute to developing a predictive model of treatment resistance.

Nevertheless, the work emerging from this thesis provides a platform for these future studies, which may hopefully lead to a discovery of much needed new treatments for this disabling and intractable condition.

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Article

Dopamine Synthesis Capacity in Patients With Treatment-Resistant Schizophrenia

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Objective: Elevated presynaptic striatal dopaminergic function is a robust feature of schizophrenia. However, the relationship between this dopamine abnormality and the response to dopamine-blocking antipsychotic treatments is unclear. The authors tested the hypothesis that in patients with schizophrenia the response to antipsychotic treatment would be related to the severity of presynaptic dopamine dysfunction, as indexed using $[^{18}F]$-DOPA uptake positron emission tomography (PET).

Method: Twelve patients with treatment-resistant schizophrenia, twelve patients with schizophrenia who had responded to antipsychotics, and twelve healthy volunteers matched for gender, age, ethnicity, weight, and cigarette smoking underwent $[^{18}F]$-DOPA PET scanning. $[^{18}F]$-DOPA influx rate constants (K_i^{cer} values) were measured in the striatum and its functional subdivisions.

Results: Patients who had responded to antipsychotic treatment showed significantly higher K_i^{cer} striatal values than did patients with treatment-resistant illness (effect size=1.11) and healthy volunteers (effect size=1.12). The elevated $[^{18}F]$-DOPA uptake was most marked in the associative (effect size=1.31) and the limbic (effect size=1.04) striatal subdivisions. There were no significant differences between patients with treatment-resistant illness and healthy volunteers in the whole striatum or any of its subdivisions.

Conclusions: In some patients with schizophrenia, antipsychotic treatment may be ineffective because they do not exhibit the elevation in dopamine synthesis capacity that is classically associated with the disorder; this may reflect a different underlying pathophysiology or a differential effect of antipsychotic treatment.

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Between 20% and 35% of patients with schizophrenia show only a limited response to antipsychotic treatment (1). This treatment resistance is a major clinical problem, impairing the lives of these patients.

The dopamine hypothesis of schizophrenia proposes that dysregulated dopaminergic function underlies the positive psychotic symptoms of the disorder (2). Supporting this hypothesis, eight radiolabeled DOPA positron emission tomography (PET) studies have reported that dopamine synthesis capacity is elevated in schizophrenia, with effect sizes ranging from 0.63 to 1.89 (3). Furthermore, elevated dopamine synthesis capacity predates the onset of psychosis (4, 5) and progressively increases as patients move from the prodromal phase to the first episode of illness (6). Other molecular imaging studies in schizophrenia indicate that both dopamine release and baseline dopamine levels are elevated (7, 8).

There is substantial evidence that the efficacy of antipsychotic drugs is related to dopamine D_2 receptor blockade (9). All currently approved antipsychotic medications block dopamine receptors (10), and their relative clinical potency closely parallels their binding and blocking affinity for the dopamine D_2 receptor subtype (11). PET studies have demonstrated a significant association between striatal D_2 occupancy and short-term clinical response to antipsychotic treatment (12) and have indicated that at least 50% occupancy of D_2 receptors is necessary to achieve clinical response (13). Wolkin et al. (14) have investigated D_2 receptor occupancy in relation to treatment resistance in schizophrenia. They found almost identical levels of striatal dopamine receptor occupancy in patients who responded to antipsychotic treatment and those with treatment-resistant illness, suggesting that while adequate D_2 blockade may be necessary for a therapeutic response, it does not guarantee it.

Dopamine levels have also been linked to treatment response. It has been shown that the higher the level of striatal synaptic dopamine, the better the subsequent response to antipsychotic treatment (7). However, to our knowledge no study has examined dopamine synthesis capacity specifically in relation to treatment resistance.

The limited data available suggest that patients who do not respond to current dopamine-blocking treatments may have a lower level of $[^{18}F]$-DOPA uptake than those who respond to dopaminergic blockers (7, 15). However, it could also be argued that patients who do not respond to antipsychotic treatment have an exceptionally hyperactive dopaminergic system that is not blocked by current

antipsychotics. We sought to address this issue by using [18F]-DOPA PET to compare dopamine synthesis capacity in patients who have shown a good response to antipsychotic treatment with those who have shown a poor response, as well as with healthy comparison subjects.

Method

The study protocol was approved by the research ethics committee of the Institute of Psychiatry, King's College London, and permission to administer radioactive substances was granted by the Administration of Radioactive Substances Advisory Committee, United Kingdom. All participants gave written informed consent to participate after receiving a full description of the study.

We recruited two groups of patients, defined according to their response to antipsychotic treatment. All met DSM-IV criteria for schizophrenia, paranoid subtype, determined by using OPCRIT (the Operational Criteria Checklist) (16). The group with treatment-resistant illness (treatment-resistant group; N=14) comprised patients who met modified Kane criteria for treatment resistance (17). All of these patients had previously received at least two sequential antipsychotic trials, each of at least 4 weeks' duration at a daily dose of 400–600 mg of chlorpromazine equivalents, but continued to have persistent psychotic symptoms, which was defined as having a rating of at least moderate severity on one or more items on the positive symptom subscale of the Positive and Negative Syndrome Scale (PANSS) (18), having a total PANSS score ≥75 (19), and having a score <59 on the Global Assessment of Functioning (corresponding to at least moderate functional impairment) (20). The group of patients who responded to antipsychotic treatment (responder group; N=12) comprised patients who met the Remission in Schizophrenia Working Group criteria for treatment remission (21). These patients scored ≤3 on all items of the PANSS (corresponding to mild severity or no symptoms) and had not experienced a symptomatic relapse in the 6 months prior to the study. All patients were recruited from the South London and Maudsley NHS Trust. A group of healthy comparison subjects (N=12) with no previous or current history of psychiatric illness (as assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders and the Structured Clinical Interview for DSM-IV Personality Disorders) and no family history of psychosis were recruited through advertisements in the press. The groups were matched for age, gender, ethnicity, weight, and smoking. Patients in the treatment-resistant and responder groups were matched, in addition, for duration of illness and for antipsychotic dosage (in chlorpromazine equivalents).

All patients were receiving antipsychotic medication other than clozapine at the time of scanning. Two patients in the treatment-resistant group had previously been treated with clozapine, but it had been discontinued because of side effects without reaching therapeutic blood levels. Adherence to medication was determined by measuring antipsychotic drug serum levels and by reviewing pharmacy and medical records. Patients were excluded if there was evidence of nonadherence at any point in the 6 months prior to the scan or if serum levels were not at adequate levels. Exclusion criteria for all groups were pregnancy (all women received a pregnancy test prior to scanning), contraindication to imaging, history of neurological or active medical illness or head injury, or substance abuse or dependence. All patients received a urine drug screen prior to scanning and were excluded if it was positive for illicit substances.

One patient was excluded from the treatment-resistant group because he did not complete the scanning, and one patient in the treatment-resistant group withdrew from the study. The data analysis was therefore restricted to 12 patients in the treatment-resistant group, 12 patients in the responder group, and 12 healthy volunteers.

PET Protocol

Participants were instructed to fast and to refrain from caffeine, tobacco, and alcohol for at least 12 hours before scanning. One hour before the start of each scan, all subjects received 150 mg p.o. of carbidopa, a peripheral aromatic acid decarboxylase inhibitor, and 400 mg p.o. of entacapone, a peripheral catechol-O-methyltransferase inhibitor, to increase specific signal detection, as these compounds reduce the formation of radioactively labeled metabolites that may cross the blood-brain barrier and thus confound the measurements.

PET imaging data were acquired on a Siemens/CTI ECAT HR+ 962 PET scanner (Erlangen, Germany) in three-dimensional mode, with an axial field of view of 15.5 cm. Participants were positioned in the scanner with the orbitomeatal line parallel to the transaxial plane of the tomograph. Head position was monitored via laser crosshairs and video camera. The initial transmission scan was followed by administration of approximately 180 MBq of the radiotracer [18F]-DOPA, a radioactive analog of L-dopa, as a bolus intravenous injection over 30 seconds. Emission data were obtained as 26 frames of increasing duration over 90 minutes (comprising a 30-second background frame, four 60-second frames, three 120-second frames, three 180-second frames, and finally fifteen 300-second frames). In addition, structural MRI was conducted to exclude intracranial abnormalities. No gross abnormalities were detected in any participant in a review by a neuroradiologist blind to the subject group.

Image Analysis

Both automated region-of-interest and voxel-based statistical image analyses with the cerebellum as reference region were performed to examine striatal [18F]-DOPA uptake, as previously described by our group (4–6). The region-of-interest analysis (performed by A.D.) included the whole striatum and its associative, limbic, and sensorimotor subregions, delineated as described by Martinez et al. (22). These functional striatal subdivisions reflect the differential striatal-cortical connectivity and the functional organization of the striatum. The limbic striatum, anatomically equivalent to the ventral striatum, receives input from limbic structures such as the hippocampus and amygdala and includes the nucleus accumbens. The associative striatum comprises the precommissural dorsal caudate, precommissural dorsal putamen, and postcommissural caudate and receives input from associative cortical regions, including the dorsolateral prefrontal cortex. The sensorimotor striatum includes the postcommissural putamen and receives projections predominantly from motor and premotor areas (22). The region-of-interest map thus comprised the whole striatum, its subdivisions, and the cerebellum, defined using a probabilistic atlas.

To correct for head movement, the nonattenuated dynamic image was denoised and realigned frame-to-frame to a single reference frame acquired 7 minutes after [18F]-DOPA injection. Next, the transformation parameters were applied to the corresponding attenuation-corrected frames, and the realigned frames were summated to create a movement-corrected dynamic image ready for analysis. The region-of-interest map was then normalized together with an [18F]-DOPA template to each individual PET summation image using SPM5 (www.fil.ion.ucl.ac.uk/spm), which allows region of interest to be placed

automatically and without observer bias on individual [^{18}F]-DOPA PET dynamic images. A Patlak graphical analysis was used to calculate striatal [^{18}F]-DOPA influx rate constants (K_i^{cer} values) (23) to index striatal dopamine synthesis capacity relative to uptake in the reference region (the cerebellum) for left and right sides combined.

Voxel-based statistical image analysis was performed to independently confirm the results derived from region-of-interest analysis and determine whether there were subregional differences. Parametric maps of the influx rate constants for [^{18}F]-DOPA were constructed from movement-corrected images by using a wavelet-based kinetic modeling approach that increases the signal-to-noise ratio without significantly affecting resolution (see Howes et al. [4]). After normalization of the parametric images, statistical analyses were performed using SPM5, restricted to the striatum using a mask, to examine differences between groups. The results presented were analyzed with correction for multiple comparisons (p<0.05, family-wise error rate) and, in a further sensitivity analysis, without correction.

Statistical Analysis

We conducted preliminary tests to explore homogeneity of variance, regression slopes, normality, and reliable measurements of covariates. The Kolmogorov-Smirnov test confirmed that the data were normally distributed. To determine whether there was an effect of group on striatal K_i^{cer} values and on demographic, striatal volume, and clinical data, analysis of variance and independent t tests were performed as appropriate. When there were significant group effects, planned independent t tests were performed using Bonferroni correction for multiple comparisons to examine differences in K_i^{cer} values between groups. To assess whether the effect was influenced by medication, an additional analysis of covariance was performed with daily medication dose (in chlorpromazine equivalents) added as a covariate. The independent variable was group, and the dependent variables were the K_i values for the striatum and its three subdivisions. A two-tailed significance threshold of 0.05 was used throughout.

Results

Demographic and clinical characteristics are presented in Table 1, and antipsychotic use is summarized in Table 2. No between-group differences were observed for age, gender, ethnicity, weight, radiation dose received, cigarette smoking, duration of illness, or medication dosage. In addition, there were no differences across the groups for the whole striatal volume or any of its subdivisions.

Figure 1 shows the mean dopamine synthesis capacity for the three groups. The analysis of variance identified a statistically significant effect of group on K_i^{cer} values for the whole striatum (F=5.4, df=2, 33, p=0.01) and for each of its associative (F=6.7, df=2, 33, p=0.004), limbic (F=4.0, df=2, 33, p=0.03), and sensorimotor subdivisions (F=3.4, df=2, 33, p=0.05). Mean K_i^{cer} values are listed in Table 3.

Because one of the patients in the responder group had particularly high K_i^{cer} values, we repeated the analysis with that subject excluded. The group effect remained significant for the whole striatum (F=4.2, df=2, 32, p=0.02) and its associative subdivision (F=6.4, df=2, 32, p=0.005), but not the other striatal subdivisions.

To assess the effect of antipsychotic medication, the analysis was repeated with the addition of medication dose (in chlorpromazine equivalents) at the time of scanning as a covariate. The effects of group on K_i^{cer} values from the whole striatum (F=7.2, df=1, 22, p=0.01) and the associative (F=10.2, df=1, 22, p=0.005) and limbic (F=6.4, df=1, 22, p=0.02) subdivisions remained significant, but there was no longer a significant difference in the sensorimotor subdivision.

Between-Group Comparisons

Treatment-resistant versus responder group. After adjustment for multiple comparisons, K_i^{cer} values were significantly greater in the responder group than in the treatment-resistant group in the whole striatum (p=0.02, corrected; effect size=1.11) and the associative (p=0.008, corrected; effect size=1.31) and limbic subdivisions (p=0.03, corrected; effect size=1.04). There was no significant difference in the sensorimotor subdivision.

Greater K_i^{cer} values in the responder group than in the treatment-resistant group were also observed in the corresponding voxel-based analysis, with a peak in the head of the caudate (p=0.039), which lies within the associative subdivision of the striatum (Figure 2). The difference was significant at p<0.05, corrected for multiple comparisons using the family-wise error rate. The treatment-resistant group > responder group contrast showed no significant differences, even at an uncorrected statistical threshold (p<0.05, uncorrected).

Responder group versus healthy volunteers. K_i^{cer} values were significantly elevated in the responder group compared with the healthy volunteer group, after multiple comparison adjustments, in the whole striatum (p=0.02, corrected; effect size=1.12) and the associative subdivision (p=0.01, corrected; effect size=1.24), but not in the limbic or sensorimotor subdivisions. Similarly, the voxel-based analysis revealed significantly greater K_i^{cer} values in the responder group compared with the healthy volunteer group, with a peak in the caudate (p=0.037, corrected at the family-wise error rate). The voxel-based contrast of the healthy volunteers with the responder group showed no significant differences, even at an uncorrected threshold (p<0.05 uncorrected).

Treatment-resistant group versus healthy volunteers. There was no significant difference in mean striatal K_i^{cer} values between the treatment-resistant group and the healthy volunteers, in the whole striatum or its subdivisions. This was confirmed with the subsequent voxel-based analysis for the contrast of treatment-resistant group > healthy volunteer group and for the contrast of the healthy volunteer group > treatment-resistant group, even at an uncorrected threshold (p<0.05, uncorrected).

Discussion

To our knowledge, this is the first study to provide direct evidence that dopamine synthesis capacity in schizophrenia is lower in patients with treatment-resistant illness

TABLE 1. Demographic and Clinical Characteristics of Patients With Treatment-Resistant Schizophrenia, Treatment Responders, and Healthy Volunteers[a]

Variable	Patients With Treatment-Resistant Illness (N=12)		Treatment Responders (N=12)		Healthy Volunteers (N=12)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	45.7	9.8	44.0	11.9	44.2	8.9
Weight (kg)	80.9	19.2	83.0	20.7	80.9	23.3
Radioactivity injected (MBq)	180.0	5.5	183.6	4.3	183.4	5.6
Specific activity (GBq/μmol)	0.025	0.017	0.033	0.015	0.035	0.019
Duration of illness (years)	16.1	8.6	16.2	10.1		
Medication dosage (mg/day chlorpromazine equivalents)	396.1	157.5	283.9	159.14		
Positive and Negative Syndrome Scale						
Total score	104.3	10.6	50.7	5.8		
Positive symptom score	26.2	3.8	11.9	2.4		
Global Assessment of Functioning score	47.5	3.9	67.5	4.5		
Whole striatal volume (mm^3)	15,590.9	1,675.2	16,424.1	1,818.9	16,773.6	1,870.1
Associative striatal volume (mm^3)	9,644.0	1,165.5	10,185.0	1,164.1	10,005.0	1,768.2
Limbic striatal volume (mm^3)	1,953.1	262.3	1,965.5	348.0	2,013.1	269.3
Sensorimotor striatal volume (mm^3)	4,260.2	928.6	4,189.6	513.5	4,394.4	459.6
	N	%	N	%	N	%
Male	5	41.6	6	50.0	5	41.6
Smoker	3	25.0	4	33.3	3	25.0
Ethnicity						
White	5	41.6	4	33.3	6	50.0
Black	6	50.0	7	58.3	4	33.3
Asian	1	8.3	1	8.3	2	16.7

[a] No significant differences between groups on any variable.

than in those who show a good response to antipsychotic medication. This suggests that treatments that involve the blockade of dopamine receptors may be effective in patients who have an elevation of dopamine synthesis capacity but less useful in patients in whom dopamine synthesis capacity is relatively normal.

Limitations

One potential limitation of our study is that the patients were chronically medicated, which could have influenced presynaptic dopamine synthesis capacity (24). The two patient groups were matched for both the current dosage of medication and the total duration of treatment. The mean daily dose was higher in the treatment-resistant group (reflecting their poor response to treatment), but the difference in dose was not statistically significant, and group differences in K_i^{cer} values remained significant after covarying for dose. Another potential limitation is that our patients received various types of antipsychotic drugs that could have differentially affected dopamine synthesis. However, the groups were relatively well matched in terms of generation and type of antipsychotics (see Table 2). Two of our patients, one in the treatment-resistant group and the other in the responder group, were taking amisulpride, which at dosages lower than 200 mg daily may increase dopamine transmission via a preferential blockade of presynaptic D_2-like autoreceptors (25). However, as these two patients received much higher dosages—800 mg and 600 mg daily, respectively—than the level for a preferential
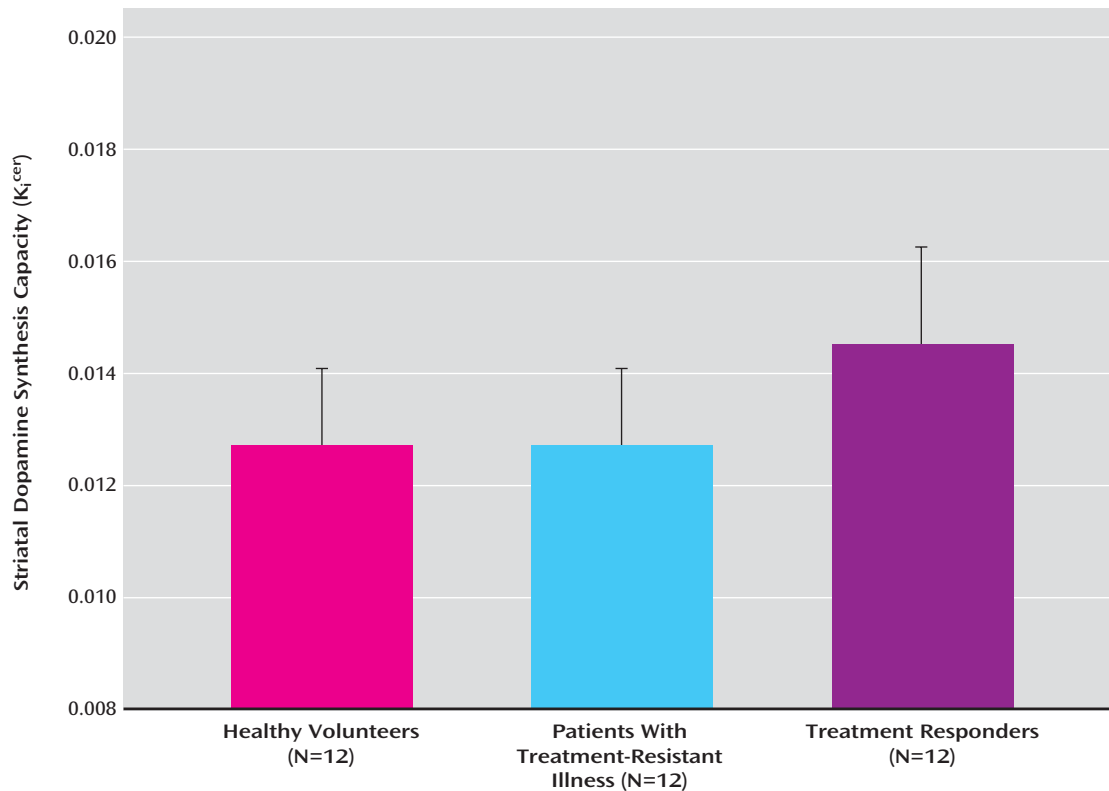
TABLE 2. Antipsychotic Use in Patients With Treatment-Resistant Schizophrenia and in Treatment Responders[a]

Antipsychotic	Patients With Treatment-Resistant Illness (N=12)		Treatment Responders (N=12)	
	N	%	N	%
Olanzapine	3	25.0	2	16.7
Quetiapine	2	16.7	1	8.3
Amisulpride	1	8.3	1	8.3
Risperidone	1	8.3	0	0.0
Risperidone depot	2	16.7	3	25.0
Aripiprazole	0	0.0	1	8.3
Chlorpromazine	1	8.3	0	0.0
Zuclopenthixol decanoate	1	8.3	3	25.0
Flupenthixol decanoate	1	8.3	1	8.3

[a] No significant differences between groups.

presynaptic action and were in different groups, it is unlikely that our results were affected. Only one of our patients (in the responder group) was taking aripiprazole, which has the unique property of being a partial dopamine receptor agonist (25). Theoretically, partial agonists should reduce dopamine synthesis capacity, reflected in a lower the K_i^{cer} value, although in practice little net effect has been observed during chronic aripiprazole administration in rats (26). As the aripiprazole-treated patient was in the responder group, a reduction in K_i^{cer} value would reduce rather than account for the group differences we observed

FIGURE 1. Mean Dopamine Synthesis Capacity for the Whole Striatum in Patients With Treatment-Resistant Schizophrenia, Treatment Responders, and Healthy Volunteers[a]

[a] The treatment-resistant group showed significantly lower dopamine synthesis capacity than the treatment responders (p=0.02, corrected for multiple comparisons). There were no significant differences between treatment-resistant patients and healthy volunteers. Error bars indicate standard deviation.

TABLE 3. Comparison of Mean Presynaptic Striatal K_i^{cer} Values Between Patients With Treatment-Resistant Schizophrenia, Treatment Responders, and Healthy Volunteers

Brain Structure	Patients With Treatment-Resistant Illness (N=12)		Treatment Responders (N=12)		Healthy Volunteers (N=12)		Analysis	
	Mean	SD	Mean	SD	Mean	SD	F (df=2,33)	p
Whole striatum	1.3×10^{-2}	0.14×10^{-2}	1.4×10^{-2}	0.18×10^{-2}	1.3×10^{-2}	0.14×10^{-2}	5.38	0.01
Associative striatum	1.2×10^{-2}	0.14×10^{-2}	1.4×10^{-2}	0.15×10^{-2}	1.2×10^{-2}	0.14×10^{-2}	6.71	0.004
Limbic striatum	1.3×10^{-2}	0.17×10^{-2}	1.5×10^{-2}	0.23×10^{-2}	1.3×10^{-2}	0.17×10^{-2}	4.02	0.03
Sensorimotor striatum	1.4×10^{-2}	0.2×10^{-2}	1.6×10^{-2}	0.24×10^{-2}	1.4×10^{-2}	0.15×10^{-2}	3.38	0.05

between the responder and treatment-resistant groups. Furthermore, as the K_i^{cer} values for this individual were similar to the mean K_i^{cer} value for the group, it is unlikely that including the aripiprazole-treated individual had a major effect on the overall results.

As the group sizes were relatively modest, the possibility that the absence of differences in dopamine synthesis capacity between the treatment-resistant group and the healthy volunteers could reflect limited statistical power should be also considered. However, data from previous studies in schizophrenia suggest that the effect size for the elevation in dopamine synthesis capacity measured using the same PET protocol is relatively large (>1) (4, 27), and a formal power calculation indicated that a sample size of

12 per group had 80% power to detect an effect size of >0.7, using a two-group two-sided t test and a significance threshold of 0.05.

Presynaptic Dopamine Synthesis Capacity in Treatment-Resistant Schizophrenia

The lack of an elevation in presynaptic striatal dopamine synthesis capacity in patients with treatment-resistant illness could provide an explanation for the ineffectiveness of antipsychotic treatment in this group. Our findings are in agreement with studies of plasma homovanillic acid in patients prior to treatment with antipsychotics: levels were higher in the responder group than in the treatment-resistant group (15). Furthermore, Abi-Dargham et al. (7)

found that higher synaptic dopamine levels, as indexed by D_2 receptor occupancy, were associated with a better response to antipsychotic treatment. There is evidence from animal studies that chronic treatment with dopamine-blocking antipsychotics induces D_2 receptor up-regulation, which can reduce the efficacy of antipsychotic treatment and may lead to breakthrough dopamine supersensitivity (28, 29). Whether these elevated D_2 receptors may then affect dopamine synthesis in patients with treatment-resistant illness is not entirely clear. Our study was cross-sectional and therefore cannot determine whether presynaptic dopamine synthesis capacity was normal in patients in the treatment-resistant group at the onset of their illness, or whether it was initially abnormal but then some change occurred so that the persistent psychotic symptoms were no longer related to a striatal dopamine excess. Kolakowska et al. (30) observed that in most of their patients with treatment-resistant illness, the response to antipsychotic treatment had been insufficient throughout the illness, which led them to conclude that the treatment response was linked to the type rather than the stage of schizophrenia. Establishing whether differences in the severity of dopamine dysfunction predate exposure to antipsychotics will require long-term prospective studies of therapeutic response in patients who are initially medication naive.

Higher Presynaptic Dopamine Synthesis Capacity in Responders

We observed significantly higher presynaptic dopamine synthesis capacity in patients who showed a good response to antipsychotics, with the strongest effect observed in the associative subdivision of the striatum, consistent with previous evidence (4, 5, 31). While the elevation in the responder group is consistent with some previous PET studies in chronic patients (27, 32), there are conflicting reports from other studies in chronic stable patients with schizophrenia that found no significant dopamine elevation (33, 34). These studies, however, did not distinguish specifically between patients with a good response to antipsychotics and those with treatment-resistant illness, which may explain the discrepancy in results. Thus, the studies that found a dopamine elevation could have included predominantly responders, and those that reported no elevation may have included more patients with treatment-resistant illness.

One tentative explanation for the paradoxically high dopamine synthesis capacity in the face of relative symptomatic remission could be that in the context of chronic exposure to D_2 receptor blockade, these patients do not have enhanced transmission, because of the ambient postsynaptic D_2 blockade. However, it is not clear whether antipsychotic drugs do normalize dopamine synthesis capacity. In one study (35) acute treatment was found to increase dopamine synthesis capacity, although in another study (36) no overall effect was observed. On the

FIGURE 2. Greater Striatal Dopamine Synthesis Capacity in Schizophrenia Treatment Responders Relative to Patients With Treatment-Resistant Illness[a]
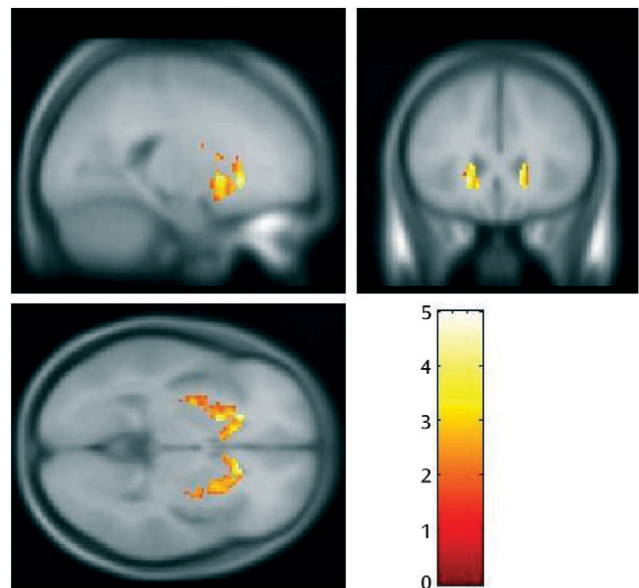
[a] The images show increased dopamine synthesis capacity in responders (N=12) relative to patients with treatment-resistant illness (N=12). The most significant increase was in the head of the caudate nucleus (p=0.039, corrected at the family-wise error rate).

other hand, Gründer et al. (37) have reported that longer-term antipsychotic treatment reduces presynaptic dopamine synthesis capacity, and preclinical studies (38, 39) have shown that antipsychotics induce delayed depolarization block of presynaptic dopamine neurons, an effect that is more rapidly induced in a rat schizophrenia model showing increased dopamine neuron activity than in wild-type rats. However, the Gründer et al. study (37) did not include healthy volunteers; thus, while antipsychotics reduced dopamine synthesis capacity, it remains unknown whether antipsychotic treatment normalized dopamine synthesis or not. Our study and some other studies (4, 27), although not all (34), involving antipsychotic-treated patients suggest that dopamine synthesis capacity is not completely normalized by antipsychotic treatment. Finally, the effect size seen in our responder group approximates that observed in previous studies of dopamine synthesis capacity in schizophrenia, including those involving medication-free or medication-naive patients (4). Thus, overall, these findings suggest that medication does not explain the elevation seen in the responder group.

Conclusions

These data indicate that schizophrenia patients whose illness is resistant to antipsychotic treatment have relatively normal levels of dopamine synthesis capacity, compared with levels in patients whose symptoms respond to treatment. This suggests either that patients with treatment-resistant illness start with a different underlying

pathophysiology or that antipsychotics have an effect on their dopamine synthesis capacity, albeit one that does not reduce symptoms. Since our study was cross-sectional and comprised a small sample of medicated patients, our results require replication, ideally in prospective studies of large samples of antipsychotic-naive patients. Future studies need to determine the involvement of other neurotransmitters. In particular, evidence that glutamate dysfunction may contribute to the pathophysiology of schizophrenia and to the efficacy of clozapine in patients who have not responded to treatment with stronger D_2 antagonists suggests that changes in the glutamate system and its interaction with other neurotransmitters may be an important factor in this subgroup (40).

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