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## Dealing with treatment resistance to clozapine : characteristics of treatment response in schizophrenia

Matthiasson, Pall

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UNIVERSITY OF LONDON  
INSTITUTE OF PSYCHIATRY

**Dealing with treatment resistance to clozapine:  
characteristics of treatment response in  
schizophrenia.**

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A thesis submitted to the University of London for the degree of  
Doctor of Philosophy

Prepared under the supervision of Professor Robert W. Kerwin and  
Dr. Michael J. Travis

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*To my patients.*

*Their fortitude and courage in the face of adversity  
are a constant inspiration to me.*

## **Abstract**

**Background:** Clozapine, the treatment of choice in treatment-resistant schizophrenia, is not effective in up to half of patients. Aims of this thesis were: to verify whether clozapine augmentation with amisulpride, an atypical antipsychotic with preferential affinity at dopaminergic D<sub>2</sub>-like receptors, is clinically effective; to test the prediction that changes in D<sub>2</sub>-like receptor availability might explain that improvement; to explore clinical and receptor availability characteristics of good clozapine responders.

### **Methods:**

**Study 1:** Thirty-three patients with schizophrenia, partially or non-responsive to clozapine, had augmentation with amisulpride using an open label design.

**Study 2:** Ten patients recruited from study 1 underwent <sup>123</sup>I-IBZM SPET scans at baseline and after 10-12 weeks on amisulpride augmentation, to assess striatal D<sub>2</sub>-like receptor binding potential. Ten matched controls had one <sup>123</sup>I-IBZM scan. Scanning was carried out using a Picker Prism 3000XP triple headed SPET camera.

**Study 3:** Ten “good” responders to clozapine monotherapy were matched to patients in study 2 and had one <sup>123</sup>I-IBZM scan.

### **Results:**

**Study 1:** Twenty-eight subjects (85%) completed 6 months’ augmentation. There was a statistically significant improvement from baseline in clinical rating scales and no change in side-effects. 71% and 32% of patients showed a 20% and 50% reduction in BPRS respectively.

**Study 2:** Patients had mean striatal D<sub>2</sub>-like receptor occupancy of 47% at baseline, which increased with amisulpride augmentation to 59%.

**Study 3:** Clozapine responders were on much lower doses of clozapine (331 mg/day) with lower s-clozapine levels (0.26 ng/L). Their D<sub>2</sub>-like occupancy was 45%.

**Conclusion:** The augmentation led to substantial improvement in both positive and negative symptoms and was well tolerated. It raised D<sub>2</sub>-like binding to likely “threshold levels” for response. Some patients require both the broad receptor occupancy profile of clozapine and a higher degree of D<sub>2</sub>-like receptor occupancy than can be provided by clozapine alone.

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## Description of thesis

The first chapter of this thesis is an introduction which reviews definitions, epidemiology and characteristics of treatment-resistance in schizophrenia. A detailed review is made of recent studies on clozapine and other treatment options in treatment-resistance. It then reviews the dopamine hypothesis of schizophrenia and neurochemical aspects of dopamine receptors. The basics of SPET neuroimaging and its role in schizophrenia treatment are described. The chapter ends with the aims of the thesis.

The second chapter discusses general methods. Ethical aspects, study samples, assessment scales and statistical methods are described and discussed. SPET neuroreceptor imaging is discussed in some detail, along with the radioligand used and its in vivo quantification. The imaging procedure is described as well as data processing and image analysis.

The third chapter describes a naturalistic study of 33 partially responsive treatment-resistant patients on clozapine monotherapy. Their treatment was augmented with another antipsychotic: amisulpride and the patients then followed up for 6 months. The augmentation showed a significant improvement in clinical state with no worsening of side-effects.

Chapter four describes a  $^{123}\text{I}$ -IBZM SPET study undertaken to clarify why amisulpride augmentation might work. Ten patients from the larger group underwent a scan at baseline and after 10 weeks on amisulpride. Dopamine  $\text{D}_2$ -like<sup>1</sup> receptor occupancy was significantly increased.

In chapter five, the characteristics of good response to clozapine monotherapy are explored. Ten “responders” were recruited and underwent a  $^{123}\text{I}$ -IBZM SPET scan. The results show the responders to be much more similar to

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<sup>1</sup>  $\text{D}_2$  is not the only receptor assessed with the ligands commonly used for  $\text{D}_2$  quantification. In particular  $\text{D}_3$  is similar to  $\text{D}_2$  in many aspects and although there are now pharmacological ligands that do differentiate between them, this is rarely done. In this thesis the distinction between  $\text{D}_2$  and  $\text{D}_3$  receptors is only made occasionally. Generally “ $\text{D}_2$ -like” receptors is used as a general term, emphasising that  $\text{D}_2$  is the focus of interest but not the only dopamine receptor measured.

treatment-resistant patients at baseline, than after augmentation, in terms of D<sub>2</sub>-like binding characteristics.

In chapter six, results are summarised and possible explanations for findings discussed. The findings are then put into context within current knowledge of the pathophysiology and treatment of schizophrenia. Finally future directions are predicted.

# **Chapter 1. General introduction**

Schizophrenia is a serious mental illness. It is common, affecting close to 1% of the world's population and one of the world's major causes of morbidity (Murray et al. 1997). Schizophrenia causes 50% excess mortality over the rate in the general population (Brown 1997). Despite antipsychotic medication many patients have a poor outcome (Hegarty et al. 1994) and only a minority are able to work (Foster et al. 1996). Stigma, side-effects, social isolation, poverty and homelessness all add to the disabilities suffered by these patients (Sartorius 2002). Poor response to the treatments available mars attempts to improve outcomes of people suffering from this chronic illness and treatment-resistance is a major concern in modern psychiatry.

## **1.1. Treatment-resistant schizophrenia**

### **1.1.1. The concept of treatment-resistant schizophrenia**

Treatment-resistant schizophrenia is an important operational term, as it allows the identification of potentially the most chronic group of patients with schizophrenia. Having a clear definition facilitates research into the area and recognition of characteristics and treatment options that work.

It is difficult to separate the concept of treatment-resistant schizophrenia from the story of clozapine. Clozapine came into clinical use in the early 1970's and there were soon indications that it might have superior efficacy to other antipsychotics (see 1.1.6.1 below). It was later withdrawn from the market due to deaths related to agranulocytosis. Further reports of its efficacy and safety profile led to attempts by the pharmaceutical company Sandoz (now Novartis) to get a new licence for it in the 1980's. For it to consider a new licence the United States Food and Drug Administration (FDA) requested a double-blind efficacy trial. The trial had to show the superiority of clozapine with regard to psychopathology reduction in patients not or poorly responding to typical



antipsychotics (Meltzer et al. 2001a). The criteria for treatment-resistance developed for the study (Kane et al. 1988) were:

1. The presence of very high levels of psychopathology, according to the BPRS and Clinical Global Impression Scale, including persistent positive psychotic symptoms.
2. No history of good social or occupational functioning in the last 5 years.
3. A failure to improve despite at least three periods of treatment with at least two different classes of typical neuroleptics at doses > 1000 mg/day chlorpromazine equivalents for 6 weeks or more and following a prospective trial of haloperidol at 10-60 mg/day.

The Kane study showed clozapine's superiority to chlorpromazine in treatment-resistant cases, with 30% of patients responding to clozapine and 4% to chlorpromazine. This finding was replicated in other patient cohorts and against other medications. Clozapine received a licence for treatment-resistant schizophrenia, both in the United States and Europe.

The criteria used in the above study were later widely criticised. The criticisms mainly focused on the arbitrary definitions which were potentially not in line with current evidence (Meltzer 1990; Brenner et al. 1995; Marder 1995; Meltzer 1997a). The focus on positive symptoms was felt to be too narrow as negative and cognitive symptoms often cause more morbidity and are possibly improved by clozapine. The requirement of 5 years without adequate response was regarded as too long as non-response is often established by 3-4 months of treatment and there is evidence that the longer a patient is inadequately treated the worse the outcome (May et al. 1976; Wyatt 1991; Loebel et al. 1992). Additionally there is some evidence that the longer the delay until clozapine is started, the worse the response to clozapine (Kerwin et al. 1993) while early use of clozapine predicts better response (Talmon et al. 1995). Finally, it was argued that it would be unnecessary to require 3 trials of different antipsychotics before starting clozapine. Two and probably one trial should be adequate, as long as it is of adequate length. It was also felt that the antipsychotics dose in the criteria was unnecessarily high, based on the view in the late 1980's that a higher dose of antipsychotic medication would be more

effective in treatment-resistance. This view is no longer held and now far lower doses are advocated, based on evidence from clinical and neuroreceptor scanning studies.

Current guidelines vary, but in the UK, advice from the National Institute of Clinical Excellence gives the following definition:

“Treatment-resistant schizophrenia is suggested by a lack of a satisfactory clinical improvement despite the sequential use of the recommended doses for 6 to 8 weeks of at least two antipsychotics at least one of which should be an atypical.” (NICE 2003)

These are the criteria that have to be met before clozapine can be prescribed within its licence in the United Kingdom and this was the definition used in this thesis for treatment-resistance in schizophrenia. Other current definitions include the one by the American Psychiatric Association (APA 1997), which is widely used (Meltzer 1997a) and includes:

- a) A failure to respond to two previous antipsychotic trials,
- b) Intolerable side effects, such as severe EPSE or tardive dyskinesia,
- c) Persistent psychotic symptoms despite treatment,
- d) Violent behaviour unresponsive to antipsychotic medications.

The most recent guidelines addressing this issue are: expert consensus guidelines from 2003 which are ambivalent about the definition of treatment-resistance and at which stage a move to clozapine is warranted (Kane et al. 2003), as well as the most recent update of the Texas Medication Algorithm Project (Miller et al. 2004).

In defining treatment-resistance it is important to give clozapine adequate time to work. An open study showed that 25 of 31 patients (81%) met response criteria within 6 months of starting treatment (Meltzer 1989a). The rate of patients who responded at 9 and 12 months was 16% and 3% respectively. Another open study showed 14 of 14 or 100% of patients meeting response criteria within 6 months (Miller et al. 1994a). Two prospective controlled studies showed 4% and 0% of patients responding after 12 weeks of treatment

(Lindenmayer et al. 1994; Breier 1994a). Another study came to the conclusion that there was no reason to wait for response beyond 3 months (Carpenter et al. 1995). A double-blind randomised controlled trial (RCT) compared response to clozapine (n=122) and to typical antipsychotics, mainly haloperidol (n=123) over a 1 year period (Rosenheck et al. 1999a). The conclusions were that the superior response to clozapine was exclusively gained during the first 6 weeks of treatment. Other studies have concluded that while some patients seem to respond very quickly to clozapine treatment (<6 weeks) (Stern et al. 1994) other patients need longer (6 weeks to 6 months) (Lieberman et al. 1994). Few patients seem to respond after 6 months. The duration of a trial before it can be regarded as ineffective may therefore be as long as 6 months (Safferman et al. 1991; Lieberman et al. 1994; Meltzer 1995).

A cut-off point on clinical rating scales is the definition sometimes used. That cut-off point is generally at a level where a patient would still have major symptoms in more than one domain (e.g. Shiloh et al. 1997). This was used to guide definitions in this thesis and a cut-off point of 25 was used on the 18 point BPRS (0-6) (Overall et al. 1961). Another way to define treatment-response is to look at a reduction in a clinical rating scale by a pre-determined percentage from baseline. This was used in this thesis as a definition of treatment response in the augmentation studies (studies 1 and 2). The cut-off point for a response used was a reduction of 20% (the same as in Shiloh's study) or 50% on the BPRS scale. This is in line with a study reporting that a 21% reduction in the PANSS was detected by clinicians and that a major gain of "much better" was associated with a 45% reduction (Cramer et al. 2001).

### **1.1.2. Epidemiology of treatment-resistant schizophrenia**

The prevalence of treatment-resistance is dependent on the criteria used. Juarez-Reyes et al. (1995) highlighted this by using the 1988 criteria in 293 patients in a defined catchment area and finding a prevalence rate of 30%. However, when less stringent criteria were used (two trials of antipsychotics, < 600 mg/ day chlorpromazine, tardive dyskinesia etc.) the rate rose to 42.9%. In another study 29% (28 out of 95) of patients with schizophrenia were found to

suffer from treatment-resistance to conventional antipsychotic treatment (McEvoy et al. 1991). A study from Iceland followed up 107 patients 20 years after their first presentation and found that only 31% had a good outcome in terms of symptoms and functioning (Helgason 1990). Finally a study by Hegarty (1994) looked at 320 reports on 51800 patients from 1895 to 1991. The proportion with a good outcome was 40.2% over the whole period, worse before 1950 (34.9%) and worryingly again worse (36.4%) in the 1990's. In conclusion a cautious estimate would put the cumulative prevalence of treatment-resistance at 30-40% of those diagnosed with schizophrenia.

### **1.1.3. Characteristics of treatment-resistant schizophrenia**

Once established, true treatment-resistance is permanent. The patients who do not respond to treatment tend to be males according to some studies, while others identify females as worse responders (Lieberman et al. 1994). The onset is earlier in poor responders (Meltzer et al. 1997b). Obstetric complications have been reported more frequently (Robinson et al. 1999) and more disturbance described in late adolescence psychosexual functioning (Findling et al. 1996).

No demographic difference was found between primary (those who have never responded to antipsychotics) and late-onset treatment-resistance in a study from 1998 (Meltzer et al. 1998a). The primary treatment-resistant group in that study had a higher number of suicide attempts, more admissions, worse treatment-compliance and shorter duration of treatment. Perhaps identifying this group earlier might lead to earlier use of clozapine and improved outcome.

A special group of treatment-resistant patients is those who become non-responsive following withdrawal of treatment, especially with clozapine (Gerlach et al. 1974; Meltzer et al. 1996) but also possibly other atypicals. A case series published in 1999 indicated that patients who had previously responded to clozapine didn't respond as well when given clozapine again to treat relapse following discontinuation (Grassi et al. 1999). A recent retrospective case notes review (n=55) found higher clozapine doses and worse global remission scores

in those who had previously stopped clozapine abruptly but been restarted (Miodownik et al. 2005). This adds further support to the idea that discontinuation of clozapine leads to more resistant illness needing higher doses of clozapine, although these studies do not rule out the possibility that non-compliance and treatment-resistance are two manifestations of more severe illness, rather than being causally related.

#### **1.1.4. Biological basis of treatment-resistance**

There is by now ample evidence that schizophrenia is associated with cerebral changes, indicative of aberrant brain development (Murray et al. 1992; Pantelis et al. 2003). Post-mortem studies of patients' brains have shown a reduction of brain volume of 4-6% (Pakkenberg 1987), larger lateral ventricles and smaller limbic temporal lobe structures (Brown et al. 1986; Falkai et al. 1986). Neuroimaging studies have shown enlarged ventricles (Johnstone et al. 1976; Chua et al. 1995; Lawrie et al. 1997), reduced cerebral volume (Nasrallah HA et al. 1990) and smaller mesial temporolimbic structures (Marsh et al. 1994). Monozygotic twins, discordant for schizophrenia can be distinguished from their co-twins based on the size of their ventricles and temporal cortical volume (Suddath et al. 1990).

These findings are seen at the onset of illness (Weinberger et al. 1982), but the level of progression over time, while initially reported as minimal (Illowsky et al. 1988; Marsh et al. 1994) has in more recent studies been found to continue (Saijo et al. 2001; Ho et al. 2003; DeLisi et al. 2004). The correlation of brain changes to treatment is not clear with some studies showing no change with treatment (Weinberger et al. 1980) while others show a change, including a recent study describing a reduction in grey matter volume over time with haloperidol which was not seen with olanzapine treatment (Lieberman et al. 2005). Additionally histopathologic studies report an absence of gliosis, indicating that neither inflammatory nor progressive degenerative processes are involved (Roberts et al. 1987; Falkai et al. 1999; Harrison 1999). Genetic studies have attracted much interest but these will be discussed further in chapter 5.1. where characteristics of good treatment response are reviewed.

Various studies have explored the biological characteristics of poor treatment response. Ventricular enlargement has in some cases been associated with treatment response in that the larger the ventricular enlargement the worse the response (Katsanis et al. 1991; DeLisi et al. 1992; Lieberman et al. 1993). Some recent studies have failed to replicate this (DeLisi et al. 2004). Regarding cerebral atrophy the issue is again not clear with conflicting results but taken together, the better designed studies do not show an association with treatment response (Crosthwaite et al. 2000).

### **1.1.5. Duration of untreated psychosis and treatment resistance**

The duration of untreated psychosis (DUP) and duration of untreated illness (DUI)<sup>2</sup> at the onset of illness have been linked to poor treatment response. A number of prospective studies have addressed this issue. A study by Craig et al. (2000) and a study by Ho (2000) found no association between DUP and course of illness or clinical functioning at either 2 years or 6 months follow-up respectively. Yet another study (Barnes et al. 2000), found no association between DUP or DUI to any clinical variables, including response to treatment. However, patients with a longer DUP were more likely to be unemployed, to be living alone or homeless and performed worse on an executive function test. It was suggested that the symptoms that predict worse response and outcome in schizophrenia, also lead to later first presentation. This is an interesting notion and a recent study shows evidence that longer DUP is associated with refusal of treatment (Friis et al. 2004).

Some older and most recent studies conclude that a longer DUP is associated with worse outcome in many treatment domains (Bottlender et al. 2000; Bottlender et al. 2003; Harrigan et al. 2003; Addington et al. 2004; Perkins et al. 2004; Oosthuizen et al. 2005). This association is also seen when patients go untreated for many years, as was shown in a small study (n=49) from Chennai

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<sup>2</sup> DUP = the time from start of a full positive syndrome to starting antipsychotic medication. DUI = the time from onset of first symptoms (including prodromal symptoms) to starting antipsychotic medication.

in India where chronic but antipsychotic-naive patients were given antipsychotics (Tirupati et al. 2004). Their response was worse the longer their DUP. The Bordeaux first episode study showed an association between DUP and a continuous course of illness at two year follow-up, although this was greatly reduced when premorbid functioning, severity of illness and negative symptoms at first admission were adjusted for (Verdoux et al. 2001).

Some studies indicate that minor neurological abnormalities are more common in those with a long DUP (Emsley et al. 2005) as well as poor prefrontal cortex (PFC) function (Joyce et al. 2002), while others have found no association between DUP and neurocognitive dysfunction (Hoff et al. 2000; Rund et al. 2004).

Overall, the evidence favours long DUP as one of the potential causes of treatment-resistance. It is still not fully clear whether this is because of a “neurotoxic” element to being psychotic and untreated, a “sociotoxic” element to being unwell and not in treatment or a confounding factor, such as a more severe illness leading to longer DUP and worse response to treatment.

### **1.1.6. Research evidence regarding treatment options in treatment-resistant schizophrenia**

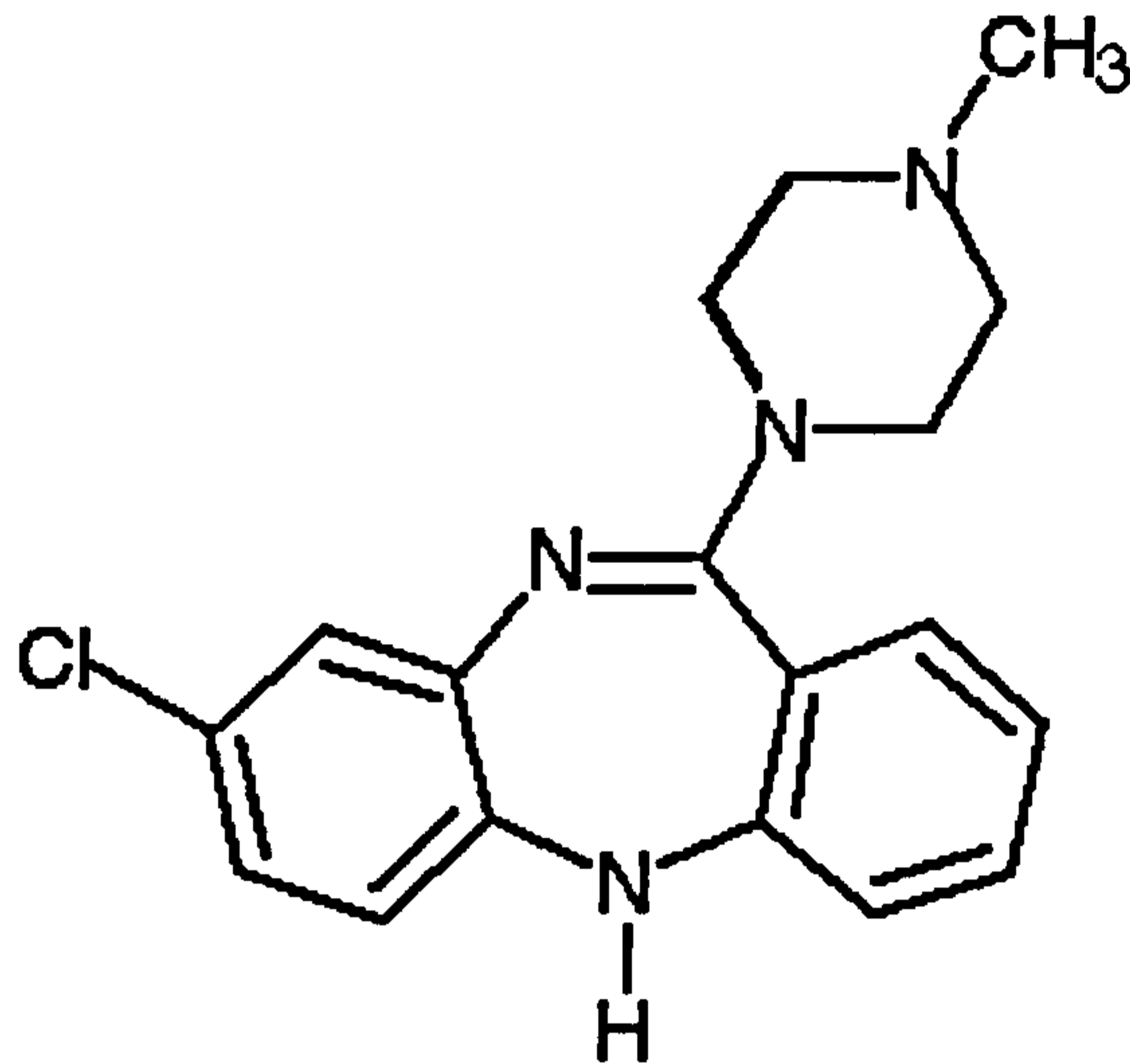
A review of the pharmacological management of treatment-resistant schizophrenia will most naturally start with a discussion of clozapine, which is generally still viewed as the “gold standard” in treatment of non-responsive schizophrenia (Remington et al. 2005).

#### **1.1.6.1. Clozapine**

Clozapine or 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e)(1,4)diazepine is a dibenzodiazepine (fig.1.1.) first synthesised in 1959 and made available in Europe for clinical use in 1971. It was classified as

“atypical”<sup>3</sup>, the first of its kind and was identified as a promising antipsychotic with little propensity to cause extrapyramidal side-effects (Stille et al. 1971; de Maio 1972). Soon its superior efficacy compared with typical antipsychotics was reported in controlled studies (Rodova et al. 1973; Ekblom et al. 1974; Gerlach et al. 1974; Fischer-Cornelissen et al. 1976).

*Fig 1.1. The chemical structure of clozapine.*



In 1975 16 cases of neutropenia ( $< 1,000/\text{mm}^3$ ) were reported from Finland within 4 months of starting clozapine treatment. 13 developed agranulocytosis ( $< 500/\text{mm}^3$ ) and eight patients died from agranulocytosis (Amsler et al. 1977)<sup>4</sup>. Following this Clozapine was taken off the market, but many of those who had responded well to clozapine experienced a relapse and didn't get better on other antipsychotics. Clozapine's use was therefore allowed on a limited basis in several countries. Over the next years low level evidence continued to gather regarding its use (Juul Povlsen et al. 1985) with nearly 600 publications to its name between 1977 and 1988. It seemed not only to be more effective but also to have no propensity for causing tardive dyskinesia (Casey 1989). The risk of

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<sup>3</sup> Pharmacologically the term “atypicality” as used today means that a drug is able to block amphetamine-induced locomotor activity, without producing catalepsy in rodents (the main animal model for extrapyramidal side-effects in humans). Clinically, “atypical” antipsychotics are associated with a much lower propensity for extrapyramidal side effects and, with some exceptions, a lack of sustained prolactin elevation (Kapur et al. 2001a).

<sup>4</sup> This sort of clustering has not been seen in Finland or anywhere else since. The patients were taking other agents as well and the deaths clustered in a small area and certain hospitals within that area. The role of clozapine in the deaths is not certain.



agranulocytosis could be managed with regular and frequent full blood count checks and the current estimate of the cumulative incidence is 0.80% after 1 year and 0.91% after 1.5 years (Alvir et al. 1994) (Honigfeld 1996). Following the study by Kane et al. (1988) its use was approved again with some restrictions (see 1.1.1. above).

#### *1.1.6.1.1. Mechanism of action*

Clozapine's receptor binding profile is unlike its predecessors in that it has a low affinity for D<sub>2</sub> receptors and a much higher affinity for 5-HT<sub>2A</sub> than D<sub>2</sub> (Farde et al. 1992; Nordstrom et al. 1993a; Meltzer 1994a; Kapur et al. 1999). Its affinity for dopamine receptors is generally described as being in the order D<sub>4</sub> > D<sub>2</sub> > D<sub>3</sub> > D<sub>1</sub> > D<sub>5</sub> (Nordstrom et al. 1995; Schotte et al. 1996). Although the D<sub>4</sub> receptor binds clozapine with a 10-fold higher affinity than the D<sub>2</sub> receptor does (Van Tol et al. 1991), D<sub>4</sub> blockade is not thought to be important in clozapine's action and it is probably a poor functional antagonist (Semba 2004). Clozapine has higher D<sub>1</sub> binding than is seen with other antipsychotic medications (Farde et al. 1989; Farde et al. 1992) and recent data indicate that the D<sub>2</sub> and D<sub>1</sub> receptors show equivalent occupancy by clozapine, although it is not clear whether its binding at D<sub>1</sub> causes agonism or antagonism (Tauscher et al. 2004). Clozapine has limbic selectivity with much higher binding in the limbic temporal lobe than the striatum (Pilowsky et al. 1997; Bigliani et al. 2000; Stephenson et al. 2000). Therefore disruption of striatal receptor activity may be less marked than with typical antipsychotics. Clozapine significantly increases noradrenenergic function by blocking the reuptake of norepinephrine (Breier 1994a). Clozapine has significant inhibiting effects on adrenergic, histamine and acetylcholine receptors (Schotte et al. 1996). Clozapine does not increase serum prolactin levels in man (Meltzer et al. 1979).

#### *1.1.6.1.2. Pharmacokinetics*

Clozapine is absorbed rapidly. Peak plasma concentration is reached within 1-6 hours. Systemic bioavailability is not affected by food and is reported to be 27%. Clozapine is 95% plasma protein bound with an average distribution volume of 1.6 to 7.3 L/kg. It is much metabolised by the liver and a key drug-metabolizing enzyme is a cytochrome P450 (CYP450) enzyme called 1A2.

Clozapine has three primary metabolites, one of which, desmethylclozapine is slightly active. Terminal elimination half-life is 9-17 hours (Jann et al. 1993; Byerly et al. 1996)(Clozaril product data , 2005).

Smoking, sex and age have noteworthy effects on serum clozapine concentration. In one study plasma concentration in males was 69% of females' levels adjusted for weight (Haring et al. 1989). The average plasma concentration for male and female smokers was 82% of non-smokers' concentration. Male smokers' clozapine levels were only 68% of non-smokers but smoking status didn't affect levels in women. Another report confirmed clozapine levels in smokers as 77% of non-smokers (Hasegawa et al. 1993). Patients already suffering from tardive dyskinesia when starting clozapine treatment, will have plasma clozapine levels 70% higher than those without the side-effect (Pollack et al. 1993). Clozapine levels in patients aged 18-26 are double the levels in those 45-54 years old. A relationship between body weight and clozapine concentration has not been consistently reported. Hence it is not possible to calculate the dose of clozapine on a mg/kg/day basis (Jann et al. 1993). Low plasma levels are seen in CYP1A2 rapid metabolisers and CYP1A2 genotyping could predict response to clozapine (Eap et al. 2004).

#### *1.1.6.1.3. Plasma levels*

In a trial by Miller et al. (1994b) it was shown that serum clozapine levels above 0.350 ng/L (=350 ng/mL) predicted better response and 5 of 7 patients with poor results became responders when plasma levels were increased above 0.350 ng/L. One study reported a response rate of 8% if levels at 4 weeks were less than 0.420 ng/L, compared with 60% rate if more than 0.420 ng/L (Potkin et al. 1994). Other studies have reported response rates when levels were above 0.350 ng/L (Perry et al. 1991; Kronig et al. 1995). Yet another study showed that even if plasma levels were below 0.420 ng/L, 29% of 4 week non-responders had responded at 12 weeks. Levels of 0.20-0.45 ng/L were superior to levels below 0.15 ng/L (n=56) (VanderZwaag et al. 1996). An open-label study (n=52) showed that 63.5% responded (by a >two-point improvement on CGI score) when levels were 0.132 ng/L (Kurz et al. 1995). Individuals show up to 52% variation in their plasma levels from one measurement to another

without a change in psychopathology (Kurz et al. 1998). One paper argues that maximum receptor occupancy is obtained at clozapine concentrations of 0.20 ng/L and no further occupancy is obtained by increasing it above 0.40 mg/L (Olesen 1998). The issue of accurate measurement of clozapine plasma concentration is important. In one study a split sample difference of 61% was found between two laboratories (Potkin et al. 1994). If clozapine levels are measured in whole blood rather than plasma, clozapine levels will be 10% lower than plasma clozapine concentrations (Flanagan et al. 2003).

#### *1.1.6.1.4. Dosage*

In the British National Formulary (2005) the maximum dose of clozapine is 900mg/day. The average clozapine dose in US trials was 450 mg/day and a possible gradual dosage reduction every 6 months was suggested (Fleischhacker et al. 1994). In central Europe an average dose of 200-300mg per day is generally used (Olesen 1998). A large meta-analysis reviewed available studies and concluded that patients with higher plasma levels had a better response and that many patients needed a higher dose than 400 mg per day (Davis et al. 2003). It also reported that when non-responders' dose was increase they generally improved. A large study (n=3782) did a multiple regression analysis and reported on the effect that dose, smoking, age, sex and metabolic activity have on serum clozapine concentrations (Rostami-Hodjegan et al. 2004). The paper produced nomograms of the clozapine dose needed to reach threshold serum clozapine levels, but conceded that large individual variability exists. As clozapine's half life is 16 hours, twice daily administration is recommended (Jann et al. 1993).

#### *1.1.6.1.5. Efficacy compared to typical antipsychotics*

The landmark study for clozapine was the Kane et al. study (1988) (see 1.1.1. above). Clozapine was found to be superior to chlorpromazine in patients resistant to treatment with typical antipsychotics. Significant improvements occurred within 1-2 weeks. The superiority of clozapine over typical antipsychotics has been confirmed in a number of other studies, both regarding efficacy and safety (in terms of reduced extrapyramidal side effects). A crossover placebo-controlled double-blind comparison with fluphenazine and

placebo showed clozapine to be significantly superior in reducing psychopathology with a response rate (defined as BPRS decrease of > 20% and BPRS < 36) of 38% for clozapine vs. 4.8% for fluphenazine (Pickar et al. 1992). A double-blind randomized controlled trial (RCT) (n=39) by Breier et al. (1994b) compared clozapine and haloperidol over 10 weeks. 42% of those treated with clozapine responded by at least a 20% decrease in BPRS compared with 5% on haloperidol. A study by Hong et al. (1997) compared clozapine and chlorpromazine (n=40) over a 3 month period. This double blind RCT used a BPRS decrease of > 20% as response criterion and found that 29% responded to clozapine but no one to chlorpromazine (mean dose of 1163 mg/day). A double-blind 29 week RCT compared clozapine and haloperidol (n=71) (Kane et al. 2001). Response was defined as >20% decrease in BPRS. 57% of clozapine treated patients responded compared with 25% of those on haloperidol. A large (n=423), 52 week double-blind RCT by Rosenheck et al. (1997) compared clozapine (mean dose 552 mg/day) to haloperidol (mean dose 28mg/day). It found clozapine to be significantly better at 6 months (26% improved (> 20% reduction in PANSS) vs. 12%) but at 1 year the difference was non-significant. The drop-out rate was much higher in haloperidol treated patients (57% of clozapine treated patients remained in the study vs. 28% of haloperidol treated patients), but that might partly be due to the very high doses of haloperidol used. Another long-term open-label study followed patients (n=227) for 2 years (Essock et al. 1996). Patients on clozapine were compared to patients on various typical antipsychotics. No significant difference in effectiveness was detected. A naturalistic one year follow-up study of inpatients started on clozapine (n=227), showed 19% to be improved at 6 weeks and 29% at 12 weeks but response was found to show "impersistence" (Zito et al. 1993).

Not all reports have been favourable. A meta-analysis by Geddes et al. looked at 12649 patients in 52 RCT's and did not find a significant difference between any typical and atypical antipsychotics, including clozapine, in terms of efficacy and side-effects (apart from extrapyramidal side-effects (EPSEs)), that could not be explained by an unfavourable comparison by using unnecessarily high doses of typical antipsychotics (> 12mg haloperidol equivalents per day) (Geddes et al. 2000). A meta-analysis by Moncrieff (2003), included 10 studies.

It discussed the problem of heterogeneity between studies, different definitions of treatment-resistance and high doses of typical antipsychotics used. The conclusion was that the benefits of clozapine compared with typical antipsychotics were “not substantial”, except in patients with a high baseline symptom score.

*Table 1.1. Randomised controlled trials (RCTs) comparing clozapine and typical antipsychotics.*

Drug comparison	Study	n	Scales	Response definition	Outcome
CLZ vs chlorpromazine	Kane 1988	268	BPRS CGI	Stat. significant improvement on both scales + on 2 of 4 predefined BPRS items	30% responded to CLZ 4% to CPZ
CLZ vs fluphenazine	Pickar 1992	21	BPRS	>20% reduction	38% responded to CLZ 4.8% to fluphenazine
CLZ vs haloperidol	Breier 1994	39	BPRS	>20% reduction	42% responded to CLZ 5% responded to halop.
CLZ vs chlorpromazine	Hong 1997	40	BPRS	>20% reduction	29% responded to CLZ 0% responded to CPZ
CLZ vs haloperidol	Kane 2001	71	BPRS	>20% reduction	57% responded to CLZ 25 % responded to halop.
CLZ vs haloperidol	Rosenheck 1997	423	PANSS	>20% reduction	26% responded to CLZ, 12% to halop. but non-sign. Difference at 1 year

Other meta-analyses have reached different conclusions. A meta-analysis by Chakos et al. (2001) looked at 12 RCT's comparing typical and atypical antipsychotics and came to the conclusion that clozapine had superior efficacy (in overall symptomatology, based on total BPRS score), as well as reduced EPSEs (reduced Simpson-Angus Rating Scale Score) and improved compliance rate. However, the magnitude of effect was variable, as fewer than half of patients in most of the studies showed a response of at least 20 - 30% reduction in total psychopathology score. The study didn't have enough evidence to evaluate other atypical antipsychotics.

A meta-analysis done by Wahlbeck et al. (1999) looked at studies comparing clozapine and typical antipsychotics. 30 trials (n=2530) were included and the conclusion was that clozapine had superior clinical efficacy, especially in treatment-resistant cases. It was however noted that the improvement was not reflected in improved functioning. Four shorter trials (6-12 weeks, n=370)

analysed in Wahlbeck's study found clozapine to be more effective but relapse rate was the same. Two longer-term studies (12-24 months) showed a less clear difference, indicating reduced clinical difference over time. However the relapse rate was reduced at 1 year compared with typical antipsychotics.

The largest meta-analysis to date included 124 RCTs including unpublished data (e.g. from electronic data bases, pharmaceutical companies and Food and Drug Agency data) (Davis et al. 2003). This study found clozapine to have an effect size 0.49 larger than typical antipsychotics which was a highly significant finding ( $p=2 \times 10^{-8}$ ) and also clinically relevant (Numbers needed to treat (NNT) =2), corresponding to approximately a 10 point reduction on the PANSS.

In conclusion in terms of overall efficacy, there is strong evidence for clozapine's superior effect on symptoms of schizophrenia, compared with typical antipsychotics.

#### *1.1.6.1.6. Effect on hospital stay*

There are indications that clozapine treatment improves discharge rates from hospital. A retrospective study of 96 patients with refractory schizophrenia showed that after a year of treatment with clozapine, 85% were discharged from hospital (Lindstrom 1989), while the number in employment (full- or part-time) was 24 of 62 (39%) of patients still receiving clozapine at 2 years (compared to 3% employment rate prior to clozapine initiation). Another study following 64 patients treated for 2 years with clozapine found that 64% were able to live independently (Revicki et al. 1990). Other reports indicate reduced rehospitalisation rates after 2-2.5 years of clozapine treatment (Reed 1994; Meltzer et al. 1994b).

#### *1.1.6.1.7. Effect on costs*

Cost savings have been reported in various studies, mainly through reduced hospitalizations in clozapine responders (Revicki et al. 1990; Meltzer et al. 1993; Meltzer et al. 1994b; Aitchison et al. 1997; Oh et al. 2001; Hayhurst et al. 2002; Magnus et al. 2005). No savings have also been reported (Laker et al.

1998). The overall conclusion seems to be that clozapine is a cost-effective treatment, particularly in high hospital users (Rosenheck et al. 1999b).

#### *1.1.6.1.8. Effect on negative symptoms*

Whether clozapine has an effect on negative symptoms is controversial, but increasingly the evidence does not support a specific effect. There are studies which support clozapine's efficacy against negative symptoms (Kane et al. 1988; Pickar et al. 1992; Volavka et al. 2002). A study by Miller (1994a) looked at 29 patients started on clozapine and saw a 31% improvement in core negative symptoms<sup>5</sup> on SANS over a six-week period. A critical review found only a benefit for secondary negative symptoms (Carpenter et al. 1995). A 29 week double blind RCT comparing clozapine (n=37) with haloperidol (n=34) found no benefit of clozapine for negative symptoms (Kane et al. 2001). The same was found in a study comparing the outcome of 75 patients randomised to clozapine or haloperidol, but it concluded that no benefit was evident of clozapine treatment for negative symptoms, neither after 10 weeks in a double blind RCT nor a 1 year open label follow-up (Buchanan et al. 1998). A double blind RCT comparing clozapine (n=205) and haloperidol (n=217) over 1 year found an improvement in positive but not negative symptoms (Rosenheck et al. 1999c). A systematic review of RCT's published did not support an effect for clozapine on negative symptoms (Tuunainen et al. 2002).

#### *1.1.6.1.9. Effect on cognitive function*

Cognitive deficits are increasingly identified as a core feature of schizophrenia and tests of cognitive function have been found to be a good predictor of long-term outcome in patients with poor cognitive function. Cognitive deficits are a leading cause of morbidity in schizophrenia (Wykes et al. 1992; Weinberger et al. 1997; Sharma et al. 1998; Keefe et al. 1999; Peuskens 1999a). Cognitive function has been correlated with negative symptoms and social functioning (Galletly et al. 1997; Breier et al. 1999; Heydebrand et al. 2004). Improved

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<sup>5</sup> "Core" or "primary" negative symptoms are symptoms such as alogia and blunting of affect which are thought to form an enduring deficit state. "Secondary" negative symptoms, such as social or emotional withdrawal are those that may be caused by adverse effects of medication, mood symptoms or as a consequence of primary symptoms. In particular sedation and the bradykinesia of Parkinsonism, caused by medication can overlap with the negative symptoms of schizophrenia with few facial expressions and gestures and monotonous speech being perceived as blunting of affect (Barnes et al. 1995).

cognitive function with clozapine use has been reported in a number of studies, particularly in terms of verbal fluency and possibly motor speed (Hagger et al. 1993; Galletly et al. 2000). In an RCT Lee et al. (1999) reported on 64 patients with recent onset schizophrenia who were randomised to clozapine (n=35) or typical antipsychotics (n=29) and followed for 12 months. The results of a detailed cognitive test battery showed improved psychomotor speed and attention with clozapine compared to typicals, independent of improvement in psychopathology. Meltzer (1999), reviewed 12 studies reporting cognitive effects of clozapine and concluded that there was strong evidence that clozapine improved attention and verbal fluency and moderate evidence that it improved elements of executive function. Other studies point generally in the same direction (Buchanan et al. 1994; Grace et al. 1996), although one study did find cognitive function unchanged or worse and hypothesised that this was secondary to anticholinergic effects (Goldberg et al. 1993). Overall, the evidence suggests a modestly positive effect of clozapine on various aspects of cognitive function.

#### *1.1.6.1.10. Effect on suicidality*

Suicide is a major cause of excess mortality in schizophrenia, and one meta-analysis found it to be responsible for 10% of deaths (Meltzer 2002) and another study for 28% of the excess mortality associated with the illness (Brown 1997). Clozapine treatment has been reported to reduce the risk of suicide in schizophrenia, with a reduction in observed mortality by as much as 75-85% (Kerwin 1995b; Meltzer 1998b; Meltzer 2001b). The reason for this apparent drop in suicide rate is not clear, but a reduction in positive symptomatology has been suggested (van Os et al. 1999), as well as improved cognitive function and insight, reduced negative symptoms and a possible direct antidepressant effect (Meltzer 2002). A recent large prospective multi-centre study comparing olanzapine and clozapine found clozapine to be more effective in reducing suicide risk (Potkin et al. 2003; Meltzer et al. 2003a). A further paper from that study reports less need for concomitant medication in patients receiving clozapine (Glick et al. 2004).



#### ***1.1.6.1.11. Effect in non-refractory schizophrenia***

A small open study started 19 non-resistant patients with mild to moderate symptom severity on clozapine and reported benefits in positive, negative and cognitive symptoms (Galletly et al. 1999). Another better designed study started 34 first episode patients with schizophrenia and schizo-affective disorder on clozapine and followed them up for 4 years (Woerner et al. 2003). It found no benefit of clozapine over typical antipsychotics and the high rate of discontinuation (only 6 patients stayed on clozapine through the study period) made an assessment of long-term benefits impossible.

#### ***1.1.6.1.12. Effect on quality of life***

Numerous studies have reported an improvement in quality of life with clozapine use (Meltzer et al. 1993; Cramer et al. 2001; Oh et al. 2001; Awad et al. 2004). Fewer negative symptoms and fewer EPSEs predict a better quality of life (Strejilevich et al. 2005). On the other hand an open study of 75 patients by Buchanan et al. (1998) (following a 10 week double-blind RCT) found no improvement in overall quality of life at one year, despite improvements in social and occupational functioning.

#### ***1.1.6.1.13. Effect in substance abuse***

Clozapine has in a number of studies been suggested as having a specific effect in patients with co-morbid alcohol or substance abuse (Volavka 1999; Green et al. 2002; Noordsy et al. 2003). Suggested mechanisms behind this effect include an action on “meso-limbic reward dysfunction” or better control of symptoms, hence reducing the need for “self-medication”. A review of 204 patients with schizophrenia found that rates of substance use disorders decreased from 57% to none in the 35 patients taking clozapine and from 50% to 13% among the 169 patients taking other antipsychotics, thus suggesting that clozapine was more effective in reducing substance use (Lee et al. 1998). Drake et al. (2000) evaluated in a 3-year follow-up naturalistic study the effects of clozapine on alcohol or other drug use disorders among 151 patients with schizophrenia or schizoaffective disorder in a dual-disorder treatment program. In the 36 patients receiving clozapine, severity of alcohol abuse and number of drinking days decreased significantly. At the end of the study, 79% of patients

on clozapine were in remission from their alcohol use disorder, as compared with 33.7% of those not receiving clozapine.

A history of substance abuse does not appear to influence response to clozapine in dually diagnosed patients. In an open-label study looking at 29 substance abusers treated with clozapine, the rate of response to clozapine in patients with a history of previous or current substance abuse was no different from that in non-substance-abusing patients (Buckley et al. 1994) . Another open-label study followed up clozapine treated patients with (n=19) or without (n=26) substance abuse and after one year the outcome was similarly beneficial in both groups (Kelly et al. 2003a).

#### *1.1.6.1.14. Side effects*

Clozapine's side effect profile is characterised by a complete lack of EPSEs (apart from occasional myoclonus) (Lindstrom 1988; Gerlach et al. 1989). However, 60-70% of patients taking clozapine experience one or more side-effects. Some of these are transient, such as those caused by cholinergic blockade and increases in liver enzymes. Others are more persisting. EEG abnormalities, epileptic seizures, fatigue, tachycardia and hypersalivation are dose-related (Treves et al. 1996). Myocarditis is a rare but often fatal condition that has been associated with clozapine, especially in the first 6 weeks of treatment (Killian et al. 1999; Hagg et al. 2001).

Serious side effects that cause discontinuation happen in 6-9 % of started treatments (Lindstrom 1989; Peacock et al. 1994). The risk of haematological toxicity is estimated at 0.8% after one year of treatment (Alvir et al. 1994).

An issue that is receiving more attention lately is the risk of metabolic disturbances. Worldwide, "the metabolic syndrome"<sup>6</sup> is increasingly being described and it seems likely that clozapine increases the risk of this syndrome, at least partly through weight gain (Alberti et al. 2005; Eckel et al. 2005).

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<sup>6</sup> The International Diabetes Federation Epidemiology Task Force Consensus Group has agreed on a definition of the Metabolic Syndrome which includes central obesity, plus any two of: raised triglycerides, reduced HDL-cholesterol, raised blood pressure, and raised fasting plasma glucose. These increase the risk of Diabetes and cardiovascular disease (Alberti et al. 2005).

Recent studies have identified the rate of the syndrome as between 37% (Heiskanen et al. 2003) and 63% (Kato et al. 2003).

*Table.1.2. Prevalence of side-effects under clozapine (from Ellenbroek et al. 2000).*

Side-effect	Clinically relevant	Reason for discontinuation
EEG-alterations	20-40	0.5
Fatigue	15-40	1.5
Leucocytosis	15-40	-
Increase of liver enzymes	10-20	1
Postural hypotension	5-20	1.5
Weight gain	8-20	2
Tachycardia	5-20	0.5
Hypersalivation	2-30	1
Fever	2-20	1
Nausea/vomiting	2-20	0.5
Constipation/ obstruction	5-15	0.5
ECG-alterations	2-13	2
Confusion/delirious states	2-5	1
Seizures	1-4	0.5
Dermatological	1-2	-

An association was already recognised between schizophrenia and diabetes mellitus (DM) in the pre-antipsychotics era and schizophrenia is an independent risk factor for DM (Kohen 2004). Antipsychotics, in particular atypical ones, increase the risk of DM and the prevalence of Type 2 DM was in a consensus statement 2 years ago estimated at 15-18% of patients with schizophrenia (Expert consensus meeting 2004) . A recent rodent study showed acutely increased whole-body insulin resistance after a single dose of clozapine and olanzapine but not risperidone and ziprasidone (Houseknecht et al. 2005). Looking at individual human studies, a retrospective cohort study by Lund et al.

(2001) compared medical and pharmacy claims to an insurer (Medicaid) in the U.S. In younger patients, aged 20-34, the relative risk of developing diabetes mellitus was 2.5 and of developing hyperlipidaemia 2.4 in clozapine (n=552) compared to typical antipsychotics (n=2461). A German register of adverse effects reported from over 86000 reports regarding antipsychotics. The rate of hyperglycemia was 0.013% for clozapine (Kropp et al. 2004b). To enter the register, an adverse effect has to be reported by the clinician and the true rate is likely to be higher, although serum glucose levels were taken regularly. A study by Sernyak et al. (2002) looked at all U.S outpatients of the veterans health administration treated with antipsychotics (n= 38632) over a 4 month period. A diagnosis of DM was looked for through the administration's ICD-9 codes. Patients on atypical antipsychotics were 9% more likely to have DM than those on typicals. Another study looked at patients on clozapine treatment in 8 veterans affairs centres who did not have diagnosed DM (n=121) (Sernyak et al. 2003). A fasting plasma glucose test showed that 23%, 17% and 6% had elevated plasma glucose, impaired fasting glucose and DM respectively. A population-based nested case-control study (Koro et al. 2002) using the UK's General Practice Research Database found the odds ratio of DM for olanzapine to be 5.8 compared with non-users of antipsychotics and 4.2 compared to typical antipsychotics. For risperidone the odds ratio was non-significantly different for non-users (2.2) and those on typical antipsychotics (1.6). A prospective study of 20 patients followed them up for 2.5 months after starting clozapine. 11 developed abnormal glucose control with mean fasting and 2 hour glucose levels raised significantly by 0.55 and 1.4 mmol/L respectively. This rise was independent of weight gain and insulin resistance (Howes et al. 2004). However, a very recent prospective study looked at chronic inpatients (n=60) and randomised them after a two week wash-out period to either risperidone, clozapine or haloperidol (Lee et al. 2005). After 12 weeks no significant differences were seen in body weight and fasting glucose levels and it was suggested that clozapine and risperidone don't cause additional weight gain in chronic patients with a long history of antipsychotic treatment.

Hyperlipidaemia is another emerging issue, particularly associated with clozapine, olanzapine and quetiapine (Koro et al. 2005), as well as low potency

conventional antipsychotics (Meyer et al. 2004). Weight gain, dietary changes and glucose intolerance are likely causes of dyslipidaemia which is an independent cardio-vascular risk factor. A recent cross-sectional study of patients who had been treated with clozapine (n=12), olanzapine (n=21) or risperidone (n=16) monotherapy for more than one year found fasting blood glucose levels to be high in 38.8%, HgA1c high in 46.9% and total cholesterol high in 22.4% (Yurtsever et al. 2005). No difference was seen between individual drugs.

#### **1.1.6.2. Alternatives to clozapine**

Clozapine is not an option for some patients who because of poor response to other antipsychotics might benefit from it. This is because of non-compliance issues, the side-effects of clozapine and the need for regular blood monitoring. It is therefore important to establish whether there are alternatives to clozapine in treatment-resistant schizophrenia.

##### *1.1.6.2.1. Clozapine compared with different antipsychotics*

A double blind RCT of treatment-resistant patients (n=157) randomised them to clozapine, olanzapine, risperidone or haloperidol (Volavka et al. 2002). It found atypical antipsychotics to be significantly but modestly more effective in both positive and negative symptoms. A further analysis of the data showed atypical antipsychotics to be effective for positive, cognitive and depression/anxiety symptoms (Lindenmayer et al. 2004). Clozapine and olanzapine improved negative symptoms, while clozapine was the only medication to improve excitement symptoms. A 3 year observational multi-centre open study, the "SOHO study" of 10000 patients compared outcomes of various antipsychotics (Haro et al. 2005). 6-month results show that patients on clozapine and on olanzapine had better outcomes on positive, negative, cognitive and depressive symptom scales than other antipsychotics.

##### *1.1.6.2.2. Clozapine versus risperidone*

A number of studies have compared clozapine and other atypicals. Looking at risperidone, an open study found clozapine to be superior to risperidone,

although risperidone performed better than typical antipsychotics (Flynn et al. 1998). A study by Bondolfi et al. (1998) (n=65) found both drugs equally effective but the dose of clozapine was low (291.2 mg/day on average) while the risperidone dose was within standard treatment range (6.4 mg/day). This might have attenuated the effect of clozapine. A 6 week long double-blind RCT comparing risperidone with clozapine or baseline fluphenazine treatment (n=29) found clozapine to be more effective than risperidone for positive symptoms, while risperidone was no better than typical antipsychotics (Breier et al. 1999). However treatment-resistance was not clearly established in all patients. A study by Klieser et al. (1995) (n=59) found clozapine equal to risperidone as measured by BPRS. However more than half the patients were neuroleptic naive, so the group was far from being treatment-resistant. A study by Azorin et al. (2001) (n=273) found clozapine to be more effective than risperidone. An RCT of 19 patients comparing risperidone and clozapine treatment for 10 weeks concluded that they were equally effective (Wahlbeck et al. 2000).

#### *1.1.6.2.3. Clozapine versus olanzapine*

An 18 week, double blind RCT (n=147) compared the response of treatment-resistant patients to clozapine and olanzapine and found no difference in response, but the the study group was only required to have failed to respond to 4-6 weeks on one typical antipsychotic and the dose of clozapine was low (216 +/- 107.9 mg/day), while the olanzapine dose was 17.2 +/- 4.8 mg/day (Bitter et al. 2004). A double-blind comparison of clozapine and olanzapine (n=40) found equal efficacy on the PANSS (Tollefson et al. 2001). The dose of clozapine was low however (303.6 mg/day) while the olanzapine dose was 20.5 mg/day on average. A double-blind RCT compared olanzapine (mean dose 16.2 mg per day) and clozapine (mean dose 209 mg per day) over 26 weeks in 114 patients (Naber et al. 2005). Outcomes were similar on clinical, quality of life and subjective wellbeing scores, but the clozapine dose was in many cases very low.

A study changed 20 clozapine-responders to olanzapine and found equivalent efficacy in 90% (Littrell et al. 2000). An open label 14 week trial of 43 treatment

resistant patients who were treated with olanzapine, found a non-significant improvement on PANSS total scores with better improvement in those treated with higher dose (25-40 mg/day) compared to standard dose olanzapine (Lindenmayer et al. 2001), which supported an earlier report to the same effect (Sheitman et al. 1997). A study compared high-dose olanzapine with clozapine and found a similar effectiveness but poor tolerability for olanzapine (Kelly et al. 2003b). A single case study using blood-flow PET found that high dose olanzapine (50 mg per day) was less effective (BPRS) and gave different pattern of brain activation than in a patient treated with clozapine (Conley et al. 2004). Another open label study followed patients (n=8) on high-dose olanzapine (mean dose 33 mg/day) for 40 weeks (Bronson et al. 2000). It concluded that at high doses olanzapine wasn't atypical, as it raised prolactin and increased EPSE. Additionally the mean weight gain was 8.0 kg. Another trial took patients (n=27) treated and not responding to normal dose olanzapine (Conley et al. 1999). They were consequently given clozapine and in an open trial 41% of those met response criteria after the clozapine trial, indicating that in treatment-resistant patients high-dose olanzapine does not match clozapine's efficacy. Another fixed-dose study by the same group compared high-dose olanzapine (50 mg/day) and clozapine (450 mg/day) in a small (n=13) double blind 18 week crossover trial of treatment-resistant patients (Conley et al. 2003). The discontinuation rate for olanzapine was 46% while no one stopped clozapine (but 2 patients had their dose reduced to 300 mg/day). The response was significantly better for clozapine with effect sizes >0.5 while they were 0.1-0.5 for olanzapine.

#### *1.1.6.2.4. Clozapine versus other atypicals. Meta-analyses*

A review and meta-analysis of 8 studies (Tuunainen et al. 2002), found clozapine to be marginally more effective than other atypical antipsychotics in terms of positive symptoms, but no better and possibly worse in terms of negative symptoms. The meta-analysis did however lack power to answer the question of difference between various antipsychotics. Another meta-analysis looked at 12 studies involving 1916 patients and again lacked power to compare clozapine and other atypicals (Chakos et al. 2001). A large prospective study, "InterSePT", found clozapine to be more effective than

olanzapine in reducing the risk of suicide (Potkin et al. 2003). A meta-analysis looking at the use of atypicals in schizophrenia included 12649 patients in 52 randomised trials (Geddes et al. 2000). It came to the conclusion that atypicals had no clear benefit over typicals when lower doses of typical antipsychotics were used, apart from an effect on EPSEs. Another meta-analysis on the other hand reported a one year relapse rate of 15% with atypical antipsychotics treatment, compared with a 23% relapse rate in patients on typical antipsychotics (Leucht et al. 2003). That study pointed out however that different adherence (on which information was generally lacking) to treatment might have affected the outcome, and also that the relatively high doses of typical antipsychotics (generally haloperidol) used might have exaggerated the benefit of atypicals, partly through differences in adherence. That meta-analysis included studies using clozapine but did not compare it directly to the other atypicals.

The largest meta-analysis to date included 124 RCTs and incorporated previously unpublished studies (Davis et al. 2003). Its conclusions were that clozapine had an effect size of 0.49 over and above typical antipsychotics, which was significantly better than for any of the other atypicals (effect sizes: 0.29 (amisulpride), 0.25 (olanzapine), 0.21 (risperidone)), with the effect sizes for the other atypicals lower and not significantly different from those for typicals.

A number of open-label naturalistic studies not mentioned here have been published, looking at atypical antipsychotics effectiveness. Generally they suffer from various methodological problems including a wide or unclear definition of treatment-resistance (Sebastian et al. 2004).

#### *1.1.6.2.5. Combining two antipsychotics*

Antipsychotic polypharmacy is common practice in treatment-resistant schizophrenia, with 52% of patients in a recent Spanish study receiving more than one antipsychotic (Garcia Mahia et al. 2005). This is despite polypharmacy only being advocated in selected cases where good evidence for the combination exists (Stahl et al. 2004). A number of case reports and small



studies have looked at combination antipsychotic treatment, particularly combining a drug with broad spectrum activity similar to clozapine (such as olanzapine or quetiapine) with a D<sub>2</sub> antagonist (Mazeh et al. 2004). An open study combined olanzapine and sulpiride treatment with a favourable clinical response (Raskin et al. 2000). Combination of olanzapine and amisulpride (up to 800 mg/day) has been reported favourably in a case report where lower dose of each medication could be used (Zink et al. 2004a). An open label retrospective case notes review (n=15) augmented treatment-resistant patients taking either clozapine, olanzapine, ziprazidone or risperidone with amisulpride (mean dose 693 mg per day) and found an improvement in CGI in every case (Lerner et al. 2005). A case report (n=1) combined olanzapine and aripiprazole and found a favourable effect (Duggal 2004). An open study of patients on olanzapine monotherapy (n=17) randomised half the group to sulpiride augmentation with no significant improvement in positive and negative symptoms but a significant improvement in depressive symptoms (Kotler et al. 2004).

#### *1.1.6.2.6. Combining an antipsychotic and ECT*

Electro-convulsive therapy (ECT) as augmentation to antipsychotic treatment is supported by open studies which suggest a moderate effect, but no RCTs have been done. One open label study reported on 293 treatment-refractory patients on flupenthixol who received ECT and showed an improvement in positive but not negative symptoms (Chanpattana et al. 2001). A closer look and 1 year follow-up with maintenance ECT of 46 of these patients who had not responded to at least two antipsychotics showed a significant improvement in BPRS (from 48.5 points at baseline to 17.1 points at 1 year) and quality of life (from 36.7 points at baseline to 67.8 points at 1 year on the Quality of Life Scale) as well as other scales (Chanpattana et al. 2003). A prospective, open study compared 15 treatment-resistant patients who had their usual treatment augmented with ECT and another 15 who didn't (Tang et al. 2003). The ECT augmentation had at 1 month a modest but significant effect on GAS and CGI but overall it was concluded that the results compared unfavourably to augmentation with another antipsychotic. It is worth mentioning that recent

NICE guidelines came out strongly against the use of ECT in the general management of schizophrenia (NICE 2003b).

#### *1.1.6.2.7. Combining an antipsychotic and a mood stabiliser*

Augmenting atypical antipsychotics (other than clozapine) with mood stabilisers is widely used despite little evidence to support it, except where mood features are prominent in the presentation (Travis 2002). An exception seems to be valproate but recent data from a double-blind multi-centre RCT (n=249) comparing olanzapine or risperidone with or without divalproex show significant improvements on PANSS total as early as by day 3 in those receiving divalproex augmentation (Casey et al. 2003). This and other smaller studies indicate that valproate may enhance global antipsychotic response in acute exacerbations of schizophrenia and in treatment-resistance (Wassef et al. 2001). 17 non-responsive patients were given lamotrigine augmentation with no improvement in symptoms when it was added to haloperidol, flupenthixol, olanzapine or risperidone – although it helped when added to clozapine (Dursun et al. 2001). A recent 24 week cross-over RCT (n=26) had topiramate titrated to 300 mg/day added to current antipsychotic treatment (clozapine, olanzapine, risperidone or quetiapine) in treatment-resistant patients (Tiihonen et al. 2005). The results showed an effect on both positive and negative symptoms on PANSS (effect size 0.7).

#### *1.1.6.2.8. Combining an antipsychotic and other agents*

A systemic review of benzodiazepines in schizophrenia summarised the results of 30 double-blind studies (Wolkowitz et al. 1991). Benzodiazepines were used alone in 14 and as addition to typical antipsychotics in 16 studies. The response was described as very variable but the effect was mainly on psychotic agitation when benzodiazepines were added to antipsychotics. Effects have been described for Ginkgo biloba with haloperidol, where significant improvements were described in positive symptoms when compared with placebo augmentation in an RCT of 54 patients (Zhou et al. 1999). Adding omega-3 triglycerides (2-4 gr eicosapentanoic acid (EPA)) daily to treatment has possible effects (see 1.1.6.3.2. below). A case report reported favourably on using transcranial magnetic stimulation for auditory hallucinations (Franck et

al. 2003). This study has now been followed up by a double blind crossover design trial where either sham or real left temporoparietal repetitive transcranial magnetic stimulation (rTMS) were given (Poulet et al. 2005). The conclusions were that treatment-resistant verbal auditory hallucinations improved by more than half after 5 days of rTMS, but not with the sham treatment.

#### *1.1.6.2.9. Summary of the evidence*

Overall the evidence for clozapine's superiority is not as conclusive against atypical as against typical antipsychotics. Well designed convincing studies exist both for and against clozapine's superior effect but many studies suffer from small numbers, being open label and from poor definition of entry criteria. What can be concluded is that using another atypical as monotherapy is a viable option when clozapine treatment is not possible. High-dose olanzapine could be considered. A combination of two antipsychotics (neither of them being clozapine) does not have much evidence for it at the moment. ECT augmentation could be considered as well as valproate augmentation.

#### **1.1.6.3. Augmentation of clozapine treatment**

Up to 50% of treatment-resistant patients with schizophrenia still fail to respond satisfactorily to clozapine (Kane et al. 1988; Kane 1992; Buckley et al. 2001). Data is limited on how to proceed in these cases and therapeutic nihilism is often a problem (Williams et al. 2002). Psychological treatments, augmentation with antidepressants, mood stabilizers or ECT have all been tried and augmentation of some sort seems common. In Denmark 40% of clozapine patients receive monotherapy, 35% a combination with another antipsychotic, 28% a combination with a benzodiazepine and 11% with an antidepressant (Juul Povlsen et al. 1985; Peacock et al. 1994). The best current evidence supports augmenting clozapine with another antipsychotic.

The common use of polypharmacy in treatment-resistant schizophrenia, with little data to support its efficacy and scant information on adverse effects has led to recent criticism. In particular Centorrino et al. (2004) recently published a retrospective case notes review where they compared 70 subject pairs where

one patient was treated with a single and the other with multiple antipsychotics. The final antipsychotic dose was 78% higher, the length of stay in hospital 55% longer and the risk of adverse effects 56% higher for those on polypharmacy compared to monopharmacy, while clinical improvement scores were similar. The study warns of the risks of polypharmacy. While it is important to keep in mind the risk of adverse effects when using polypharmacy and bear in mind the lack of research evidence within the field, Centorrino's paper makes no attempt to separate "responders" from "non-responders". This undermines the conclusions as many of the patients in the polypharmacy group must have been treatment-resistant and therefore inherently a different group from "responders" (Lerner et al. 2005). This is a reminder of the importance of a clear definition of the group under investigation, not only in terms of diagnosis, but also treatment response. It is also worth keeping in mind that polypharmacy is, at least in western Europe and the United States, generally used in established treatment-resistance and for clear target symptoms (Sernyak et al. 2004), although this may not be the case in other settings, as a recent study from Japan highlights (Ito et al. 2005).

#### **1.1.6.3.1. Switching from clozapine to another antipsychotic**

Before discussing augmentation strategies, it is important to establish whether switching from clozapine to another atypical can lead to improvement. No RCTs exist on this topic. Case reports and open studies cite different reasons for change in medication.

Looking first at reports on switching where intolerance to clozapine or patients' request was the reason for change, rather than treatment-resistance to clozapine: A case report described a man who remained well after switching from a successful clozapine treatment to olanzapine (Rafal et al. 1999). A case series (n=5) described a successful switch to olanzapine (mean dose 15.5 mg/day) in four of the patients, who were intolerant to clozapine. (Weiss et al. 1999). Another study is a case series where patients (n=6) with psychotic disorder were switched from clozapine to risperidone while another group (n=12) were switched from risperidone to clozapine (Gardner et al. 1997). Two

(33%) in the former group experienced relapse within a week of stopping clozapine. In the latter group no one experienced clinical deterioration. A study of 19 outpatients switched to olanzapine (mean dose 17 mg/day) showed 8 doing well and 11 requiring return to clozapine, with 7 of those needing hospitalisation (Henderson et al. 1998). Another open label study of clozapine responders showed 18 of 20 patients respond to a switch to olanzapine (mean dose: 21.7 mg/day) (Littrell et al. 2000).

Studies where treatment-resistance to clozapine led to switch also exist. A case series switched patients (n=5) with chronic water intoxication over to olanzapine 20-25 mg/day and followed them up for four months (Millson et al. 1999). The water intoxication did not improve and four of the patients deteriorated so much (from 91.4 to 125.8 mean PANSS scores) that olanzapine had to be stopped. Another series reported on five patients switched from clozapine to risperidone with all patients relapsing within a month (Lacey et al. 1995). An 18-week open label trial switched clozapine resistant patients (n=48) to olanzapine (mean dose 22 mg/day) and showed 18 (40%) respond by more than 20% reduction in PANSS (Dossenbach et al. 2000).

Taken together the level of evidence for switch from clozapine is sub-optimal with no RCTs existing. There are trials describing relapse when patients are switched from clozapine to other atypicals but equally other studies come to the conclusion that the switch is successful. Given how inconclusive the evidence is, the risk of relapse must be regarded as high with a switch from clozapine, causing delay if clozapine needs restarting. This risk, few conclusive studies on the topic and clozapine's established position as "gold standard" seem to be the reasons experts continue to recommend clozapine augmentation rather than switch (Remington et al. 2005).

### **1.1.6.3.2. Augmentation of clozapine – not using an antipsychotic**

#### ***1.1.6.3.2.1. Clozapine and antidepressants***

Augmentation with antidepressants has been tried in a number of studies, mainly Selective Serotonin Reuptake Inhibitors (SSRI). Often treatment-

resistance is not the reason for augmentation, but rather depressive symptoms, obsessive-compulsive symptoms etc. and those studies are less useful in guiding approaches to treatment-resistance. In a small study, plasma levels of clozapine increased by 60%, 30% and 20% by paroxetine, fluoxetine and sertraline respectively (Centorrino et al. 1994). This seems to be through SSRIs inhibiting isoenzymes of the hepatic cytochrome P450 (CYP), especially CYP1A2, as well as CYP2D6 and CYP3A4, which can raise plasma clozapine levels, at times to toxic levels (Centorrino et al. 1996). This is particularly the case with fluvoxamine; a potent inhibitor of the CYP1A2 system (Brosen et al. 1993). A case study (n=1) reported an eight-fold increase in clozapine plasma levels when fluvoxamine was added (Hiemke et al. 1994). An open study (n=16) reported an average 2-3 fold increase in plasma clozapine levels (Szegedi et al. 1999). A study by Lu et al. (2000), reported that a reduced dose of clozapine could be used with fluvoxamine. They gave patients with treatment-resistant schizophrenia (n=18) clozapine (titrated to 100 mg/day) and fluvoxamine (mean dose 50 mg/day) and followed them up for a month. An improvement was seen on CGI and GAF but side-effects were problematic although they got better towards the end of the study. Various studies have reported that adding an SSRI, at least fluvoxamine and fluoxetine, may improve the negative symptoms of schizophrenia (Silver 2004). This is an effect that is often seen within 2 weeks of starting augmentation. An 8-week double-blind parallel group RCT (n=33) showed no difference between augmentation with fluoxetine (mean dose 48.9 mg/day) and with placebo (Buchanan et al. 1996). An 8-week double-blind RCT of mirtazapine augmentation (n=24) reported improved negative symptoms (a reduction in total SANS and BPRS scores, no effect on SAPS) in an 8-week trial (Zoccali et al. 2004).

In conclusion, the effect of antidepressant augmentation seems modest at best and it may only be secondary to raised serum clozapine levels in the case of SSRIs (Buchanan et al. 1996; Williams et al. 2002).

#### *1.1.6.3.2.2. Clozapine and mood stabilizers*

Mood stabilisers have also been suggested but with the exception of lamotrigine they are generally felt to be of doubtful effect. Carbamazepine

increases the risk of bone marrow depression (Williams et al. 2002) and there may be increased risk of agranulocytosis when it is combined with clozapine (Junghan et al. 1993). Carbamazepine can also induce hepatic isoenzymes, including CYP1A2, and cause a drop in clozapine plasma levels (Jerling et al. 1994). A meta-analysis of 10 studies (n=283) of carbamazepine use in schizophrenia did not find a clinical effect, whether it was used on its own or in combination (Leucht et al. 2002a).

Valproate in addition to clozapine is commonly prescribed, mainly to protect against seizures, when high doses of clozapine are used. Positive effects have been reported in case reports and chart reviews (Kando et al. 1994; Suppes et al. 1996; Hofer et al. 2003). A meta-analysis of studies comparing valproate augmentation of antipsychotics with placebo included 5 RCTs (n=379) and did not find clinical benefits of its use in augmentation (Basan et al. 2004). Increased sedation was seen and a tendency for faster response to treatment. However four of the five studies were small and poorly reported so overall the evidence is as yet inconclusive.

A meta-analysis (n=611) of lithium augmentation of antipsychotics looked at 20 studies (Leucht et al. 2004a). 11 of those studies looked at lithium augmentation (not necessarily to clozapine) and didn't find a significant improvement when patients with mood symptoms were excluded. A double-blind crossover RCT of patients resistant to clozapine (n=20, 10 with schizophrenia, 10 with schizoaffective disorder) showed lithium augmentation to be effective in schizoaffective patients but not in those with a diagnosis of schizophrenia (Small et al. 2003). Additionally total white blood cell count (WBC) and granulocytes increased with lithium but not placebo. Adverse effects, including neurological symptoms, diabetic ketoacidosis and neuroleptic malignant syndrome have been reported with lithium augmentation (Remington et al. 2005).

A case series described 6 cases of lamotrigine augmentation which all improved (Dursun et al. 1999). Three further studies have supported a role for lamotrigine in clozapine augmentation: A small open label study augmented

various antipsychotics with lamotrigine and topiramate and found clozapine with lamotrigine to be the only useful combination (Dursun et al. 2001). Another study was a double-blind crossover RCT over 12 weeks (n=34) where patients had 200 mg lamotrigine added to their clozapine treatment (Tiihonen et al. 2003). A significant improvement was seen in positive symptoms (effect size 0.7) and general psychopathology (effect size 0.6) on the PANSS, but not in negative symptoms. The most severely ill patients responded best. A third study, a double-blind RCT (n=38) where two thirds received 400 mg/day lamotrigine as augmentation to various antipsychotics, showed lamotrigine to be a useful augmentation strategy for positive and general symptoms and to work equally well in both typical and atypical drugs (Kremer et al. 2004). As lamotrigine has been reported to cause bone marrow depression (Solvason 2000), it is prudent to exercise caution in combinations with clozapine. Additionally a case report describing tripling of plasma clozapine levels after lamotrigine was added, gives added reason for caution (Kossen et al. 2001).

Topiramate augmentation of clozapine has been reported from a few cases. In an open label study, Dursun et al. added topiramate (300 mg/day) to clozapine (n=9) and reported no improvement in clinical ratings over 2 weeks (Dursun et al. 2001). A case series reported on four patients where clozapine was augmented with topiramate (250 mg/day) (Millson et al. 2002). All patients deteriorated clinically.

#### *1.1.6.3.2.3. Clozapine and ECT*

Using electroconvulsive therapy with clozapine has been reported to be both safe and effective (Bhatia et al. 1998), although concerns have been raised that the effects may not be sustained once ECT is withdrawn (Tang et al. 2001). A case series described four treatment-resistant cases and one clozapine-intolerant case. ECT augmentation was effective in all patients, with marked improvement in three cases (Kales et al. 1999). Another study reported a more than 40% improvement in BPRS in three of four treatment-resistant patients (Benatov et al. 1996). A study by Kho et al. (2004), reported on 11 treatment-resistant patients who had received uni- and later bilateral ECT. Eight of nine who completed the study responded (>30% reduction in PANSS total and



positive subscale). Five suffered a relapse within 3-19 weeks but three responded again with another course of ECT followed by maintenance ECT. A meta-analysis of 36 cases reported a 67% response rate, defined as marked clinical improvement (Kupchik et al. 2000). The diagnosis was however unclear and at times clozapine was added to ECT treatment rather than the other way around, so it is hard to draw conclusions from that study. Reversible adverse effects were seen in 16.6% of cases.

#### *1.1.6.3.2.4. Clozapine and benzodiazepines*

Benzodiazepines are frequently used in combination with clozapine with 28% of clozapine patients on that combination in one study (Peacock et al. 1994). However, no systematic assessment of the clinical efficacy and safety of this combination has been done. One case report reported relapse in a patient on clozapine and lorazepam when lorazepam was discontinued, but an improvement when it was reinstated (Kanofsky et al. 1993). Concerns have been expressed regarding the toxicity of clozapine and benzodiazepines. These concerns have mainly concerned respiratory depression and cardio-respiratory collapse (Sassim et al. 1988; Chong et al. 2000), although this was not seen in a large study (Naber et al. 1992) where benzodiazepines were well tolerated.

#### *1.1.6.3.2.5. Clozapine and glutamatergic agents*

Interestingly, although glycine site agonists on the NMDA receptor may reduce positive and negative symptoms when added to olanzapine and risperidone (Heresco-Levy et al. 2004), this does not seem to happen with clozapine. On the contrary there is evidence that the agonists serine, glycine and the partial agonist D-cycloserine interfere with clozapine's efficacy (Goff et al. 1999; Potkin et al. 1999).

#### *1.1.6.3.2.6. Clozapine and fatty acids*

Adding omega-3 triglycerides to clozapine has been reported to have a modest effect in treatment-resistant patients, including those taking clozapine (Peet et al. 1998; Puri et al. 1998; Berger et al. 2002).

### **1.1.6.3.3. Augmentation of clozapine with another antipsychotic**

Augmentation of clozapine with another antipsychotic is common practice with between 15% (US outpatients) and 50% of patients on clozapine receiving antipsychotic augmentation (Freudenreich et al. 2002). A study from Denmark looked at information on all hospital clozapine forms. This indicated that 35% of patients receive an additional antipsychotic (Peacock et al. 1994). The figure has been even higher in previous reports or up to 60% (McCarthy et al. 1995). A recent Spanish study found the rate to be 52% with higher rates in those who had many previous admissions and longer time in hospital (Garcia Mahia et al. 2005). For an overview of the discussion that follows, please refer to table 1.3. on page 51.

#### ***1.1.6.3.3.1. Clozapine and typical antipsychotics***

An open study (n=7) used pimozide (mean dose 4 mg/day) added to an average of 425 mg/day clozapine and showed a mean BPRS reduction of 24 points from 51 points (Friedman et al. 1997). A prospective study (n=7) added loxapine (25-200 mg/day) to clozapine for 18-50 weeks and reported an improvement in BPRS total scores from 57 to 38 points, with a swift improvement in the best responders (Mowerman et al. 1996). A case report (n=1) reported favourably on using sulpiride (Stubbs et al. 2000). A case series (n=3) augmented patients resistant to clozapine (mean dose 666 mg/day) with typical antipsychotics (one patient had haloperidol 2 mg/day; one haloperidol decanoate 50 mg/month; and one fluphenazine decanoate 50 mg/month) (Rajarethinam et al. 2003). After 8 weeks all three had reduced BPRS scores. A PET study (n=5) added haloperidol (4 mg/day) to clozapine and reported an increase in D<sub>2</sub> binding from 55 to 79% and a high rise in prolactin levels, but didn't mention the clinical effect (Kapur et al. 2001b).

#### ***1.1.6.3.3.2. Clozapine and atypical antipsychotics***

A case report (n=2) reported favourably on using olanzapine as augmenting agent (Gupta et al. 1998). A case report (n=1) described a patient who did better on a combination of clozapine (100 mg/day) and olanzapine (10 mg/day), than on either agent alone (Rhoads 2000). Another report (n=3) described

delayed improvement of clozapine-induced neutropenia when olanzapine was started immediately after clozapine was stopped (Flynn et al. 1997). This may be a combined effect and caution may be needed when augmenting with olanzapine, especially as other reports exist of olanzapine-induced neutropenia (Tolosa-Vilella et al. 2002; Stergiou et al. 2005). A report (n=3) by Lim et al. (2004) described aripiprazole augmentation of low dose clozapine as beneficial. However, as clozapine was used to augment aripiprazole treatment and the patients responded swiftly and at low doses of clozapine, it is quite likely that they would have responded to clozapine monotherapy. An open label study (n=11) added ziprasidone 160 mg/day to clozapine treatment at very high doses (mean starting dose 854 mg/day) (Kaye 2003). Clinical improvement was seen and the clozapine dose could on average be reduced by 40-50% (mean final clozapine dose 459 mg/day). The combination was well tolerated and there was an improvement in weight and lipid profiles. An open label study by Reinstein et al. (1999) took clozapine patients (n=65) with diabetes and added quetiapine while reducing the clozapine dose (25% of the clozapine dose was changed to quetiapine, using a ratio of 1 mg clozapine to 2 mg of quetiapine). Mean weight loss through the 10 month study period was 4.2 kg. The patients were not necessarily resistant to clozapine but 20% (13 patients) showed clinical improvement. No one withdrew from the study.

#### *1.1.6.3.3.2.1. Clozapine and risperidone*

A number of case reports, case series and small open studies have described the beneficial effects of using risperidone. McCarthy (1995), reviewed augmentation practices in Denmark and described successful augmentation of clozapine with risperidone (n=2) which was well tolerated. Henderson et al. (1996), described 10 out of 12 patients improving by at least 20% on BPRS total by 4 weeks. The mean dose of clozapine was 479 mg/day and that of risperidone 3.8 mg/day. Serum clozapine was non-significantly increased by 2% with risperidone augmentation. De Groot et al. (2001) added risperidone (mean dose 5.3 mg/day) to clozapine (n=12) and of the 11 who remained in the study no one had improved by 20% by 4 weeks. Serum clozapine levels were therapeutic and not affected by the augmentation. In another open study, 7 out of 13 patients' BPRS improved by more than 20% over 12 weeks (mean dose

of risperidone 3.0 mg/day) (Taylor et al. 2001) . Raskin et al. (2000), showed an improvement on PANSS total by 20-30% in 3 patients as did a case report (n=2) by Morena et al. (1999) showing a BPRS total reduction of 9 and 10 points. Other case series and case reports have generally reported a positive effect (Tyson et al. 1995; Chong et al. 1996; Adesanya et al. 2000; Raju et al. 2001).

*Table 1.3. Studies of clozapine augmentation in schizophrenia.*

Antipsychotic combination	Study	Study type	n	Scales, dose, efficacy type	Side-effects
Clozapine + pimozide	Friedman 1997	RCR	7	Scale : BPRS. Pimozide dose : 4 mg/day Mean 24 point BPRS decrease	Not reported
Clozapine + loxapine	Mowerman 1996	OPT	7	Scale : BPRS. Loxapine dose range 25-200 mg/day. Range of BPRS improvement 19-38 points	Not reported
Clozapine + sulpiride	Stubbs 2000	CR	1	No scale. "Improved"	
Clozapine + haloperidol	Rajarethinam 2003	CR	3	Scale: BPRS "Improved"	Not reported
Clozapine + haloperidol	Kapur 2001	OPT	5	Not reported. Haloperidol dose:4 mg/day	High increase in s-prolactin
Clozapine + olanzapine	Gupta 1998	CR	2	No scale. "Improved"	
Clozapine + olanzapine	Rhoads 2000	CR	1	No scale. "improved". CLZ dose: 100 mg/d	Not reported
Clozapine + aripiprazole	Lim 2004	RCR	3	BPRS. 34% improvem. Aripiprazole dose: 25 mg/d CLZ dose: 167 mg/d	Not reported
Clozapine + ziprasidone	Kaye 2003	OPT	11	No scale. "Improved" Ziprasidone dose 160 mg/d	None reported
Clozapine + quetiapine	Reinstein 1999	RCR	65	Side-effect rating scales. No deterioration. 25% of CLZ dose changed for quetiapine	Drowsiness
Clozapine + risperidone	McCarthy 1995	CR	2	No scales. "Successful Augmentation".	
Clozapine + risperidone	Henderson 1996	OPT	12	Scale : BPRS R dose: 3.8 mg/day CLZ dose 479 mg/day. 10/12 >20% improvement BPRS (mean decrease 11.9 points)	Akathisia (n=4) Hypersaliv. (n=5)
Clozapine + risperidone	deGroot 2001	OPT	12	Scale: BPRS. R dose: 5.3 mg/day No improvement by > 20% BPRS	Orthostatic hypotension(n=1)
Clozapine + risperidone	Taylor 2001	OPT	13	Scale: PANSS. R dose: 3.0 mg/day 7/13 showed > 20% improvement in PANSS scores	Worsening compulsion (n=1)
Clozapine + risperidone	Raskin 2000	CR	3	Scale : PANSS. 20-30% improvement	
Clozapine + risperidone	Morena 1999	CR	2	Scale : BPRS. 9 and 10 point improvement	
Clozapine + risperidone	Raju 2001	CR	2	CLZ dose 800 & 600mg/day.R dose 10 mg/day	
Clozapine + risperidone	Adesanya	CR	2	CLZ dose 600 & 900 mg/	

Clozapine + risperidone	2000 Tyson 1995	CR	1	day.R dose 6 mg/day. CLZ dose 600 mg/day, R 2 mg/day. Increased s-clz (344-598 ng/ml)	None
Clozapine + risperidone	Chong 1996	CR	1	CLZ dose 600 mg/day. R dose 6 mg/day.	Hypersalivation
Clozapine + risperidone	Chong 1997	CR	1	CLZ dose 175 mg/day, R dose 1.5 mg/day	Atrial ectopics
Clozapine + risperidone	Godleski 1996	CR	1	CLZ dose 900 mg/day R dose 6 mg/day	Agranulocytosis
Clozapine + risperidone	Koreen 1995	CR	1	CLZ 500 mg/day, R 4 mg/day	Oculogyric crisis
Clozapine + risperidone	Beauchemin 2002	CR	1	CLZ dose 750 mg/day, R dose 4 mg/day	NMS
Clozapine + risperidone	Kontaxakis 2002	CR	1	CLZ dose 100 mg/day, R dose 16 mg/day	NMS
Clozapine + amisulpride	Cook 2004	CR	6	Olanzapine dose: 10 mg/d No scale. 5 "Improved"	None
Clozapine + amisulpride	Agelink 2004	OPT	7	Scale:BPRS. CLZ dose: 293 mg/day AMI dose:543 mg/day BPRS reduced by 4.2 points by 17 days and 16.4 points by 9 months.	Not reported
Clozapine + amisulpride	Zink 2004b	RCR	15	No scale. CLZ dose 375 mg/day AMI dose 527 mg/day "Major" benefit in 6, "marked" benefit in 8	Akathisia (n=1)
Clozapine + amisulpride	Kampf 2005	OPT	14	Scale: CGI. 11"much improved" after 20 weeks. CGI reduced from 5.6 to 3.9	None
Clozapine + amisulpride	Croissant 2005	CR	1	CLZ dose 800 mg/day down 400 mg/day. AMI dose 600 mg/day	Weight gain
Clozapine + amisulpride	Lerner 2005	RCR	5	Scale: CGI. CLZ dose:490 mg/day AMI dose:1000 mg/day CGI reduced from 5.4 to 2.4	Not reported
Clozapine + amisulpride	George	CR	1	CLZ dose: 400 mg/day AMI dose: 400 mg/day	Not reported
Clozapine + amisulpride	Kalaitzi	CR	1	CLZ dose: AMI dose:	Hypertension tachycardia
Clozapine + chlorpromazine	Potter 1989	RCT	57	Scale: BPRS. CLZ dose; not reported Combination treatment Similar to clz monotherapy	Not reported
Clozapine + sulpiride	Shiloh 1997	RCT	28	Scales: BPRS, SANS,SAPS Sulpiride dose: 600 mg/day 50% improved >20% BPRS 50% showed no improve- ment	Worse sialorrhea (n=1) Worse TD (n=1) Increased prolactin
Clozapine + risperidone	Anil Yagcioglu 2005	RCT	30	Scales: PANSS, CGI, CDS R dose < 6 mg.day	Sedation, raised prolactin
Clozapine + risperidone	Josiassen 2005	RCT	40	Scales: BPRS, PANSS, SANS R dose :<6 mg/day 7 of 20 in clz + risp group improved >20% on BPRS vs. 2 of 20 in clz + placebo group	None reported

RCT – randomised controlled trial; OPT – open prospective treatment; RCR – retrospective chart review; CR – case report; CLZ – clozapine; R – risperidone; AMI – amisulpride.

The combination of clozapine and risperidone is generally well tolerated but adverse effects have been reported. Hypersalivation and akathisia were common in one trial (Henderson et al. 1996). Other reported adverse effects are worse obsessive-compulsive symptoms (Chong et al. 1996), atrial ectopic beats (Chong et al. 1997), oculogyric crisis (Koreen et al. 1995), agranulocytosis (Godleski et al. 1996) and neuroleptic malignant syndrome (Beauchemin et al. 2002; Kontaxakis et al. 2002).

#### *1.1.6.3.3.2.2. Clozapine and amisulpride*

Eight small recent studies have reported augmentation with amisulpride: A case series described the addition of amisulpride to clozapine treatment (n=6), with “a favourable outcome” in 5 cases and no side-effects reported (Cook et al. 2004). Despite the patients being described as treatment-resistant, it is unclear to what extent their resistance was established. Clozapine was reduced by an average of nearly 50% in all cases. An open study by Agelink et al. (2004) described amisulpride augmentation of 7 patients, with an average amisulpride dose of 543 mg/day. BPRS was reduced from 50.1 points at baseline to 45.9 by 17 days and 33.7 after 9 months’ treatment. Although described as treatment-resistant to clozapine, some of the patients had only been treated with it for 8 weeks (range 8-52 weeks). Therefore the definition of the study group is blurred and it is doubtful that they were all truly resistant to clozapine treatment. Additionally, the average dose of clozapine was quite low (293 mg/day). QTc was unchanged and plasma clozapine did not change significantly. A larger (n=15) open retrospective case notes review by Zink et al. (2004b), reported a “major” benefit in 6 and “marked” benefit in another 8 cases. The definition of outcome was unclear however, the follow-up period short and improvement assessed based on unstructured entries in clinical notes. The mean dose of clozapine was 375 mg/day (s-clozapine 0.38 mg/L) and the mean dose of amisulpride 527 mg/day. A reduction of 24% was possible in the clozapine dose, reducing clozapine side-effects. Akathisia was a problem in one case. In another open study, 14 patients were treated with amisulpride augmentation (Kampf et al. 2005). 11 were “much improved” after 20 weeks’ treatment with a reduction in CGI from 5.6 to 3.9. However, they had only received clozapine monotherapy for 4 weeks prior to augmentation. A case report (n=1) described

reduced side-effects, especially less hypersalivation, when clozapine was reduced by half and replaced by amisulpride (Croissant et al. 2005). An open retrospective case records' review (n=15) described the augmentation of various antipsychotics with amisulpride (Lerner et al. 2005). 5 of the patients were on clozapine (mean dose = 490 mg/day) and were given a mean of 1000 mg/day of amisulpride. The effect was assessed from clinical notes at baseline and after 3 months using the CGI scale, with a reduction from 5.4 to 2.4. No side-effects were noted with this combination. George et al. (2005), reported on a patient who did not improve on either amisulpride or clozapine on its own, but got dramatically better on a combination of both drugs (clozapine 400 mg/day, amisulpride 400 mg/day). A case report (n=1) described hypertension and tachycardia when amisulpride was added to clozapine (Kalaitzi et al. 2005). Urinary catecholamines were raised, but phaeochromocytoma was ruled out. The symptoms disappeared when amisulpride was withdrawn.

#### *1.1.6.3.3.3. RCTs*

To date there are four published RCTs looking at the augmentation of clozapine with another antipsychotic. The first one was an addition of chlorpromazine to clozapine in a mental health centre in China (n=57) (Potter et al. 1989). Those on clozapine fared better than those on chlorpromazine but no additional benefit was observed when chlorpromazine was added to clozapine in this 8-week study which used BPRS to measure psychopathology.

Another study was a double-blind RCT by Shiloh et al. (1997), which investigated partial/non-responders (n=28) to clozapine. Half of them were given sulpiride (600 mg/day), a selective D<sub>2</sub> antagonist, in addition to clozapine. The augmentation led to marked improvement (defined as more than 20% reduction in BPRS baseline scores) both in total (mean reduction 8.7 points in those who responded), positive and negative symptoms and general psychopathology on the BPRS in 50% of patients. The mean score reduction in responders was 42.4% and 50.4% on the BPRS and SANS respectively. No improvement was seen in 37.5% (defined as < 5% change in BPRS, mean reduction in those 2.3 points on BPRS total) over the 10 weeks of the trial. Plasma clozapine levels were not reported on. One case of increased

hypersalivation and one of aggravation of previous tardive dyskinesia were reported, as well as a significant increase in serum prolactin levels without clinical manifestations of hyperprolactinaemia.

A recent six week double-blind RCT (n=30) of partial responders to clozapine compared augmentation with up to 6 mg/day of risperidone (mean dose 5.1 mg/day) or placebo (Anil Yagcioglu et al. 2005). Those who had risperidone showed significant improvement on PANSS positive but no changes either in other clinical measures or on a quality of life scale. Another recent double-blind RCT by Josiassen et al. (2005), looked at treatment-resistant patients (n=40) on clozapine (treated for > 3 months, mean time on clozapine=396.9 weeks) with half the group augmented with placebo and the other half with up to 6 mg/day of risperidone (mean dose of risperidone at endpoint 4.43 mg/day). By week 12 both groups had improved but the risperidone treated group showed significantly more improvement on BPRS total, the positive subscale and SANS. 7 out of 20 patients in the clozapine / risperidone group showed a reduction of at least 20% on the BPRS total score, compared to 2 out of 20 in the clozapine/placebo group. Side-effects were similar in both groups with no additional weight gain, agranulocytosis or seizures.

#### *1.1.6.3.3.4. Summary*

To date at least 37 studies with more than 365 patients have been reported. 4 have been randomised (with 107 patients receiving active augmentation). Most studies have used risperidone (n=125), followed by chlorpromazine (n=57), amisulpride (n=50), and sulpiride (n=29). The studies indicate an overall benefit of augmenting clozapine with another antipsychotic with a response generally seen within a few weeks and in about half of cases. Not all the trials are positive however.

With the exception of olanzapine, there is no indication from these reports that augmenting clozapine with another antipsychotic increases the risk of agranulocytosis. The larger trials have not reported increases in serum clozapine levels with augmentation, although some case reports have found that.

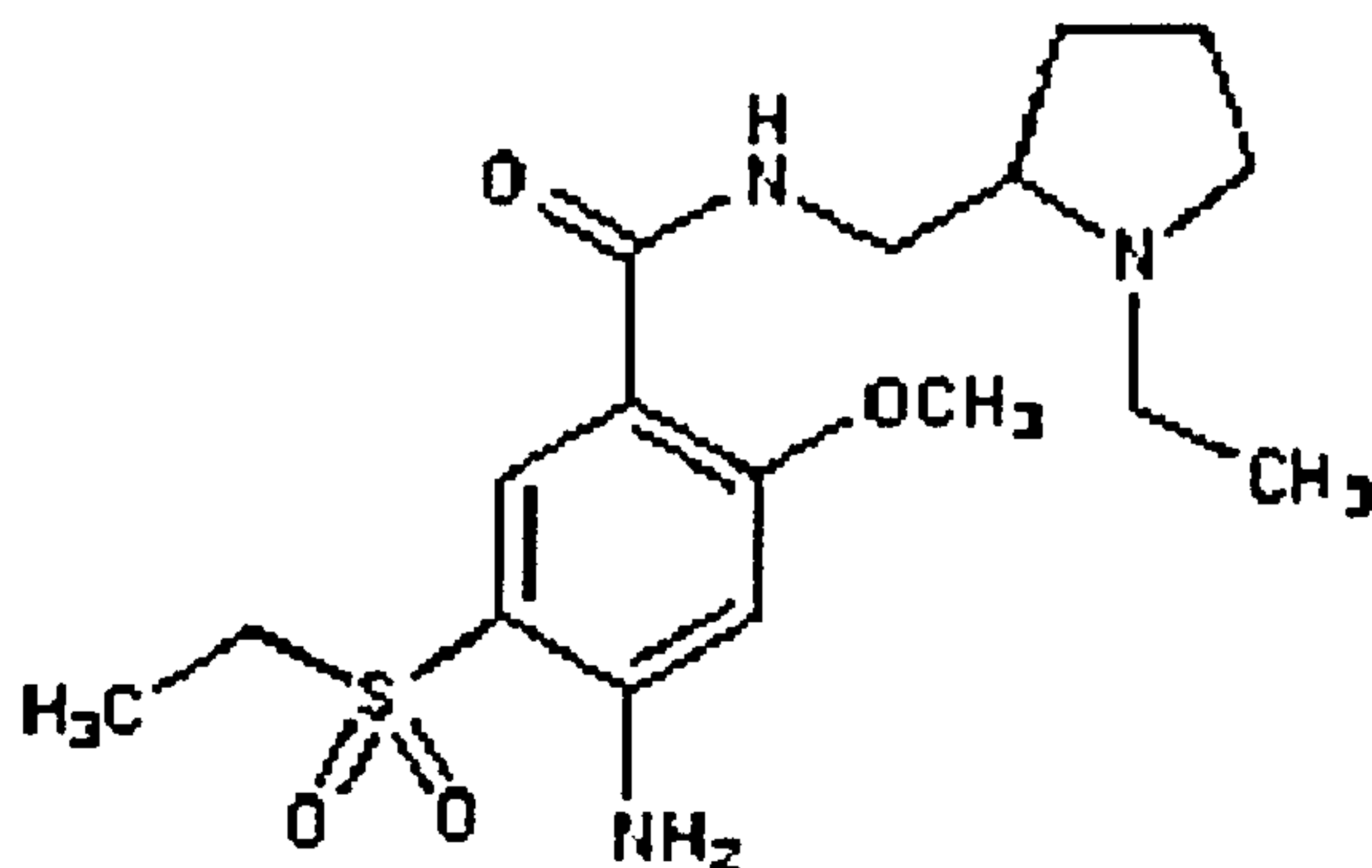


Overall, the level of evidence for augmenting with another antipsychotic is sub-optimal, because of the small number of RCTs. However, there is growing evidence for a role for antipsychotic augmentation in truly treatment-resistant cases. This augmentation seems to be generally reasonably well tolerated. More reported adverse effects with risperidone augmentation may reflect the higher number of studies. The strongest evidence is for risperidone augmentation.

## 1.2. Amisulpride – an unusual antipsychotic

Amisulpride has been available in France since 1988, in other European countries only since 1997 or later and it is not licensed in the US. It is a substituted benzamide, structurally related to the older, typical antipsychotic sulpiride.

*Fig.1.2. The chemical structure of amisulpride.*



### 1.2.1. Pharmacodynamics

Amisulpride has high and similar affinities for the dopamine D<sub>2</sub> (binding constant,  $k_i=2.8$  nM) and D<sub>3</sub> receptor ( $k_i=3.2$  nM) subtypes but no affinity ( $k_i >1$  micromol/L) for D<sub>1</sub>, D<sub>4</sub> and D<sub>5</sub> receptors (Scatton et al. 1997). It also has little affinity for serotonergic, alpha-adrenergic, H<sub>1</sub> histaminergic or cholinergic receptors (Schoemaker et al. 1997; Trichard et al. 1998; Castelli et al. 2001). The fact that it is an atypical antipsychotic despite its receptor profile is one of

the reasons for a renewed interest in the dopamine hypotheses of schizophrenia.

Amisulpride at lower doses (< 10 mg/kg) has a preferential effect on D<sub>2</sub> and D<sub>3</sub> presynaptic autoreceptors that control dopamine production and release in rats. At higher doses (40-80 mg/kg in rats) the traditional postsynaptic dopamine D<sub>2</sub> receptor occupancy is seen (Perrault et al. 1997). At lower doses it increases dopamine release and hence prefrontal cortical activity, which is thought to explain its modest effect on negative symptoms (Cudennec et al. 1997; Danion et al. 1999; Leucht 2004b), as well as an effect on dysthymia (Pani et al. 2002; Racagni et al. 2004). At higher doses it blocks D<sub>2</sub>-like receptor activity. It has preferential binding in limbic cortical areas over striatal areas (Bressan et al. 2003a) and there is an apparent higher affinity for extra-striatal areas at lower doses, but for striatal areas at higher doses (Xiberas et al. 2001a).

Amisulpride has a high dissociation constant,  $K_{off}$ , which is thought to play a role in its lack of EPSEs (Seeman 2002; Mortimer 2004a). Amisulpride reaches high striatal (but still less than 80%) D<sub>2</sub>-like blockade at a wide range of plasma amisulpride levels, although there is some correlation between plasma levels and Dopamine D<sub>2</sub>-like binding (Vernaleken et al. 2004). Dosages of 630 – 910 mg/day in humans are associated with 70-80% striatal D<sub>2</sub>-like occupancy (Martinot et al. 1996). It has been suggested that amisulpride's preferential action on limbic D<sub>2</sub>-like receptors and its preferential blockade of presynaptic D<sub>2</sub>-like receptors may explain its atypicality (Moller 2003).

### *1.2.2. Pharmacokinetics*

Amisulpride has two absorption peaks; 1 and 4 hours after the dose is taken. Elimination half-life is 12 hours and absolute bioavailability 48%. Its volume of distribution is 5.8 L/kg and very little is bound to plasma proteins (17%). It crosses the blood brain barrier (BBB) poorly, with in vitro studies showing porcine brain endothelial cells to be nearly impermeable to amisulpride (permeation coefficient,  $P < 1 \times 10^{-7}$  cm/s) in the resorptive direction, transport in the secretory direction was  $P$  (+/-SD) of  $5.2 \pm 3.6 \times 10^{-6}$  cm/s, indicating active transport across the BBB (Harterter et al. 2003). Rodent studies have

confirmed much higher central to peripheral D<sub>2</sub> receptor blockade for amisulpride indicating poor BBB permeability (Kapur et al. 2002a). Amisulpride is weakly metabolised by the liver into two inactive metabolites. It is mainly eliminated unchanged by the kidney (Rosenzweig et al. 2002). In the elderly (>65 years old) elimination is prolonged by 10-30% after a single dose (Solian product data, 2005). Excretion is slowed in renal impairment. Amisulpride has few interactions with other drugs and does not affect the activity of the cytochrome P450 system.

A study used high-performance liquid chromatography to assess plasma amisulpride levels in 85 patients with schizophrenia (Bergemann et al. 2004). Inter-individual difference in plasma-amisulpride levels was high and plasma levels increased linearly with increased dose. Older patients and women had higher dose-related plasma levels. Cigarettes and benzodiazepines did not affect plasma levels. Lithium and clozapine co-administration increased plasma levels significantly.

### *1.2.3. Side effects*

Amisulpride is overall well tolerated. A pooled analysis of 11 trials with doses of 100-1200 mg amisulpride per day gave these frequencies for side-effects in those with predominantly positive symptoms (n=579, mean dose 670 mg/day) (Coulouvrat et al. 1999): EPSEs 15%, insomnia 11%, hyperkinesias 9%, anxiety 9%, bodyweight increase 7%, agitation 6%. In those with predominantly negative symptoms (n=342, mean dose 118 mg/day) these were the side-effects seen: insomnia 7%, bodyweight increase 4%, agitation 3%, anxiety 3%, EPSEs 4%. Those who received placebo (n=202) had the same frequency of side-effects as the ones treated for negative symptoms. A drug utilisation observation study (n=570) compared compliance in outpatients on typical antipsychotics and three months after switching to amisulpride (Linden 2005). 43.7% were noncompliant with the typicals compared to 14.2% on amisulpride. This is likely to be partly attributable to a preferential side-effect profile.

Prolactinaemia is very commonly seen. Clinical signs of prolactinaemia are rare though, but more common in females (Coulouvrat et al. 1999; Grunder et al. 1999; Colonna et al. 2000; Kropp et al. 2004a).

Amisulpride is associated with relatively low weight gain which is maintained in long term studies (Newcomer 2005). A pooled analysis of 11 studies showed a 0.8kg weight gain after 10 weeks of treatment (Prakash et al. 1998). During long-term treatment a study showed 1.37kg weight gain after one year of treatment (Leucht et al. 2004c). It compares favourably with both risperidone (Peuskens et al. 1999b) and olanzapine (Mortimer et al. 2004b). Regarding metabolic effects, there is little information available but preliminary data from one prospective 16-week study of glucose metabolism showed neither an effect on fasting glucose levels nor changes in insulin resistance levels in the 12 patients studied. This was in contrast to 7 out of 13 patients treated with clozapine (Rettenbacher et al. 2004). Amisulpride appears to have less risk of treatment-related dyslipidaemia than olanzapine, although there is a paucity of information available (Peuskens et al. 2004a).

Data from a pooled analysis of studies where patients had had at least one ECG during treatment (n=341) gave an incidence of prolonged corrected QTc (>450ms in males, >470ms in females) of 3.5%, which was similar to haloperidol and risperidone. A reduction was seen in both heart rate and blood pressure but this was not clinically significant (Coulouvrat et al. 1999). A study comparing QTc times in a number of patients with schizophrenia (n=51) showed no increase with amisulpride after 14 days treatment (Agelink et al. 2001).

Amisulpride seems to have little effect on cognitive function, relative to haloperidol (Peretti et al. 1997), and clozapine (Adler et al. 2004) and a similar neuropsychological outcome to olanzapine (Joyce et al. 2004). A SPET study showed an increase in cerebral blood flow in frontal and dorsolateral frontal regions after 4 weeks' treatment with amisulpride 100mg/day in 19 patients with schizophrenia, characterised by primary negative symptoms (Vaiva et al. 2002; McKeage et al. 2004).

#### *1.2.4. Efficacy*

Amisulpride seems more effective than typical antipsychotics and some atypicals (quetiapine, aripiprazole, ziprazidone) in reducing overall psychopathology scores. It is equivalent to risperidone and olanzapine in this respect and less effective than clozapine (Davis et al. 2003). In its effect on predominantly positive symptoms, amisulpride is at least as effective as haloperidol (Carriere et al. 2000; Sechter et al. 2002), and olanzapine (Mortimer et al. 2004b). Looking at predominantly negative symptoms, amisulpride did better than haloperidol on the PANSS negative symptom scale, but doses of both medications were high (haloperidol 10-33 mg/day, amisulpride 400-1200 mg/day) (Carriere et al. 2000). Low doses (50-300 mg/day) of amisulpride have shown greater efficacy than placebo on negative symptoms in 3 double-blind RCTs (Boyer et al. 1995; Loo et al. 1997; Danion et al. 1999). It failed however to show a statistical difference from haloperidol (haloperidol given in low doses, 58% had doses of 3-4.5 mg/day) in a 12-month study (Speller et al. 1997). A trend was seen, with changes that approached significance for affective flattening and apathy/avolition. This lack of response might possibly be explained by the age of the patient group (median age 63 years) and long duration of illness (median 441 months).

In terms of quality of life, amisulpride has not been compared with other atypical antipsychotics. It has however done better than haloperidol in a double-blind RCT on the Heinrichs Quality-of-Life Scale, but these findings should be interpreted with some caution because of the high doses of haloperidol (Carriere et al. 2000). In an open study where lower doses of haloperidol were used (5-20 mg/day) amisulpride also did significantly better in inter-personal relations, "instrumental role" and "intrapsychic foundations" (Colonna et al. 2000).

#### *1.2.5. Pharmacoeconomics*

A few studies exist, indicating a reduction in direct treatment costs compared to haloperidol (Souetre et al. 1992) and risperidone (Nicholls et al. 2003) as a result of lower resource utilisation and lower drug acquisition costs. A more

recent study showed cost savings with the use of amisulpride, with less time on acute wards but more time in rehabilitation wards (Surguladze et al. 2005).

#### *1.2.6. Dosage*

For acute episodes a dose of 400-800 mg/day is recommended. For patients with predominantly negative symptoms 50-300 mg/day are recommended. Maintenance doses are individually adjusted and doses above 300 mg/day are given twice daily (Solian product data, 2005).

### **1.3. The dopamine hypothesis of schizophrenia**

All available antipsychotics share a propensity to antagonise Dopamine D<sub>2</sub> and D<sub>2</sub> like receptors (Jones et al. 2002). The original dopamine hypothesis of schizophrenia stated that antipsychotic medication ameliorated the most prominent symptoms of schizophrenia, via blockade of dopamine receptors. The hypothesis was supported by the finding that a log-linear correlation exists between D<sub>2</sub> antagonist affinity for the dopamine D<sub>2</sub>-receptors and the average daily dose of antipsychotic needed to control the symptoms of schizophrenia, a surrogate for “antipsychotic potency” (Burt et al. 1977; Peroutka et al. 1980). The D<sub>2</sub> receptor was acknowledged as a major site for antipsychotic action (Seeman et al. 1976; Johnstone et al. 1978). There was reported to be a threshold dose of antipsychotics required for clinical response equal to approximately 400 mg of chlorpromazine equivalents (Davis et al. 1986). Later it was postulated that a threshold of D<sub>2</sub>-occupancy would be needed for the antipsychotics to work (Nordstrom et al. 1993b). More recent work with PET supports this by showing that less than approximately 60% striatal D<sub>2</sub> occupancy is associated with poorer clinical response to typical antipsychotics (Remington et al. 1998). There seems to be a therapeutic “band” as raised prolactin and especially extrapyramidal side-effects become a problem once a threshold of approximately 80% D<sub>2</sub> blockade is reached (Farde et al. 1989; Kapur et al. 2000).

The dopamine hypothesis was however insufficient to fully explain the clinical effects of antipsychotics. In brief, some patients were demonstrated to have

very high striatal D<sub>2</sub> blockade (up to 95%) without clinical benefits (Wolkin et al. 1989; Coppens et al. 1991). More recently the characteristics of clozapine led to a reappraisal of theories of the mechanism of action of antipsychotics (Kane et al. 1988). The relationship between D<sub>2</sub> receptor blockade and clinical efficacy seemed less simple than believed previously. A study comparing two groups of schizophrenic patients, non-responders on typical antipsychotics and responders on clozapine demonstrated that those on clozapine responded better clinically, at a lower level of D<sub>2</sub> blockade (Pilowsky et al. 1992). Other studies showed clozapine to be clinically effective with a range of dopamine D<sub>2</sub> receptor occupancy from 20-67% (Nordstrom et al. 1995).

These findings led to a search for alternative explanations for antipsychotic action based on the knowledge that clozapine has a high affinity for a range of receptors, including 5-HT<sub>2A</sub>, H<sub>1</sub>, M<sub>1</sub>, D<sub>4</sub>, α<sub>1</sub> and 5-HT<sub>2C</sub> (Kerwin 1994). It has been argued that maybe this “rich” pharmacology could explain the almost unique therapeutic action of clozapine. In particular, the idea that the ratio of 5-HT<sub>2A</sub> to D<sub>2</sub> receptor affinities might be important attracted considerable interest (Meltzer et al. 1989b; Meltzer 1992).

Using SPET and PET it was shown that atypicals show high 5-HT<sub>2A</sub> binding. However blockade seems unrelated to clinical response (Travis et al. 1998). Additionally amisulpride is an atypical antipsychotic, at least equivalent to other atypicals in efficacy (Sechter et al. 2002; Davis et al. 2003), although it does not block 5-HT<sub>2A</sub> at all (Schoemaker et al. 1997; Seeman 2002). It now seems unlikely that 5-HT<sub>2A</sub> receptor blockade alone can explain antipsychotic action (Kapur et al. 1999; Natesan et al. 2005).

Interest in dopamine as an important or possibly sufficient explanatory model for antipsychotic drug action has increased with the observation that amisulpride, a selective D<sub>2/3</sub> blocker is an atypical antipsychotic, as well as with the marketing of a partial dopamine agonist, aripiprazole as an antipsychotic. Recent dopamine theories will be discussed in chapter 6.3.1.1.

## 1.4. Neurochemical aspects of Dopamine receptors

Chemical neurotransmission is at the core of psychopharmacology and neuroreceptors are key factors in neurotransmission. In exploring aspects of treatment response in schizophrenia, this thesis looks at dopamine D<sub>2</sub>-like receptors and changes in their occupancy through medication. It is therefore worthwhile discussing the D<sub>2</sub> receptor, as well as other members of the dopamine receptor family.

The dopamine family of neuroreceptors is located at neuronal synapses where the neurotransmitter dopamine binds to them and starts a G-protein coupled intra-cellular cascade.

Neurotransmitter receptors are polypeptides which extend through neuronal membranes. As neurotransmitters are located extracellularly, the binding site of the receptor is generally external to the membrane or in the trans-membrane region. The intracellular part of the receptor generally interacts with effector units. These can be ion channels giving a more rapid response through changes in trans-membrane ion transport. The other main effector unit is G proteins (guanyl nucleotide binding proteins), of which at least four families exist. These are large polypeptides with 7 trans-membranic domains which are coupled to neuroreceptors and mediate between them and “second messengers<sup>7</sup>”, a role called “receptor-effector coupling”. Dopamine receptors are bound to G proteins and the binding of dopamine to receptors triggers an intra-cellular signal transduction cascade. Biological activity most commonly results from kinase-mediated phosphorylation of a substrate protein although de-phosphorylation sometimes happens and self-moderating processes exist as well. The two main “second messengers” are cyclic AMP and inositol-1,4,5-trisphosphate, although others, such as arachidonic acid and tyrosine kinases can also be found. Other proteins that regulate signal transduction cascades include protein kinase C. (Wilcox et al. 1998; Shiloh et al. 1999).

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<sup>7</sup> Second messengers are low-weight diffusible molecules that are used in signal transduction within a cell. They are synthesised or released by specific enzymatic reactions as a result of an external signal and can be synthesised/released and broken down again in specific reactions by enzymes.



An example of a cascade is the activation by a G protein of adenylate cyclase enzymes. Adenylate cyclase is a trans-membranic enzyme that synthesises cAMP from ATP. cAMP binds to specific locations on the regulatory units of a cAMP-dependent protein kinase, and causes dissociation between its regulatory and catalytic subunits, thus activating the catalytic units and enabling them to phosphorylate substrate proteins, leading to changes in their activity (Wilcox et al. 1998; Shiloh et al. 1999).

Apart from the effects on cytoplasm proteins and ion channels, “second messengers”, in particular cAMP, also cause transcription in the cell nucleus. Another role is desensitisation of receptors. There is also evidence that dopamine increases not only cAMP levels intracellularly but also in extracellular space (Lazareno et al. 1985; Newman et al. 2000).

At least five types of dopamine receptors have been identified in the human central nervous system. These form two families; the D<sub>1</sub> and the D<sub>2</sub> family. The D<sub>1</sub> family (D<sub>1</sub> and D<sub>5</sub> receptors) stimulates formation of cAMP by activation of stimulatory G proteins. The D<sub>2</sub> family (D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> receptors) on the other hand acts by activating an inhibitory G-protein and through that inhibiting the formation of cAMP. D<sub>2</sub> receptors exist as post- and presynaptic autoreceptors (Missale et al. 1998).

The D<sub>2</sub> receptor is found in two isoforms which differ in 29 amino acid residues and are formed by alternative splicing which happens during maturation of the D<sub>2</sub> receptor pre-mRNA (Giros et al. 1989). These two forms of the D<sub>2</sub> receptor co-exist in all tissues but their ratio varies greatly. Both forms inhibit cAMP accumulation but their effects are somewhat variable (Hayes et al. 1992). It has been hypothesised that differential splicing of the D<sub>2</sub> receptor may account for regional “limbic” selectivity of certain antipsychotics for striatal and extrastriatal sites (Malmberg et al. 1993).

D<sub>2</sub> receptors are more widely distributed than D<sub>3</sub> and D<sub>4</sub> receptors and are present in abundance in the striatum and nucleus accumbens, but in low density in the prefrontal cortex and the cerebellum. Within the striatum, D<sub>2</sub> receptors are mainly found in GABAergic neurones that participate in indirect

pathways (Hersch et al. 1995). D<sub>3</sub> and D<sub>4</sub> receptors are most densely distributed in the limbic forebrain. D<sub>3</sub> is also present in high density in the ventral striatum while D<sub>4</sub> is virtually absent from the nigrostriatal system (Sokoloff et al. 1990; Van Tol et al. 1991). The D<sub>5</sub> receptor is present in low amounts and is restricted to certain areas in the hippocampus, the hypothalamus, and the parafascicular nucleus of the thalamus (Meador-Woodruff et al. 1992). D<sub>1</sub> receptors are widespread in the neocortex, including the prefrontal cortex and are in high numbers in the striatum.

In schizophrenia only a few studies have shown a significant elevation of D<sub>2</sub> receptors in schizophrenia. Currently the view is that untreated patients with schizophrenia show a minor increase in D<sub>2</sub> receptor density in the striatum (12%) of unclear clinical relevance (Abi-Dargham 2003a). Results from post-mortem and gene expression studies are not decisive regarding D<sub>3</sub> receptors but there is indication that their density is slightly increased as well (Seeman et al. 1994). Studies have been inconclusive regarding the density of D<sub>4</sub> receptors in schizophrenia with some studies showing much increase compared to controls (Seeman et al. 1994; Lahti et al. 1998; Stefanis et al. 1998), but others no difference (Seeman et al. 1993). A PET study showed D<sub>1</sub> receptors to be reduced in the prefrontal cortex but not in the striatum of patients with schizophrenia (Okubo et al. 1997). Other studies have found an up-regulation of D<sub>1</sub> receptors in the cortex in schizophrenia, which in the dorsolateral prefrontal cortex is correlated with poor performance on the n-back task<sup>8</sup> (Abi-Dargham 2004). This may reflect compensation to a dopamine deficit in the cortex, due to hypoactive mesocortical dopamine projections to the prefrontal cortex (Abi-Dargham et al. 2003b).

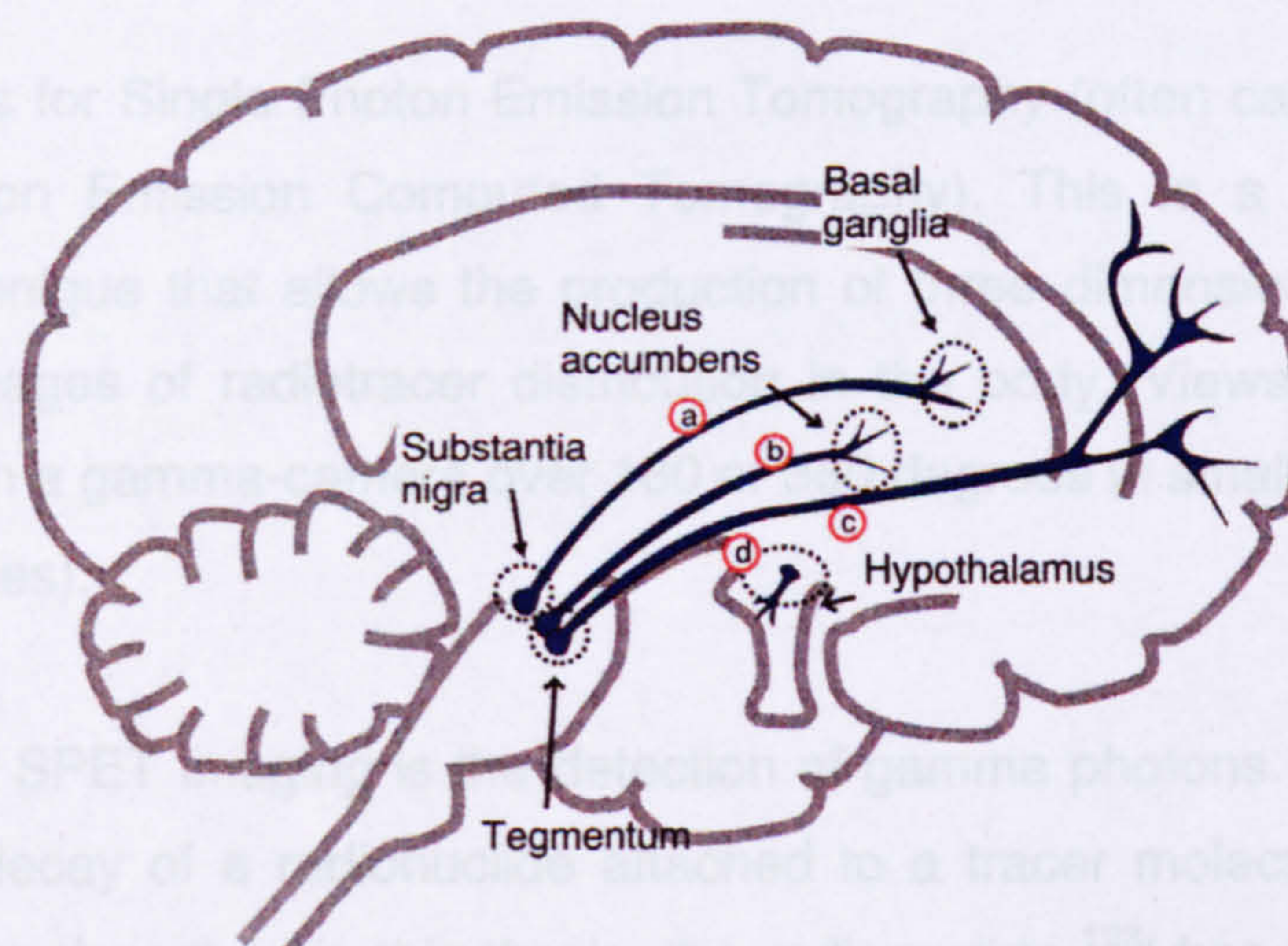
There are four main dopamine tracts in the brain (Fig. 1.3.): (a) The nigrostriatal tract projects from the substantia nigra (area A9) in the midbrain to the dorsal striatum / putamen. Its role is mainly in motor control. Blocking of D<sub>2</sub> receptors at the dorsal striatal end of this pathway leads to extrapyramidal (Parkinsonian)

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<sup>8</sup> The n-back task is a task "in which subjects are asked to monitor the identity or location of a series of verbal or nonverbal stimuli and to indicate when the currently presented stimulus is the same as the one presented n trials previously." (Owen et al. 2005) It is thought to engage working memory and functional neuroimaging shows activation of mainly areas of the prefrontal cortex when subjects are engaged in this task.

side-effects. (b) The mesolimbic and (c) mesocortical tracts have their origins in a few thousand cell bodies in the ventral tegmental area (area 10) close to the substantia nigra. The mesolimbic tract projects into the limbic system, mainly to the nucleus accumbens (a part of the ventral striatum) and the mesocortical tract to the prefrontal cortex, especially the medial surface of the frontal lobes in the neocortex. Blockade of these tracts is thought to be where antipsychotic medication exerts its antipsychotic action. When these tracts are stimulated dopamine is diffusely released in the prefrontal cortex. Dopamine release in the nucleus accumbens triggers neurons to release endogenous opioids in the prefrontal cortex. There is growing evidence that the ventral striatum has an important function in mediating natural rewards and in goal-directed behaviours and it is likely to have relevance for the concept of salience (see chapter 6.3.1.1) (Joel et al. 1997; Stefan et al. 2002; Spitzer 2003). (d) The tuberoinfundibular tract has its cell bodies in the arcuate nucleus and periventricular area of the hypothalamus. The neurons project to the infundibulum and anterior pituitary. When antipsychotics block these neurons the inhibitory effect of dopamine on prolactin secretion is lifted and causes prolactinaemia. (2000; Franke et al. 2004)

*Figure 1.3. Dopamine pathways of the brain: a. Nigro-striatal pathways; b. Meso-limbic pathways; c. Meso-cortical pathways; d. Tubero-infundibular pathways (from Stahl 2000).*



Dopamine is synthesised as part of the common pathway for catecholamines. It is metabolised intraneuronally by monoamine oxidase type B and extraneuronally by catechol-O-methyl transferase. The main metabolite is homovanillic acid.

The number of dopamine receptors in the brain is dynamic. There is evidence that they are subject to both “up” and “down-regulation”. Dopamine D<sub>2</sub> receptor up-regulation in the striatum is not a feature of schizophrenia per se (Farde et al. 1990), but rather of antipsychotic treatment. Up-regulation has in animal experiments been associated with the administration of typical antipsychotics. This has also been shown in PET studies in humans, using <sup>11</sup>C raclopride and is associated with the development of tardive dyskinesia (Silvestri et al. 2000). On the other hand patients with Parkinson’s disease treated with L-Dopa show evidence of down-regulation of striatal D<sub>2</sub> receptors (Brucke et al. 1993).

There is evidence from dopamine depletion studies that dopamine occupies a larger proportion of striatal D<sub>2</sub> receptors in schizophrenia than controls (Abi-Dargham et al. 2000; Frankle et al. 2004).

## **1.5. In vivo SPET studies in schizophrenia**

### **1.5.1. The basics of SPET imaging**

SPET stands for Single Photon Emission Tomography (often called SPECT or Single Photon Emission Computed Tomography). This is a single photon imaging technique that allows the production of three dimensional and cross-sectional images of radiotracer distribution in the body. Views are generally collected with a gamma-camera over 180 or 360 degrees in small angular steps (e.g. 3 degrees).

The basis of SPET imaging is the detection of gamma photons emitted during radioactive decay of a radionuclide attached to a tracer molecule. In studies such as those described in this thesis, the radionuclide <sup>123</sup>I has been attached to a benzamide derivative drug which binds to specific receptors. The drug

iodobenzamide (or IBZM) which has an affinity for dopamine D<sub>2</sub>-like receptors, thus becomes the radioligand <sup>123</sup>I-IBZM. The <sup>123</sup>I-IBZM binds to available D<sub>2</sub>-like receptors in the body. The proportion of the radiotracer bound to the basal ganglia of the brain is quantified by the amount of gamma rays emitted by the <sup>123</sup>I in that region, at specified times following the injection of the radioligand in comparison with regions which have little or no binding sites for <sup>123</sup>I-IBZM.

The gamma camera was first developed by Anger in 1958 (Anger 1958). The camera consists of a detector which is mounted on a gantry (Fig. 1.4.) and is connected to a computer. The detector detects the gamma photons and contains a collimator, a scintillation crystal, photomultiplier tubes and preamplifiers. It is well shielded from stray radiation. The computer is used for further image processing and display.

Gamma photons are emitted from the patient in all directions. A collimator made up of lead septa, makes sure that only gamma rays with a defined angle go through (Fig. 1.5.) and this way keeps some of the scattered gamma rays away, although photons can still be scattered into the angle of acceptance of the collimator. The gamma rays passing through reach a scintillation crystal, which consists of thallium-activated sodium iodide (NaI(Tl)). The crystal converts the gamma rays to light flashes. Photomultiplier tubes (PMTs) are optically connected to the crystal and give out electrical signals with amplitudes proportional to the amount of light they detect. Information is obtained about the position of the gamma photon from interaction in the crystal from the relative signals in different PMTs. The total signal from all PMTs is proportional to the amount of energy deposited in the crystal. The energy signal is analysed by the pulse height analyser and an output signal is only produced when the detected gamma photon energy is within a selected range, reducing the amount of scattered gamma photons registered.

Figure 1.4. The main components of a gamma camera (from Murray et al. 1998)

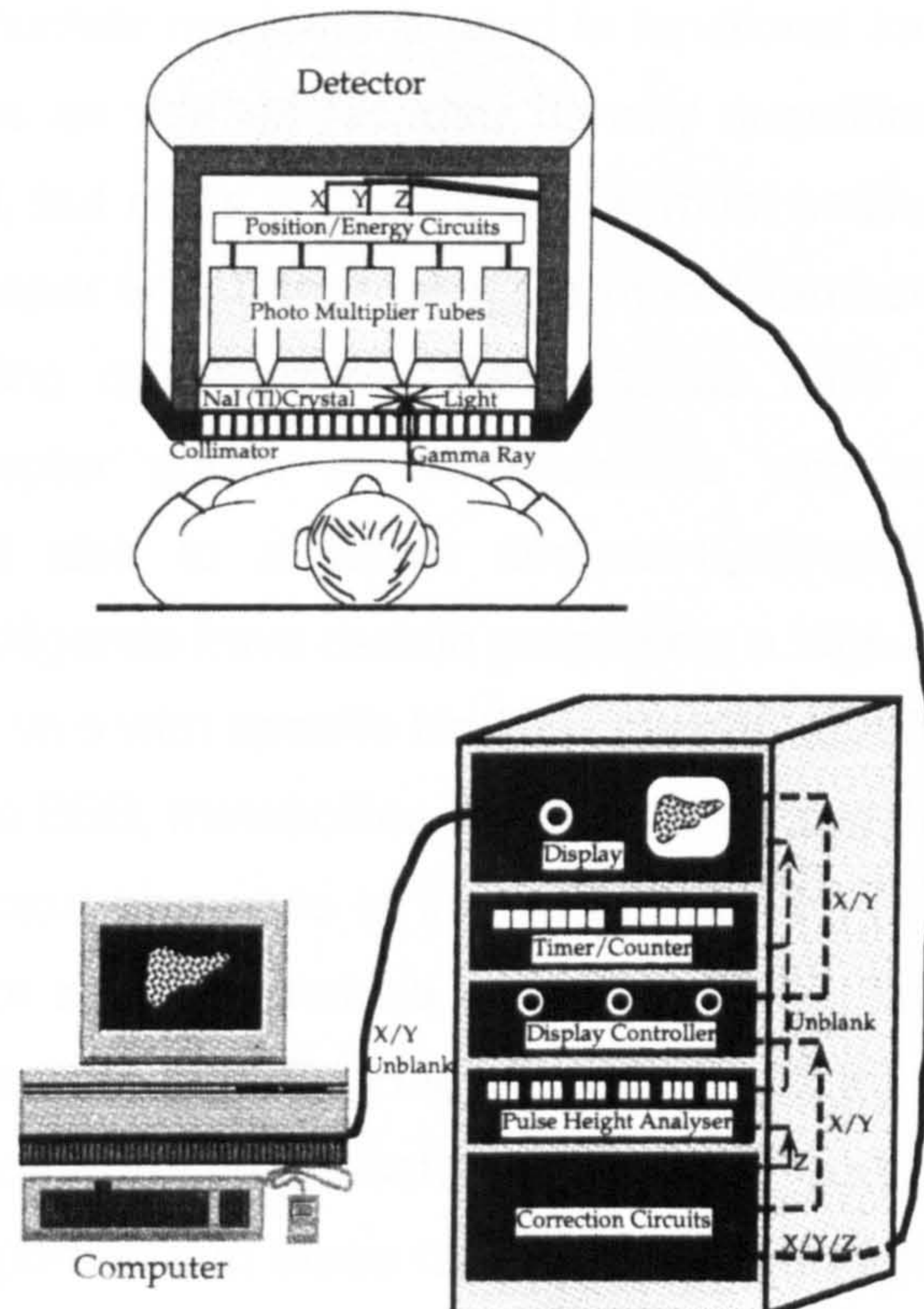
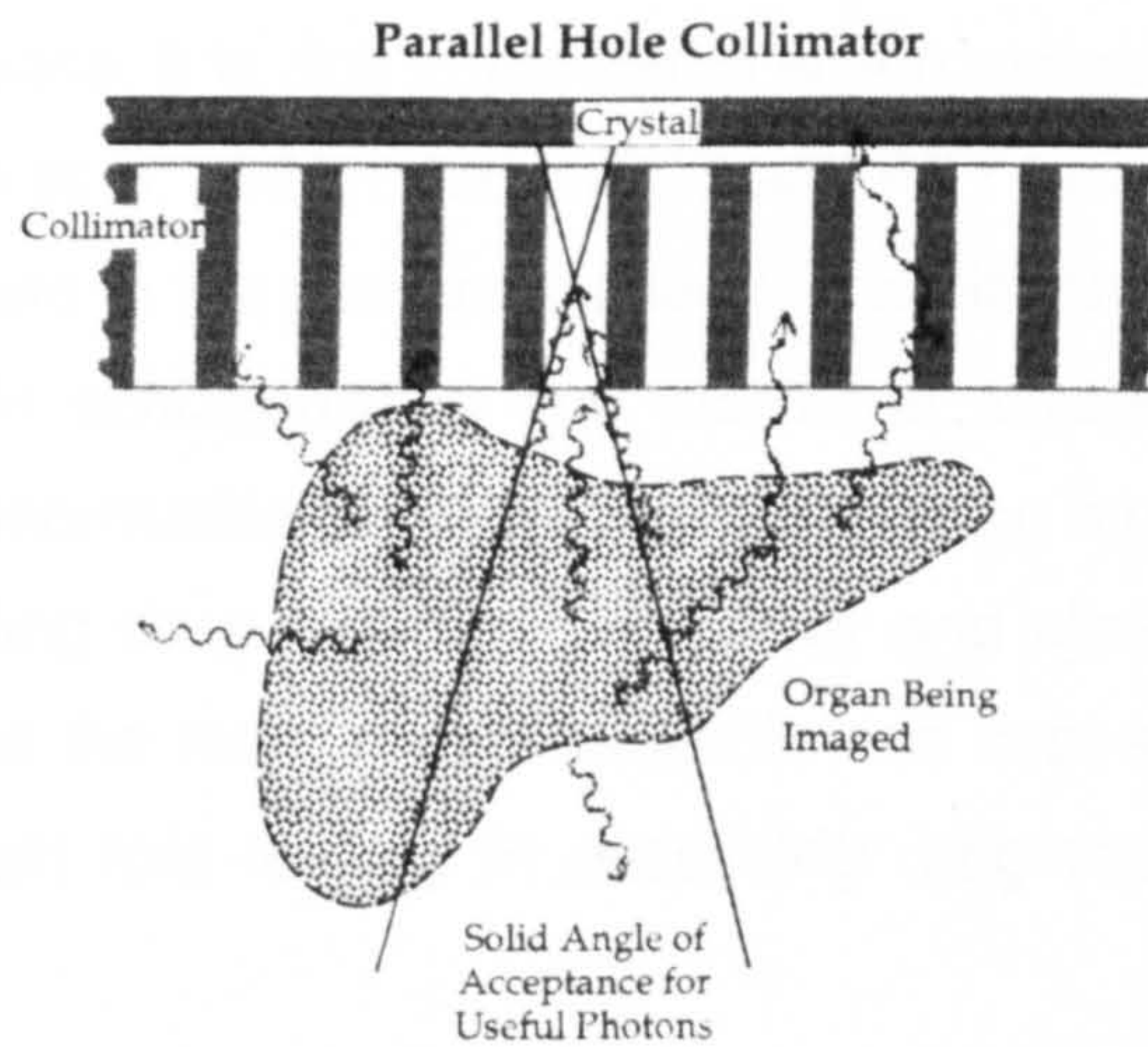


Figure 1.5. Image formation with a parallel-hole collimator (from Murray et al. 1998).



### 1.5.2. SPET in the treatment of schizophrenia

In schizophrenia nuclear medicine is used in functional imaging, anatomic and localisation studies as well as receptor density quantification. PET scanning has proved useful, but costs hinder its use in most settings. The more widely available and cheaper SPET technology is more commonly used, particularly for receptor binding quantification. Radioligands have been developed to explore neuroreceptor action in schizophrenia with studies focusing on dopaminergic and also to a lesser degree GABAergic and serotonergic systems. The radioligands have certain properties; a high affinity for the D<sub>2</sub> (or other) receptors in vivo with specific binding, low non-specific binding, an ability to pass through the BBB, metabolites which do not cross the BBB and interfere with binding and rapid clearance of the tracer from the blood. The ones used now for D<sub>2</sub> receptor scanning include raclopride (PET), fallypride (PET), IBZM (SPET) and epidepride (SPET). Radioligands used for GABA include <sup>123</sup>I- iomazenil (a benzodiazepine) (Busatto et al. 1995) and for 5-HT<sub>2A</sub> the <sup>123</sup>I- 5\_R91150. It is important that these radioligands have high specific activity<sup>9</sup> so that when given in minute amounts they do not influence the mass action of the endogenous agent at the receptor site. Most radioligands currently used do not distinguish well between D<sub>2</sub> and D<sub>3</sub> receptors.

The basal ganglia is the site of most interest when studying D<sub>2</sub> receptors using PET and SPET scans. It is rich in receptors which makes precise quantification easier (Andreasen et al. 1988). Both SPET and PET have been used to explore the role of dopamine in the pathophysiology of schizophrenia. The brain is only subtly abnormal in schizophrenia and activation studies<sup>10</sup> in particular have helped localise abnormalities in this illness. Scanning studies have made clear revelations regarding drug receptor occupancy and clinical state. In particular SPET scanning, as the more widely available and cost-effective method has a potentially important role to play in assessing drug receptor occupancy and clinical response.

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<sup>9</sup> Not normally a problem for SPET, but can be for PET with <sup>11</sup>C labelled tracers.

<sup>10</sup> Activation studies use neuroimaging to explore the relationship between activity in certain brain regions and specific mental functions. Specific methods are used to measure localised changes in blood flow and hence patterns of brain activity, related to neural activity.

The question of the relationship between clinical response and Dopamine D<sub>2</sub> blockade is an important one. With the exception of clozapine there is a threshold of approximately 55-65% D<sub>2</sub> striatal occupancy above which a clinical response to an antipsychotic is seen (Remington et al. 1998; Seeman 2002). A <sup>123</sup>I-IBZM SPET study of 22 chronic patients with schizophrenia suggested that SPET might have a role in predicting response (Volk et al. 1994). The study looked at D<sub>2</sub>-like binding correlated with response. Response (measured by a reduction in CGI scores) was seen in 9 patients, and this response was predicted by higher D<sub>2</sub>-like binding. However, marked improvement (defined as > 30% reduction in BPRS) was seen in only 2 patients. Blockade of D<sub>2</sub> receptors up to or above this threshold is not always adequate. This can be seen in a study where 6 chronic, treatment-resistant patients had over 95% striatal D<sub>2</sub>-like blockade without response (Coppens et al. 1991). Another PET study measured the uptake of [18F]N-methylspiroperidol (a D<sub>2</sub> tracer) in 10 treatment-resistant patients before and after haloperidol treatment. The indices of [18F]N-methylspiroperidol uptake were identical in both responders and non-responders after treatment, and the authors concluded that neuroleptic uptake or binding in the brain did not predict clinical response (Wolkin et al. 1989).

To sum up, SPET has a role in understanding the pathophysiology of schizophrenia, especially when combined with other methods. It has an important role in quantifying receptor blockade and that way aiding understanding of treatment response and side-effects. To date it has not been conclusively used to predict treatment response to guide choice of antipsychotics, although this may change as more suitable radioligands and techniques are developed.

## **1.6. Aims of the thesis**

The aim of this thesis is to elucidate further the relationship between D<sub>2</sub> receptor occupancy and pharmacological treatment-resistance in schizophrenia. Clinically it tests a strategy for dealing with the lack of treatment response when clozapine, the best available antipsychotic for patients resistant to traditional pharmacotherapy, is not effective. The literature indicates a



possible step forward through augmenting clozapine with another antipsychotic, in particular with sulpiride<sup>11</sup> (Shiloh et al. 1997). The main objectives of this thesis are to;

- a) explore whether augmenting treatment with another antipsychotic is a clinically valid approach to overcoming treatment-resistance to clozapine through using a medication closely related to sulpiride, amisulpride<sup>12</sup>,
- b) if a response is seen, to explore whether this response might be secondary to increased dopamine D<sub>2</sub>-like blockade in the striatum,
- c) to see whether those who respond to the augmentation differ from those who remain treatment-resistant,
- d) to determine whether treatment-resistant patients differ from those known to be particularly responsive to clozapine, in terms of Dopamine D<sub>2</sub>-like receptor blockade of clozapine.

### **1.6.1. Main hypotheses under investigation:**

1. Amisulpride augmentation of clozapine treatment in patients suffering from schizophrenia, who are partially or non-responsive to clozapine, will lead to an improvement in clinical ratings.<sup>13</sup>

2. Amisulpride augmentation of clozapine treatment in patients with schizophrenia, who are partially or non-responsive to clozapine, will lead to increased striatal D<sub>2</sub>-like receptor occupancy.

3. An increase in D<sub>2</sub>-like receptor occupancy to approximately 60% striatal blockade is associated with response.

4. Subjects with low, (<50 %) striatal D<sub>2</sub>-like occupancy on clozapine alone, will show an enhanced response to amisulpride augmentation in comparison to patients with a higher striatal (>50 %) D<sub>2</sub>-like occupancy on clozapine alone.

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<sup>11</sup> At the time the studies underpinning this thesis were planned, the best evidence for augmentation was using sulpiride.

<sup>12</sup> The argument for using amisulpride rather than sulpiride is discussed in chapter 3.1.1.

<sup>13</sup> At the time this thesis was planned no information was available regarding whether amisulpride would be an effective medication for augmentation.

5. Clozapine non-responders will have lower D<sub>2</sub>-like receptor occupancy at baseline than good responders. Thus the augmentation will make non-responders' D<sub>2</sub>-like profile more like that of responders.

## **Chapter 2. General methods.**

### **2.1. Ethical aspects**

#### **(Consent, radiation exposure, confidentiality)**

Approval for the study was obtained from the Ethics Committee of the Maudsley Hospital (05/99), The East London and the City Health Authority Research Ethics Committee, The Bexley Local Research Ethics Committee and The Ethics Committee of Burnley General Hospital.

Administration of radioactive material carries with it a risk which must be balanced against the benefits of the exposure. Permission for the study was obtained from The UK Administration of Radioactive Substances Advisory Committee (ARSAC) (RPC 141-431 (13088)).

Following a full description of the study to the subjects, written informed consent was obtained. All subjects in the scanning studies (studies 2 and 3) received a payment equivalent to 8 times the minimum national hourly wage for participation in each scan. Participants in the clinical study (study 1) received no payment for their participation.

Confidentiality was carefully guarded. Each participant was given a unique number and the key to that system kept in a locked cabinet. All questionnaires, image analysis files etc. were only identified with the unique number.

### **2.2. Study samples**

#### **(Inclusion and exclusion criteria)**

#### **2.2.1. The control sample**

Healthy volunteers were recruited to act as controls in the scanning studies. Ten healthy controls (8 males, 2 females, mean age =35.8 years, race; 7 Caucasian, 2 Asian and 1 African-Caribbean subject) were recruited through

personal contacts. An attempt was made to match them with the patients in study 2 in terms of sex, age and ethnicity. However they were not matched for IQ and no attempt was made to match them for educational level etc. This was not felt necessary because of lack of any evidence that IQ and social position affects striatal D<sub>2</sub>-like occupancy. The exclusion criteria were: left-handedness, a history of seizures, a history of primary psychiatric or physical illness, drug or alcohol dependence syndrome, and being prescribed any psychotropic medication at the time of study.

### **2.2.2. The treatment-resistant group**

Patients were recruited through recommendations from psychiatrists and nurses, especially in “Clozapine Clinics” at The Maudsley Hospital, St. Clement’s Hospital, London, The Bracton Centre, Bexley, Kent and Burnley General Hospital, Burnley. After agreement from the responsible psychiatrists, potential subjects were approached and written informed consent to participate in the study obtained.

The inclusion criteria in the clinical study (study 1) were: a diagnosis of schizophrenia according to DSM-IV criteria, age between 18 and 65 years, treatment with clozapine for at least 6 months (to ensure that a reasonable opportunity to respond to clozapine monotherapy had been given), the clozapine treatment was in the view of the patients’ Consultant Psychiatrists clinically optimised, and partial or non-responsiveness to treatment defined as a score of more than 25 on the 18-point BPRS (0-6) scale (Overall et al. 1961).

The exclusion criteria were: a history of seizures, contra-indications to amisulpride, being prescribed any antipsychotic medication in addition to clozapine, other primary psychiatric or physical illnesses, drug or alcohol dependence syndrome. Change in clozapine dose during the trial also led to exclusion. An additional exclusion criterion for the scanning study (study 2) was left-handedness.

### **2.2.3. The responders' group**

Consultant psychiatrists at The Maudsley Hospital were approached and asked to suggest patients who had responded exceptionally well to clozapine. These were then approached. The inclusion criteria were: a diagnosis of schizophrenia according to DSM-IV criteria, age between 18 and 65 years, treatment with clozapine for at least 6 months, which the patients' Consultant Psychiatrists felt was clinically optimised, and a good response to treatment defined as a score of less than 25 on the 18-point BPRS<sub>(0-6)</sub> scale (Overall et al. 1961). Exclusion criteria were: a history of seizures, being prescribed any antipsychotic medication in addition to clozapine, other primary psychiatric or physical illnesses, as well as drug or alcohol dependence syndrome, left-handedness.

## **2.3. Clinical ratings and assessment schedules**

### **2.3.1. Clinical rating scales**

#### *2.3.1.1. BPRS*

The Brief Psychiatric Rating Scale is a widely used scale for assessing the severity of a range of psychiatric symptoms (Overall et al. 1961). The version used here was the 18-item version with each item consisting of a 7-point scale varying from 0 = 'not present' to 6 = 'extremely severe'. Scores range from 0 - 108 with a higher score indicating increased severity. In this study the definition of a score higher than 25 was used as a definition of treatment-resistance. This is the cut-off point used in the study by Shiloh et al. (1997). To score higher than 25 a patient has to have significant illness symptoms in more than one domain.

#### *2.3.1.2. PANSS*

The Positive and Negative Syndrome Scale for Schizophrenia was developed from the BPRS and the Psychopathology Rating scale (Kay et al. 1987). It is a 30 item scale used to assess presence of symptoms, both positive (7 items), negative (7 items) and general (16 items). Each item consists of a 7-point scale

varying from 1 = “not present” to 7 = “extremely severe”. Scores range from 30 – 210.

#### **2.3.1.3. SANS**

Scale for the assessment of negative symptoms (Andreasen 1983). Used to assess changes in negative symptoms over the treatment period. Including SANS in the study, in addition to PANSS, which has a negative subscale, was done as it groups negative symptoms and helps in identifying the more persistent ones. SANS assesses five symptom complexes to obtain clinical ratings of negative symptoms in patients with schizophrenia. Assessments are conducted on a six-point scale (0=“not at all” to 5=“severe”). Maximum total possible score is 100. The lower the score the lower the severity of negative symptoms. In this thesis the raw scores from each category were added together to give a composite sub-score. This was done to capture variance in the data better.

#### **2.3.1.4. GAS**

The Global Assessment Scale (Endicott et al. 1976). Used to assess overall psychosocial functioning and symptom level. Maximum total score is 90. The higher the score the better the patient’s psychosocial functioning and symptoms.

#### **2.3.1.5. Calgary Depression Scale / Anxiety Scale**

Scales validated to assess depressed and anxious mood in schizophrenia (Addington et al. 1990). The depression scale is a 9-item scale specifically developed for the assessment of depression in patients with schizophrenia. Items do not focus on weight change and initial insomnia, both of which can be confounded by the drug treatment of schizophrenia.

### **2.3.2. Side effects rating scales**

The purpose for choosing the following different side effects rating scales was to get a broad view of all the different types of side effects likely to occur. This was important as a proxy for the tolerability of augmentation.

#### ***2.3.2.1. Simpson & Angus***

An extrapyramidal side-effects rating scale (Simpson et al. 1970). It contains 10 items, with each item rated between 0 and 4. A total score is obtained by adding the scores and dividing by 10. A score below 0.3 is regarded as normal. It has good clinical validity and high inter-rater reliability (Lejoyeux et al. 1993).

#### ***2.3.2.2. Barnes Akathisia Scale***

This scale which is a highly valid and reliable 4 item scale assesses the presence and severity of akathisia including the subjective and objective presence of akathisia together with a global clinical assessment (Barnes 1989; Barnes 2003; Janno et al. 2005).

#### ***2.3.2.3. AIMS***

AIMS or the Abnormal Involuntary Movement Scale is a 12-item instrument to assess abnormal involuntary movements (Guy 1976). Scoring the AIMS consists of rating the severity of movement disturbance in three main anatomic areas (facial/oral, extremities, and trunk), based on a five-point scale (0="none", 4="severe").

#### ***2.3.2.4. CAERS***

Clozapine Adverse Effects Rating Scale (CAERS). The CAERS is an interview based scale, designed and used locally, in which patients and clinicians rate the presence and severity of clozapine induced side effects and the amount of distress caused. The CAERS has now been developed further into the ANNSERS (The Antipsychotic Non-Neurological Side Effects Rating Scale) which covers both side effects seen with typical and atypical side-effects (Yusufi et al. 2005).

### **2.3.3. Other scales**

#### ***2.3.3.1. The ANNETT handedness scale***

The standard scale to assess handedness in people (Annett 1970).

### ***2.3.3.2. The Lancashire Quality of Life Profile (LQoLP)***

A well validated, reliable and widely used quality of life assessment tool (Oliver et al. 1997; Gaite et al. 2000; van Nieuwenhuizen et al. 2001). It is used to assess changes in the patient's well-being and level of functioning. It comprises the following subscales: general well-being, work / education / leisure / participation, religion, finances, living situation, legal & safety issues, family relations, social relations, health, and self-concept.

## **2.3.4. Assessment schedules**

### **2.3.4.1. Controls**

The control group had a baseline assessment, comprising a General Health Questionnaire (including questions regarding relevant past medical and psychiatric history), The Annett Handedness Scale, a full blood count (FBC), electrolytes, urea and liver function tests (LFTs).

### **2.3.4.2. Patients in the clinical study (study 1)**

Patients had a baseline assessment: a full medical and psychiatric history, a review of medical notes, the clinical and side-effect rating scales, the LQoLP, and a blood test (FBC, electrolytes, urea, glucose, LFTs, TFTs, s-prolactin, s-clozapine and s-norclozapine). Follow-up evaluations using the same rating scales as at baseline were undertaken at 3 and 6 months when blood tests, including s-clozapine and s-prolactin levels were repeated as well.

### **2.3.4.3. Patients in scanning study (study 2)**

These patients were also a part of study 1. Following the baseline assessment (which included The Annett Handedness Scale in addition to what was required in study 1), the patients had a <sup>123</sup>I-IBZM SPET scan. After 10-12 weeks they had a second SPET scan as well as undergoing the 3 month study 1 assessment.



#### **2.3.4.4. Patients in the responders' study (study 3)**

These patients had the same baseline assessment as patients in study 2. Following this they had one  $^{123}\text{I}$ -IBZM SPET scan.

### **2.4. Neuroreceptor imaging with SPET**

#### **2.4.1. The ligand: $^{123}\text{I}$ -IBZM**

##### **2.4.1.1. General characteristics**

$^{123}\text{I}$ -IBZM ( $^{123}\text{I}$ -iodobenzamide) is a radioligand widely used in  $\text{D}_2$ -like receptor SPET studies. IBZM is a benzamide and as such structurally related to both sulpiride and amisulpride to which it has similar dopamine receptor affinities. Its affinity for dopamine receptors is thought to be in the order  $\text{D}_2 > \text{D}_3 > \text{D}_4 > \text{D}_1 > \text{D}_5$  with a  $K_i$  value for  $\text{D}_2$  of 1.6 nM and for  $\text{D}_3$  of 2.2 nM (Videbaek et al. 2000). According to another study IBZM has only a 2-3 fold higher affinity for  $\text{D}_2$  than for  $\text{D}_3$ , but as  $\text{D}_3$  density in the striatum is negligible this does not markedly affect the  $^{123}\text{I}$ -IBZM signal for  $\text{D}_2$  receptors (Sokoloff et al. 1990). IBZM's preference for  $\text{D}_2$  over  $\text{D}_4$  is in the magnitude of 1.1 to 100 (Van Tol et al. 1991). The  $^{123}\text{I}$ -IBZM signal depends on the availability of endogenous dopamine. It is conceivable that although only one  $\text{D}_2$  receptor exists structurally it may have the ability to couple to different signal transduction mechanisms, a term called "receptor promiscuity" which gives the target cells much flexibility (Drukarch 1991).

##### **2.4.1.2. Preparation**

$^{123}\text{I}$ -IBZM was prepared by Amersham, UK and delivered to the Radiopharmacy at the Institute of Nuclear Medicine, The Middlesex Hospital on Mortimer Street. Radiopharmaceutical purity and quality control were the responsibility of Amersham, UK. The dose administered was dispensed by the radiopharmacy at the Middlesex Hospital.

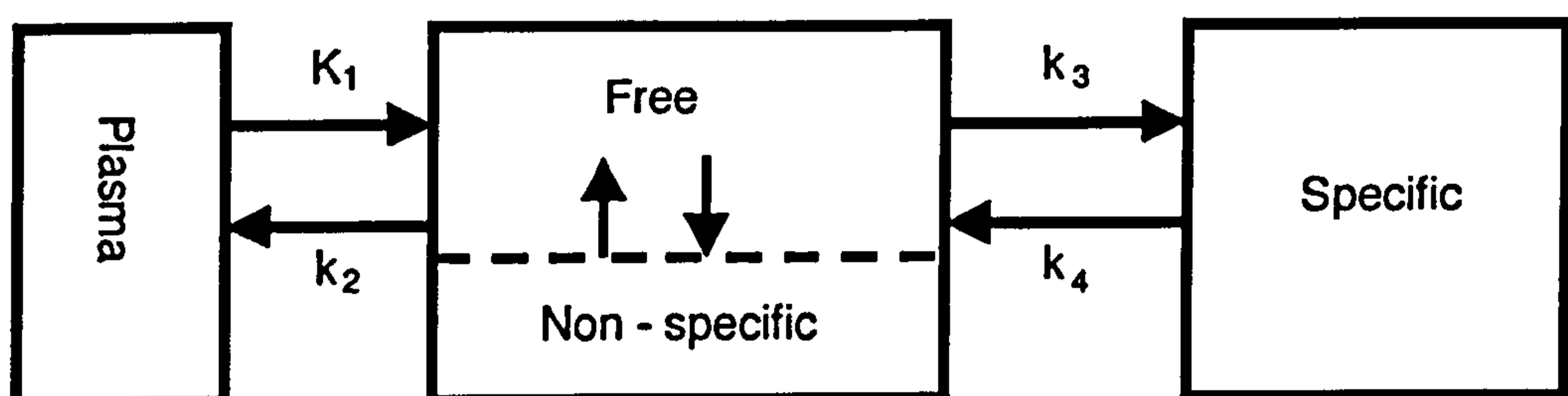
### 2.4.1.3. Radiation dosimetry

A bolus injection of 185 MBq of  $^{123}\text{I}$ -IBZM was given. Having two SPET scans gave subjects approximately 8.8 mSv or the equivalent of three to four years background environmental radiation in London (each member of the UK population is exposed, on average, to 2.2 mSv (range 1.5-7.5 mSv) of unavoidable ionizing radiation every year). The total dose was about one third of the maximum amount of radiation allowed to be absorbed in one year by a radiation worker and under the 10mSv limit suggested for individuals who are not gaining a direct benefit from the scan (Health Protection Agency 2005).

### 2.4.2. In vivo neuroreceptor quantification

In vitro neuroreceptor pharmacology is the basis of in vivo neuroreceptor imaging. The pharmacology is based on the Michaelis-Menten kinetic. This is founded on the law of mass action (Kerwin et al. 1995a), which states that the unbound ligand reversibly binds to the unbound receptor at a rate dependent on the concentration of the two reactants and the ligand-receptor complex dissociates at a rate that is proportional to the concentration of the complex. At steady state the rate of association is equal to the rate of dissociation and it is assumed that other factors are not acting.

*Figure 2.1. A two-tissue compartment model.*



In a two-tissue compartment model, such as the one used in this thesis, it is assumed that free and non-specifically bound compartments equilibrate rapidly

and can be considered a single compartment.  $K_1$  (plasma to tissue influx constant),  $k_2$  (tissue to plasma efflux constant),  $k_3 = f_2 k_{on} B_A$  ( $k_3$  pseudo first order association rate constant,  $f_2$  is the tissue free fraction of the tracer,  $k_{on}$  is the first order bimolecular association rate constant and  $B_A$  is the concentration of available binding sites) and  $k_4 = k_{off}$  (dissociation rate constant).  $V_T$  represents the volume of tracer from the plasma that is extracted by tissue. For the two-tissue compartment model,  $V_T$  is estimated by the formula  $V_T = k_1/k_2 (1 + k_3/k_4)$ . Additionally, the equilibrium dissociation constant ( $K_D$ ) and receptor density ( $B_{max}$ ) can be determined.  $K_D$  is the ratio of the rate of dissociation constant ( $k_{off}$ ) and the rate of association constant ( $k_{on}$ ),  $K_D = k_{off}/k_{on}$ . This is determined by the concentration of ligand that binds specifically to half of the receptors in equilibrium conditions. The lower the  $K_D$ , the greater the binding affinity.  $B_{max}$  is the density of receptors and corresponds to the ligand concentration specifically bound to the receptor in saturation conditions.

Neuroreceptor imaging is based on the principle that the regional uptake of radiotracer is related to the number of receptors which the tracer has affinity for. However, in vivo many factors affect ligand availability in the brain, such as: BBB permeability, regional cerebral blood flow (rCBF), rate of peripheral clearance, binding to plasma proteins and non-specific binding in the brain, concentration of endogenous competing substance, and partial voluming due to cross-contribution from nearby brain regions (Laruelle 2000).

Receptor information can be obtained in vivo with SPET using either semi-quantitative or quantitative methods. Initially the region of interest (ROI) based simplified reference tissue model (SRTM) method was tried to analyse the images (Lammertsma et al. 1996). This method didn't give robust results and was abandoned. The method finally used in the studies described in this thesis was a quantitative method; a voxel-based graphical analysis. The main difference and the likely explanation for the difficulties in the SRTM method lies in the use of ROIs. The SRTM method was based on ROIs being drawn individually, while the latter method used a single set of ROIs drawn on a common template.

Maps of binding potential for drug treated groups and controls were generated using a voxel-based graphical analysis called Logan plot (Logan et al. 1990). It uses a rearrangement of the model equations to generate a parametric image of BP values at each voxel<sup>14</sup> of a PET image. The nonlinear SPET time-activity curve (consisting of bound + free tracer) is linearised by integral transformation. The Logan method plots the integral of the tissue curve divided by the tissue concentration against the integral of the plasma curve, divided by the tissue concentration from each time point. As <sup>123</sup>I-IBZM is a reversibly bound tracer a point in time is reached when the change in plasma concentration is approximately equal to the change in tissue concentration. This time corresponds to a transition point beyond which the Logan plot is linear. With a plasma input function, the slope of the plot will be constant and will equal the total volume of distribution ( $V_T$ ). With the reference tissue method, the slope is  $V_T / V_R$  where  $V_R$  is the  $V_T$  of the reference region. The binding potential is then obtained as the slope minus one:  $BP = V_T / V_R - 1$ . The cerebellum is used as a non-displaceable reference region to obtain the input function.

The plasma input Logan equation is as follows:

$$\frac{\int_0^t C_T(\tau) d\tau}{C_T(t)} = V_T \frac{\int_0^t C_p(\tau) d\tau}{C_T(t)} + L_1; t > t^*$$

where  $C_T(t)$  and  $C_p(t)$  are the activity concentrations in the target region and plasma, respectively,  $V_T$  is the total volume of distribution in the target region and  $L_1$  is a constant.

The reference region Logan equation is as follows:

$$\frac{\int_0^t C_T(\tau) d\tau}{C_T(t)} = DVR \frac{\int_0^t C_R(\tau) d\tau}{C_T(t)} + L_2; t > t^*$$

where  $C_T(t)$  and  $C_R(t)$  are the activity concentrations in the target and reference

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<sup>14</sup> Voxel = volume element (3D pixel)

region, respectively,  $DVR=V_T/V_R$ , and  $L_2$  is a constant.

Many factors can influence the quality of receptor imaging data. This includes the sensitivity of SPET cameras, scatter, the determination of ROIs, patient motion between scans and the suitability of analytical imaging techniques. This was addressed by: using a brain dedicated triple headed SPET camera, fan beam collimation, fiducial markers, scatter and attenuation correction, post-acquisition realignment, alignment to a common template, and user-independent ROI analysis.

### **2.4.3. Factors affecting D<sub>2</sub> quantification**

It has been suggested that age and gender might affect D<sub>2</sub>-like imaging with <sup>123</sup>I-IBZM (Seibyl et al. 1992). Studies of healthy volunteers have shown a decrease in D<sub>2</sub> receptor activity with age. A study using <sup>123</sup>I-IBF found an age related decline in D<sub>2</sub> binding by 7-13% per decade (Ichise et al. 1998). The results could be similar for <sup>123</sup>I-IBZM, as age-related decline in D<sub>2</sub>-like binding has been reported in some studies using that ligand in rats as well as in subjects with Parkinson's Disease (Brucke et al. 1993; Cao et al. 2000). Another study using <sup>11</sup>C-raclopride found a decline of 7.9% of D<sub>2</sub> receptors per decade in healthy volunteers (Volkow et al. 1996). However, a <sup>123</sup>I-IBZM SPET study comparing age-related decline in D<sub>2</sub>-like receptor density found mild age-related decrease in controls (n=20) but not drug-free patients (n=20) (Pilowsky et al. 1994) and another study looking at schizophrenic patients did not find an effect with age (Farde et al. 1990). A study using <sup>123</sup>I-iodolisuride SPET scanning did not find a significant effect on striatal D<sub>2</sub> density with age (Chabriat et al. 1992) nor have further studies (Boulay et al. 1996). In conclusion age is not likely to have a significant influence on results.

Gender is another potential factor. Lower affinity for D<sub>2</sub> receptors is seen in women than men but the age-related decline in D<sub>2</sub> binding is similar (Pohjalainen et al. 1998). The difference between the sexes is slight and unlikely to affect results.

Ethnicity is unlikely to be important, given the lack of conclusive evidence that it has an effect on scanning results as well as the fact that in the studies presented here the groups were reasonably well matched for this variable.

Drugs and alcohol can affect D<sub>2</sub> quantification. Imaging studies have shown significant reductions in dopamine D<sub>2</sub> receptor availability in methamphetamine and cocaine abusers (Volkow et al. 2001; Volkow et al. 1993). Another study found reduced dopamine D<sub>2</sub> receptor availability in alcohol dependence compared to controls, which failed to improve with abstinence (Volkow et al. 2002). This indicates that although low D<sub>2</sub> receptor levels are associated with drug and alcohol abuse, this may be one of the causes of abuse, rather than D<sub>2</sub> receptor reduction being a consequence of abuse. In support of this there is preliminary PET evidence that dopamine D<sub>2</sub> receptor levels predict response to psychostimulants and that low D<sub>2</sub> receptor levels may contribute to psychostimulant abuse (Volkow et al. 2000). A more recent study has found a correlation between resting D<sub>2</sub> receptor availability and healthy controls' response to alcohol (Yoder et al. 2005). Keeping this in mind, it is unlikely that drug and alcohol abuse confounded D<sub>2</sub> quantification in studies 2 and 3 as drug and alcohol dependency excluded both patients and controls from entry into the studies.

## **2.5. Imaging procedure**

### **2.5.1. Positioning of subjects**

Scanning was carried out using a Picker Prism 3000XP triple headed SPET camera, situated at the Institute of Nuclear Medicine, The Middlesex Hospital on Mortimer Street, University College, London.

Subjects were brought into the department alone or accompanied by either a family member or a nurse. They had been briefed beforehand about the scanning procedure and were shown the equipment again on arrival. The accompanying person was allowed to stay in the room throughout the procedure. A urine pregnancy test was done on female subjects.

Fiducial markers (five for the first subjects, later four), each filled with approximately 0.2 MBq of  $^{123}\text{I}$  were attached to the subjects' head to make realignment of different scanning sessions easier.

The subjects were positioned comfortably on the imaging table with their head strapped in a headrest and legs on a knee rest. The subjects were then placed with their head in the camera and a position equidistant to the 3 heads of the scanner. The position of the table was recorded ("scanning position"). The subjects were then taken halfway out of the camera and two laser pointers, fixed laterally to the scanner heads were directed at the orbital fiducial markers. The position of the table was noted again ("positioning position"). Now the imaging table was moved back to the scanning position.

### **2.5.2. Blood sampling**

An intravenous cannula with a three-way tap was placed in the subject's cubital vein and a blood sample obtained. The blood sample was sent to the Maudsley Hospital's laboratory for FBC, electrolytes, urea, glucose, LFTs, s-prolactin, s-clozapine and s-norclozapine.

### **2.5.3. Image acquisition and data processing**

The three detector heads were equipped with ultra-high resolution fan-beam collimators. The scanner was adjusted to collect data in a 15% wide energy window centered at 159 keV. 120 projections were acquired over 360 degrees in 128x128 matrices with a pixel size of 3.56 mm. Scanning was done with 120 degrees rotation. Sequential whole brain images were acquired for up to 210 minutes post-injection in emission scans.

A bolus injection of 185 MBq of  $^{123}\text{I}$ -IBZM was given after 1 minute of scanning. The first ten 1 minute whole brain image scans took place from -1 to 9 minutes post injection. A second session of six 5 minute scans took place from 9-39 minutes post injection. A third scan and a fourth scan, comprising three 10

minute scans took place from 90-120 and 180-210 minutes post injection. The reconstructed resolution was 11 mm FWHM.

*Table 2.1. Scanning protocol.*

Time post-injection	Length of scan
-1 – 9 minutes	10 x 1 min. scans
9 – 39 minutes	6 x 5 min. scans
60-90 minutes	3 x 10 min. scans
180 – 210 min	3 x 10 min. scans

#### **2.5.4. Image processing**

Tomographic images were reconstructed into a 128 x 128 x 60 matrix with a voxel size of 2.03 x 2.03 x 3.56 mm. Emission images were reconstructed by fan-beam filtered back-projection with a ramp-filter. After reconstruction, emission images were filtered with a 3D Butterworth low-pass filter. Scatter correction was made using the triple energy window method. Attenuation correction was performed using the Chang method (Chang 1978).

Images from different sessions were realigned by minimising the mean square error in the centroid positions of the fiducial markers. To assess shift in the markers, the inter-scan difference in marker position was calculated after realignment. Intra-subject realignment was performed by a six-parameter rigid-body transformation. The accuracy of the realignment was estimated as ~1.5 mm.

To be able to compare different studies from the same subject and between subjects, the studies need to be oriented in space so that image regions in different studies show the same anatomic regions. BP images were aligned to a common template using a 9 parameter affine model, by a least squares procedure. The template was created by averaging the BP maps from the healthy controls, after having aligned them to one subject's BP map.



## 2.6. Image analysis

### 2.6.1. Regions of interest analysis

Logan's voxel based graphical analysis was used as discussed above. To reduce the risk of subjectivity and systematic bias, the method used was automated as much as possible. Regions of interest (ROIs) were placed around the basal ganglia (head of caudate and putamen) bilaterally on the average BP map, as well as on the head of caudate and putamen separately. ROIs gave information on mean BP and the ROIs were drawn at the 50% isocontour line. The results were compared using ROIs drawn on 3, 4, 5 and 6 slices from the basal ganglia. The results did not differ and in the end it was decided to use the results obtained by using the most parsimonious method; ROIs drawn on 3 slices.

The ROIs were then applied in an automated fashion to the same location on BP maps for each subject (aligned to the control images) in order to calculate BP for the basal ganglia in a user-independent manner (Fig 2.2).

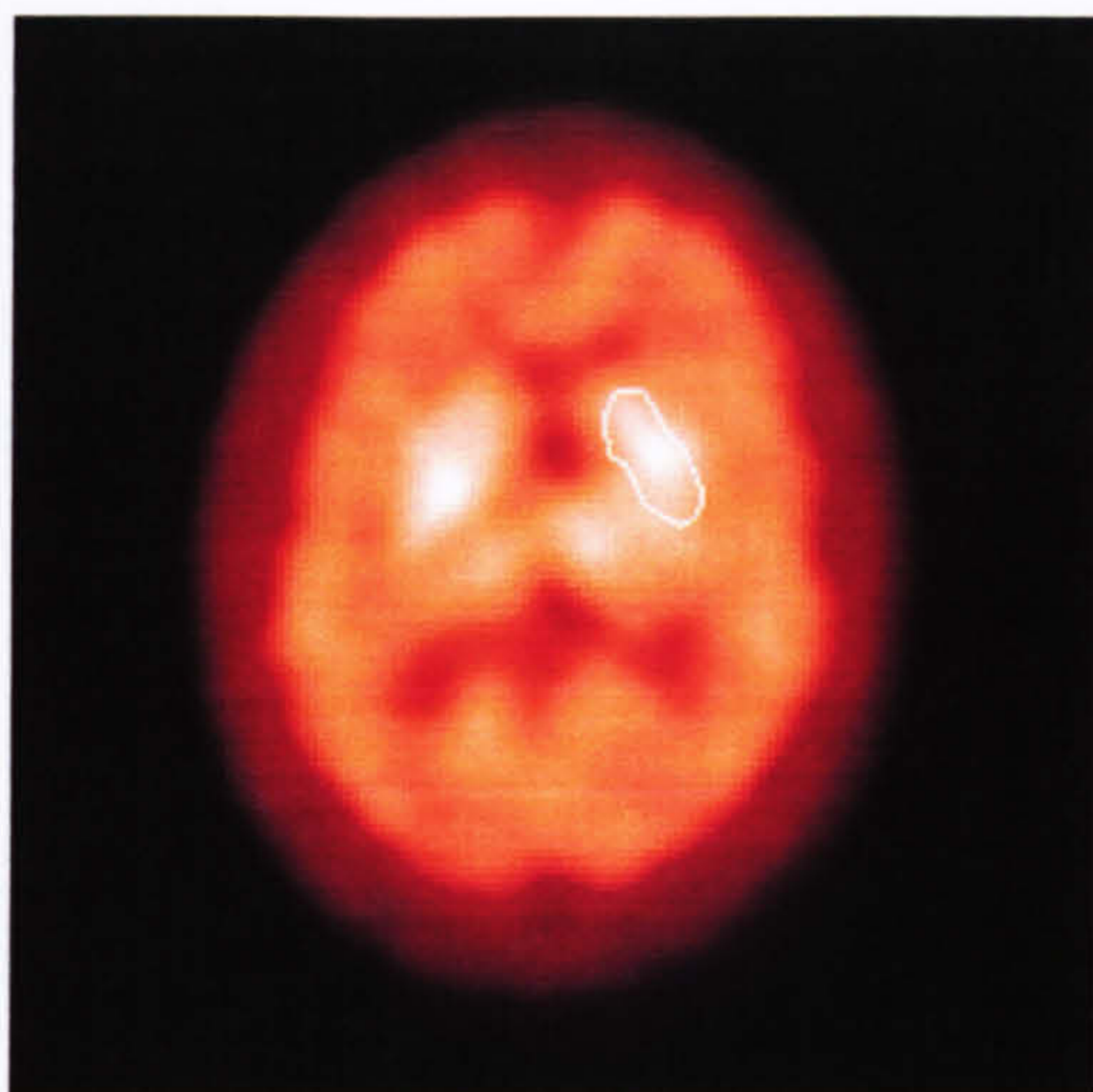
Occupancy ( $O$ ) of D<sub>2</sub>-like receptors in each ROI was determined by reference to the drug free healthy volunteer group using the formula

$$O = \left( \frac{BP_v - BP_p}{BP_v} \right) \cdot 100$$

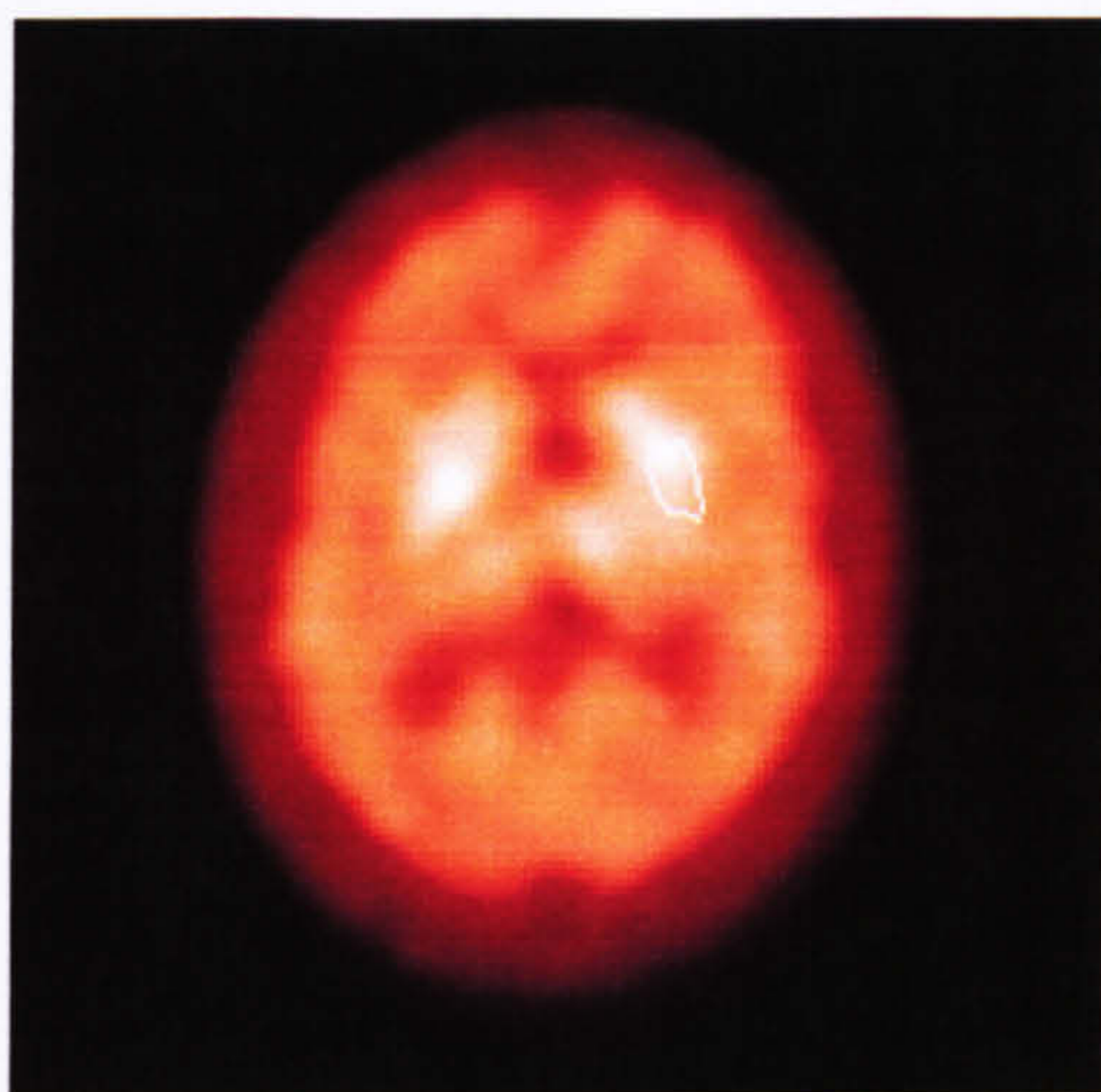
where  $BP_v$  is the mean binding potential value of the healthy volunteer group and  $BP_p$  is the binding potential value of each drug treated patient.

Figure 2.2. Regions of interest for a) the basal ganglia, b) Putamen and c) Head of caudate.

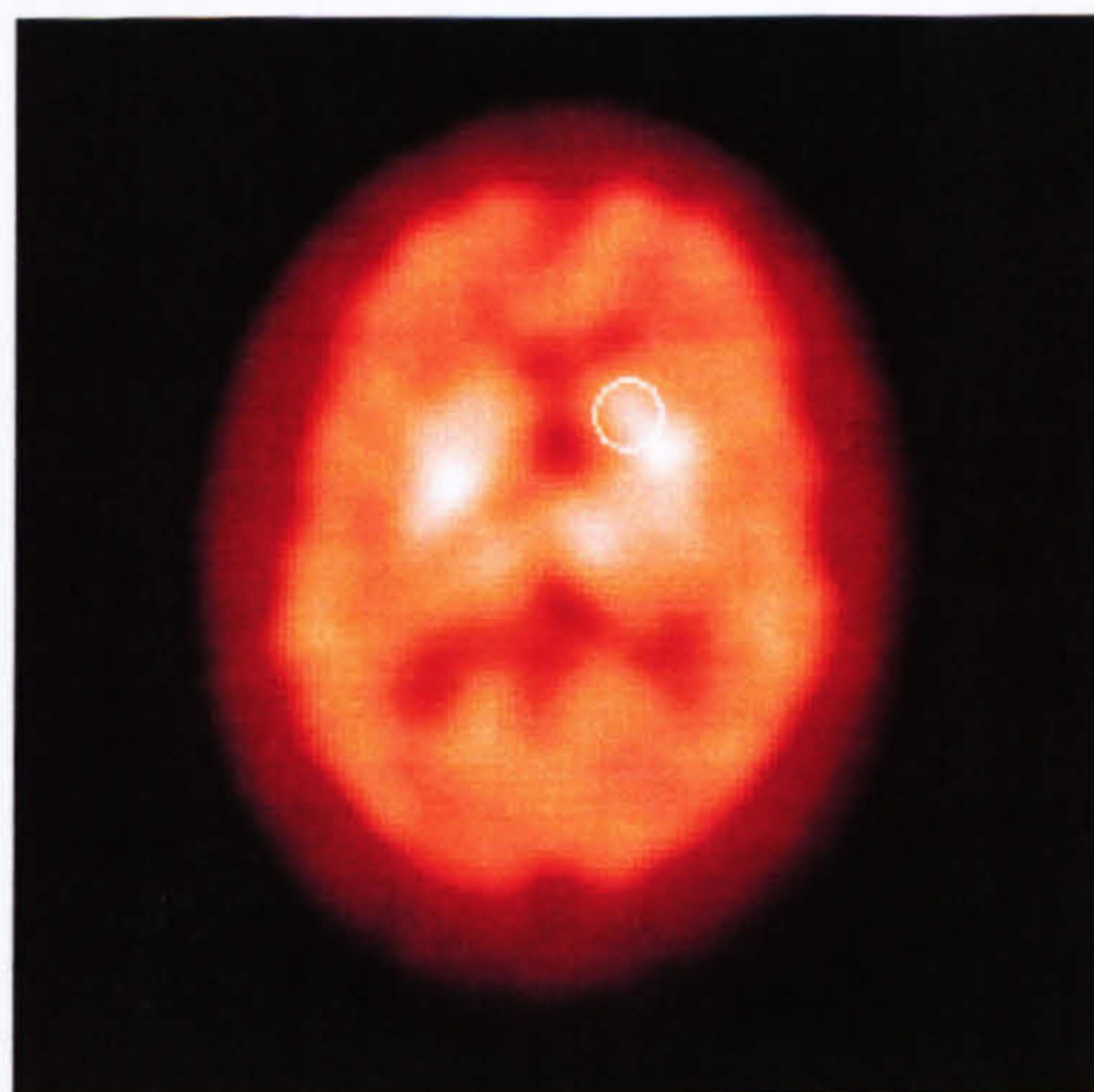
a) Basal ganglia ROI



b) Putamen ROI



c) Head of Caudate ROI



## **2.7. Statistical analysis**

### **2.7.1. Clinical study**

Data for each psychometric scale score were analysed by repeated measures analysis of variance (ANOVA-RM). The between-subject term was the individual patient ID and the repeated term was the time-point (baseline, 3 and 6 months). All F-statistics were with (2,49) degrees of freedom. Baseline epidemiological data and differences in psychometric scores as well as quality of life scores were analysed by Student's t-test. Tests were two-tailed and  $p < 0.05$  considered significant.

### **2.7.2. Scanning studies**

Comparison between occupancy values in the basal ganglia was made before and after augmentation using paired Student's t-test. Occupancy values in the head of caudate and putamen ROIs were also compared using paired Student's t-test. Comparison between occupancy values in responders and non-responders at scan 1 and at scan 2 was done using independent Student's t-test. Tests were two-tailed and  $p < 0.05$  considered significant.

# **Chapter 3. Amisulpride augmentation in patients with schizophrenia partially responsive to clozapine - an open familiarisation study (Study 1)<sup>15</sup>**

## **3.1. Introduction**

When patients fail to improve on clozapine monotherapy augmentation with another antipsychotic is common practice (Peacock et al. 1994; Wang et al. 2000). Four RCTs looking at clozapine augmentation have been reported to date as well as open label studies and case reports. One of the RCTs was a double blind study investigating whether a selective D<sub>2</sub>-like antagonist, sulpiride, would benefit partial clozapine responders (Shiloh et al. 1997). Sulpiride augmentation improved both positive and negative symptoms by at least 20% in half of the treatment resistant clozapine patients and the response was seen within a few weeks of starting treatment.

In this study the purpose was to see whether the findings from the sulpiride augmentation study of Shiloh et al. would also hold true when amisulpride is used instead of sulpiride.

### **3.1.1. Amisulpride choice**

The reason amisulpride was used instead of sulpiride, as in Shiloh's study is as follows: The atypical antipsychotic amisulpride has proven efficacy for both positive and primary negative symptoms of schizophrenia. Amisulpride is structurally related to sulpiride (a substituted benzamide). The higher selectivity and receptor binding potency of amisulpride for D<sub>2</sub>-like receptor subtypes conveys efficacy at lower doses than sulpiride and results in correspondingly fewer side-effects (Sokoloff et al. 1992; Colonna et al. 2000; Taylor et al. 2000). Amisulpride should therefore give a better opportunity to address the hypotheses tested, in particular that increased D<sub>2</sub>-like blockade explains the

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<sup>15</sup> The contents of this chapter have in part been published in *Acta Psychiatrica Scandinavica*. 2004;110(4), 202-8 (Munro et al. 2004).

effect of augmentation. Augmentation with amisulpride could also potentially be better tolerated and more likely have an effect on negative symptoms. In conclusion it was felt that amisulpride would be a similar but more suitable augmenting agent than sulpiride.

### **3.2. Aims of the study**

The aim was to test the hypothesis that amisulpride augmentation would be well tolerated and lead to an improvement in the mental state and well-being of patients with schizophrenia, partially or non-responsive to clozapine.

### **3.3. Methods**

The present study was a mirror image study designed to determine the clinical benefits and tolerability of amisulpride augmentation in patients suffering from schizophrenia who were partially responsive or non-responsive to clozapine. A large randomised controlled trial in a cohort of treatment-resistant patients is expensive and faces logistical difficulties, but it is met with less ethical, cost and patient resistance if based on a “pilot” study such as this one.

#### **3.3.1. Subjects**

Ethical Committee approval was obtained. Out-patients attending clozapine clinics and in-patients prescribed clozapine in four participating centres were screened for inclusion in the study. After agreement from the responsible psychiatrists, potential subjects were approached and written informed consent to participate in the study was obtained. A description of inclusion and exclusion criteria is given in chapter 2.2.2.

#### **3.3.2. Clinical Ratings**

Demographic and clinical details were recorded at baseline: age, sex, ethnicity, dose of clozapine, serum clozapine levels, serum prolactin levels, concomitant psychotropic medication and in- or outpatient status. At the baseline interview

mental state was rated using the Positive and Negative Syndrome Scale (PANSS), the Scale for the Assessment of Negative Symptoms (SANS) and the Global Assessment Scale (GAS). In order to distinguish between more and less persistent negative symptoms, and to detect any change in the side-effect profile after augmentation, a full assessment of side effects and movement disorders was made using the Abnormal Involuntary Movement Scale (AIMS), Barnes Akathisia Rating Scale, Extrapyramidal Side-effects Rating Scale and Clozapine Adverse Effects Rating Scale (CAERS). Mood was assessed using the Calgary Depression and Anxiety Scales (CDS, CAS). Further description of the rating scales is given in chapter 2.3.

### **3.3.3. Study Design**

During the study subjects were maintained on the pre-study, clinically optimised dose and dose timings of clozapine. Once baseline ratings were completed, amisulpride was commenced in addition to clozapine. Amisulpride was dose-adjusted against symptoms and side effects by the treating clinicians to a maximum dose of 800mg/day. Follow-up evaluations, using the same rating scales as at baseline, were undertaken at 3 months and 6 months from the start date of amisulpride. Serum clozapine and prolactin levels were repeated at follow-up. Subjects who discontinued amisulpride or clozapine before the 6 months follow-up were interviewed, when possible, with the standard follow-up ratings and the reasons for treatment discontinuation were recorded.

Response criteria were pre-determined. Treatment response was defined at two levels: a) As a reduction in the BPRS score and PANSS positive and negative subscales of greater than 20% between the baseline (clozapine only) and follow-up (clozapine plus amisulpride) ratings. This is the cut-off point used in Shiloh's study (1997) and has been reported to fit with "a clinically detectable" improvement (Cramer et al. 2001); b) As a threshold of more than 50% reduction in the same rating scales from baseline to follow-up. This would fit in with "a major" gain or the patient being described as being "much better" (Cramer et al. 2001).

### **3.3.4. Statistical Analysis**

Data for each psychometric scale score were analysed by repeated measures analysis of variance (ANOVA-RM). The between-subject term was the individual patient ID and the repeated term was the time-point (baseline, 3 and 6 months). All F-statistics were with (2,49) degrees of freedom. Baseline epidemiological data and differences in psychometric scores as well as quality of life scores were analysed by Student's t-test. Tests were two-tailed and  $p < 0.05$  considered significant.

## **3.4. Results**

### **3.4.1. Baseline demographic and clinical results**

33 subjects were augmented with amisulpride; of these, 28 (84.8%) completed the study and 5 (15.2%) discontinued amisulpride before 6 months follow-up. There were no associations between any of the demographic and baseline clinical variables (table 3.1), including the treatment centre. 11 of the patients were recruited at the Maudsley Hospital, 9 at St. Clement's Hospital, 6 in Burnley General Hospital and 2 in The Bracton Centre, Bexley Hospital.

Table 3.1. Baseline demographic and clinical data of subjects completing the study (n=28)

<b>Age mean years</b>	36.1 (sd 7.4, range 21-49)
<b>Sex</b>	
Male	24 (85.7%)
Female	4 (14.3%)
<b>Ethnicity</b>	
Caucasian	19 (67.9%)
African-Caribbean	3 (10.7%)
Asian	6 (21.4%)
<b>In/out patient status at baseline</b>	
In-patient	12 (42.9%)
Out-patient	16 (57.1%)
<b>Clozapine dose mean mg/day</b>	519 (sd 179, range 100-900)
<b>Concomitant psychotropic medication</b>	
None	17 (60.7%)
Tricyclic antidepressant	2 (7.1%)
SSRI antidepressant	1 (3.6%)
Other	8 (28.6%)

### 3.4.2. Treatment response

In the study subjects, who were partially or non-response to clozapine, co-administration of amisulpride led to a statistically significant improvement in mental state as measured by PANSS, BPRS, GAS and SANS scales over a 6 month period (table 3.2). All measures of positive and negative symptoms showed significant improvement. The improvement was equivalent over the first and second 3 month study periods (Fig. 3.1). Measures of depression and anxiety did not change significantly over time.



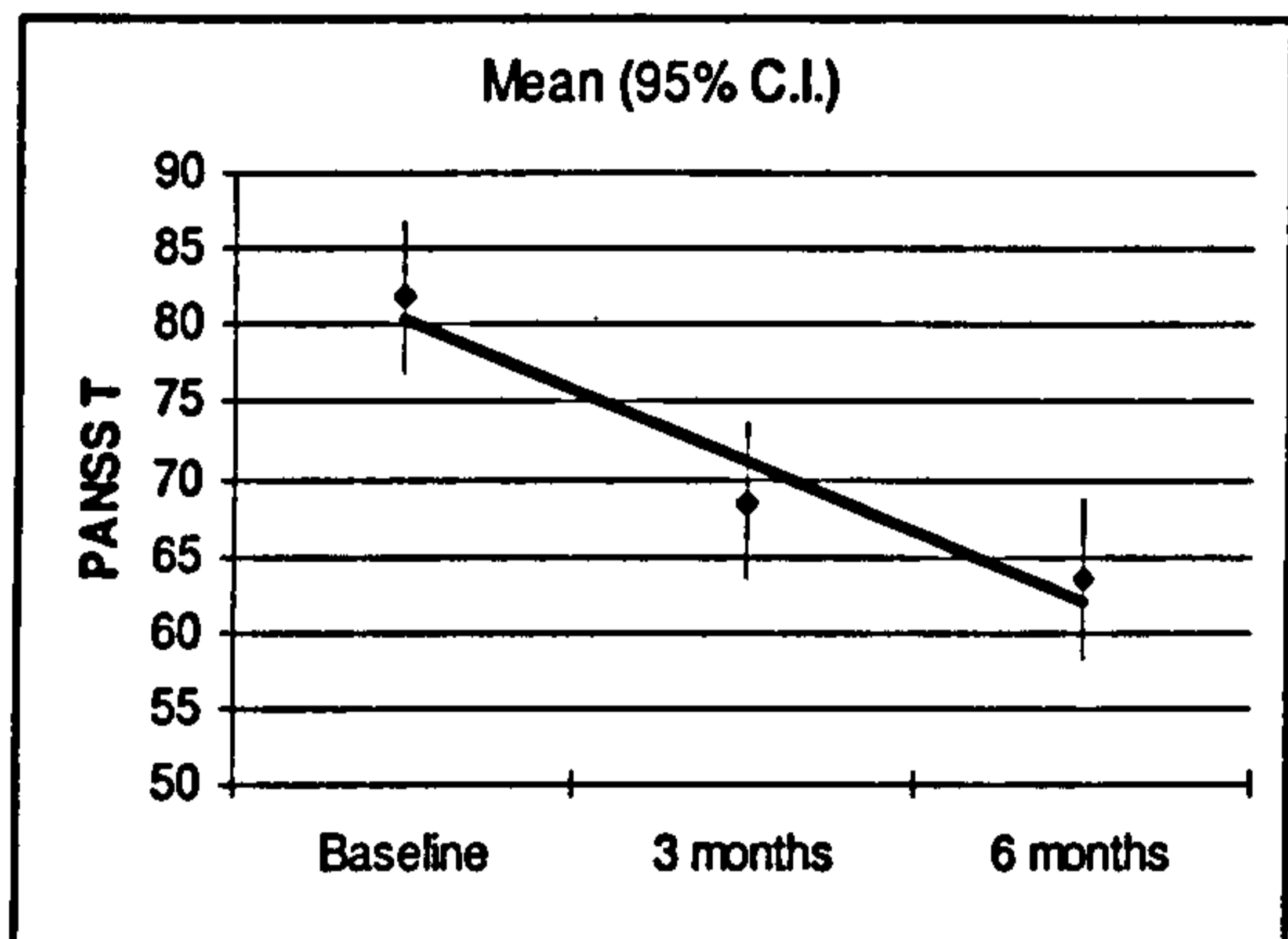
Table 3.2. Clinical ratings from baseline, 3 and 6 months assessments for subjects completing the study.

	<b>Baseline</b> <b>N=28</b>	<b>3 months</b> <b>n=24</b>	<b>6 months</b> <b>n=28</b>	<b>F statistic</b>	<b>p value</b>
<b>Amisulpride dose</b> mean mg/day	0	585 range 300-800	600 range 300-800		
<b>Clinical rating scales mean scores (95% confidence intervals)</b>					
<b>PANSS total</b>	81.78 (76.88 – 86.67)	68.5 (63.35 - 73.65)	63.46 (58.21 - 68.72)	55.11	<0.0001
PANSS positive	20.7 (19.24 – 22.17)	18.67 (16.71 - 20.63)	15.82 (13.98 - 17.66)	24.18	<0.0001
PANSS negative	22.07 (18.95 – 25.2)	18.46 (15.37 - 21.55)	16.89 (14.18 - 19.61)	42.94	<0.0001
PANSS general	39 (36.94 – 41.06)	31.38 (29 - 33.75)	30.75 (28.22 - 33.28)	33.95	<0.0001
<b>SANS total</b>	<b>42</b> <b>(34.39 - 49.61)</b>	<b>32.13</b> <b>(25.13 - 39.13)</b>	<b>27.56</b> <b>(20.67 - 34.44)</b>	<b>30.40</b>	<b>&lt;0.0001</b>
SANS blunting	14.68 (11.95 - 17.4)	12.36 (8.83 - 15.89)	10.56 (7.5 - 13.61)	9.93	0.0003
SANS alogia	5.11 (3.17 - 7.04)	4.24 (2.88 - 5.6)	3.44 (2.22 - 4.67)	3.68	0.0324
SANS avolition	8.15 (6.48 - 9.81)	5.96 (4.38 - 7.54)	4.52 (3.32 - 5.71)	20.46	<0.0001
SANS anhedonia	11.56 (9.63 - 13.48)	9 (6.97 - 11.03)	7.37 (5.29 - 9.45)	25.16	<0.0001
SANS attention	2.7 (1.48 - 3.92)	2.24 (1.08 - 3.4)	1.67 (0.65 - 2.68)	5.05	0.0102
<b>BPRS</b>	30.18 (28.30 - 32.06)	22.32 (19.98 – 24.66)	19.59 (16.68 - 22.50)	41.47	<0.0001
<b>GAS</b>	36.25 (32.31 – 40.18)	40.6 (36.29 – 44.91)	45.74 (41.09 – 50.39)	26.77	<0.0001
<b>Calgary depression</b>	4.26 (2.86 – 5.66)	2.87 (1.49 – 4.26)	3.65 (2.18 – 5.13)	2.17	0.1256
<b>Calgary anxiety</b>	4.08 (2.89 – 5.26)	2.79 (1.70 – 3.88)	3.27 (2.12 – 4.41)	3.17	0.0511

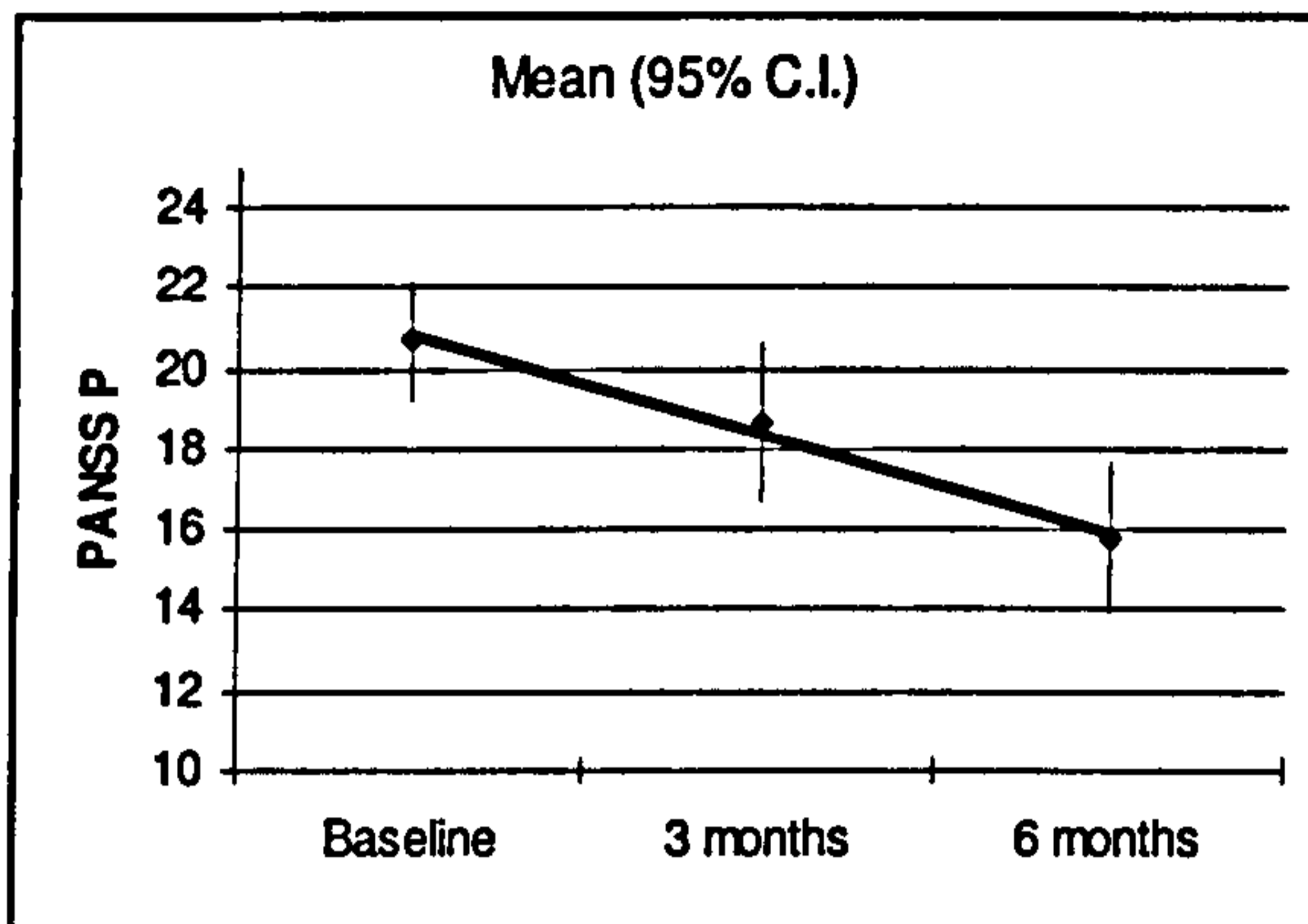
Note: A decrease in score indicates a clinical improvement for all scales except GAS

Figure 3.1. Graphs of clinical rating scales, at baseline and follow-up, with error bars.

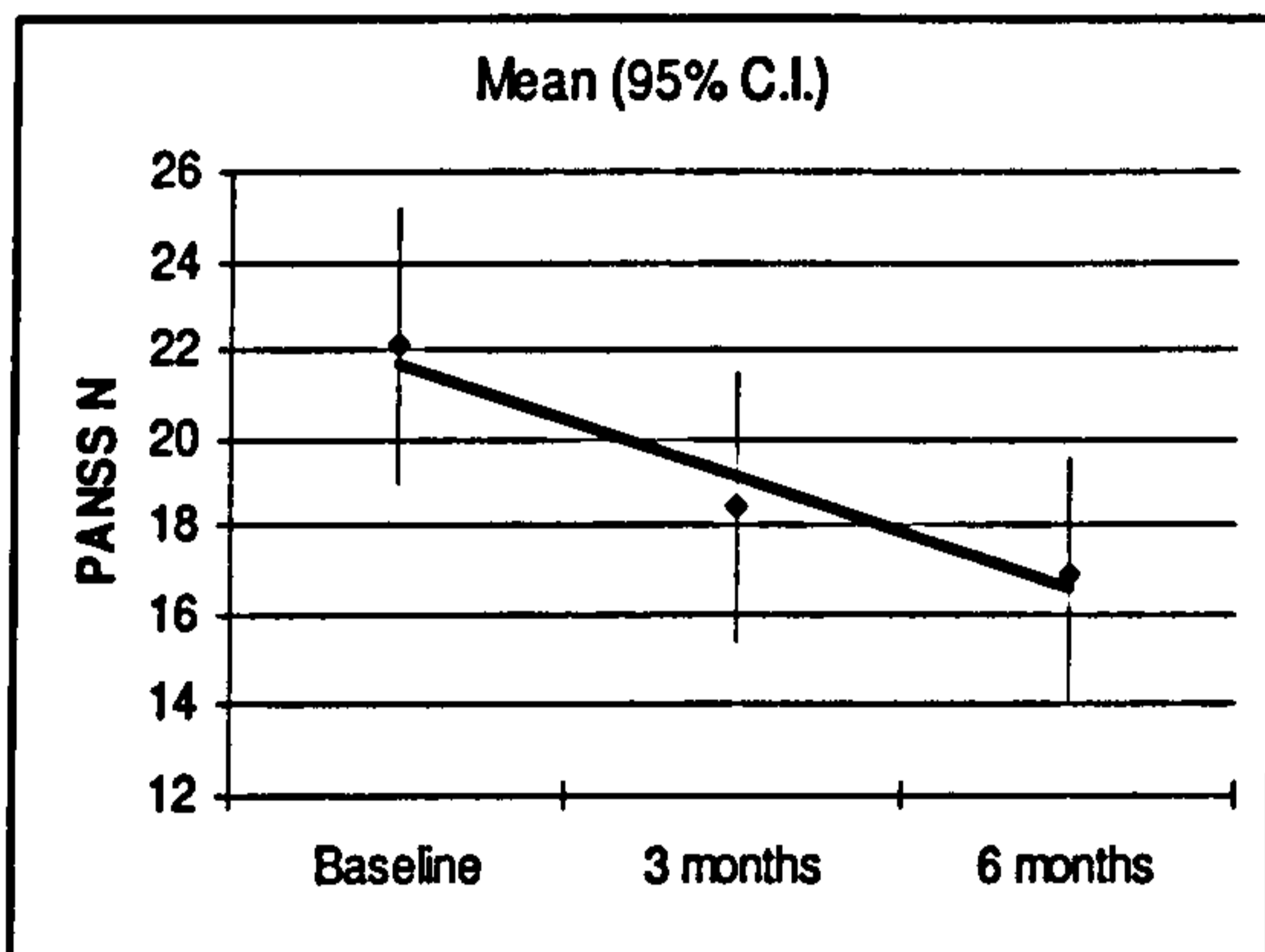
i) PANSS total



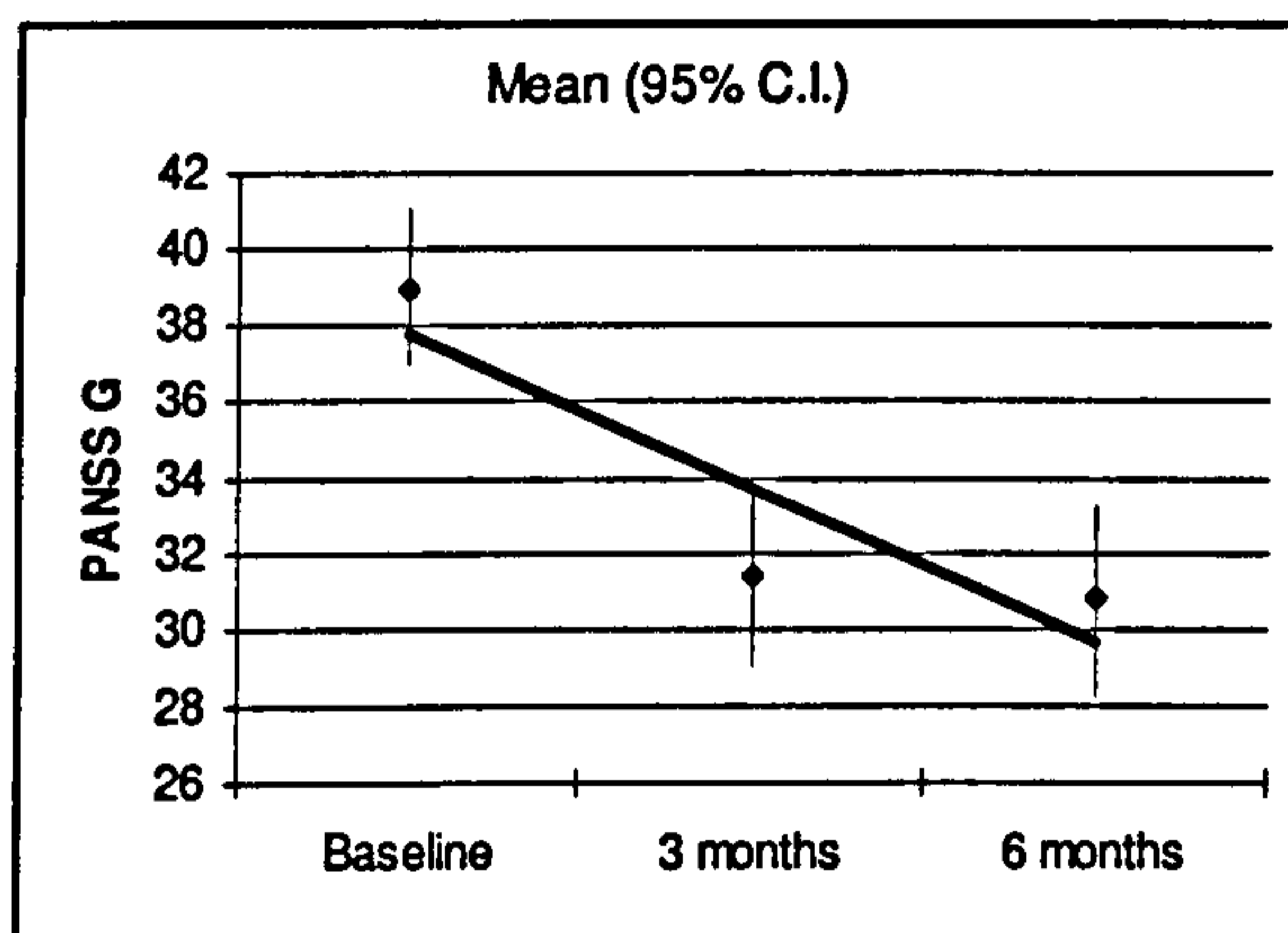
ii) PANSS positive symptoms



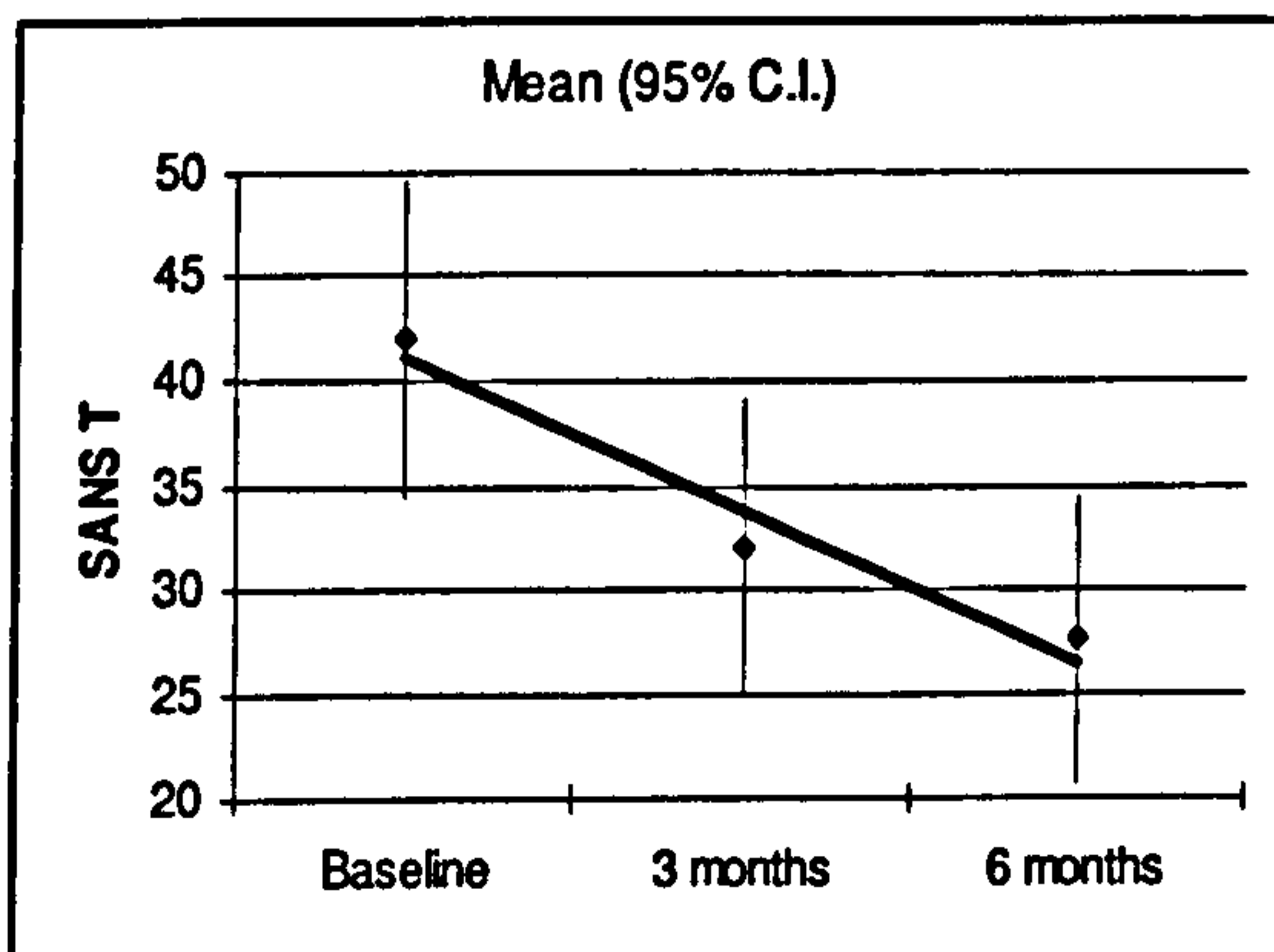
iii) PANSS negative symptoms



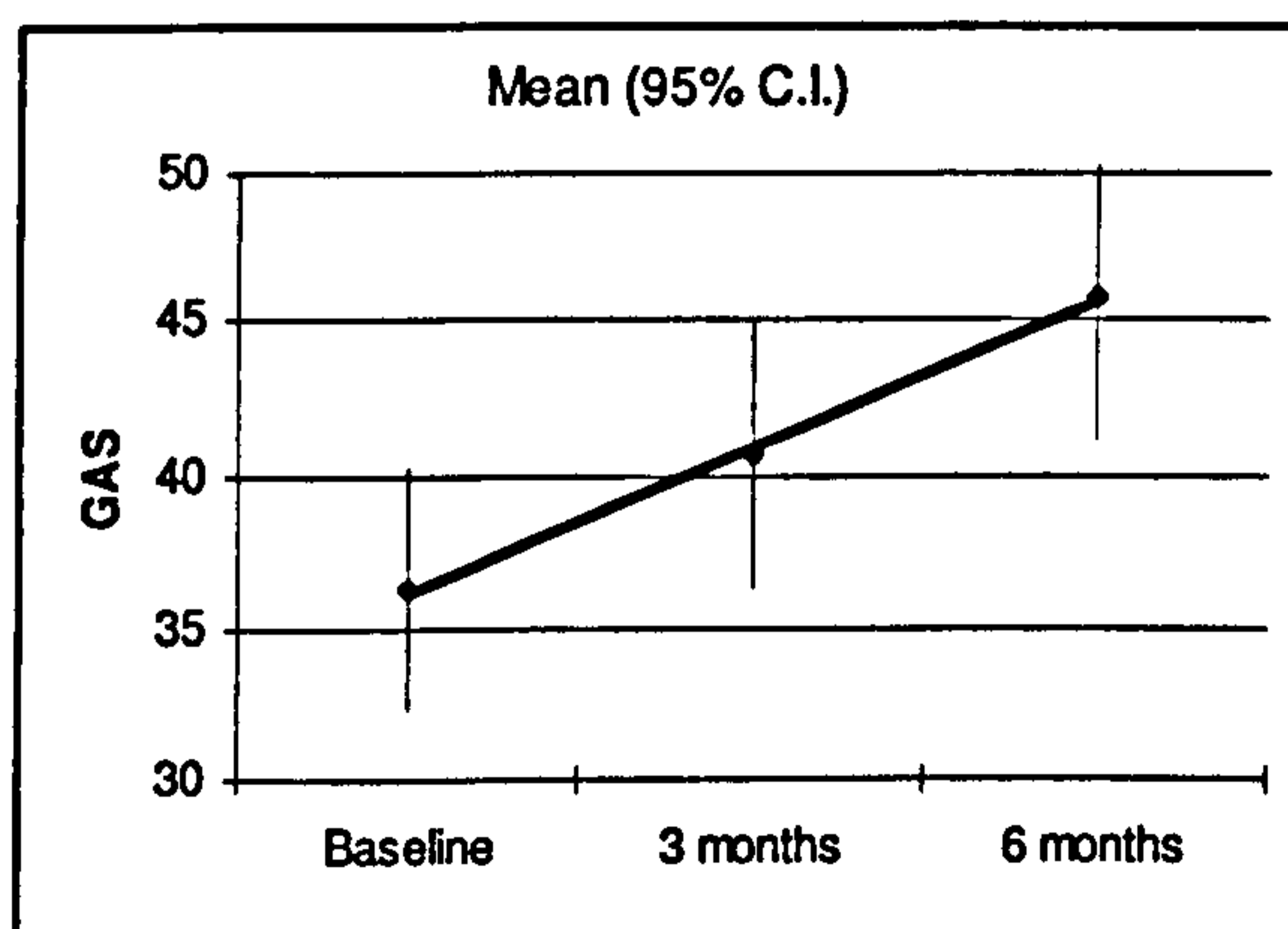
iv) PANSS general subscore



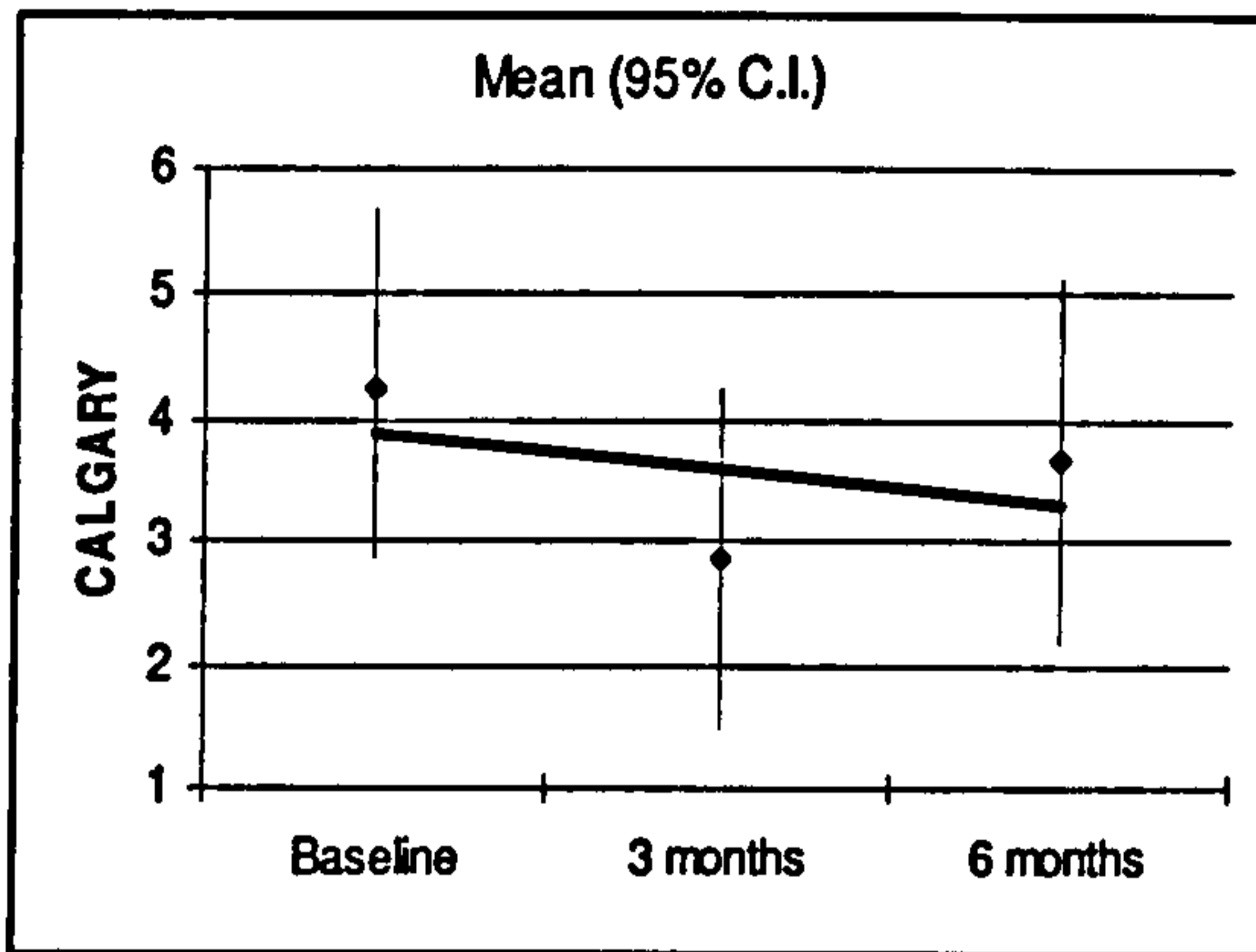
v) SANS total



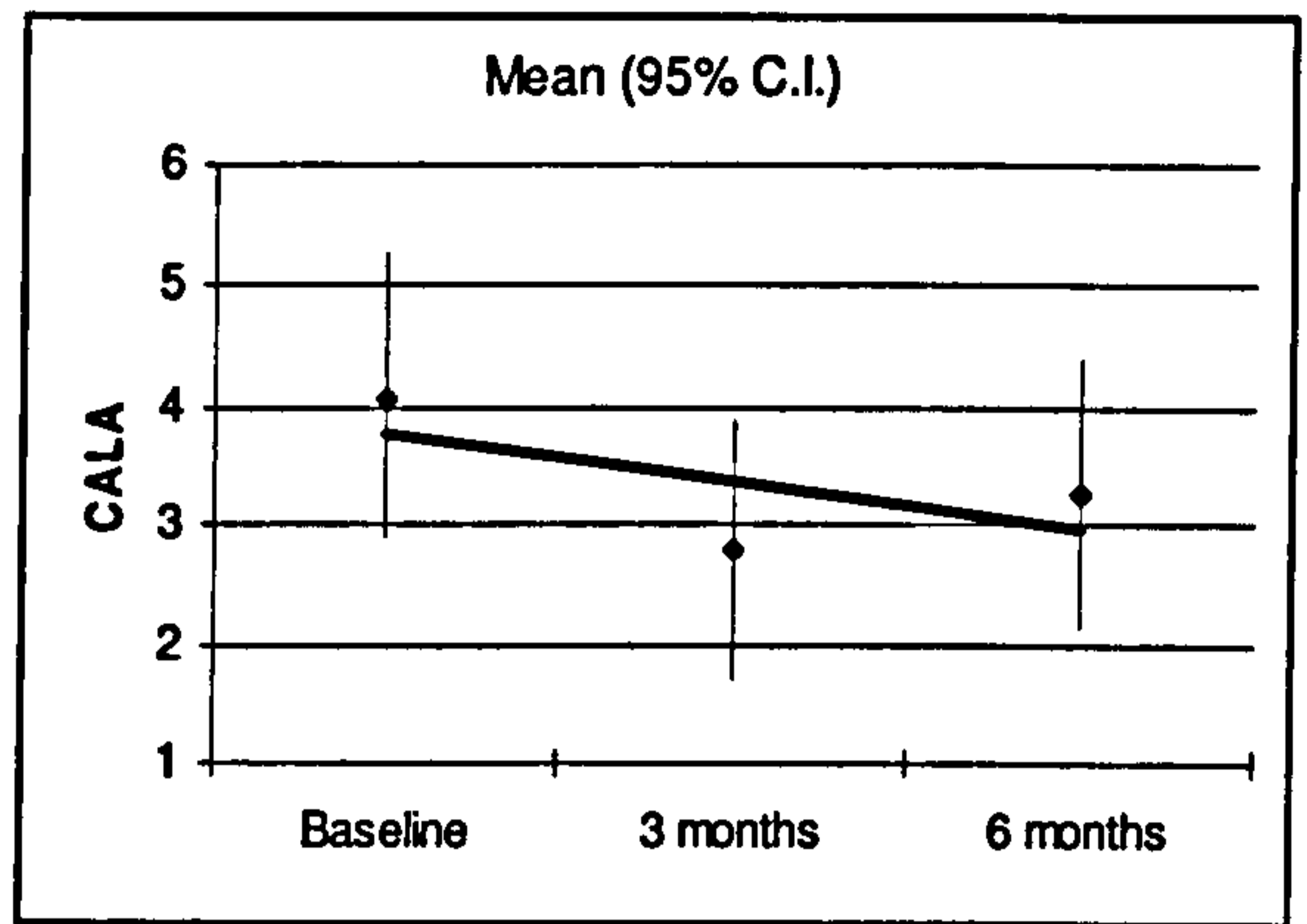
vi) Global assessment of symptoms \*



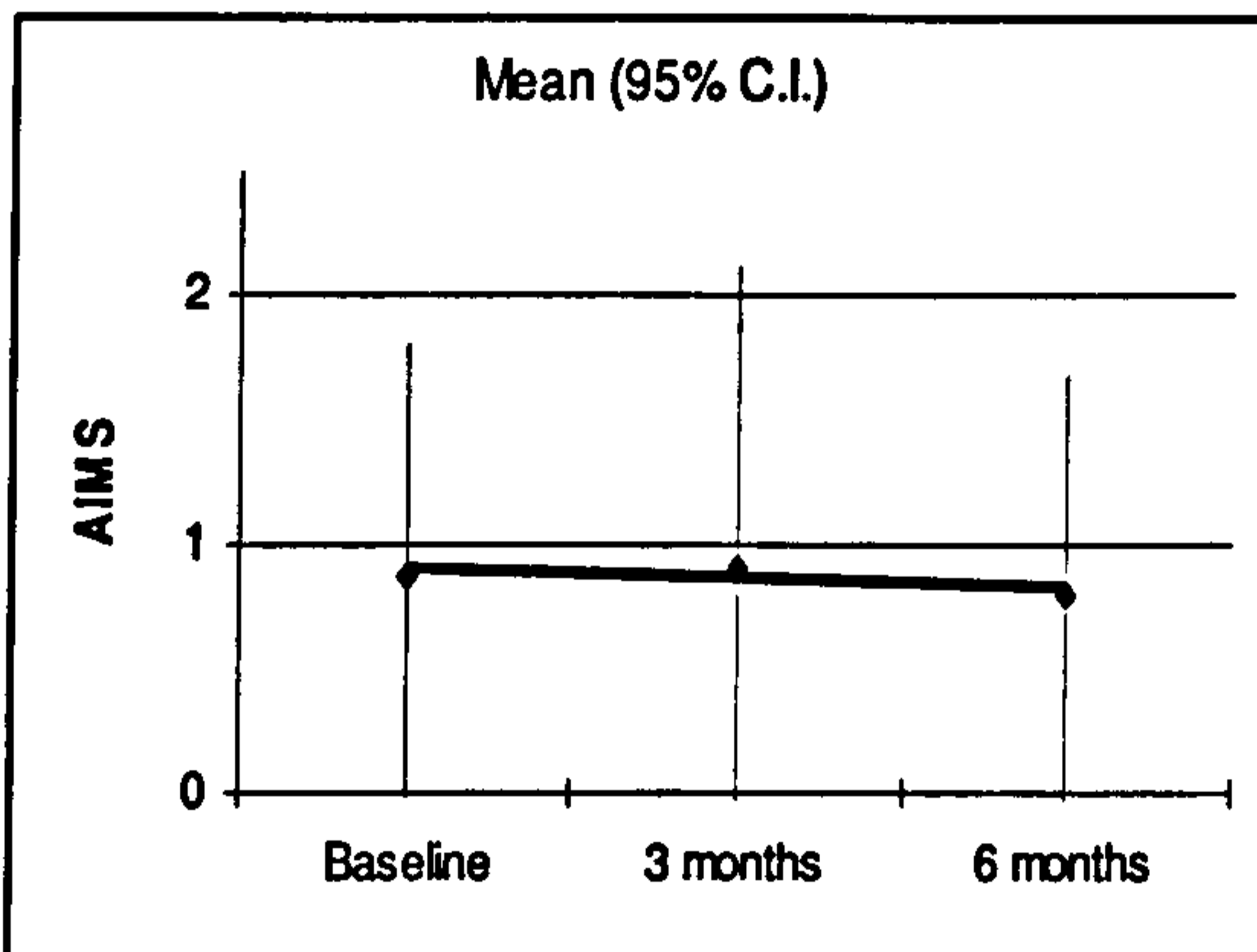
vi) Calgary depression scale



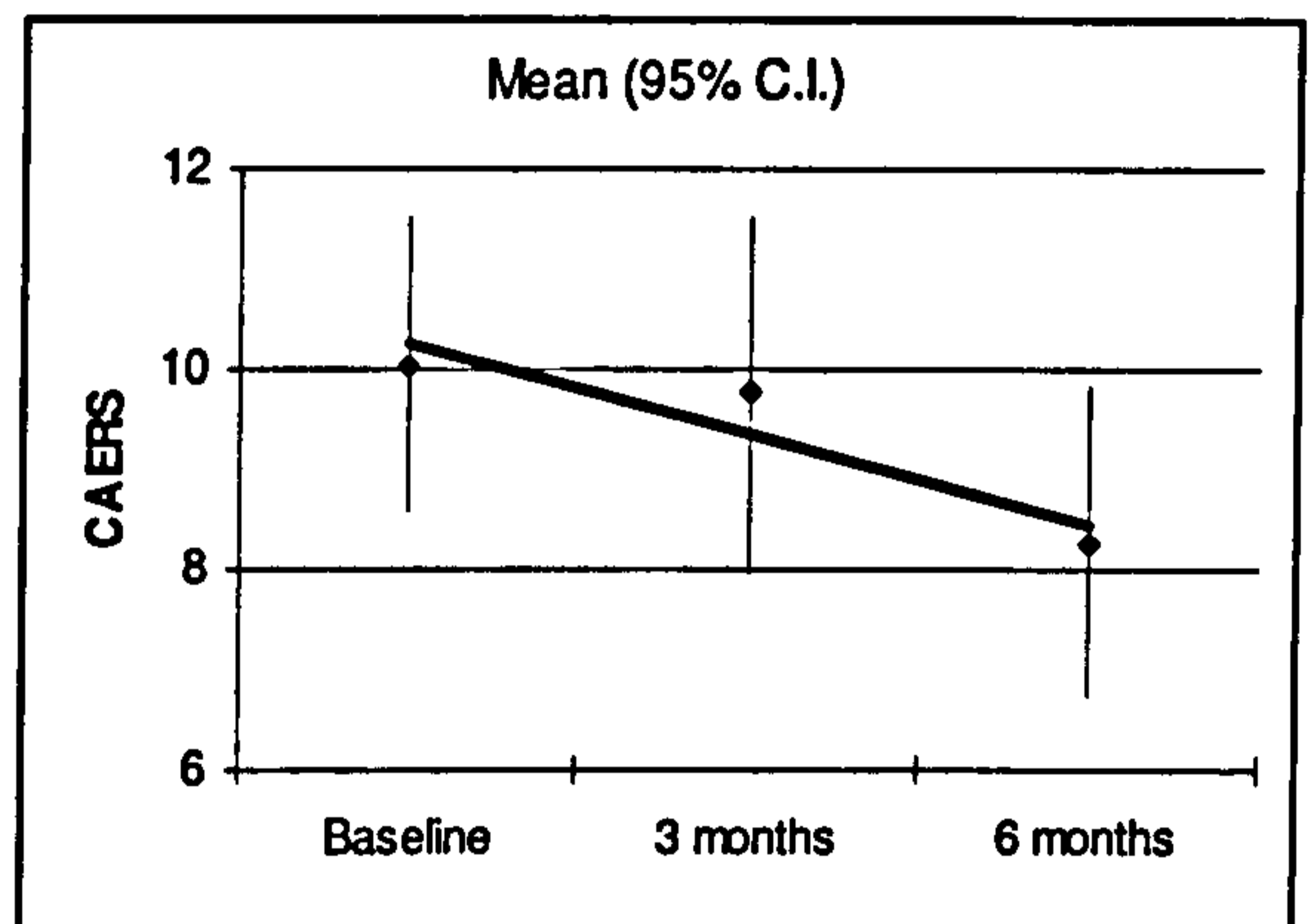
viii) Calgary anxiety scale



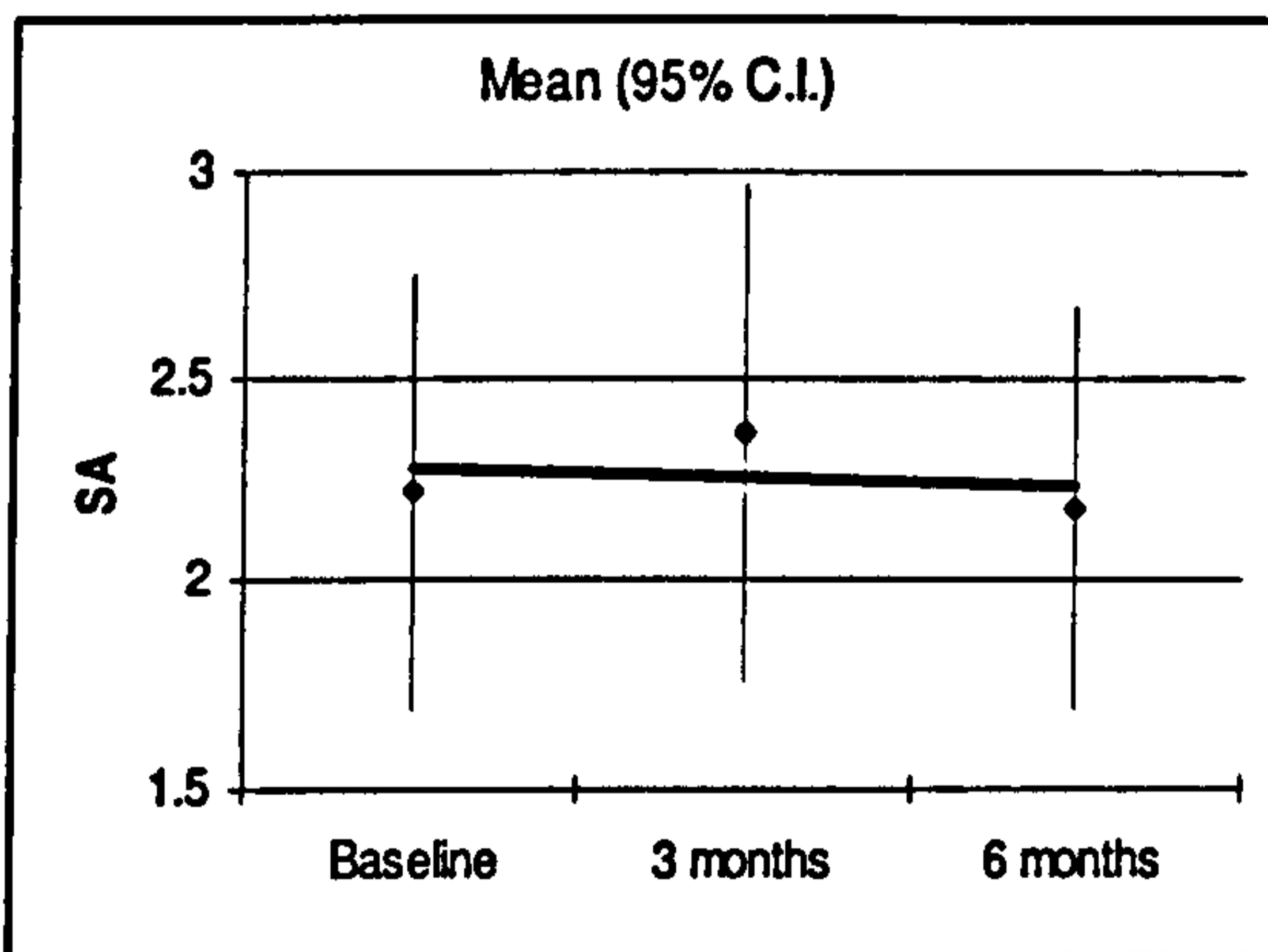
ix) AIMS



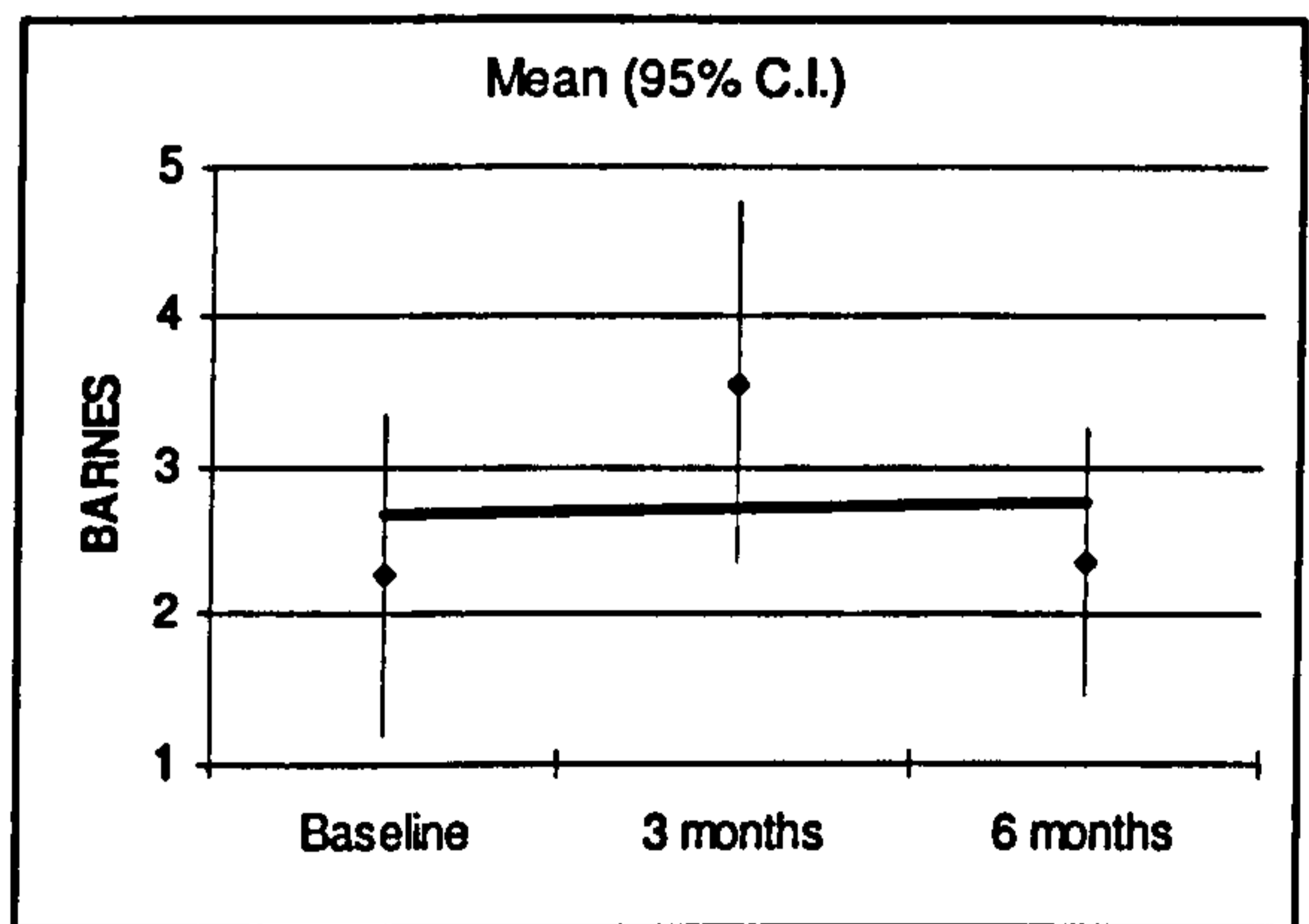
X) CAERS



XI) Simpson & Angus scale



XII) Barnes akathisia scale



\* For all other scales a decrease in score indicated a clinical improvement. For GAS an increase in score indicated an improvement.

### 3.4.3. Response Rate

Using the pre-determined response criteria (>20% reduction in BPRS score), of the 28 subjects completing the 6 months study, 20 (71%) responded to co-administration of amisulpride (Table 3.3). This represented a response rate of 61% in the total cohort of 33 subjects who commenced the study, including those who dropped out. 15 subjects (46% total cohort) responded in terms of positive symptoms and 17 (52% total cohort) in terms of negative symptoms, as defined by a greater than 20% reduction in PANSS positive or negative subscale scores. When more stringent response criteria of >50% reduction in BPRS score were used, 9 (32%) responded or 27% of those starting the study.

*Table 3.3. Response rate of patients completing the study, according to 20% and 50% improvement in BPRS, and positive and negative PANSS subscales.*

	Number of patients responding to treatment (%)				Mean improvement in scores (range)
	n=28				
	20% response rate		50% response rate		
	3 months	6 months	3 months	6 months	
BPRS	15 (53.6)	20 (71.4)	2 (7)	9 (32)	35.4 (-25.9-81.5)
PANSS pos Symptoms	8 (28.6)	15 (53.6)	0 (0)	2 (7)	24.3 (-27.3-50)
PANSS neg Symptoms	8 (28.6)	17 (60.7)	1 (3.6)	2 (7)	25.2 (-6.25-50)

### 3.4.4. Drop-outs

5 subjects (15%) failed to complete 6 months of amisulpride treatment. The demographic and baseline clinical characteristics did not differ significantly between the subjects completing the study (n=28) and those who dropped out. An attempt was made to interview study drop-outs 6 months after baseline. 4 subjects consented to at least a part of a follow up interview.

Table 3.4. Baseline demographic and treatment characteristics of drop-outs.

<b>Age mean years</b>	32.4 (SD 14.0, range 19-52)
<b>Sex</b>	
Male	5 (100%)
Female	0 (0%)
<b>Ethnicity</b>	
Caucasian	2 (40%)
African-Caribbean	0 (0%)
Asian	3 (60%)
<b>In/out patient status at baseline</b>	
In-patient	3 (60%)
Out-patient	2 (20%)
<b>Clozapine dose mean mg/day</b>	580(sd 152.5, range 400-800))
<b>S-clozapine (ng/L)</b>	0.99(sd 0.56, range 0.34-1.62)
<b>Concomitant psychotropic medication</b>	
None	4 (80%)
Tricyclic antidepressant	0
SSRI antidepressant	0
Other	1 (mood stabiliser) (20%)

Table 3.5. Baseline and 6 month clinical characteristics of drop-outs. Mean (SD, Range)

	<b>Baseline (n=5)</b>	<b>6 months (n=4)</b>
<b>PANSStotal</b>	84.2 (17.2, 65-103)	73 (23.2, 50-104)
<b>PANSSpos</b>	18.4 (5.9, 10-24)	16.5 (5.3, 10-23)
<b>PANSSneg</b>	23.6 (6.5, 13-29)	21.7 (7.7, 11-29)
<b>SANStotal</b>	43.4 (19.5, 16-63)	39.2 (24.7, 10-68)
<b>GAS</b>	35 (15.8, 15-55)	38.3, (20.8, 15-55)
<b>Simpson &amp; Angus</b>	2.4 (1.7, 1-5)	1.5 (1.0, 0-2)
<b>Barnes Akathisia Scale</b>	3.2 (3.1, 0-7)	3.75 (2.6, 0-6)
<b>AIMS</b>	0.2 (0.45, 0-1)	0.25 (0.5, 0-1)
<b>CAERS</b>	9.6 (5.8, 4-16)	7.5 (4.1, 4-12)
<b>Calgary depr. Scale</b>	1.6 (2.1, 0-5)	0.25 (0.5, 0-1)

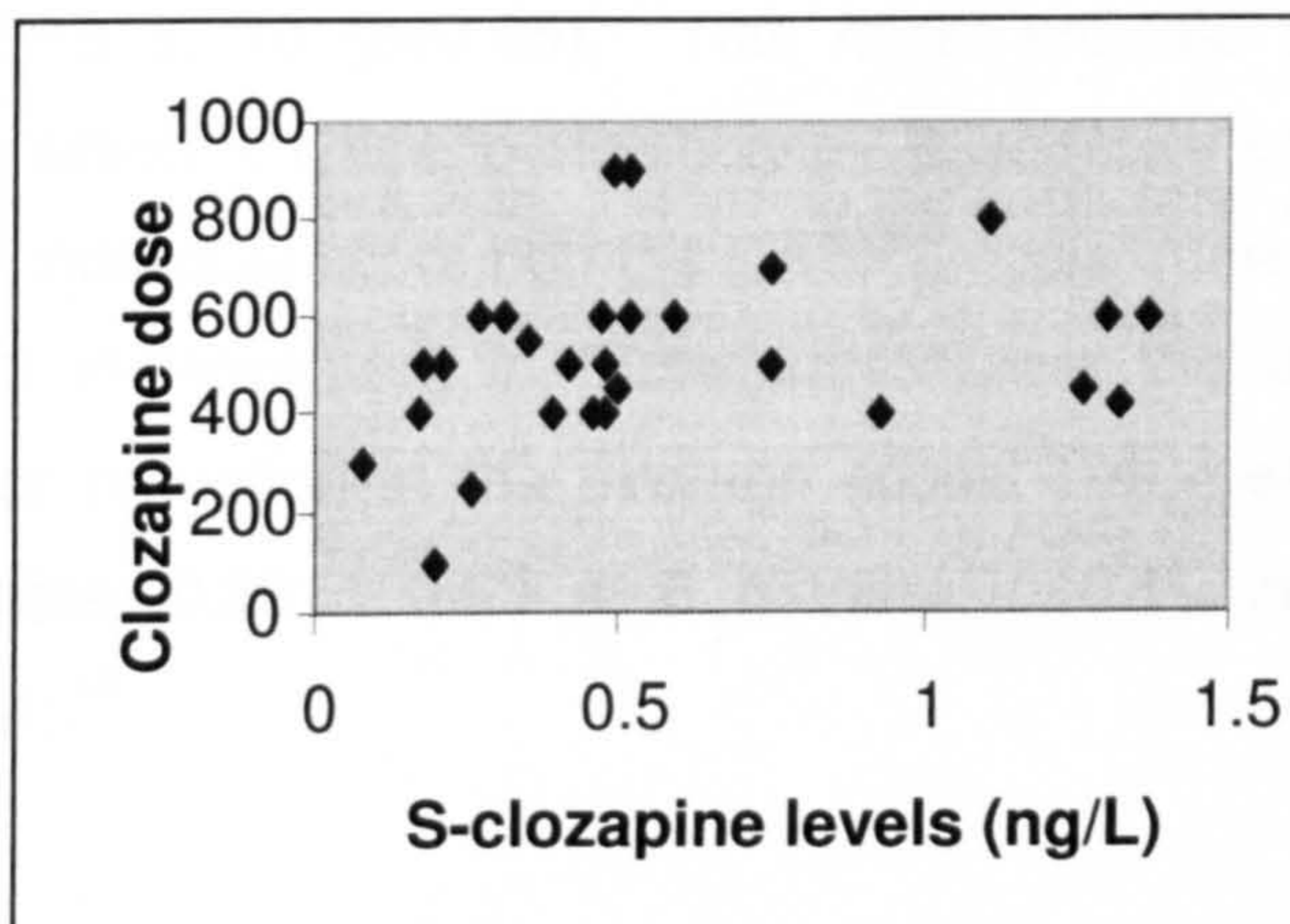
The subjects discontinued treatment for the following reasons: 1 subject agitation; 1 subject stopped all medication; 1 subject reduced clozapine to 100mg/day; 2 subjects , unclear complaints.

Numbers are small so it is hard to draw any definite conclusions but the drop-outs did not seem different in most demographic or clinical characteristics. It is worth noting however that there. There were slightly more inpatients amongst the drop-outs, their plasma clozapine levels were higher and they scored lower on the Calgary depression scale. Asians were under-represented amongst the drop-outs.

### 3.4.5. Clozapine levels and white blood cell counts

The mean serum clozapine levels (0.64 ng/L) were above the recommended minimum therapeutic level (0.35 ng/L), did not vary significantly over time and did not correlate significantly with clozapine dose (Pearson Correlation Coefficient 0.273,  $p=0.160$ ) or amisulpride dose. There was no association between serum clozapine levels and any baseline or demographic variables, or the improvements in clinical rating scales. No changes were observed in white blood cell counts.

*Fig. 3.2. Serum clozapine levels (ng/L) plotted against clozapine dose (mg/day).*



### 3.4.6. Adverse effects

The co-administration of amisulpride in clozapine treated patients did not result in a corresponding increase in side effects over the 6 months period (Table 3.6.). There was a significant increase in prolactin levels over time.

Table 3.6. Side effect ratings at baseline, 3 and 6 months (n=28)

<b>Side-effects rating scales mean scores (95% CI)</b>					
	<b>Baseline</b>	<b>3 months</b>	<b>6 months</b>	<b>F statistic</b>	<b>p value</b>
<b>Serum prolactin</b> <i>mean mg/l</i>	248 (182 - 314)	1376 (881 - 1872)	1025 (739 - 1311)	36.41	<0.0001
<b>Simpson Angus</b>	2.22 (1.69 – 2.75)	2.36 (1.75 – 2.97)	2.18 (1.69 – 2.67)	0.24	0.78
<b>Barnes Akathisia</b>	2.26 (1.17 – 3.35)	3.54 (2.33 - 4.76)	2.35 (1.43 – 3.26)	1.81	0.17
<b>AIMS</b>	.88 (-.01 – 1.8)	.92 (-.28 – 2.11)	.81 (-.07 – 1.68 )	1.48	0.24
<b>CAERS</b>	10.04 (8.57 – 11.51)	9.75 (7.97 – 11.52)	8.27 (6.73 – 9.81)	2.70	0.07

There was a significant correlation, as expected, between the GAS and all PANSS subscale scores and also between the Calgary Anxiety Scale score and the CAERS score ( $p < 0.05$ ). This might indicate that the reduction in CAERS seen which is close to significance is because of reduced anxiety. An exploration of individual scores on the CAERS scale interestingly showed that a non-significant improvement in “drowsiness” was the item most commonly responsible for reduction in the average score. The mean score was  $1.14 \pm 0.59$  at baseline,  $0.89 \pm 0.69$  at 6 months ( $p = 0.15$  in a paired two-tailed Students t-test).<sup>16</sup>

<sup>16</sup> The score for drowsiness on the CAERS scale is: 0 =drowsiness absent; 1 = mild drowsiness, resistible; 2 = drowsiness, irresistible; 3 = asleep most of day.

### 3.4.7. Quality of life

Due to difficulties in maintaining the attention of this very unwell group of individuals for a sufficient period, data was lacking for some patients from the Lancashire Quality of Life Questionnaire (LQoLP) (it was administered at the end of the scoring session and is quite a lengthy questionnaire which some patients refused to complete). In total, information was obtained for at least some parts of the scale for 21 out of the 28 responders who completed the study.

On the LQoLP, when only patients who had scores at baseline and 6 months were included, there were no significant differences between scores at start and at 6 months, either on the total score or on any of the subscales.

*Table 3.7. Lancashire Quality of Life results at baseline and 6 months, paired scores (\*= $p < 0.05$  comparing baseline and 6 month score with paired t-test).*

LQoLP (n)	Baseline mean (SD)	6 months mean (SD)
Aggregate score (21)	4.46 (1.09)	4.41 (0.56)
General well-being (20)	4.27 (1.71)	4.8 (1.21)
Work (16)	3.99 (1.74)	3.84 (1.41)
Leisure (17)	4.51 (1.03)	5.15 (0.84)
Religion (16)	5.03 (1.43)	4.56 (1.21)
Finances (16)	3.5 (2.1)	3.69 (1.75)
Living/accommod.(16)	4.33 (1.21)	4.38 (1.29)
Legal/safety (15)	4.63 (1.36)	4.63 (1.45)
Family relationships (15)	4.93 (1.18)	5.1 (1.33)
Social relationships (15)	4.63 (1.03)	4.6 (1.04)
Health (15)	4.4 (1.67)	4.8 (1.13)

When results at baseline were “pooled” and compared to results at 6 months, a significant improvement was seen in the “leisure” subscale.



Table 3.8. Lancashire Quality of Life results at baseline and 6 months, pooled scores (\*= $p < 0.05$  comparing baseline and 6 month score with unpaired t-test, except in the aggregate scores where paired t-test was used).

<b>LQoL subscales</b>	<b>Baseline mean (n)(SD)</b>	<b>6 months mean (n)(SD)</b>
<b>Aggregate score</b>	4.46 (21) (1.09)	4.41 (21) (0.56)
<b>General well-being</b>	4.40 (21) (1.77)	4.8 (20) (0.41)
<b>Work</b>	3.71 (18) (1.83)	3.84 (16) (1.41)
<b>Leisure</b>	4.47 (19) (1.1)	5.15 (17) (0.84)*
<b>Religion</b>	4.72 (18) (1.64)	4.56 (16) (1.21)
<b>Finances</b>	3.19 (19) (2.16)	3.69 (16) (1.75)
<b>Living/accommodation</b>	4.27 (19) (1.43)	4.38 (16) (1.29)
<b>Legal/safety</b>	4.88 (19) (1.38)	4.63 (15) (1.45)
<b>Family relationships</b>	4.89 (19) (1.32)	5.1 (15) (1.33)
<b>Social relationships</b>	4.47 (18) (0.94)	4.6 (15) (1.04)
<b>Health</b>	4.13 (19) (1.58)	4.8 (15) (1.13)

The risk of type 1 error is great with these multiple comparisons, however. When a Bonferroni correction<sup>17</sup> is used to adjust for this risk, all findings are non-significant.

### 3.5. Discussion

This study provided an opportunity, in a naturalistic setting, to investigate the hypothesis that those patients partially or non-responsive to clozapine treatment would show an improvement in mental state when clozapine was augmented with amisulpride. The results indicate that the combination of clozapine and amisulpride is both effective and well tolerated.

<sup>17</sup> The Bonferroni correction adjusts for the risk that multiple tests of statistical difference will find a difference where none exists. The correction is  $\alpha / N$ , where  $\alpha$  = statistical significance level,  $N$  = independent hypotheses on a set of data. Here the p-value should be set at  $0.05 / 11 = 0.0045$ .

### **3.5.1. Clinical efficacy**

Clozapine is an effective treatment for positive symptoms. It is controversial whether it affects negative symptoms and recent well designed studies do not support this (Buchanan et al. 1998; Kane et al. 2001; Tuunainen et al. 2002). Additionally there remains controversy as to whether an effect on primary (more persistent / “core”) or secondary negative symptoms is principally responsible when an effect is reported (Carpenter et al. 1995; Javitt 2001). Amisulpride has proven efficacy in both positive and negative symptoms of schizophrenia (Freeman 1997; Leucht et al. 2002b). In this study, the significant improvement in all ratings of negative symptoms, without changes in side-effect ratings, suggested that amisulpride was effective against more persistent negative symptoms, which often prove particularly difficult to treat in treatment-resistant schizophrenia. There was some, although not total, overlap between the cohort of patients who responded in terms of positive symptoms and those who improved in the negative subscale scores. It is possible that there are some individual patient factors which pre-determine good treatment response in one or more symptom domains. This individual variance may be genetically determined (see chapter 5.1.2), with some combinations of receptor polymorphisms increasing the likelihood of a good treatment response (Collier et al. 2001).

When 50% improvement on BPRS total was used to define response, 32% of those who completed the study met those criteria. The improvement took a long time to materialise, with only 7% meeting the response criteria at 3 months. The improvement was also mainly due to general psychopathology scores with only 7% meeting the 50% response criteria on the positive and negative subscales of PANSS, which is interesting. It indicates that although there is a solid, clinically noticeable improvement in positive and negative symptoms, the main cause for more dramatic improvement may be changes in the general psychopathology subscale which includes items such as anxiety, depression, ambivalence and insight.

The number of patients (32%) who meet the 50% improvement criteria indicates a robust response and it is interesting to compare it to the response rate in the Shiloh et al. study (1997). In that study a 20% improvement threshold was used to define response, but the mean improvement in that study amongst responders (who were half of those treated) was actually 50% and 42% for BPRS and SANS respectively.

### **3.5.2. Adverse effects and tolerability**

The addition of amisulpride to clozapine did not result in a worsening of adverse drug effects (other than an increase in serum prolactin levels). In particular there was no aggravation of movement disorders. Despite the increase in serum prolactin levels, no clinical manifestations of raised prolactin levels were noted. Studies have found a rate of endocrine side-effects of approximately 4% (Colonna et al. 2000). Given the number of patients in this study and the fact that the majority of patients were males it is therefore not surprising that no clinical signs of prolactin rise were reported. At 6 months there was a non-significant reduction in serum prolactin from 1376 to 1025 mg/L. This is in line with the findings that a continuous decline in prolactin occurs over time following an initial spike (Schlosser et al. 2002; Bressan 2004).

The addition of amisulpride was well tolerated and the rate of drop-outs less than expected. The majority of patients (25/28) expressed a wish to continue with amisulpride, in addition to clozapine, at the end of the 6 months study period. 1 subject wanted to stop amisulpride and 2 were ambivalent about future treatment.

### **3.5.3. Quality of life changes**

Quality of life has in the last decade attracted more attention in evaluating the success of treatment in schizophrenia. The concept refers to the individual's general well-being, but no universally agreed definition exists (Fernandez-Ballestos 1998). Quality of life is used to represent a broad range of dimensions

both connected to psychological well-being and behavioural functioning. Within the remit of psychopharmacology this would encompass both medical and non-medical aspects of life, such as a sense of well-being, social and occupational functioning, financial status and feeling safe. Apart from the effect changes in the severity of illness can have on these factors, medication causes side-effects that can influence quality of life. There is some evidence that increased D<sub>2</sub> receptor occupancy is related to lower scores on the 'Subjective Well-being under Neuroleptic treatment scale' (SWN) (De Haan 2005). The questionnaire used in this study; The Lancashire Quality of Life Profile (LQoLP), has been widely validated (Oliver et al. 1997; Gaite et al. 2000; van Nieuwenhuizen et al. 2001). It has been found particularly useful in chronic patients (Meijer et al. 2002), although not everyone has found it helpful (Ritsner et al. 2002).

The LQoLP is affected by many factors, including an individual's pre-morbid level of functioning, expectations of the individual and his environment, insight etc. The results presented here indicate that the addition of another medication did not make a significant difference to quality of life. This may be due to the nature of morbidity in patients included in this study. Most of the patients were severely disabled by their illness, many in hospital or residential care. Factors that affect people's quality of life, particularly in that situation, may not be amenable to change during the relatively short period of observation here.

#### **3.5.4. Limitations**

The limitations of this study include those inherent upon a naturalistic investigation. Although the cohort was small, the rate of drop-outs was low, and the improvement in clinical ratings highly significant. The cohort was followed up over a 6 month period, which was sufficient for response to be evaluated, especially given reports of a rapid response in augmentation with a similar high potency D<sub>2</sub>-like blocker (Shiloh et al. 1997). However, the risk / benefit evaluation of combining the two antipsychotics may require a longer period of investigation to fully assess side effects and long-term risk, but combination antipsychotic therapy has been correlated with reduced survival in patients

(Waddington et al. 1998). A longer follow-up would likely have increased the rate of drop-outs however.

The sample was fairly homogenous as patients were all treatment-resistant (treated with at least two antipsychotics from different classes in an adequate dose for an adequate length of time before starting clozapine) and were confirmed as having residual symptoms on clozapine (18 point BPRS<sub>(0-6)</sub> score of at least 25). However, patients who had failed to respond to clozapine at all, may have been withdrawn from treatment by the clinical teams, and thus resulted in a cohort of, at worst, partial clozapine responders. It is acknowledged that the time taken to respond to clozapine for some individuals may be up to, or beyond, a year. Our minimum requirement of at least 6 months in clozapine therapy may thus have included some late responders. Additionally, there was no protocol driven attempt to encourage clozapine response by increasing the clozapine dose prior to study recruitment. Thus, it could be argued that some patients may have been on sub-optimal doses, irrespective of serum clozapine levels, and responded to an increase in the overall level of antipsychotic prescribed. The clinicians were adamant on every occasion that clozapine was being given at the highest feasible dose, but a further investigation of the level of response to optimal clozapine monotherapy prior to augmentation is merited.

It has been pointed out that improvement following the addition of drug B to drug A doesn't necessarily mean that the combination works. It is also possible that drug B alone could cause improvement (Stahl 2002). This study does not address the possibility that amisulpride might on its own do what clozapine has failed to do. A small (n=7) eight week open-label trial described treatment-resistant patients switched from various antipsychotics to amisulpride (Kontaxakis et al. 2005). All clinical ratings were significantly reduced (PANSS total reduced from 123.4 to 65.7 points, PANSS positive from 34.5 to 16.2 points and PANSS negative from 32.4 to 20.1 points). In the study it is unclear however whether the patients were truly treatment-resistant, as clozapine had not been tried. A recent meta-analysis indicates that when compared with a typical antipsychotic, clozapine is more effective than amisulpride (Davis et al.

2003). That study does not directly compare the effectiveness of clozapine and amisulpride however, and the patients were not necessarily treatment-resistant. The role of amisulpride on it's own in treatment-resistant schizophrenia needs further investigation.

The exacerbation of extrapyramidal side-effects was a primary consideration in the rating of adverse drug effects in this study. Adverse effects in atypical antipsychotics are different and the CAERS scale used in this study attempts to some extent to address those. It may be that in future studies more specific tools could be used as they become available.

### **3.6. Conclusions**

In a chronically unwell group of patients non-responsive to clozapine monotherapy, augmentation with amisulpride significantly reduced the symptoms in the majority of patients. The improvement was seen both in positive and negative symptoms of schizophrenia as well as general functioning. The augmentation was well tolerated with no increase in side-effects.

# **Chapter 4. Amisulpride augmentation of clozapine and striatal dopamine D<sub>2</sub> receptor binding potential. A <sup>123</sup>I-IBZM SPET study (Study 2)**

## **4.1 Introduction**

In the previous chapter it was shown that augmenting clozapine treatment with amisulpride can be clinically helpful in patients partially or not responding to clozapine on its own (Munro et al. 2004). Other studies confirm these findings and indicate that in particular using as augmenting agent a medication with high D<sub>2</sub> receptor binding can be helpful (Agelink et al. 2004; Zink et al. 2004b; George et al. 2005; Kampf et al. 2005; Lerner et al. 2005). The explanation for this observed improvement is unclear but one suggested rationale is that augmentation works through increased Dopamine D<sub>2</sub> blockade (Freudenreich et al. 2002). Other explanations for the mechanism of augmentation exist, but this is the one most likely to be relevant when explaining the effect of antipsychotics that mainly block D<sub>2</sub>/D<sub>3</sub> (such as amisulpride and sulpiride) (Stahl 2002).

It has been postulated that a certain threshold of D<sub>2</sub>-occupancy is needed for antipsychotics to be effective. Work with PET supports this by showing that less than 55 – 60 % striatal D<sub>2</sub> occupancy is associated with poorer clinical response to typical antipsychotics (Nordstrom et al. 1993b; Kapur et al. 1996; Remington et al. 1998). There seems to be a therapeutic “band” as raised prolactin and especially extrapyramidal side-effects become a problem once a threshold of approximately 80% D<sub>2</sub> blockade is reached (Farde et al. 1989; Kapur et al. 2000). Clozapine is unique in having a clinical effect at D<sub>2</sub> occupancy levels that tend to be lower (38-63%) than those needed in most other antipsychotics for a therapeutic response (Farde et al. 1992). However, it is possible that in some patients not responding to clozapine increasing D<sub>2</sub> binding to the “therapeutic threshold” would be beneficial.

A recent paper reports that amisulpride (as well as risperidone), binds

preferentially to D<sub>2</sub>-like receptors in the head of caudate compared to the putamen (Stone et al. 2005). The nigro-striatal pathway converges on the putamen while the mesolimbic dopamine pathway connects to the ventral striatum and associative regions connect to the caudate nucleus generally (Joel et al. 1997). The intrastriatal selectivity observed by Stone et al. is suggested as an additional mechanism by which antipsychotics could convey therapeutic benefits while avoiding side-effects. Whether this selectivity plays a part in the response seen with augmentation strategies is unclear and it is interesting to explore whether regional selectivity is enhanced by the augmentation.

Hardly any imaging studies of clozapine augmentation have been reported. Kapur et al. showed that augmenting clozapine with haloperidol increases striatal D<sub>2</sub> occupancy, but the clinical response was not reported (Kapur et al. 2001b).

## **4.2. Aims of the study**

In an attempt to clarify why clozapine augmentation may be helpful D<sub>2</sub>-like occupancy was quantified before and after augmentation with amisulpride, a selective D<sub>2</sub>-like antagonist. This was done: Firstly, to investigate whether augmentation leads to increased D<sub>2</sub>-like receptor occupancy; Secondly, to see whether a “threshold effect” exists in that an increase in D<sub>2</sub>-like blockade above approximately 60% striatal blockade is associated with a response; Thirdly to see whether there is a regional difference in binding within the basal ganglia with increased binding; Fourthly to see whether subjects with low (<50%) striatal D<sub>2</sub>-like occupancy on clozapine alone, will show an enhanced clinical response to amisulpride in comparison to patients with a higher (>50%) striatal D<sub>2</sub>-like occupancy on clozapine alone. Finally to see whether changes in D<sub>2</sub>-like binding correlate with changes in clinical state as an association has been shown in some studies between increased D<sub>2</sub>-like binding and clinical response (Volk et al. 1994), especially in terms of positive symptoms (Lavalaye et al. 1999; Abi-Dargham 2004).



### 4.3. Methods and Materials

Ethics and ARSAC approval for the study was obtained. Following a full description of the study to the subjects, written informed consent was obtained. All subjects received a payment equivalent to 8 times the minimum national hourly wage for participation in each scan.

#### 4.3.1. Subjects

Patients who were partially responsive to clozapine were recruited as part of a larger study (Munro et al. 2004). The inclusion and exclusion criteria were described in chapter 2.2.2.

A group of 10 healthy controls (8 males, 2 females, mean age =35.8 years, race: 7 Caucasian; 2 Asian; 1 African-Caribbean subject), subject to the same exclusion criteria were recruited through personal contacts. Each had one IBZM SPET scan. An attempt was made to match patients and controls for sex and age. (Table 4.1)

*Table 4. 1. Demographic variables of subject group completing two scans and healthy controls.*

Group	Number (male/female)	Mean age	Race		
			African- Caribbean	Asian	Caucasian
Patients	7 (5/2)	33.6 ± 7.9	1	2	4
Controls	10 (8/2)	35.8 ± 7.2	1	2	7

#### 4.3.2. Study design

33 patients with sub-optimal response to clozapine were commenced on amisulpride in addition to clozapine. 10 of these patients (8 males, 2 females), agreed to participate in the imaging study and immediately prior to starting

amisulpride they had a  $^{123}\text{I}$ -IBZM SPET scan. After 10-12 weeks on the augmentation regime, 8 of these patients had a second IBZM SPET scan as well as a clinical assessment. The 2 patients who dropped out did not do so for side-effect related reasons and their profile was similar to those who stayed in the study. A third patient was excluded from analysis due to confirmed non-compliance with medication. The 7 patients left in the study (5 males, 2 females) had a mean age of 33.1 years  $\pm$  8.6 yrs. There were 4 Caucasian, 2 Asian and one African-Caribbean subject. The mean dose of clozapine = 562 mg  $\pm$  226mg, mean s-clozapine levels = 0.79  $\pm$  0.45 ng/L, mean s-clozapine at 3 months = 0.95 $\pm$ 0.53 ng/L (paired Student's t-test comparing s-clozapine at baseline and scan 2 at 3 months was non-significant; p=0.23). Mean dose of amisulpride at 2<sup>nd</sup> scan = 583mg  $\pm$  160mg.

### **4.3.3. Clinical measures**

Clinical status was evaluated at the time of both scans using the Positive And Negative Syndrome Scale (PANSS), Scale for the Assessment of Negative Symptoms (SANS), Global Assessment Scale (GAS), Calgary Depression Scale, Calgary Anxiety Scale and various side effect rating scales.

### **4.3.4. Image and statistical analysis**

The image analysis was performed as described in chapters 2.5 and 2.6.

Comparison between occupancy values in the basal ganglia, head of caudate and putamen was made before and after augmentation using paired Student's t-test. Clinical comparison was made using paired Student's t-test. Tests were two-tailed and p<0.05 considered significant.

## **4.4. Results**

### **4.4.1. D<sub>2</sub> receptor occupancy**

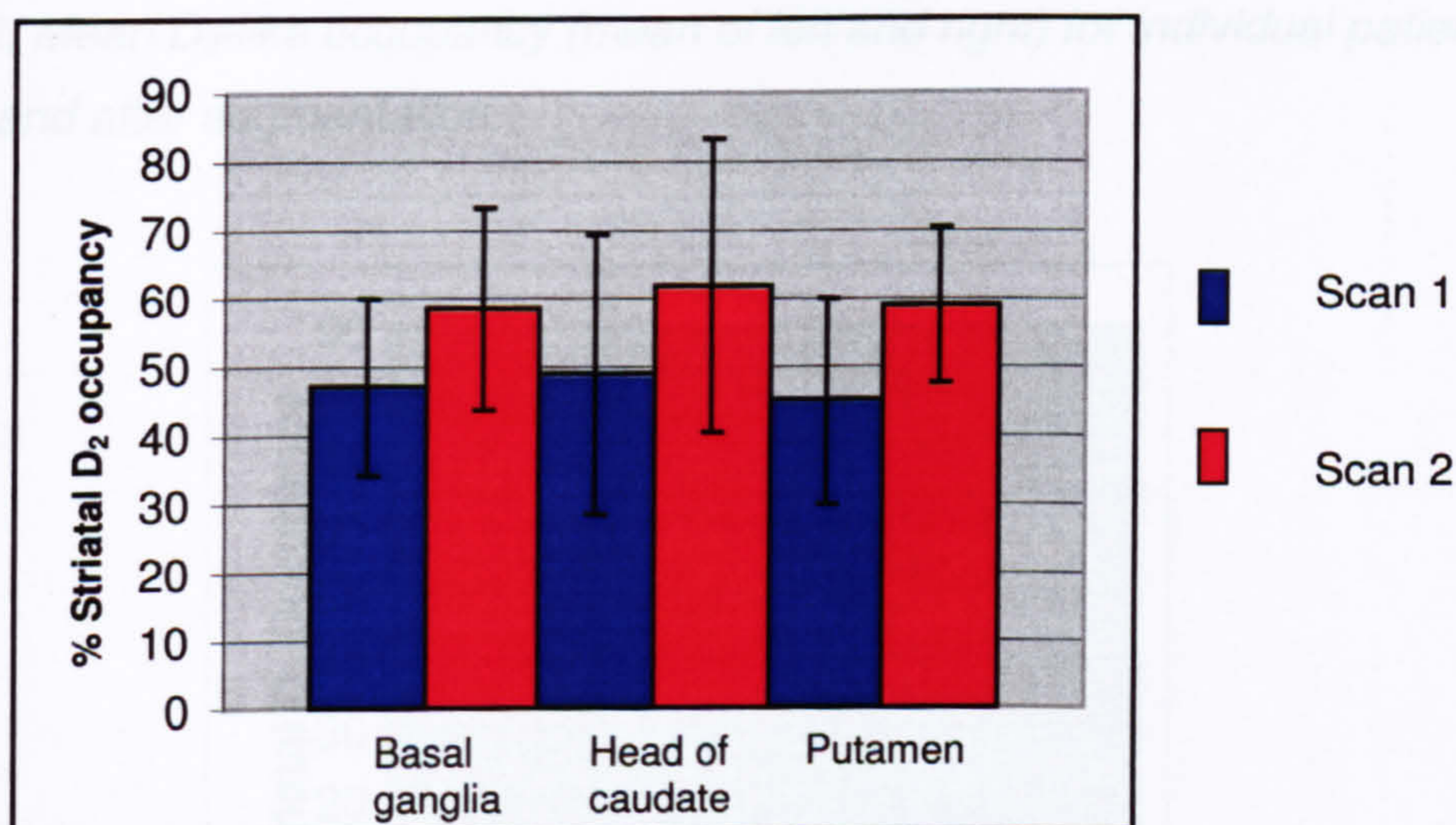
The mean striatal Dopamine D<sub>2</sub>-like receptor binding potential in the basal

ganglia of controls was 0.85. The mean D<sub>2</sub>-like binding occupancy in patients at baseline was 46.97% ( $\pm$ 12.84% (scan 1). 10-12 weeks later the mean occupancy value was 58.65% ( $\pm$ 14.72%) (scan 2) (p=0.007) (Table 4.2). Individual patients showed increased occupancy in each case but inter-patient variation was great (Table 4.3) (Fig.4.1).

*Table 4.2. Dopamine D<sub>2</sub> % occupancy in patients before (scan 1) and after (scan 2) augmentation of clozapine with amisulpride (SD=standard deviation).*

Area	Occupancy % scan 1 (SD)	Occupancy % scan 2 (SD)	p
Basal ganglia	46.97 (12.84)	58.65 (14.72)	=0.007
Head of caudate	49.21(20.49)	62.02 (21.38)	<0.05
Putamen	45.14 (15.03)	59.01 (11.33)	=0.02

*Fig. 4.1. Dopamine D<sub>2</sub>-like % occupancy in patients before (scan 1) and after (scan 2) augmentation of clozapine with amisulpride.*



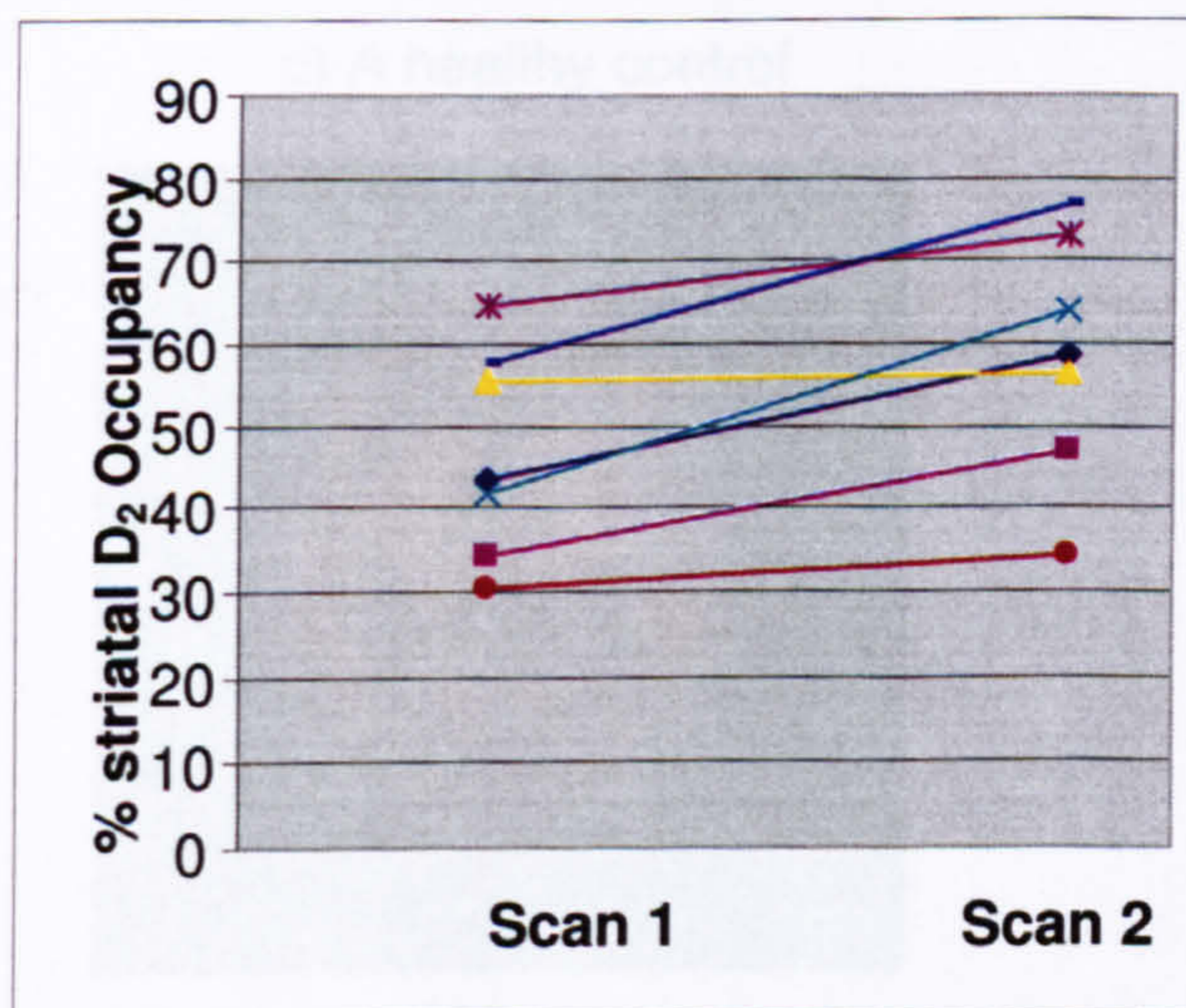
The D<sub>2</sub>-like occupancy rate of two separate ROIs within the basal ganglia (head of caudate and putamen) was measured (Table 4.2). The changes in occupancy rate between scans 1 and 2 mirrored those seen for the whole basal

ganglia. Occupancy on the left, compared with the right basal ganglia was also compared for each group and no difference seen. No difference was seen either for the putamen or head of caudate between the right and left.

*Table 4.3. Individual % occupancy rates; scan 1 and scan 2.*

Patient No.	Scan 1			Scan 2		
	Left BG	Right BG	Mean BG	Left BG	Right BG	Mean BG
1	54.89	61.10	57.99	80.85	72.57	76.71
2	40.59	46.60	43.60	55.71	61.97	58.84
3	32.10	36.35	34.22	47.20	46.90	47.05
4	51.74	59.68	55.71	55.31	57.63	56.47
5	48.17	35.90	42.04	57.56	59.95	63.80
6	60.62	69.03	64.83	67.35	79.34	73.35
7	33.75	27.06	30.41	30.06	38.56	34.31

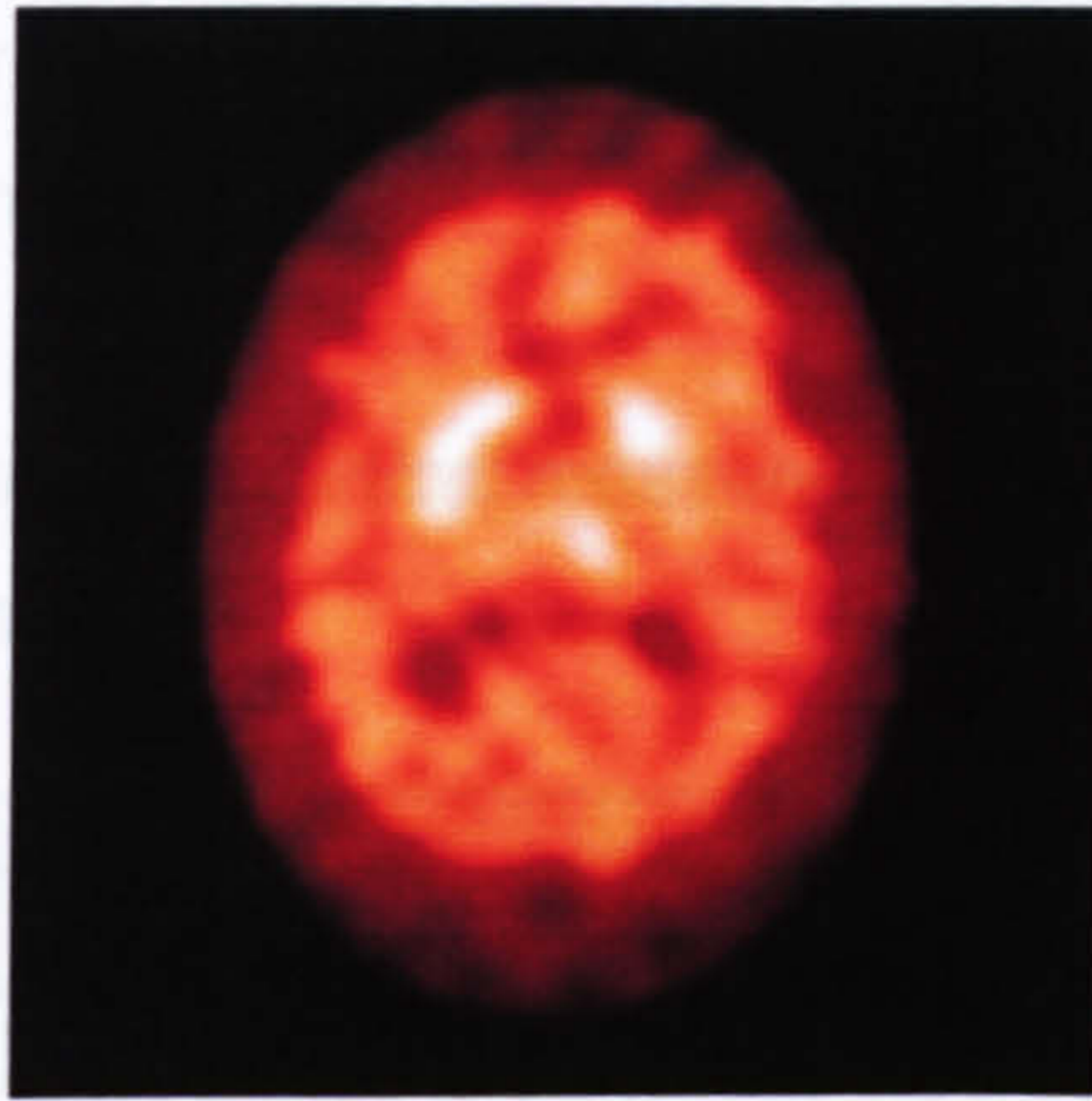
*Fig. 4.2. Mean D<sub>2</sub>-like occupancy (mean of left and right) for individual patients, before and after augmentation.*



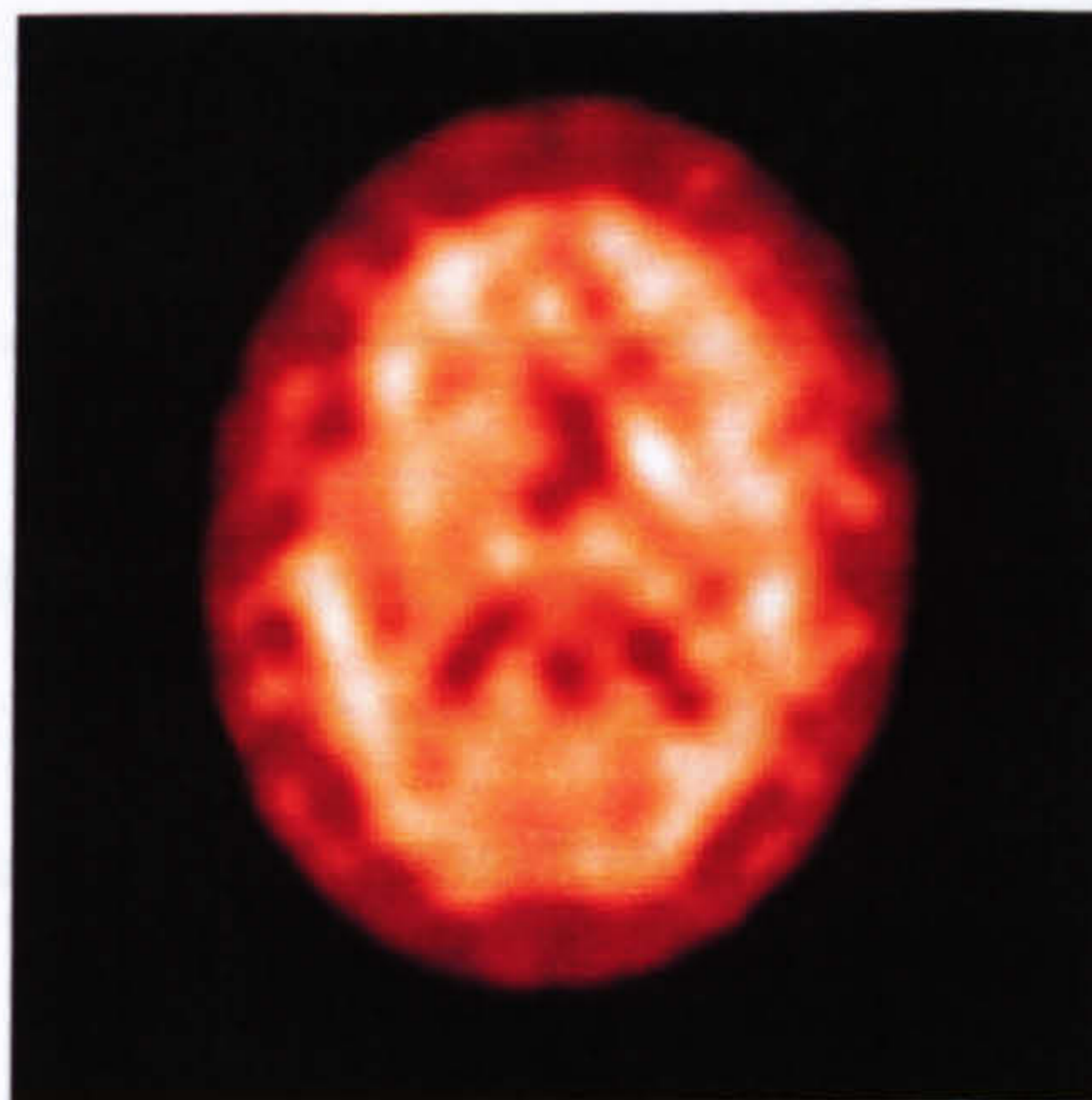
#### 4.4.2. Clinical rating scales

Fig. 4.3. A SPET image for patient 2: a) at scan 1, b) at scan 2, c) A SPET image from a healthy control.

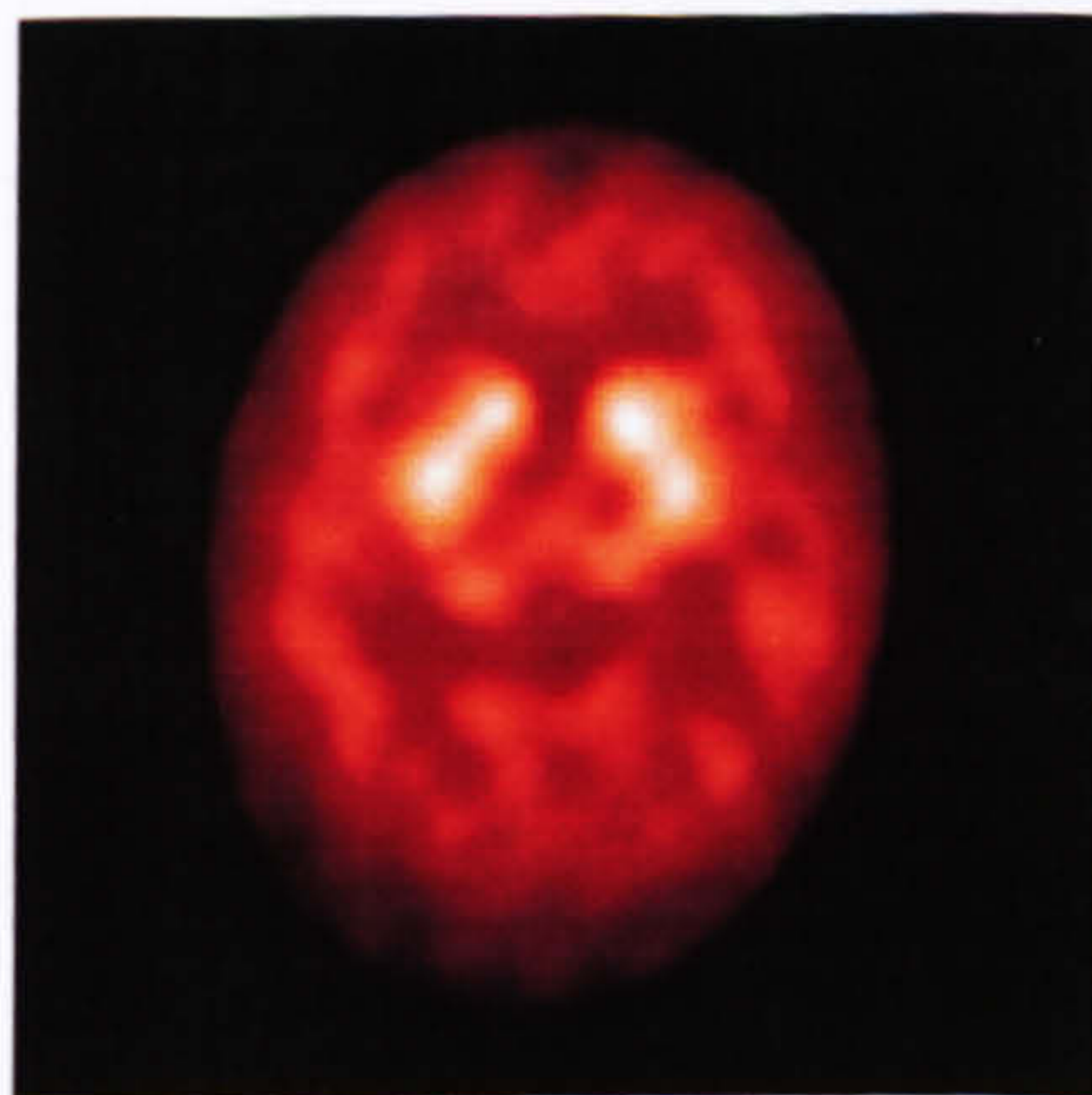
a) Patient 2, scan 1



b) Patient 2, scan 2



c) A healthy control



#### 4.4.2. Clinical rating scales

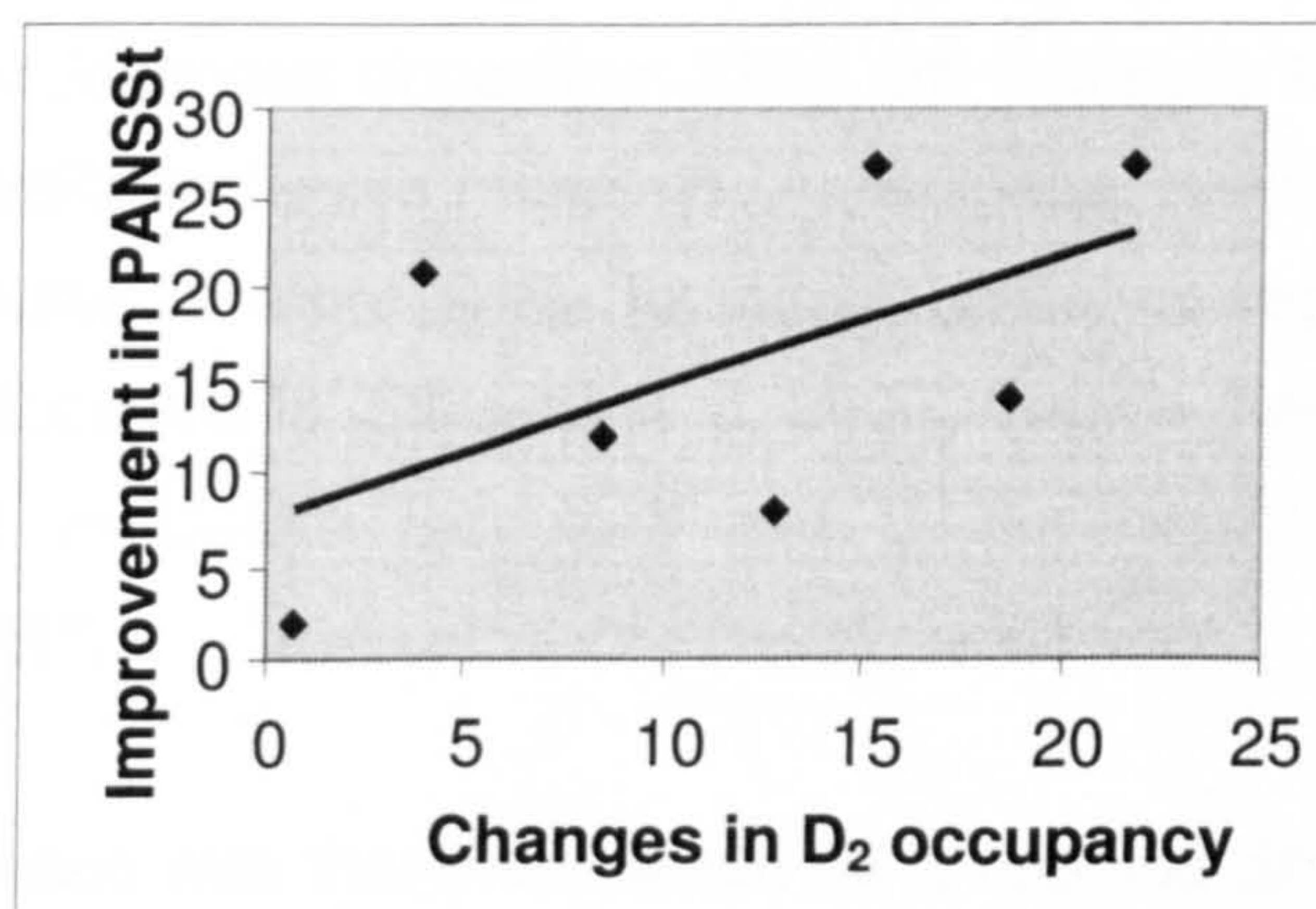
A significant improvement was seen in the patients on the PANSS (total, positive and negative subscales) rating scales (Table 4.4.), which corresponds with the findings for the larger group which were described in chapter 3 above. The same was seen for BPRS.

Table 4.4. Improvement in clinical measures before and after augmentation (SD=standard deviation).

Rating scale	Score at scan 1 (SD)	Score at scan 2 (SD)	P
PANSS total	78.6 (11.7)	67.0 (10.8)	<0.001
PANSS pos	20.0 (2.9)	17.6 (3.0)	<0.05
PANSS neg	20.9 (7.4)	16.7 (6.0)	=0.02
BPRS <sub>(0-6)</sub>	29.7 (3.8)	22.1 (3.6)	<0.001

The change in D<sub>2</sub>-like occupancy rate was calculated but although a trend was seen, a significant correlation was not found between the amount of change in D<sub>2</sub>-like occupancy and improvement in clinical parameters (Fig. 4.4).

Fig. 4. 4. Changes in D<sub>2</sub>-like occupancy (x-axis) versus improvement in PANSS total (y-axis).



## 4.5. Discussion

This study explored what happens in successful augmentation of clozapine with another antipsychotic medication.

The first hypothesis was confirmed. Adding amisulpride to clozapine significantly increases D<sub>2</sub>-like occupancy in the basal ganglia. As amisulpride is a highly selective D<sub>2</sub>-like dopamine receptor blocker this is not surprising.

Regarding the second hypothesis, the findings are not conclusive as to whether a threshold effect can be used to explain the clinical response to augmentation. Average occupancy post augmentation was 59%, which is within the threshold range of 55-60% blockade. Not all patients did however reach the putative threshold. As there is likely to be individual variation in the threshold this is hard to conclude from with certainty.

In this study the average post augmentation D<sub>2</sub>-like occupancy was 59%. This is far from the 75-80% threshold above which extra-pyramidal side effects occur and would explain their non-occurrence in our patients. This relatively low post-augmentation D<sub>2</sub>-like blockade may seem surprising as amisulpride has high affinity for D<sub>2</sub>-like receptors. A SPET study found that a mean amisulpride dose of 400 mg/day caused 56% striatal D<sub>2</sub>-like binding (Bressan et al. 2003a) while a PET study described 70-80% D<sub>2</sub>-like blockade at amisulpride doses ranging from 630 – 910 mg /day (Martinot et al. 1996). This is likely to be caused by amisulpride's much higher affinity for the D<sub>2</sub>-like receptor than both clozapine and endogenous dopamine. They are therefore left to compete for the remaining available D<sub>2</sub>-like receptors. Amisulpride, having more affinity for the D<sub>2</sub>-like receptor is more likely to cause some up-regulation of D<sub>2</sub>-like receptors than clozapine is (Silvestri et al. 2000). Hence there may be a slight overall increase in D<sub>2</sub>-like receptors and D<sub>2</sub>-like receptor occupancy as measured by SPET.

The third hypothesis was that there would be a regional difference in binding within the basal ganglia with increased blockade. No significant changes were

seen with augmentation in the distribution of binding between the head of caudate and the putamen. This is therefore unlikely to be an important factor in the effect of amisulpride augmentation. Minor, non-significant changes were however seen, so the possibility that the study might lack power to demonstrate a difference cannot be ruled out.

In schizophrenia, higher D<sub>2</sub> receptor densities have been reported in the left basal ganglia, especially the left putamen (Farde et al. 1990). Another study indicated higher D<sub>2</sub> receptor densities in the right putamen of patients with schizophrenia, with up to a 40% difference (Reynolds et al. 1987). In this study, no significant difference was seen between the right and left basal ganglia.

The fourth hypothesis was that patients with high (<50%) striatal D<sub>2</sub>-like occupancy would show an enhanced clinical response compared with patients with low (>50%) D<sub>2</sub>-like occupancy. Three patients had high and four low striatal D<sub>2</sub>-like occupancy. The improvement on the PANSS and PANSS positive subscale was not significantly different between the low and high groups. However, the numbers in the study are really too small to answer this question fully.

The final hypothesis was that changes in D<sub>2</sub>-like binding would correlate with changes in clinical symptoms. In this study there was an improvement in both positive and negative symptoms with augmentation which reflects the findings of the larger clinical study (see chapter 3). Groupwise this clinical improvement and increased D<sub>2</sub>-like blockade mirror each other. A connection between increased D<sub>2</sub>-like blockade and positive symptoms has been reported (Lavalaye et al. 1999; Abi-Dargham 2004). The effect on negative symptoms is likely to have another explanation (see chapter 6.3.1.3.).

However, there is a lack of correlation in individual patients between the change in D<sub>2</sub>-like occupancy rate and change in clinical parameters. This may indicate a confounding factor, i.e. the improvement seen may be due to factors not measured in this study. As the study can only directly answer questions relating to dopamine D<sub>2</sub>-like blockade this is a problem inherent in any study



using this particular method of investigation. Different approaches are therefore warranted to gain deeper understanding of the pharmacodynamics of augmentation.

#### **4.5.1. Limitations**

There are obvious limitations to studying this group of patients. Those relating to the cohort are discussed in chapter 3.5.4.

The presence of two women in the treatment-resistant group could influence the findings. Lower affinity for D<sub>2</sub> receptors is seen in women than men but the age-related decline in D<sub>2</sub> binding is similar (Pohjalainen et al. 1998). The difference between the sexes is slight and unlikely to affect the results, especially as only a minority of the subjects were women.

#### **4.6. Conclusions**

The augmentation of clozapine with amisulpride, a selective D<sub>2</sub>-like receptor antagonist leads to increased D<sub>2</sub>-like receptor occupancy in the basal ganglia of patients. A “threshold effect” of 55-60% D<sub>2</sub>-like blockade needed for response seems to exist although the results are not conclusive regarding this. There was no evidence of regional difference in binding within the basal ganglia. An improvement was seen in all clinical parameters.

# **Chapter 5. Investigation into how clozapine responders differ from clozapine-resistant patients. A <sup>123</sup>I-IBZM SPET study (Study 3)**

## **5.1 Introduction**

In this thesis the issue of treatment-resistance to clozapine is explored. One way of trying to understand this phenomenon is to look at the opposite end of the spectrum; at people who are known to respond well to clozapine monotherapy. Exploring characteristics of their response might give valuable insights.

### **5.1.1. Clinical markers of good response to clozapine**

In a retrospective review of 50 treatment-resistant patients (n=50) by Talmon et al. (1995), it was reported that younger patients with recent onset of illness and short total duration of hospitalisation responded better to clozapine. A short duration of hospitalisation is likely to be another marker of less severe illness so that finding isn't surprising, but the study does indicate that more chronic patients respond worse to clozapine. Stern et al. (1994) reported on 40 treatment-resistant patients with schizophrenia. They were given clozapine for 5 weeks. The approximately 30% who showed good response had higher BPRS scores at baseline and larger improvements in BPRS by the end of the first week of treatment, compared to non-responders. Another study of 86 clozapine-treated patients looked at the presence of EEG abnormalities and response to clozapine treatment (Pillay et al. 1996). Overall, there was no difference between the groups with and without EEG irregularities. However two subgroups; females and patients with major depressive episodes had significantly higher GAF (Global Assessment of Functioning) scores when this co-occurred with EEG abnormalities. This study seems to suffer from Type 1 error, as no attempt was made to correct for chance findings. A small study reported on treatment-resistant patients given either risperidone (n=6) or clozapine (n=5) and came to the bold conclusion, given the size of the study,

that men responded better to clozapine, while women responded better to risperidone (Chouinard et al. 1994). A study by Lieberman et al. (1994) started 86 treatment-resistant patients on clozapine and the results indicated that early onset of illness and female sex predicted poorer response while the predictors of good response included shorter duration of illness, a diagnosis of paranoid schizophrenia as well as extrapyramidal side effects during previous treatment with typical antipsychotics. A controlled clinical trial by Pickar et al. (1994) also found that extrapyramidal side effects during previous treatment were a good predictor of positive response to clozapine. A correlation between weight gain and good response was reported in a small study (n=21) (Leadbetter et al. 1992) but a larger study (n=90), following patients up for 90 months, did not see a correlation between weight gain and treatment response (Umbricht et al. 1994). An open study of 40 patients started on clozapine reported that a significant change in BPRS scores at 1 week predicted a response at 5 weeks with 75% accuracy (Stern et al. 1994). A recent open-label prospective study (n=104) compared characteristics of responsive (>30% reduction in PANSS total) and non-responsive (27% of sample) first onset patients at discharge (mean length of stay 44 days) (Ceskova et al. 2005). Responders had significantly higher scores on PANSS total and all subscales on admission. Non-responders showed no reduction in the PANSS negative subscale. A case series published in 1999 indicated that patients who had previously responded to clozapine didn't respond as well when given clozapine again to treat relapse following discontinuation (Grassi et al. 1999).

What can be concluded from these studies is that response to clozapine, although overall better than to other antipsychotics is still variable and certain patient characteristics seem to be associated with a better response. Characteristics connected to better response include a previous history of extrapyramidal side effects, younger age and less chronic illness, while being a woman seems to be associated with a worse response to clozapine treatment.

### **5.1.2. Genetic markers of good response to clozapine**

With the onset of pharmacogenomics, researchers were at last able to move beyond the phenotype and look at the influence of genes on drug response. The benefits hoped for include monitoring enzyme polymorphisms associated with deficient drug metabolism as well as pre-treatment prediction of drug response. Given the large difference in treatment response between individuals this is likely to be a complex trait, influenced by a combination of genes. Research has mainly been in two areas; on the one hand the effect of polymorphisms in metabolic enzymes, affecting drug transformation and elimination and on the other hand the effect of polymorphisms in neurotransmitter receptors targeted by antipsychotic drugs.

Numerous studies have found associations between particular alleles and clozapine response (Arranz et al. 2001; Basile et al. 2002). The problem is that these findings are very often followed by negative studies by different groups, which undermines their credibility and potential clinical usefulness, although various methodological reasons such as sample size and ethnic origin may explain the inconsistency (Arranz et al. 2000a). Several strategies have been employed to address this problem (Nebert 2000).

Additionally, the findings currently reported have small predictive value which further limits their clinical usefulness. As treatment response is likely to be a complex trait, attempts have been made to combine information from several genes to increase their predictive value. A study by Arranz et al. (2000b), showed that combining six mutations on 4 genes could predict response to clozapine to some degree (>78% success). The polymorphisms were in genes coding for three neurotransmitter receptors (H<sub>1</sub>, 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>) and a mutation in a serotonin transporter protein. This work has now been developed into a commercial kit, by the company LGC, to predict clozapine response (LGC 2005). Other studies have been published which still need to be validated but indicate a promising venue for finding predictors of clinical response to clozapine and other antipsychotics.

### **5.1.3. Markers of good response from neuroimaging**

A study by Lauriello et al. (1998) followed schizophrenic men (n=21) during treatment with typical antipsychotics and then clozapine treatment (mean dose 562 mg/day) over on average of 6.2 months, in an attempt to identify what predicted good response to clozapine. 47.6% improved by at least 20% on the BRPS. The improvement scores were correlated to MRI values. The only significant finding was that patients with larger anterior superior temporal lobe cerebrospinal fluid volumes showed greater improvement on clozapine. A study by Lawrie et al. (1995) took 40 demographically matched patients, 20 treatment-resistant and 20 treatment responsive and compared them with MRI brain scans. Brain structure was not related to treatment response but poor responders in general had lower volumes of most brain structures. A further study by the same group compared 22 poor responders, 20 good responders and 50 controls (Lawrie et al. 1997). Controlling for age patients with schizophrenia showed an elevated rate of atrophy for all brain structures (OR=11.7), which had a tendency to be greater in treatment-resistant patients than good responders (OR=2.8, p=0.06). Taken together these studies do not unequivocally show a clear relationship between brain structures and good response.

Within the area of neurochemistry, both PET and SPET studies have been used to investigate the effect of clozapine on neuroreceptors. Many of those were reviewed in chapter 1 of this thesis. Although it is likely that many of the subjects in these studies were good responders to clozapine, that relationship is rarely the main purpose of investigation. Often the subjects are somewhat heterogeneous. The study by Lawrie et al. (1997), mentioned above, used SPET as well as MRI to compare 20 “good responders” and 20 “treatment-resistant” patients on various antipsychotics. It is possible that these patients would have responded to clozapine, so the definition of the treatment-resistant group is uncertain. No difference was seen on SPET between the two groups. In summary no study could be located that specifically and clearly targeted people, known as “good responders”.

Dopamine D<sub>2</sub> receptor occupancy, although not the only determinant of antipsychotic response, plays a major role in it. Clozapine is effective at lower levels of D<sub>2</sub> blockade than typical antipsychotics (Nordstrom et al. 1995), but no studies have looked at the relationship between the level of D<sub>2</sub> occupancy in responders versus non-responders specifically.

## **5.2. Aims of the study**

The response to clozapine varies between individuals. It would be interesting to know whether people who respond well to clozapine clinically share some characteristics in terms of pharmacodynamics. In particular, in this current exploration, it would be useful to know whether their D<sub>2</sub>-like blockade is different from the D<sub>2</sub>-like blockade of clozapine-resistant patients.

This is a SPET study of particularly good responders to clozapine (referred to as “responders”). The specific prediction of the study was that patients known to respond well to clozapine monotherapy would have higher D<sub>2</sub>-like receptor occupancy than treatment-resistant patients on clozapine monotherapy. If that turned out to be the case, good responders would be closer in D<sub>2</sub>-like receptor occupancy to treatment-resistant patients, post amisulpride augmentation. This would help explain why treatment-resistant patients improve with amisulpride augmentation.

## **5.3. Materials and Methods**

### **5.3.1. Ethics**

Approval for the study was obtained from the Ethics Committee of the Maudsley Hospital as an extension to study 2 (05/99) and from the UK Administration of Radioactive Substances Advisory Committee (ARSAC). Following a full description of the study to the subjects, written informed consent was obtained. All subjects received a payment equivalent to 8 times the minimum national hourly wage for participation.

### 5.3.2. Subjects

Patients selected were good responders to clozapine. Consultant psychiatrists at the Maudsley Hospital were approached and asked to suggest patients who had responded exceptionally well to clozapine and were still receiving the drug. These were then approached. The inclusion and exclusion criteria were described in chapter 2.2.3.

The same group of control subjects was used as in study 2. These were 10 healthy controls (8 males, 2 females, mean age =35.8 years). They were subject to the same exclusion criteria and were recruited through personal contacts. Each had one  $^{123}\text{I}$ -IBZM SPET scan. (Table 5.1). None of the responders were on other psychotropic medication, but 4 used hyascine hydrobromide.

Table 5.1. Demographic variables of subject group.

Group	Number (male/female)	Mean age	Race		
			African- Caribbean	Asian	Caucasian
Responders	8 (8/0)	42.8 ± 5.1	3	1	4
Controls	10 (8/2)	35.8 ± 7.2	1	2	7

### 5.3.3. Study design

Ten patients with optimal response to clozapine (8 males, 2 females), agreed to participate in the imaging study and 9 underwent a  $^{123}\text{I}$ -IBZM SPET scan as well as a clinical assessment. One patient was excluded as she had claustrophobia and one patient as she had a bad cold on the day of the scan and movement artefacts from coughing made image analysis impossible. The 8 patients left in the study (8 males) had a mean age of 42.8 years ± 5.1 yrs,

mean dose of clozapine = 331 mg  $\pm$  75.3 mg and their mean BPRS<sub>(0-6)</sub> scale score was 5.75  $\pm$ 2.8 and hence well under the defined upper cut-off point of 25.

#### **5.3.4. Clinical measures**

Clinical status was evaluated at the time of the scan using the 18-point BPRS(0-6) scale, Positive And Negative Syndrome Scale (PANSS), Scale for the Assessment of Negative Symptoms (SANS), Global Assessment Scale (GAS), Calgary Depression Scale, Calgary Anxiety Scale and various side effect rating scales. A fuller description of these is given in chapter 2.3.

#### **5.3.5. Image and statistical analysis**

Scanning was carried out using a Picker Prism 3000XP triple headed SPET camera, at the Institute of Nuclear Medicine, UK. Following a bolus injection of 185MBq of <sup>123</sup>I-IBZM, sequential whole brain images were acquired up to 210 minutes post-injection. Maps of binding potential for drug treated groups and controls were generated using a voxel-based graphical analysis (Logan et al. 1990). For more details refer to chapters 2.4. – 2.7. above.

Comparisons between responders and patients in study 1 and 2 on demographics and clinical parameters were done using two-tailed independent Student's t-test and  $p < 0.05$  considered significant.

Comparisons between occupancy values in the basal ganglia were made between responders and clozapine-resistant patients before and after augmentation using independent Student's t-test. Tests were two-tailed. To reduce the risk of Type 1 error the Bonferroni correction was made and  $p < 0.025$  considered significant<sup>18</sup>.

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<sup>18</sup> The Bonferroni correction reduces conservatively the risk of Type 1 error. It is  $\alpha / N$ , or here  $0.05 / 2 = 0.025$ .



## 5.4. Results

### 5.4.1. Clinical rating scales

The outcome of clinical rating scales confirmed that the patients had few symptoms of schizophrenia (table 5.2.). Apart from some instances of weight gain no side effects were picked up on side-effects rating scales.

*Table 5.2. Outcome of clinical rating scales.*

Rating scale	Score (SD)
BPRS	5.75 (2.8)
PANSS total	40.2 (3.6)
PANSS pos	8.9 (2.8)
PANSS neg	9.5 (1.1)
GAS	74 (3.5)

### 5.4.2. D<sub>2</sub> receptor occupancy

The mean Dopamine D<sub>2</sub>-like receptor occupancy value in the basal ganglia of patients was 45.47 ( $\pm$ 12.14), 46.6 ( $\pm$  9.85) in the caudate and 44.80 ( $\pm$ 14.45) in the putamen. There was great inter-subject variation in binding (Table 5.3).

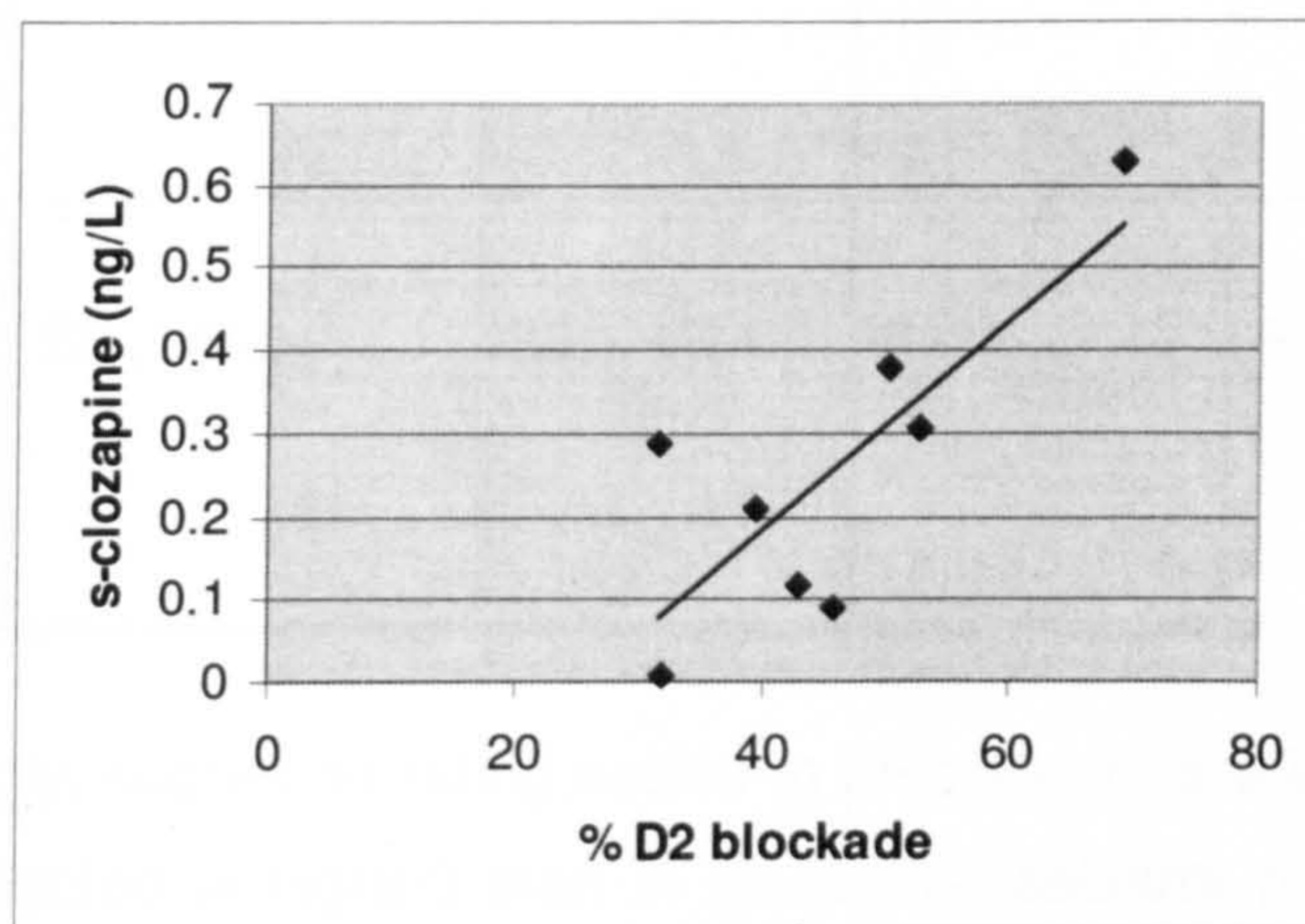
### 5.4.3. Serum clozapine levels

Average s-clozapine levels were 0.26 ng/L ( $\pm$  0.20). There was a significant correlation between s-clozapine levels and % Dopamine D<sub>2</sub>-like occupancy (Pearson Correlation coefficient = 0.787,  $p=0.021$ ) (Fig.5.1.). In one case s-clozapine was at the lowest measurable level (0.01 ng/L) but D<sub>2</sub>-like blockade just above 30%.

Table 5.3. Individual D<sub>2</sub>-like occupancy in clozapine responders.

Patient No.	Left BG	Right BG	Mean BG
1	49.48	50.86	50.17
2	69.26	68.50	68.88
3	46.26	58.96	52.61
4	36.16	49.64	42.90
5	39.21	39.86	39.53
6	44.37	47.42	45.90
7	33.13	31.16	32.14
8	31.92	31.37	31.65

Fig. 5.1. Relationship between s-clozapine and % striatal D<sub>2</sub>-like blockade (Pearson Correlation coefficient =0.787, p=0.021)



## 5.4.4. Comparing results in responders and clozapine-resistant patients

### 5.4.4.1. Clinical parameters

The demographics of responders are comparable to the clozapine-resistant patients (Table 5.4). No correlation was found, between age and D<sub>2</sub>-like occupancy, when the results of both groups were pooled (Pearson Correlation Coefficient = -0.399, p=0.140 (2-tailed)).

*Table 5.4. Demographics of responders compared to a) the clozapine-resistant patients in study 2 and b) the larger study 1. (\*p<0.05, responders vs. study2)*

Group	Number (male/female)	Mean age	Race		
			African- Caribbean	Asian	Caucasian
Responders	8 (8/0)	42.7 ± 5.0	3	1	4
Clozapine-resistant patients (study 2)	7 (5/2)	33.6 ± 7.9*	1	2	4
Clozapine-resistant patients (study 1)	28 (24/4)	36.1 ± 7.4	3	6	19

Not surprisingly, scores on rating scales in responders are lower (except GAS, which as expected is higher) than in clozapine-resistant patients (Table 5.5). This reflects the fact that responders have far fewer clinical symptoms than those who don't respond to treatment.

*Table 5.5. Improvement in clinical measures before and after augmentation (\*p<0.01, \*\*p<0.001), comparing responders to patients before (scan1) and after (scan2) augmentation respectively (SD=standard deviation)*

Rating scale	Responders (SD)	Clozapine-resistant patients – study 2	
		Score at scan 1 (SD)	Score at scan 2 (SD)
BPRS <sub>(0-6)</sub>	5.75 (2.8)	29.7 (3.8)**	22.1 (3.6)**
PANSS total	40.2 (3.6)	78.6 (11.7)**	67.0 (10.8)**
PANSS pos	8.9 (2.8)	20.0 (2.9)**	17.6 (3.0)**
PANSS neg	9.5 (1.1)	20.9 (7.4)**	16.7 (6.0)*
GAS	74 (3.5)	36.4 (4.0)**	40.7 (4.3)**

Looking at results from the larger group of clozapine-resistant patients in study 1, a similar pattern emerges.

*Table 5.6. Improvement in clinical measures before and after augmentation, comparing responders to the larger group of patients at baseline and after 6 months on amisulpride augmentation respectively.*

Rating scale	Responders (SD)	Clozapine-resistant patients-study 1	
		Score at baseline	Score at 6 months
BPRS <sub>(0-6)</sub>	5.75 (2.8)	30.18 (28.30-32.06)	19.59 (16.68-22.50)
PANSS total	40.2 (3.6)	81.78 (76.88-86.67)	63.46 (58.21-68.72)
PANSS pos	8.9 (2.8)	20.7 (19.24-22.17)	15.82 (13.89-17.66)
PANSS neg	9.5 (1.1)	22.07 (18.85-25.2)	16.89 (14.18-19.61)
GAS	74 (3.5)	36.25 (32.32-40.18)	45.74 (41.09-50.39)

#### 5.4.4.2. Dopamine D<sub>2</sub>-like receptor occupancy

Dopamine D<sub>2</sub>-like occupancy was not significantly different between responders and clozapine-resistant patients before augmentation (table 5.7.), either in the basal ganglia or its component parts (putamen and caudate head). When comparing responders and clozapine-resistant patients after augmentation (table 5.8.) there was a non-significant trend towards higher D<sub>2</sub>-like binding in the augmented patients. Responders' D<sub>2</sub>-like occupancy is therefore similar to D<sub>2</sub>-like occupancy in treatment-resistant patients at baseline and lower than D<sub>2</sub>-like occupancy post-augmentation (Fig.5.2.).

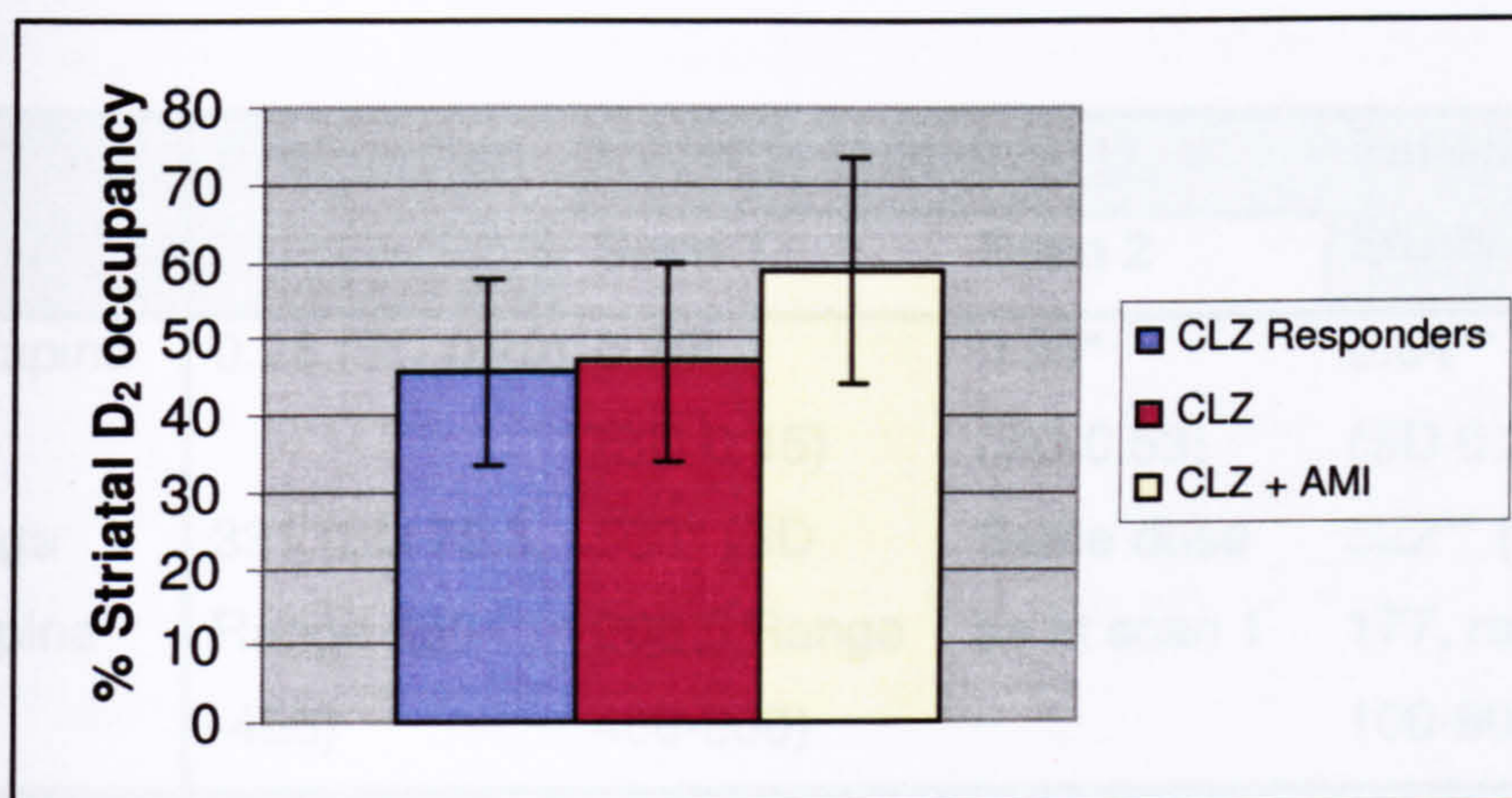
*Table 5.7. Dopamine D<sub>2</sub>-like % occupancy in Responders compared with patients before (scan 1) augmentation of clozapine with amisulpride (using independent t-test).*

Area	Occupancy % Responders	Occupancy % non-responders (scan 1)	p
Basal ganglia	45.47	46.97	= 0.8 Not significant
Head of caudate	46.60	49.21	=0.8 Not significant
Putamen	44.80	45.14	=1.0 Not significant

*Table 5.8. Dopamine D<sub>2</sub>-like % occupancy in Responders compared with patients after (scan 2) augmentation of clozapine with amisulpride*

Area	Occupancy % Responders	Occupancy % non-responders (scan 2)	p
Basal ganglia	45.47	58.65	= 0.08 Not significant
Head of caudate	46.60	62.02	=0.12 Not significant
Putamen	44.80	59.01	=0.06 Not significant

Figure.5.2.  $D_2$ -like occupancy in the basal ganglia of (1) responders, (2) treatment-resistant patients before augmentation, (3) clozapine-resistant patients, after augmentation with amisulpride.



The inter-patient variation was similar to non-responders.

#### 5.4.4.3. Serum clozapine levels

There is a significant difference between mean s-clozapine levels in clozapine-resistant patients on the one hand and in responders on the other hand (Table 5.9.). When looking at the clozapine dose, the same pattern can be seen. The clozapine dose is significantly lower in responders, compared with the smaller group of non-responders in study 2 ( $p=0.02$ ) and in the larger group of clozapine-resistant patients in study 1 ( $p<0.001$ ). Patients were on the same clozapine dose at scan 2. Changes in clozapine dose would have excluded them from the study.

*Table 5.9. S-clozapine levels in ng/L for responders, clozapine-resistant patients at scan 1 and scan 2 and patients in the larger group (study 1). T-tests between responders and study 2, scan 1, study 2, scan2 and study 1 respectively. (\*= $p < 0.05$  \*\*= $p < 0.01$ ).*

	Responders	Patients in study 2		Patients in Study 1
		Scan 1	Scan 2	
s-clozapine ng/L	0.26 (SD 0.20)	0.79* (SD 0.45)	0.95* (SD 0.53)	0.64** (SD 0.38)
Average Clozapine Dose	331 (SD 75.3, Range 250 -450)	568* (SD 206.5, Range 400-900)	Same dose as at scan 1	522** (SD 177, range 100-900)

## 5.5. Discussion

### 5.5.1. Clinical findings

The results of clinical rating scales demonstrate clearly how the responders have a significantly better clinical profile than the non-responders. The difference is both statistically and clinically significant on the PANSS rating scale, both the total score and the score of the positive and negative subscales, as well as the GAS scale (and the BPRS). For example a difference in the PANSS positive subscale of 11 points (20-8.9) is equivalent to an average difference in each item on the subscale of 1.5 point and the results are similar for the negative subscale. Studies have indicated that for a change in a clinical rating scale to be visible a reduction of more than 20% in that scale is needed pre- and post-treatment (Cramer et al. 2001). The difference here far outweighs that requirement.

### **5.5.2. Scanning findings**

The null hypothesis was that responders would have higher D<sub>2</sub>-like receptor occupancy than non-responders on clozapine monotherapy and be closer to the post-augmentation group. The D<sub>2</sub>-like % occupancy of responders (45%) is much closer to the clozapine-resistant group pre-augmentation (47%) than post-augmentation (59%). Clearly there is no difference between responders and non-responders at baseline. Using independent Student's t-test there is not a significant difference between responders and non-responders post augmentation, although a trend is seen. Independent Student's t-tests are rightly more stringent than paired t-tests and it is not unlikely that a larger study with more power would show a significant difference.

The clozapine-resistant patients become less like good responders after augmentation, rather than more like them, in terms of striatal D<sub>2</sub>-like binding. This is an interesting finding. It could be that the non-responders are so unlike responders that different treatments are needed to improve their clinical state. Responders to clozapine may need only relatively low D<sub>2</sub>-like binding. On the other hand, in non-responders as discussed in chapters 1 and 4, bringing D<sub>2</sub>-like occupancy above the "therapeutic threshold" is necessary for clinical improvement to take place.

In conclusion the difference between responders and non-responders is not due to a difference in striatal D<sub>2</sub>-like binding.

### **5.5.3. Serum clozapine findings**

The difference in s-clozapine levels is large and significant ( $p < 0.05$ ) between responders and non-responders and is even more marked when compared to the larger group of non-responders in study 1 ( $p < 0.01$ ). The difference in levels reflects the clozapine dose difference between responders and non-responders. While some studies have been unable to find a correlation between clozapine dose and s-clozapine, others have described this. A study by Palego et al. (2002) recruited 50 patients on clozapine. A relationship was



reported between clozapine dose per kilogramme body weight and clozapine levels. Of note neither age of patient nor body weight influenced this correlation. A much larger study (n=3782) did a multiple regression analysis and reported on the effect of dose, smoking, age, sex and metabolic activity on serum clozapine concentrations (Rostami-Hodjegan et al. 2004). It produced nomograms of the clozapine dose needed to reach threshold serum clozapine levels, but conceded that considerable variability exists regarding the relationship.

It is interesting that the mean s-clozapine levels in responders (0.26 ng/L  $\pm$ 0.2ng/L) are below what is normally regarded as the plasma threshold level for increased probability of a good clinical response; 0.35 – 0.42 ng/L (Kronig et al. 1995; Bell et al. 1998). The difference in clozapine dose and s-clozapine levels is yet another characteristic that separates good responders from poor responders. At a relatively low dose and plasma levels, good responders already have reached a D<sub>2</sub>-like blockade that requires a much higher dose of clozapine in non-responders. More importantly the former group is actually responding to this dose.

It is of course quite possible that the binding of clozapine to other receptors is responsible for this good response. Looking at other receptors, the 5-HT<sub>2A</sub> receptor is heavily occupied by clozapine. Could this explain the response of clozapine at a low level of D<sub>2/3</sub> blockade? This is unlikely. Travis et al. (1998), used SPET and the selective ligand <sup>123</sup>I-5-I-R91150 to assess 5-HT<sub>2A</sub> binding in clozapine and risperidone. They found no correlation between 5-HT<sub>2A</sub> binding indices and changes in the Global Assessment Scale (GAS). Another study used PET and [<sup>18</sup>F] Setoperon and found high 5-HT<sub>2A</sub> blockade at all doses of clozapine as well as at high doses of chlorpromazine, which suggested that high 5-HT<sub>2A</sub> binding was not specific to clozapine (Trichard et al. 1998). For clozapine, as well as risperidone and olanzapine, 5-HT<sub>2A</sub> receptors are already saturated at doses of medication below what's needed for therapeutic response (Kapur et al. 1999; Seeman 2002).

Another possible explanation for the good response is the binding of clozapine to cortical D<sub>2</sub>-like receptors. That issue is not addressed by this study but the possible mechanism of such a response is discussed in Chapter 6.

The finding that one patient had a plasma clozapine level of 0.01 ng/L at the time of his scan is worthy of a comment. The patient's D<sub>2</sub>-like occupancy was 32% and he had minimal clinical symptoms. He claimed to take his clozapine by "gnawing" at the tablet throughout the day and was a heavy smoker. The half-life of clozapine is 9 to 17 hours and clearance varies greatly (Jann et al. 1993). It is unlikely that the pharmacokinetics of clozapine can explain such low plasma clozapine levels. The patient is likely to have been largely non-compliant before the scan or a mix-up of his blood sample could have taken place. A D<sub>2</sub>-like occupancy of 32% is conceivable without an antipsychotic, particularly as patients with schizophrenia tend to have higher D<sub>2</sub>-like occupancy by endogenous dopamine (Frankle et al. 2004). Additionally this subject was 52 years old. There is an age-related decline in the number of D<sub>2</sub> receptors (Volkow et al. 1996). It is possible that the "occupancy" quoted may actually be related to an age related decline in the density of striatal D<sub>2</sub>-like receptors or perhaps that a smaller amount of clozapine is needed to occupy the appropriate level of the smaller number of available D<sub>2</sub> receptors.

#### **5.5.4. Limitations**

Possibly the difference in % D<sub>2</sub>-like occupancy could be explained by a demographic difference between the groups compared. The difference in age is nearly 10 years between the responders (42.7 ± 5.0 years) and non-responders (33.6 ± 7.9 years), which is a significant difference (p=0.02). This may have an influence on the findings, as studies of healthy volunteers have shown a decrease in D<sub>2</sub> receptor activity with age. A study using <sup>123</sup>I-IBF found an age related decline in D<sub>2</sub> binding by 7-13% per decade (Ichise et al. 1998). The results are likely to be similar for <sup>123</sup>I-IBZM, as age-related decline in D<sub>2</sub>-like binding has been reported using that ligand in rats as well as in subjects with Parkinson's Disease (Brucke et al. 1993; Cao et al. 2000). Another study using <sup>11</sup>C-raclopride found a decline in D<sub>2</sub> receptors by 7.9% per decade in healthy

volunteers (Volkow et al. 1996). However, an IBZM SPET study comparing age-related decline in D<sub>2</sub>-like receptor density found mild age-related decrease in controls (n=20) but not drug-free patients (n=20) (Pilowsky et al. 1994) and another study looking at schizophrenic patients did not find an effect with age (Farde et al. 1990). In conclusion the difference in age may possibly influence the results. The responders' % D<sub>2</sub>-like binding could be expected to be higher if they were of the same younger age as the clozapine-resistant group. In that case the results would be nearer the post-augmentation group of non-responsive patients. The exact effect of age on D<sub>2</sub>-like binding is hard to ascertain though due to lack of studies looking at age-related change in schizophrenic patients and the suggestion from the studies of Pilowsky et al. (1994) and Farde et al. (1990) that this age-related change may not exist in patients. In the subjects in studies 2 and 3 in this thesis, no correlation was seen between age and D<sub>2</sub>-like occupancy. There are clearly different ways of analysis the effect of the age factor but on balance I felt that age was not an important issue here.

The effect of ethnicity is unlikely to matter, given the lack of conclusive evidence that it has an effect on scanning results and the fact that the groups were reasonably well matched for this variable.

The difference in D<sub>2</sub>-like binding between responders and non-responders before as well as after augmentation is non-significant. Although a clear trend is seen the prediction cannot be fully refuted unless a larger study is done to see whether the trends seen here are true findings.

## **5.6. Conclusions**

This study explored characteristics of good clozapine responders and found them to be unlike clozapine-resistant patients in many aspects. Responders are less unwell on a range of clinical rating scales. They respond at significantly lower doses of clozapine and at serum clozapine levels that are below what is generally regarded as optimal therapeutic levels. The study lacked power to find a significant difference in % D<sub>2</sub>-like occupancy between responders and

treatment-resistant patients pre and post augmentation with amisulpride. The trend was however very clear and showed D<sub>2</sub>-like occupancy to be similar in responders and non-responders before augmentation. Therefore the prediction that responders would have similar D<sub>2</sub>-like blockade to non-responders post augmentation is very unlikely to be correct. The effect of amisulpride augmentation of clozapine does not mimic D<sub>2</sub>-like blockade in good responders to clozapine.

## **Chapter 6. General discussion**

### **6.1. Summary of results**

The main aim of this thesis is to explore aspects of treatment refractory schizophrenia, a research field which until recently has received less attention than it deserves, despite the large proportion of patients who prove resistant to treatment. Three studies were undertaken for this purpose.

The first study is described in chapter 3. When this work was planned, only one RCT had looked at the augmentation of clozapine treatment, a study by Shiloh et al. (1997) which used the antipsychotic sulpiride to augment clozapine treatment. Study 1 was a follow-on from Shiloh et al's study, albeit using a different but related antipsychotic, amisulpride. This was an open-label study, which followed for six months in a naturalistic setting, 33 well characterised chronically unwell, non- or partial responders to clozapine. 28 out of 33 patients completed the study and received an average of 600 mg/day (range 300-800 mg/day) of amisulpride in addition to their mean dose of 519 mg/day of clozapine (range 100-900 mg/day). Augmentation with amisulpride reduced symptoms in most of the patients, with 71% of those who completed the study, or 61% of those who commenced the study showing a more than 20% reduction in BPRS total. The improvement was seen in both positive (46% of total cohort responding) and negative (52% of total cohort responding) symptoms of schizophrenia with a reduction in the respective PANSS subscales by more than 5 points and a SANS scale reduction of over 14 points. An improvement was also seen in general functioning with a reduction in the PANSS general sub-scale of more than 8 points and a Global Assessment Scale score increase of over 9 points. When a more stringent criterion of 50% improvement on BPRS was used, 32% (or 27% of those started on treatment) met the response criteria. The augmentation was well tolerated with no increase in side-effects.

Chapter 4 describes the second study. Study 2 presents the analysis of a subset of 7 (out of 10 selected) of the larger group of patients described in

study 1. They underwent two  $^{123}\text{I}$ -IBZM SPET scans, at baseline and a repeat scan after 10-12 weeks on medication. The purpose of this scanning study was to elucidate the mechanism behind amisulpride augmentation. The augmentation of clozapine with amisulpride led to an increase in  $\text{D}_2$ -like receptor occupancy in the patients' striatum from 47% to 59% with no regional difference observed between the head of the caudate and putamen within the basal ganglia nor between the left and right basal ganglia. The increased binding moved the  $\text{D}_2$ -like occupancy into the proposed "threshold" occupancy for response seen with other antipsychotics. No relationship was seen in individuals between their pre-augmentation  $\text{D}_2$ -like occupancy and response to amisulpride. Changes in  $\text{D}_2$ -like binding in individuals did not correlate with their clinical improvement. Groupwise, however, an association was found between clinical improvement and increase in  $\text{D}_2$ -like like occupancy.

The final study, study 3, is described in chapter 5. It looks at a different group of patients: exceptionally good responders to clozapine. The 8 (out of 10 recruited) responders underwent a single  $^{123}\text{I}$ -IBZM SPET scan. As would be expected the responders were less unwell than refractory patients (mean BPRS total score = 5.75 versus 29.7 for non-responders at baseline; PANSS total = 40.2, versus 78.6 for non-responders at baseline). They had also responded at much lower doses of clozapine (mean dose of clozapine = 331mg/day, versus 562 mg/day for non-responders) and at plasma clozapine levels below suggested optimal levels (mean s-clozapine = 0.26 ng/L versus 0.79 for non-responders at baseline). The  $\text{D}_2$ -like occupancy was 45%, which is similar to the non-responders prior to augmentation (47%) and different from non-responders after augmentation (59%) although the study lacked power to demonstrate a significant difference. The effect of amisulpride augmentation is therefore not simply to make clozapine non-responders more like responders, in terms of  $\text{D}_2$ -like binding.

## **6.2. Outcome regarding hypotheses under investigation**

*1. Amisulpride augmentation of clozapine treatment in patients suffering from schizophrenia, who are partially or non-responsive to clozapine, will lead to an improvement in clinical ratings*

Hypothesis strongly supported. An RCT needed to fully answer the question.

*2. Amisulpride augmentation of clozapine treatment in patients with schizophrenia, who are partially or non-responsive to clozapine, will lead to increased striatal D<sub>2</sub>-like receptor occupancy.*

Data very strongly supports that augmentation increases striatal D<sub>2</sub>-like occupancy.

*3. An increase in D<sub>2</sub>-like receptor occupancy to approximately 60% striatal blockade is associated with a response.*

Strong support for hypothesis.

*4. Subjects with low, (<50 %) striatal D<sub>2</sub>-like occupancy on clozapine alone, will show an enhanced response to amisulpride augmentation in comparison to patients with a higher striatal (>50 %) D<sub>2</sub>-like occupancy on clozapine alone.*

Data does not support this hypothesis, although to be certain a larger study with greater power would be needed.

*5. Clozapine non-responders will have lower D<sub>2</sub>-like receptor occupancy at baseline than good responders. Thus the augmentation will make non-responders' D<sub>2</sub>-like profile more like that of responders.*

The data does not support this hypothesis. Rather clozapine responders seem to have similar D<sub>2</sub>-like occupancy to non-responders at baseline.

## **6.3. Relevance of the findings for the pathophysiology and management of treatment-resistant schizophrenia**

### **6.3.1. Pathophysiology**

It is important to try and understand the mechanism underlying augmentation strategies. Neuroreceptor imaging is a helpful means to that end. The only previous study looking at neuroreceptor binding in clozapine augmentation was an  $^{11}\text{C}$  raclopride PET study by Kapur et al. (2001b). The study didn't report on the clinical response to augmentation but striatal  $\text{D}_2$ -like binding increased from 55% to 79% over the 4 - 8 weeks of the study as would be expected from the pharmacodynamics of haloperidol and clozapine. The studies outlined in this thesis therefore mark one of the first attempts to use neurochemical imaging to translate the findings from a clinical trial into an evidence based pharmacological rationale for the augmentation of clozapine in clozapine partial and non-responders.

#### **6.3.1.1. Recent Dopamine (and other) theories In Schizophrenia**

All available antipsychotics share a propensity to antagonise Dopamine  $\text{D}_2$  and  $\text{D}_2$ -like receptors (Jones et al. 2002). The original dopamine hypothesis of schizophrenia stated that antipsychotic medication ameliorated the most prominent symptoms of schizophrenia via blockade of dopamine receptors. The  $\text{D}_2$  receptor was later acknowledged as a major site for antipsychotic action (Seeman et al. 1976; Johnstone et al. 1978). The dopamine hypothesis was however insufficient to explain the clinical effects of antipsychotics, particularly why up to 50% of patients failed to respond to antipsychotics despite high doses and high  $\text{D}_2$ -like occupancy. It is also inadequate to explain why a proportion of these non-responding patients gain benefit from clozapine despite its low affinity for  $\text{D}_2$ -like receptors and low occupancy of these receptors *in vivo*. The knowledge that clozapine has a high affinity for a range of receptors drove new hypotheses of antipsychotic action, in particular that of 5-HT $_2\text{A}$ . It now seems unlikely, however, that 5-HT $_2\text{A}$  receptor blockade alone can explain antipsychotic action (Kapur et al. 1999).



Whilst it is generally accepted that a reduction in limbic-striatal dopamine function is necessary for antipsychotic action, whether it is a sufficient explanation is disputed. Novel, revised versions of the dopamine hypothesis of schizophrenia have come forth in recent years, reflecting a continued interest in dopamine. Many current revisions to the dopamine hypothesis are predicated by the idea that D<sub>2</sub> action alone is adequate to explain the effects of antipsychotics (Seeman 2002), especially against the positive symptoms of psychosis. This stems in part from a lack of convincing evidence of a role for other monoamines in the genesis of psychosis and response to treatment. The focus, however, has shifted from simply considering levels of striatal D<sub>2</sub>-like occupancy to attempts to understand the effects of this occupancy on the neural and neurochemical networks involved in schizophrenia.

#### *6.3.1.1.1. Cortico-subcortical imbalance theory*

A revision of the dopamine hypothesis focusing on “cortico-subcortical imbalance” is based on the observation by different groups that cortical and subcortical dopamine systems seem in some ways reciprocal and opposite (Pycock et al. 1980; Weinberger 1987; Davis et al. 1991). Prefrontal dopamine activity inhibits subcortical dopamine activity, i.e. in mild stress. A deficiency in mesocortical dopamine function might translate into disinhibition of mesolimbic dopamine activity. Corticostriatal-thalamocortical “loops” are important targets of dopamine modulation. They are separated into “limbic” loops, “associative” loops and “motor” loops (Joel et al. 2000). Within each loop, output goes via a direct and indirect pathway, modulated by dopamine. It has been suggested that the dopamine system provides a connection by which information from the ventral limbic corticostriatal-thalamocortical loops spirals along nigrostriatal loops, feeding into the cognitive and sensorimotor loops, in that way translating drives into actions (Haber et al. 1997). This theory of cortical-subcortical imbalance links in with the discussion in section 6.3.1.1.2. below.

#### *6.3.1.1.2. Glutamate – Dopamine interaction theories*

Hypotheses accounting for glutamate dopamine interactions have been around for the last 20 years, suggesting that abnormal prefrontal cortical function might

be a primary defect in schizophrenia (Weinberger 1987). These theories are based on evidence that prefrontal cortex function in schizophrenia is abnormal and propose that dopamine transmission in schizophrenia (both mesocortical dopamine deficit and mesolimbic dopamine hyperactivity) might be related to faulty NMDA<sup>19</sup> transmission in the prefrontal cortex. NMDA antagonists (e.g. phencyclidine and ketamine) induce positive and negative symptoms of schizophrenia in patients and controls (Goff et al. 2001). There are long-term effects of NMDA antagonists on dopamine transmission, in animal studies, with both reduced mesocortical dopamine activity and excessive subcortical reactivity being observed (Jentsch et al. 1998). Although various animal studies have supported parts of this model, unequivocal proof of NMDA dysfunction in schizophrenia has proved elusive. For example, a recent study indicates that phencyclidine, ketamine and LSD in addition to blocking NMDA have a relatively high affinity for the D<sub>2</sub> receptor and through direct agonism may be responsible for a “hyperdopaminergic” state (Seeman et al. 2005), without the recruitment of glutamate-dopamine interaction.

One model of glutamate modulation of dopamine neurones in the ventral tegmental area suggests that the prefrontal cortex modulates activity of midbrain dopamine neurones through both an activating and an inhibitory pathway (Carlsson et al. 1999). The activating pathway is via glutamatergic projection to dopaminergic cells, the inhibitory pathway via prefrontal cortex glutamatergic neurons to midbrain GABAergic interneurons and striatomesencephalic GABA neurones. It is suggested that in schizophrenia reduced prefrontal cortex activity (possibly caused by NMDA transmission deficiency) could result in reduced mesocortical dopamine activity (causing prefrontal cortex related cognitive impairment) and also, under stress, a failure of the prefrontal cortex to properly regulate subcortical dopamine activity, hence leading to positive symptoms via increased mesolimbic dopamine activity. In this way the cortex could have a role as a “brake” on the striatal dopamine system (Carlsson et al. 2001). Various studies have provided support for the theory that upregulated striatal dopamine function in schizophrenia might be

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<sup>19</sup> NMDA or N-methyl-D-aspartate is an amino acid derivative that binds as an agonist at the NMDA receptor and mimics the action of glutamate, the most abundant excitatory neurotransmitter in the brain. Activation of NMDA receptors causes the opening of Ca<sup>++</sup> ion channels.

caused by abnormal cortical functioning. In particular a study showing that lower dorsolateral prefrontal cortex (DLPFC) N-acetyl aspartate (NAA), an intracellular neuronal marker that indirectly reflects neuronal synaptic abundance, predicted larger reduction in dopamine receptor availability after amphetamine administration (Bertolino et al. 2000). This showed that the more abnormal the prefrontal physiological response the greater the striatal activity. Another study by Kegeles et al. (2000), disrupted NMDA transmission with ketamine in 8 healthy volunteers and found that amphetamine-induced dopamine release was increased more than two-fold.

There is evidence for increased release of subcortical dopamine in schizophrenia under stress conditions (Abi-Dargham et al. 1998). This is an interesting finding in the light of models of schizophrenia which propose that cortical regulation of subcortical dopamine systems is interrupted (Grace 2004). It is also of interest when considering the possibility that especially amisulpride, in low doses, may be able to cause prefrontal dopaminergic facilitation (Lecrubier 2003). The increased release of dopamine in the prefrontal cortex might play a role in improving cognition and negative symptoms as well as decreasing dopamine in the mesolimbic region (Goldman-Rakic et al. 1997) if the "brake" effect of the prefrontal cortex exists in the human brain.

Coming back to the regulatory role of dopamine, there is evidence from various experiments that dopamine neurones gate excitatory transmission from associative cortical information, filter out irrelevant information and reduce responses to it while enhancing contextually important stimuli. In this way it has been suggested that dopamine neurons improve signal to noise ratios in behavioural contexts, perhaps through increased GABA neurone excitation and surrounding inhibition (Weinberger 2003).

This provides a biological framework for a recently revisited dopamine theory that attempts to explain psychosis and antipsychotic action (Gray 1995; Kapur 2003a; Kapur 2004). The theory is based on ideas about dopamine as a mediator of "life's pleasures" (Wise 1978) as well as later suggestions regarding the functional role of dopamine as a mediator of an external stimulus to a

wanted or unwanted entity (Berridge et al. 1998). This explanation is based on the concept of “salience”. Salience or “motivational salience” is a term used to describe how a reward associated stimulus captures the attention of an animal and becomes the focus of goal-directed behaviour in laboratory experiments. The theory is that an abnormal (for whatever reason) dopamine system, especially the mesolimbic dopamine system, is overactive and causes aberrant, inappropriate salience. This causes a person that’s becoming psychotic to feel unusual; new otherwise neutral things take on significance and cause the sense of anxiety and confusion sometimes seen in the prodrome of schizophrenia. Over time the person develops delusional ideas to “make sense” of the things that have taken on a new importance. When an antipsychotic is given, the D<sub>2</sub>-like receptor blockade reduces the aberrant salience, hence the drive behind the delusions is gone and they matter less to the patient or disappear altogether, although they can still linger as any other beliefs people have, but potentially amenable to argument. This is an interesting theory which attempts to explain some of the characteristics of psychosis and clinical response to antipsychotics. It gives a framework for understanding the reduced interest in the environment generally, seen at times with antipsychotic use, which can amount to secondary negative symptoms. These might be due to a reduction in not only aberrant but also normal salience of things one should show an interest in, caused by D<sub>2</sub>-like blockade. Although it is an interesting theory which attempts to bridge the biology, phenomenology and pharmacology of schizophrenia, it is not particularly useful, at this time, for understanding treatment-resistance.

It is still not clear what role exactly glutamate plays in the pathogenesis and treatment of schizophrenia and whether schizophrenia is a “hypoglutamatergic” or “hyperglutamatergic” state (Tuominen et al. 2005). Both the basal ganglia-thalamocortical and limbic systems are involved in learning, memory and goal-directed behaviour and could provide a platform for dopamine and glutamate to interact (Tamminga 2003a). As further evidence becomes available from in vitro, animal and human studies the interactions within these complex systems may be further elucidated.

#### ***6.3.1.1.3. Dopamine stabilisation***

Presynaptic dopamine autoreceptors are inhibitory on overall dopamine activity. They therefore antagonise postsynaptic D<sub>2</sub> receptors. Dopamine release instability has been suggested as a cause of psychosis rather than continuously elevated dopamine activity. Dopamine stabilisers are drugs that can occupy functionally antagonistic pre- and post synaptic receptors, depending on the level of background tone (see 6.3.1.1.5. below). The effect would be to regulate their activation and cause unchanged tonic but reduced phasic dopaminergic activity. (-)-OSU6162 is a compound that seems able to do this, by either stimulating or inhibiting behaviour based on the initial behavioural state (Hadj Tahar et al. 2001; Carlsson 2002). A recent drug, aripiprazole is also able to exert its effects through a similar principle. In an environment where there is a lack of dopamine it is a partial agonist that stimulates D<sub>2</sub> release through binding to autoreceptors, while it blocks post-synaptic D<sub>2</sub> in the striatum where dopamine is more abundant (Bolonna et al. 2005). Although this is an intriguing hypothesis, the clinical data with aripiprazole does not show any difference in efficacy compared with haloperidol (Kane et al. 2002) or other antipsychotics (El-Sayeh et al. 2005).

#### ***6.3.1.1.4. Negative symptomatology***

Unlike positive symptomatology which is associated with hyperactivity in the mesolimbic system, negative symptoms have been related to underactivity of dopamine in the mesocortical pathways and the PFC. Amisulpride is an interesting model in this regard. It increases dopamine turnover in the brain with evidence that it may block presynaptic dopamine receptors at lower doses than are needed to block post-synaptic receptors (Schoemaker et al. 1997). Amisulpride shows regional selectivity for the cortex compared to the striatum (Xiberas et al. 2001a). In the cortex amisulpride may mainly block presynaptic D<sub>2/3</sub> receptors, leading to a rise in extracellular dopamine concentration and thus to increased D<sub>1</sub> receptor activation, which may account for improvements in negative symptomatology.

#### **6.3.1.1.5. Dopaminergic tone**

The difference in pharmacological response between individuals, the sexes and patients is an emerging issue (Laruelle et al. 1996; Breier et al. 1997; Kapur et al. 2000; Melkerson et al. 2000). Both individual variation in dopaminergic tone and the indication that the underlying dopamine tone and dopaminergic response is aberrant in schizophrenia are complicated issues. There is evidence that dopamine release is both “tonic” and “phasic”. Tonic release is used to describe extracellular extrasynaptic dopamine release and is relatively impulse-independent. On the other hand phasic release is impulse-dependent synaptic dopamine discharge. It has been suggested that increased phasic dopamine activity could be caused by low tonic activity. This would lead to overstimulation of post-synaptic D<sub>2</sub> receptors and positive symptoms (Grace 1991; Grace 1993).

Recent hypotheses based on these ideas postulate that in schizophrenia phasic dopamine transmission in the limbic regions is overactive while in frontal and prefrontal cortical areas tonic dopamine transmission is underactive. Blockade of D<sub>2</sub> receptors in the former regions would help reduce positive symptoms, while D<sub>2</sub> blockade in the frontal/prefrontal areas or tracts subserving them would exacerbate negative symptoms (Moore et al. 1999). If these models are correct, cortical selectivity in D<sub>2</sub> receptor occupancy of antipsychotics is an important issue and might help to explain the effects of at least some atypical antipsychotics, (Xiberas et al. 2001b; Jones et al. 2002; Bressan et al. 2003b), including the effect of amisulpride on negative symptoms (Leucht et al. 2002b; Bressan et al. 2003a). For clozapine a PET study failed to show regional D<sub>2</sub> binding selectivity (Talvik et al. 2001).

#### **6.3.1.1.6. Striatal vs. extrastriatal D<sub>2</sub>-like binding**

For methodological reasons, D<sub>2</sub>-like binding was for a long time mainly measured in the striatum. Newer radioligands such as <sup>123</sup>I-epidepride, [18F]desmethoxyfallypride and [76Br]FLB457 have made extrastriatal D<sub>2</sub>-like estimates possible (Bigliani et al. 2000; Vernaleken et al. 2004). A PET study using [76Br]FLB457 found that both typical and atypical antipsychotics bound similarly (72-97%) to D<sub>2</sub>-like receptors in the temporal cortex while binding of

atypicals was much lower than of typicals in the striatum (Xiberas et al. 2001b). It has been suggested that a drug with high temporal D<sub>2</sub>-like binding but low striatal D<sub>2</sub>-like binding has antipsychotic effects with less propensity for EPSE.

### 6.3.1.1.7. *K<sub>off</sub>* (transient equilibrium, “loose binding”)

One recent theory of dopamine action is that low affinity and fast dissociation (high *K<sub>off</sub>*) from the dopamine D<sub>2</sub>-like receptor is an important requirement for atypicality. It is suggested that fast dissociation means that although a drug attenuates dopamine transmission it would distort the patient’s physiological dopamine transmission less than drugs with slower dissociation from the D<sub>2</sub> receptor (Kapur et al. 2001c). Although medications with faster dissociation have lower overall affinity for the D<sub>2</sub>-like receptor and are hence likely to be used in higher doses, physiological dopamine transmission would still be relatively less suppressed (Kapur 2003b). The idea that “loose” binding might be what differentiates atypicals from typical antipsychotics is not new (Hartvig et al. 1986; Seeman et al. 1998). The preservation of physiological dopamine transmission may be a part of the explanation for atypicality, i.e. why an antipsychotic effect is sometimes seen with little prolactin rise or EPSE. Whether a drug’s *K<sub>off</sub>* is higher or lower than that of dopamine may be an important factor, with those who dissociate faster not suppressing physiological dopamine transmission.

*Table. 6.1. Dissociation rate constants for antipsychotic drugs at the D<sub>2</sub> dopamine receptor ( Data from Kapur et al. (2001c), data for amisulpride from Seeman (2002).*

<b>Antipsychotic</b>	<b><i>K<sub>off</sub></i> (per minute)</b>	<b>Dissociation time (t<sub>1/2</sub>)</b>
Quetiapine	3.013	<30 seconds
Clozapine	1.386	30 seconds
Amisulpride	0.730	<60 seconds
Olanzapine	0.039	17 minutes
Sertindole	0.014	18 minutes*
Haloperidol	0.017	42 minutes
Chlorpromazine	0.022	36 minutes

\* According to Seeman (2002). Older measurements showed 49 minutes

Broadly speaking low affinity / fast  $k_{off}$  (which are generally, but not always similar for a drug, with low affinity being correlated to fast  $k_{off}$ ) is a factor that may help to explain atypicality. There are however problems with that hypothesis: A more widely accepted and better evidence-based explanation is that prolactin rise and EPSE are associated with  $D_2$  blockade above a threshold of approximately 75% for prolactin rise and 80% for EPSE (Schlegel et al. 1996; Farde et al. 1997; Nyberg 1998); Another issue is that  $k_{off}$  can only be measured in drugs that it is possible to radiolabel, so information is lacking on some medications; Yet another problem with the  $k_{off}$  hypothesis is that aripiprazole has low  $K_{off}$  and is still atypical (although that may be due to it being a partial dopamine agonist). Whether or not “loose” binding is a helpful model for atypicality, it does not again address the issue of treatment-resistance.

#### *6.3.1.1.8. Other dopamine receptors*

$D_1$  is blocked by some antipsychotics (i.e. phenothiazines) but not others (i.e. butyrophenones).  $D_1$  receptor antagonists have been developed but pilot studies have not shown any benefit in the treatment of schizophrenia (Karlsson et al. 1995). Clozapine is a  $D_1$  receptor agonist (Salmi et al. 1996). The  $D_5$  receptor has similar properties to the  $D_1$  receptor but is currently not regarded as a useful site for drug development.

$D_4$ , which clozapine has higher affinity for than other antipsychotics (Van Tol et al. 1991) was a focus of some attention as a potential site for drug action. Large randomised clinical trials however, with the  $D_4$  receptor antagonists, fananserin and L-745 870 (Kramer et al. 1997), showed no effect in schizophrenia treatment.

$D_3$  is similar to  $D_2$  in many aspects. Highly selective  $D_3$  antagonists have been developed in recent years and may eventually prove useful, but currently the distinction between  $D_2$  and  $D_3$  receptors is rarely made.



#### **6.3.1.1.9. Serotonin**

The importance of the 5-HT<sub>2A</sub> receptor to antipsychotic action has been demoted somewhat by the discovery that 5-HT<sub>2A</sub> antagonism is not dose-related to antipsychotic effects (Travis et al. 1998) and that amisulpride has atypical qualities without any 5-HT<sub>2A</sub> antagonism. It is still an important receptor, a fact reflected in its ubiquity and presence anatomically in important locations, such as pyramidal cells of the cerebral cortex. Ritanserin, a selective 5-HT<sub>2A</sub> antagonist, has little effect on dopamine release when administered on its own. When given with a D<sub>2</sub> antagonist, raclopride, it does however stimulate release of dopamine in the prefrontal cortex, while striatal dopamine release remains unaffected (Andersson et al. 1995). Haloperidol and another 5-HT<sub>2A</sub> receptor antagonist, M100907, show synergy, especially at low doses of haloperidol (Liegeois et al. 2002) and it has again been suggested that one of the explanations for clozapine's profile may lie in its 5-HT<sub>2A</sub> blockade (Meltzer 2003b), including an effect on negative symptoms (Tamminga 2003b).

5-HT<sub>2C</sub> receptors are structurally related to the 5-HT<sub>2A</sub> receptor. They are located in the ventral tegmental and substantia nigra dopamine neurones and seem to have a facilitatory effect on basal dopamine efflux from the striatum (Lucas et al. 2000) and 5-HT<sub>2C</sub> antagonists can directly increase dopamine release in the nucleus accumbens. The clinical effect of this has not been established as yet.

5-HT<sub>1A</sub> receptors are functionally antagonistic to the 5-HT<sub>2A</sub> receptors both pre- and postsynaptically and it has been suggested that 5-HT<sub>1A</sub> agonists are similar to 5-HT<sub>2A</sub> antagonists in function. This has been supported by the finding that a 5-HT<sub>1A</sub> agonist, R(+)-8-OH-DPAT, stimulated release of dopamine in the prefrontal cortex and enhanced the effect of D<sub>2</sub> receptor blockers on dopamine release (Ichikawa et al. 1999). Aripiprazole is a potent 5-HT<sub>1A</sub> partial agonist and this may potentiate its antipsychotic action (Meltzer 2003b).

#### **6.3.1.1.10. Adrenergic receptors**

Affinity for  $\alpha_1$ -adrenoceptors has for antipsychotics been associated with sedation (Hertel et al. 1999). Clozapine blocks  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors. It has

been suggested that  $\alpha_1$ -adrenoceptor blockade may suppress striatal hyperdopaminergia and reduce the positive symptoms of schizophrenia.  $\alpha_2$ -adrenoceptor blockade may augment prefrontal dopaminergic functioning and improve negative and cognitive symptoms (Svensson 2003).

#### *6.3.1.1.11. Acetylcholine receptors*

Dopamine and acetylcholine are functionally antagonistic in the basal ganglia. Clozapine and some other antipsychotics are strongly anticholinergic (muscarinic  $M_1$  receptor). This blockade may increase acetylcholine release in the prefrontal cortex, which may have beneficial effects on cognitive function and possibly negative symptoms (Parada et al. 1997; Meltzer 2003b). A very recent study showed a dose-dependent rise in extracellular levels of acetylcholine in the frontal cortex when a selective  $D_1$  receptor agonist (SKF 82958) was administered to a rat and the same effect was seen when a selective  $D_3$  receptor antagonist (S 33084) was given. Both enhanced social memory. A  $D_1$  receptor antagonist and a  $D_3$  receptor agonist had respectively the opposite effect on acetylcholine levels and social memory when given after the  $D_1$  agonist and  $D_3$  antagonist (Panayi et al. 2005).

#### *6.3.1.1.12. Conclusion*

The continued interest in dopamine's role in schizophrenia stems both from its position as a sine qua non factor in antipsychotic drug action as well as a growing understanding of the complicated interactions of various neurotransmitters and neuronal pathways in the brain. The regulation of dopamine neurotransmission is far more sophisticated than previously thought with various sub-receptors interacting both pre- and postsynaptically. Additionally, the regulation of dopaminergic tone takes place through afferents from in particular the prefrontal cortex, via other pathways. The role of glutamate and both NMDA and GABA in schizophrenia has attracted much interest and the regulatory role of not only glutamatergic systems on dopamine, but even more importantly the "gating" role of dopamine on glutamatergic neurons and limbic activity, are providing a framework to incorporate more of the complicated and initially seemingly conflicting findings that research at all levels of neuroscience and clinical work are providing.

### **6.3.1.2. Theories regarding the mechanism of action in clozapine augmentation with antipsychotics**

Augmentation strategies have in the past been driven more by trial and error than by theory. Once a strategy seemed to be working, a hypothesis might be put forth regarding the possible mechanism underlying the response. More recently conclusions have been drawn from larger numbers of studies and a number of theories constructed regarding the underlying mechanism of response in augmentation (Freudenreich et al. 2002).

#### ***6.3.1.2.1. The "rich binding" hypotheses***

Clozapine has a "rich" binding profile, i.e. it binds to various neuroreceptors, including 5-HT<sub>2A</sub>, other serotonergic receptors, various dopamine receptors, noradrenergic, muscarinic, histaminergic and possibly glutamatergic receptors. This may be the reason for its greater efficacy. Studies where other antipsychotics are added (typicals but more so various atypicals with "rich" pharmacology) frequently employ the following argument; augmentation complements the binding profile of the original drug and hence increases its effect. The 5-HT<sub>2A</sub> / D<sub>2</sub> ratio hypothesis propagated by Meltzer (1989c; 1991) and others was a variant of such a theory, used to explain the action of clozapine. Effects of clozapine on the adrenergic and glutamatergic systems have also been used to explain its superior efficacy (Baldessarini et al. 1992; Goff et al. 2001; Tamminga et al. 2003c; Tuominen et al. 2005).

Using the theory of complementary receptor binding of two antipsychotics without referring to specific mechanisms is problematic when explaining augmentation of clozapine. Clozapine already binds to most of the receptors implicated in antipsychotic action and many of the atypicals were developed as an attempt to imitate the binding profile of clozapine.

#### ***6.3.1.2.2. The "extra-D<sub>2</sub> binding" hypothesis***

A more "parsimonious" hypothesis attempts to explain D<sub>2</sub>-like receptor binding in the context of clozapine's "rich pharmacology". This hypothesis can be

regarded as an attempt to bridge separate hypotheses, but with a clear suggestion regarding the neuroreceptor involved. It is based on the observation that some of the best evidence in augmentation strategies to date involves medications which selectively bind to dopamine D<sub>2</sub> (and D<sub>3</sub>) receptors, namely sulpiride and more recently amisulpride. Work with PET has indicated that less than 60% striatal D<sub>2</sub> occupancy is associated with poorer clinical response to typical antipsychotics (Remington et al. 1998). Clozapine is unusual in that it has been found to be effective at D<sub>2</sub> blockade levels below 60%. However, clozapine is not effective in 40-50% of the treatment-resistant cases where it is tried. This reflects the heterogeneity of treatment response in schizophrenia which is as wide and varied as the heterogeneity in the clinical syndrome of schizophrenia. Perhaps some of these clozapine-resistant though partially responding patients require more and possibly "tighter" D<sub>2</sub> binding than clozapine can provide whilst still requiring the "rich pharmacology" of clozapine. This is the hypothesis upon which the work in this thesis was based.

#### *6.3.1.2.3. The "increased serum clozapine" hypothesis*

A third hypothesis to consider is whether augmenting clozapine with another antipsychotic simply works by pushing up the levels of serum clozapine. This is a possibility that must be considered in augmentation trials, as levels of clozapine above a certain threshold seem associated with a better response, although Llorca et al. (2002), have reported no correlation between clozapine plasma levels and percentage improvement in clinical symptom ratings in a clozapine treated cohort of patients. Various papers have reported on serum clozapine levels in augmentation studies and generally they do not show a significant increase in serum clozapine levels (Henderson et al. 1996; de Groot et al. 2001; Agelink et al. 2004; Josiassen et al. 2005).

In the context of this thesis, this hypothesis anyway fits the data poorly as the stability of serum clozapine levels over the study period suggests that the benefit of amisulpride is not consequent upon raised serum clozapine levels and pharmacokinetic interactions. This is different from two case reports which described a doubling of clozapine levels following augmentation with

risperidone (Koreen et al. 1995). It seems that amisulpride does not cause such rise.

### **6.3.1.3. Current findings**

Study 2 described in chapter 4 in this thesis looks at what takes place neurochemically in terms of D<sub>2</sub>-like receptor occupancy when clozapine is augmented with a D<sub>2</sub>-like antagonist, amisulpride. At the time the study was being planned the “extra D<sub>2</sub> binding hypothesis” discussed above had not been clearly elucidated and one of the aims of the study was to see what effects augmentation with a D<sub>2</sub>-like receptor antagonist might have.

The results in study 2 show an increase in D<sub>2</sub>-like receptor occupancy from 47% at baseline to 59% 10-12 weeks after augmentation. Keeping in mind the “D<sub>2</sub> threshold” for response this indicates a move from a below threshold occupancy at baseline to a post-augmentation occupancy within the threshold range. These are however average numbers. In individual cases, although an increase is always seen in D<sub>2</sub>-like blockade, it sometimes goes from a very low D<sub>2</sub>-like occupancy up to only a slightly higher one. The threshold for response is likely to show great individual variation, so it is possible that even in those cases, a post augmentation occupancy rate of e.g. 47% may be within that individual's threshold range. It must be conceded however that in one case especially, where occupancy rate goes from 30% up to 34% it is unlikely to be meeting threshold levels. That patient did respond clinically however, with a reduction in PANSS<sub>total</sub> by 17 points, in PANSS<sub>pos</sub> by 6 points and in SANSS<sub>total</sub> by 16 points. Interestingly, in this patient, s-clozapine increased from 0.17 to 0.49 ng/L.

Amisulpride selectively blocks dopamine D<sub>2</sub> and dopamine D<sub>3</sub> receptors with little or no affinity for any other relevant receptor. The preferential blockade of mesolimbic rather than nigrostriatal dopaminergic transmission, the preferential blockade of dopamine D<sub>2</sub> autoreceptors rather than postsynaptic receptors and the preferential blockade of dopamine D<sub>3</sub> receptors in limbic areas have all been proposed as explanations for the atypical activity of amisulpride (Schoemaker et al. 1997). It is likely that the efficacy of amisulpride augmentation on clozapine monotherapy can be attributed to the

complementary receptor effects of the two drugs, rather than a pharmacokinetic phenomenon. Clozapine has a rich and complex receptor binding profile with low affinity for the D<sub>2</sub>-like receptor. Clozapine causes relatively little suppression of endogenous dopamine activity, an attribute which has recently attracted some interest (Kapur et al. 2001c). Amisulpride on the other hand is a limbic selective, highly specific D<sub>2</sub>-like receptor antagonist with a higher affinity than clozapine for the D<sub>2</sub>-like receptor, but again an antipsychotic which is unlikely to suppress endogenous dopamine activity, at least if  $k_{off}$  can be used as a marker for this. In this selected group of treatment-resistant patients clozapine isn't effective. Those who do respond to the amisulpride augmentation may belong to a subgroup of patients requiring higher levels of D<sub>2</sub>-like blockade than is obtained with clozapine. This mechanism was suggested in a review article some years ago (Freudenreich et al. 2002), but this is the first study to address this question experimentally.

Additionally, revisiting the "loose binding theory" / " $k_{off}$  theory" discussed above, it is worth remembering that clozapine's dissociation constant ( $k_{off}$ ) from D<sub>2</sub> is much higher than for typical antipsychotics ( $k_{off}$  = 63 nM for clozapine,  $k_{off}$  = 0.55 nM for haloperidol). This "loose binding" may explain the low D<sub>2</sub> binding observed as well as clozapine's unique effects. Perhaps some of these clozapine-resistant patients require not only more but also "tighter" D<sub>2</sub> binding than clozapine can provide? That could explain the effect in augmentation of medications such as amisulpride ( $k_{off}$  = 1.8) with an intermediate  $K_{off}$  and also that of risperidone ( $k_{off}$  = 1.1 nM) with a low dissociation constant.<sup>20</sup> However, other medications, such as sulpiride ( $k_{off}$  = 9.9) have a high dissociation constant (Seeman 2002), not that different from clozapine, but still seem to have an effect in augmentation. This indicates that "loose binding" is not as useful a model to explain augmentation of clozapine as increased D<sub>2</sub> binding.

What can be said is that patients with schizophrenia are a heterogeneous group. Identifying treatment-resistant patients is to identify a subgroup of people with schizophrenia who may be more responsive to clozapine. In this, the selection process that precedes clozapine therapy in most countries is

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<sup>20</sup> Dopamine's antipsychotic dissociation constant,  $k_{off}$  at the dopamine D<sub>2</sub> (high) receptor is 1.75 nM (Seeman 2002).

important. A study in China where clozapine is more indiscriminately used, found no benefit for clozapine over typical antipsychotics when it was used in first episode schizophrenia (Woerner et al. 2003). This indicates that the special qualities of clozapine are particularly useful in those who don't respond to other antipsychotics. It is a well replicated fact, however, that 40-50% of treatment-resistant patients fail to respond to clozapine monotherapy. In those patients, based on our and other studies, augmentation with a selective D<sub>2</sub>-like antagonist seems to provide benefit in the majority of cases and cause a major improvement in more than one third of cases. Synthesising the complementary lines of evidence it is possible to suggest that there are some patients with treatment-resistance who require the "rich" pharmacology of clozapine (a low affinity D<sub>2</sub> antagonist) complemented by a high D<sub>2</sub> affinity medication.

Amisulpride is the most selective D<sub>2</sub>-like antagonist currently available in the UK (Schoemaker et al. 1997) and is therefore the augmentation agent used in the studies here. The augmentation is likely to change receptor occupancy in other ways, which may be beneficial in these patients. This study did not measure other receptors or other D<sub>2</sub>-like binding sites than the striatum, so one can only hypothesise what these changes might be. They do involve increased striatal D<sub>2</sub>-like binding up to "therapeutic" levels, as the "therapeutic band" of 55-65% D<sub>2</sub> occupancy is reached. Additionally amisulpride is likely, due to its preference for (temporal) cortical binding to cause a larger increase in limbic D<sub>2</sub> binding but also a more pronounced blockade of pre-synaptic D<sub>2/3</sub> receptors, especially in the prefrontal cortex. This would increase dopaminergic activity in the cortex because D<sub>1</sub> receptors which are not blocked by amisulpride would be affected by the rise in extracellular dopamine concentrations caused by the presynaptic D<sub>2/3</sub> blockade (Lecrubier 2003). This activation of cortical D<sub>1</sub> receptors may be important for amisulpride's effect on negative symptoms. Clozapine is a D<sub>1</sub> agonist (Salmi et al. 1996) and the synergic action of clozapine and amisulpride on cortical D<sub>1</sub> receptors could explain the improvement in negative symptoms with augmentation described in chapters 3 and 4.

Furthermore, it is important to keep in mind that the binding by clozapine of other receptors, including serotonin and various other receptor systems is unaffected by augmentation. It is not at all clear what makes clozapine superior to other antipsychotics, but it seems likely that amisulpride does not interfere with that special effect, but augments or as in the case of negative symptoms complements it.

Recent findings have indicated that when clozapine's D<sub>2</sub> blockade is increased in animal models, using the isomer isoclozapine (which has a 10-fold higher affinity for D<sub>2/3</sub> but otherwise the same receptor profile), it loses its atypicality (Kapur et al. 2002b). This doesn't seem to be the case here as the side-effect profile didn't worsen with increased D<sub>2</sub>-like binding after augmentation. This however could be explained by the fact that in Kapur's study D<sub>2</sub>-like receptor occupancy increased to 79% while in study 2 here, it only went up to 59%, far from the 74-80% "threshold" for EPSEs. Another explanation might be that both clozapine and amisulpride have fast dissociation ( $k_{off}$ ) while it is likely that isoclozapine dissociates slowly, as high affinity is generally accompanied by slow dissociation.

Patients show a very marked, over five-fold increase in serum prolactin levels from baseline (248 mg/L) to 3 months after augmentation (1376 mg/L), which goes down to a four-fold increase at 6 months (1025 mg/L). This finding confirms what other studies have found (Schlosser et al. 2002; Bressan 2004). The anterior pituitary lies outside the BBB (Jaber et al. 1996). Amisulpride crosses the BBB poorly. It has been suggested that this poor permeability could be the reason for its effect on prolactin (Kapur et al. 2002a). Kapur's study in rodents compared the potency ( $ED_{50}$ )<sup>21</sup> of 4 atypical antipsychotics to raise prolactin and their potency for antagonism of apomorphine-induced stereotypy. Amisulpride had a very high central to peripheral  $ED_{50}$  of 21764 (compared with 1.7 for olanzapine). This much higher peripheral to central D<sub>2</sub> receptor occupancy of amisulpride means that to reach functional D<sub>2</sub> antagonism, very high peripheral (including in the anterior pituitary) antagonism is reached, causing increased prolactin secretion. This suggests that poor BBB penetrance

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<sup>21</sup>  $ED_{50}$  = Effective dose in half of cases



could explain the wide therapeutic range of amisulpride and could be yet another reason for EPSEs only being seen at high doses.

#### **6.3.1.4. Findings in good clozapine responders**

The findings of study 3 described in chapter 5 are worthy of further discussion. Many previous studies have described clinical, structural and genetic markers of good response to clozapine. No studies looking at D<sub>2</sub> binding have however focused on this.

In study 3, the good clozapine responders showed a response at low doses and low plasma-levels of clozapine, compared to the clozapine resistant group. At these low plasma clozapine levels, their D<sub>2</sub>-like occupancy rates were however quite high and on par with D<sub>2</sub>-like occupancy levels seen for non-responders at baseline. This equal D<sub>2</sub>-like occupancy, despite very different plasma clozapine levels is interesting. It is possible that long term clozapine use causes adaptation in dopamine systems. Treatment-resistant patients may well have relatively lower D<sub>2</sub>-like occupancy for their respective plasma levels because up-regulation of D<sub>2</sub>-like receptors has led to more available D<sub>2</sub>-like receptors. This higher availability of D<sub>2</sub>-like receptors, while not a characteristic of schizophrenia as such (Farde et al. 1990), may well be one of the things that characterise clozapine-resistant patients. Up-regulation of D<sub>2</sub>-like receptors is associated with the use of typical antipsychotics and with the development of tardive dyskinesia (Silvestri et al. 2000). Studies have indicated that treatment-resistant patients who reported previous EPSEs on typical antipsychotics are more likely to respond well to clozapine (Lieberman et al. 1994; Pickar et al. 1994). Patients sensitive to EPSEs are likely to be those that reach high striatal D<sub>2</sub>-like blockade at relatively low doses of antipsychotic.

Bringing this information together, it is conceivable that a propensity for EPSEs indicates a dopamine system that has not undergone much D<sub>2</sub>-like receptor up-regulation. This lack of up-regulation could be a marker of good future response to clozapine. It is possible that those who end up being resistant to clozapine are from the start more unwell and hence liable to receive higher doses of

antipsychotics, which, particularly if the antipsychotics have good passage through the BBB and bind tightly to the D<sub>2</sub>-like receptor, could result in receptor up-regulation. Alternatively, D<sub>2</sub>-like receptor up-regulation could possibly be a more salient feature of treatment-resistance. In either case, D<sub>2</sub>-like receptor up-regulation is a plausible explanation for the higher clozapine dose and plasma clozapine levels seen at baseline in non-responsive patients compared to good responders.

More importantly the responders actually do well at a D<sub>2</sub>-like occupancy that in non-responders is not adequate for response. This underlines how different clozapine-resistant patients are from clozapine responders as well as likely other responders to antipsychotics. The findings of study 3 indicate that the augmentation strategy used is, actually, in terms of D<sub>2</sub>-like binding making the non-responders less like good clozapine responders by increasing D<sub>2</sub>-like binding occupancy.

### **6.3.2. Treatment**

The findings of this thesis add to current knowledge regarding augmentation strategies in clozapine resistance. These are commonly used but with little empirical basis. This thesis looked at augmentation with a highly selective D<sub>2/3</sub> antagonist. What was already known about the use of D<sub>2</sub> antagonist augmentation from RCTs is that sulpiride improves positive and negative symptoms quite markedly when added to clozapine treatment in people who do not respond to clozapine monotherapy (Shiloh et al. 1997). The response was seen in half of those treated. Further to that study eight case reports and open label studies with a total of 50 patients have reported a benefit in the majority of patients when amisulpride is used for augmentation (Cook et al. 2004; Agelink et al. 2004; Zink et al. 2004b ; Kampf et al. 2005; Croissant et al. 2005; Lerner et al. 2005; George et al. 2005; Kalaitzi et al. 2005).

Study 1, described in chapter 3 adds to knowledge within this field. It is the largest of the amisulpride augmentation studies so far, with 28 patients finishing the study. The group was well defined and had been on clozapine for more

than six months. The follow up period was long and a number of clinical and side-effects rating scales were employed. The problems inherent in an open-label study have to be kept in mind but naturalistic studies also have their strengths, not least that they take place in natural circumstances and answer real world questions (Roy-Byrne et al. 2003).

Most response studies and all augmentation studies published report improvement if more than 20 % improvement in clinical rating scales is seen. This is a realistic goal, reflecting the reality that although antipsychotic drugs improve psychosis they rarely cause complete remission, especially in patients where treatment-resistance is already established. Response of more than 50% is a rare and unrealistic goal of treatment (Stahl 2003). Keeping this in mind the results presented in chapter 3 are encouraging. The results show that when a response definition of an improvement of at least 20% on a clinical rating scale (the minimum improvement seen by experienced clinicians (Cramer et al. 2001) is used), 71% respond or 61% if an intention-to-treat analysis is utilised. If, however, a more stringent criterion of at least a 50% improvement is used, 32% (27% of whole group started) meet the response criteria. This indicates a marked clinical response in nearly three-fourths and a major response in one third of those who stayed the course. Although this might be taken to mean that more people respond to amisulpride than sulpiride, one cannot be certain of this. This is an open label study and it may well be that the response to amisulpride use is exaggerated, possibly by the rater, but even more so by the patients who may have been affected by the study group's enthusiasm. Hence it is quite possible that the response is smaller, and more similar to the response described to sulpiride augmentation in Shiloh et al. (1997). This will only be settled with a double-blind RCT.

The rating scales used in this study do not measure cognitive factors directly. Negative symptoms and cognitive factors have a bearing on each other, but nothing can with certainty be deduced from the study about the cognitive effects of augmentation. The item most commonly responsible for the non- (but nearly) significant reduction in the CAERS scale was a reduction in drowsiness by 6 months. If that result is genuine, then it has face validity as amisulpride is

known to alert and could perceivably counter the sedation frequently caused by clozapine, as well as potentially improve cognitive function through increased prefrontal cortex activation.

The findings indicate that the augmentation is well tolerated. This is an important aspect of amisulpride augmentation which may set it apart from many other augmentation strategies. In many case reports and case series in the literature no mention is made of side effects and it is sometimes unclear whether this is an omission or because no side effects were noticed. Best information exists for risperidone. Studies using risperidone as augmentation agent found akathisia (4 of 12 patients) and hypersalivation (5 of 12) (Henderson et al. 1996), orthostatic hypotension (1 of 12) (de Groot et al. 2001), worsening compulsion (1 of 13) (Taylor et al. 2001) and elevated prolactin levels (16 of 20 patients) (Henderson et al. 2001). A recent RCT using risperidone (n=40) found no increase in side-effects, compared with placebo augmentation however (Josiassen et al. 2005). The RCT using sulpiride as augmenting agent found worse sialorrhea (1 of 28), worse tardive dyskinesia (1) and increased prolactin (Shiloh et al. 1997). Compared with this our study did very well with no change in akathisia (Barnes Akathisia Scale), EPSEs (Simpson & Angus Scale), involuntary movements (AIMS) or clozapine side effects (CAERS).

Looking at those who responded by at least 20%, the proportion of patients who responded in terms of negative symptoms was very similar to the proportion who responded in terms of positive symptoms. There was much, but not total overlap in the cohorts who responded to positive and negative symptoms respectively. The difference in response is interesting and may be due to a number of factors. The fact that amisulpride shows a robust improvement in all subscales of the SANS and the PANSS negative scale combined with an unchanged side-effect profile indicates that the improvement may be due to a genuine effect on more persistent negative symptoms rather than being caused by a change in side-effects.

Overall, this thesis brings further support to the notion that amisulpride is a valid augmentation option when patients respond poorly to clozapine monotherapy. It is an effective option that affects all symptom domains and is well tolerated. These findings have some relevance for the treatment of schizophrenia.

### **6.3.3. Statistical issues**

When attempting to interpret the findings presented in this thesis, it is important to remember the possibility of type I and type II errors. Type I errors (finding a difference in group samples when there isn't one present) are particularly worth keeping in mind when considering the results of study 1. This is because of the risks inherent in a non-blind, non-randomised study. The findings do however fit the current literature well which adds to their face validity. Only a double-blind RCT, with all the logistic difficulties such a study entails in a very unwell patient sample, would rule out a type 1 error. Type 1 error is far less likely in studies 2 and 3. It is however worth keeping in mind, as pointed out by Stern et al. (1997) that even double-blind RCTs are marred by problems. Large patient numbers (between 40 and 100) and sticking to one question only per study were suggested by Stern et al. to reduce the likelihood of type I errors in augmentation studies.

Type II errors (failing to find a difference where one exists) are more likely to be a problem in studies 2 and 3, as the small number of patients reduces the power of each study. Therefore, although there is a clear trend towards a difference between D<sub>2</sub> occupancy in clozapine responders and non-responders post augmentation, the study fails to show a statistically significant difference. This could be addressed by repeating the scanning studies, using a larger sample.

Many augmentation studies are marred by unclear or lenient inclusion criteria. Although the sample size wasn't large, the samples investigated in these studies were well defined. Treatment-resistance was established through at least 2 types of antipsychotics tried before clozapine treatment. Clozapine had been used in a clinically optimised dose for at least 6 months and high scores

on the BPRS were needed for entry into the study. This should mean that the homogeneity of the sample is greater than in many previous studies and the patients are likely to be truly resistant to past treatment. This hopefully allows clearer conclusions to be drawn from the sample.

#### **6.4. Future directions**

Future approaches to the treatment of schizophrenia will increasingly involve a range of different approaches as the results of this thesis suggest. One approach will be tried after the other, perhaps following a flow-chart, where each consecutive step is evidence-based; all in an attempt to narrow down and define better the group being given the next treatment. There is a need for improving the evidence base for each of the pharmacological approaches available. Even more so there is a dire need for more effective antipsychotics – particularly ones that help alleviate negative and cognitive symptoms – without adverse effects which draw from their appeal and reduce quality of life.

Furthermore, there is a growing need for greater understanding of the pathophysiology and characteristics of treatment response. These characteristics include clinical, which are already somewhat recognised, phenotypical, including the relevance of receptor profiles and last but not least the genetic predictors of treatment response. Work is already under way in all of these areas, but further understanding is needed within this field, to reduce morbidity and mortality and spare financial resources, through more targeted and evidence-based treatment.

The studies in this thesis point to the need for further research into the role of amisulpride. A double-blind RCT is needed to ascertain the usefulness of amisulpride augmentation of clozapine. It may be worth heeding the advice of authors who ask for pragmatic RCTs where the patients are not too narrowly selected and the questions ones of effectiveness rather than efficacy, looking at wider aspects of effectiveness such as functional recovery, treatment adherence and patient satisfaction (Hotopf 2002; Essock et al. 2003; Roy-Byrne et al. 2003; Peuskens 2004b). It is unlikely that <sup>123</sup>I-IBZM SPET will have

any direct clinical relevance in individual cases. However, neuroreceptor studies are likely to continue to play an important role in understanding treatment response and lack of it.

The fact is that all functional antipsychotics to date block D<sub>2</sub>-like receptors and clearly fail to meet the expectations we have for a fully effective schizophrenia drug. This indicates that although D<sub>2</sub>-like blockade may be a necessary part of the solution it is only in rare cases sufficient. The real future of pharmacological strategies for schizophrenia, particularly when standard treatment fails, lies in better understanding of the psychophysiology and psychopathology of the illness and treatment strategies that work for particular patient populations.

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## **Appendix A. Word list**

**AIMS = Abnormal involuntary movements scale**

**BBB = Blood brain barrier**

**BP = Binding potential**

**BPRS = Brief psychiatric rating scale**

**CAERS = Clozapine adverse effects rating scale**

**CAS = Calgary anxiety scale**

**CDS = Calgary depression scale**

**DLPFC = Dorso-lateral prefrontal cortex**

**DM = Diabetes mellitus**

**DUI = Duration of untreated illness**

**DUP = Duration of untreated psychosis**

**ECG = Electrocardiogram**

**ECT= Electro-convulsive therapy**

**EEG = Electroencephalogram**

**EPSE = Extrapiramidal side effects**

**FBC = Full blood count**

**FWHM = Full width at half maximum**

**GAF = Global assessment of functioning**

**GAS = Global assessment scale**

**LFTs = Liver function tests**

**LQoLP = Lancashire quality of life profile**

**PANSS = Positive and negative symptom scale**

**PET = Positron emission tomography**

**PFC = Prefrontal cortex**

**PMT = Photomultiplier tubes**

**RCT = Randomised controlled trial**

**ROI = Region of interest**

**rTMS = repetitive transcranial magnetic stimulation**

**SD = standard deviation**

**SPET = Single photon emission tomography**

**SSRI = Selective serotonin reuptake Inhibitors**

**TFTs = Thyroid function tests**

## **Publications arising from this work**

Munro, J., Matthiasson, P., Osborne, S., Travis, M., Purcell, S., Cobb, AM., Launer, M., Beer, D., Kerwin, RW. "Amisulpride augmentation in patients with schizophrenia partially responsive to clozapine - an open familiarisation study." Acta Scandinavica Psychiatrica 2004. Vol. 110(4).292-8.

Matthiasson P, et.al. "Augmentation of clozapine with amisulpride. A  $^{123}\text{I}$ -IBZM SPET scanning study". In manuscript.

Matthiasson P, et.al. "Good responders to clozapine are different from clozapine-resistant patients. A  $^{123}\text{I}$ -IBZM SPET scanning study". In manuscript.

