BEHAVIOURAL, PHARMACOLOGICAL AND NEUROCHEMICAL STUDIES OF SOCIAL ISOLATION REARING IN RATS

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-TO MY SAVIOUR,

JESUS CHRIST-

Abstract

Purpose:

Schizophrenia affects approximately 1% of the population. Despite marked improvement in drug treatment, 20% of patients remain treatment resistant while motor side effects hamper compliance and outcome. A better understanding of the disorder is needed, as is the need for new and improved drug treatment. Due to shown correlation between animal models to human counterparts, validated animal models enable screening and details study of the neurobiological underpinnings and treatment of a psychiatric illness. Adverse life experiences during early development have been advocated as an important risk factor in the development of schizophrenia. The neurodevelopmental theory of schizophrenia suggests a dysfunction in glutamate, particularly in the frontal cortex, where it impacts on brain development and function. Deficits in sensory motor gating, such as altered prepulse inhibition (PPI) of startle, are a typical manifestation of schizophrenia. The aim of this study was firstly, to study PPI changes in rats following acute challenge with the glutamate NMDA antagonist, dizocilpine (MK-801), and its dose-dependent reversal by atypical and typical neuroleptics. Thereafter, we sought to determine the relationship between PPI changes and frontal cortical NMDA and D_1 receptor binding characteristics in a neurodevelopmental animal model of schizophrenia, based on the concept of isolation rearing, and its subsequent response to sub-chronic treatment with atypical and typical antipsychotics.

Methods:

PPI testing: PPI was determined using a San Diego SR-lab startle response system. 120 dB pulses were presented with or without prepulses (76 dB, 80 dB, 84 dB), using a 67 dB background noise.

MK-801 model: Animals received either saline (i.p.) pre-treatment 30 min prior to PPI testing, followed by either a second saline injection (control), or MK-801 (0,25 mg/kg i.p.) 15 min prior to PPI testing. Further groups were pre-treated with increasing dosages of either clozapine (5 or 10 mg/kg i.p.) or haloperidol (0,1; 0,2 or 0,5 mg/kg i.p.) 30 min before PPI, followed 15 min later by either saline or 0,25 mg/kg MK-801.

Catalepsy: Catalepsy was routinely assessed using a rat catalepsy box, repeated at 30 min intervals for 240 min after neuroleptic administration.

Social isolation model: Animals were group- or isolation-housed for 8 weeks and assessed for deficits in PPI, as well as effects on frontal cortical NMDA and D_1 receptor binding characteristics. Isolation groups were injected with either clozapine, haloperidol or saline in the last 11 days of isolation rearing, using dosages identified in the aforegoing Mk-801 study, and their effects on PPI and cortical receptor binding determined. In addition, the effect of 4 weeks re-socialisation during the last 4 weeks of the isolation period, and its effects on PPI, was studied. Additional validation studies were performed on NMDA receptor binding in the nucleus accumbens in isolation reared rats with and without chronic drug treatments. In order to verify the effects of injection stress on PPI and to exclude it as a possible confounding factor in the chronic drug treatment studies, we studied the effect of handling in group-housed and isolation reared rats and the resulting effects on PPI.

Results:

MK-801 evoked significant deficits in PPI that were reversed by 5 mg/kg and 10 mg/kg clozapine but not by dosages of haloperidol that did not simultaneously induce catalepsy (0,1; 0,2 mg/kg). Significant deficits in PPI, together with increased frontal cortical NMDA density (with a decrease in affinity), and decreased D_1 receptor

density (and increased affinity), were induced after 8 weeks of social isolation. The associated PPI deficits induced by social isolation stress (SIS) could not be reversed by re-socialization. NMDA receptor binding characteristics in the nucleus accumbens were not altered in the social isolation paradigm. Chronic clozapine but not haloperidol treatment blocked PPI deficits following social isolation, with haloperidol not affecting NMDA receptor density, but clozapine significantly increasing this compared to the isolation-reared animals. While clozapine did not alter D_1 receptor density compared to the isolated animals, haloperidol significantly reduced this. In both treatment groups cortical NMDA receptor affinity was, however, significantly increased compared to that of the isolation reared animals, with D_1 receptor affinity also increased by haloperidol and a tendency of increased D_1 receptor affinity was shown by clozapine in isolation reared animals. No changes were observed in the nucleus accumbens with respect to NMDA receptor binding characteristics.

Conclusion:

Rodents reared in isolation have significant deficits in sensory motor gating that are correlated with up-regulation of frontal cortex NMDA receptors and down-regulation of D_1 receptors. Moreover, chronic treatment with clozapine but not haloperidol reverses SIS induced PPI deficits, similar to that observed during hypoglutamatergia induced in the acute MK-801 model, confirming that PPI deficits evoked by either pharmacological means or by neurodevelopmental trauma represent glutamate driven mechanisms that respond selectively to atypical antipsychotic agents. Haloperidol abrogates cortical D_1 receptor density in animals subjected to isolation stress, while clozapine is conservative in this action. The atypical action of clozapine involves bolstering of glutamatergic pathways, while striving to maintain cortical dopaminergic transmission.

KEY WORDS: *schizophrenia, NMDA receptor, social Isolation, dizocilpine, clozapine, haloperidol, D_1 receptor, prepulse inhibition, animal model, frontal cortex*

Opsomming

Doel:

Skisofrenie affekteer ongeveer 1% van die bevolking. Ten spyte van duidelike vooruitgang in behandeling met geneesmiddels bly 20% van pasiënte steeds weerstandig teenoor behandeling terwyl motoriese newe-effekte meewerkendheid en die uitkoms belemmer. 'n Beter begrip van hierdie versteuring is nodig net soos wat daar 'n behoefte aan nuwe en beter behandeling is. Diermodelle maak die gedetailleerde studie van die neurobiologie en behandeling van 'n psigiese siekte moontlik. Onaangename lewenservarings vroeg tydens ontwikkeling is as 'n belangrike risikofaktor vir die ontwikkeling van skisofrenie voorgehou. Die teorie van neuro-ontwikkeling van skisofrenie stel 'n disfunksie in glutamaat, veral in die frontale korteks, voor waar dit ontwikkeling en funksie van die brein beïnvloed. Tekortkominge in die vermoë om sensoriese stimulasie te filtreer, soos veranderde prepulsinhibisie (PPI) van die skrikreaksie, is 'n tipiese manifestasie van skisofrenie. Die doel van hierdie studie was eerstens om die effek op PPI na akute blootstelling aan die glutamaat-NMDA-antanonis disokilpien (MK-801) en die dosisafhanklike omkering daarvan deur atipiese en tipiese neuroleptika, te bestudeer. Verder, het ons die verwantskap tussen veranderings in PPI en eienskappe van frontale kortikale NMDA- en D_1-reseptorbinding in 'n diermodel van neurologiese ontwikkeling van skisofrenie, gebaseer op die konsep van sosiale isolasie, en die daaropvolgende respons teenoor sub-kroniese toediening van atipiese en tipiese neuroleptika bepaal.

Metodes:

PPI-toetse: PPI is bepaal deur 'n San Diego SR stelsel vir meting van skrikrespons te gebruik.

Pulse van 120 dB met of sonder prepulse (76 dB, 80 dB, 84 dB) is toegedien deur 'n agtergrondgeraas van 67 dB te gebruik.*MK-801-model:* Diere het 30 min voor die PPI-toetse voorafbehandeling met soutoplossing (i.p.) gevolg deur 'n tweede inspuiting van óf soutoplossing (kontrole) óf MK-801 (0,25 mg/kg i.p.) 15 min voor die PPI-toetse ontvang. Verdere groepe is 30 min voor PPI met toenemende dosisse van óf klosapien (5 of 10 mg/kg i.p.) óf haloperidol (0,1; 0,2 of 0,5 mg/kg i.p.) behandel wat 15 min later met óf soutoplossing óf 0,25 mg/kg MK-801 opgevolg is.

Katalepsie: Katalepsie is roetinegewys beoordeel deur 'n katalepsiehouer vir rotte te gebruik en dit is vir 240 min na toediening van die neuroleptikum elke 30 min herhaal.

Model van sosiale isolasie: Diere is vir 8 weke in groepe of in isolasie gehuisves en vir tekortkominge in PPI asook effekte op eienskappe van frontale kortikale NMDA- en D_1-reseptorbinding beoordeel. Groepe wat in isolasie gehou is, is in die laaste 11 dae van isolasie met klosapien, haloperidol of soutoplossing ingespuit teen dosisse soos in die voorafgaande studie met MK-801 bepaal en die effekte daarvan op PPI en kortikale reseptorbinding is bepaal. Daarby is die effekte van hersosialisering vir 4 weke tydens die laaste 4 weke van die isolasieperiode en die effek daarvan op PPI bestudeer. Bykomende validasiestudies is gedoen van NMDA-reseptorbinding in die nucleus accumbens met en sonder chroniese behandeling met middels van rotte wat in isolasie grootgemaak is. Ten einde die effek van stres vanweë die inspuiting op PPI te bestudeer en om dit as 'n moontlike bydraende faktor in studies van chroniese behandeling met geneesmiddels uit te sluit, het ons die effek van hantering van rotte in groepe en in isolasie grootgemaak en die effekte daarvan op PPI bestudeer.

Resultate:

MK-801 het beduidende tekortkominge in PPI teweeggebring wat deur 5 mg/kg en 10 mg/kg klosapien omgekeer is, maar nie deur dosisse van haloperidol wat nie terselfdertyd katalepsie geïnduseer het nie (0,1; 0,2 mg/kg). Beduidende tekortkominge in PPI saam met hoër frontale kortikale NMDA-digtheid (met 'n afname in affiniteit) en laer D_1-reseptordigtheid (en hoër affiniteit) is na 8 weke van sosiale isolasie geïnduseer. Die meegaande tekortkominge in PPI wat deur stres vanweë sosiale isolasie (SSI) geïnduseer is, kon nie deur hersosialisering omgekeer word nie. Eienskappe van NMDA-reseptorbinding in die nucleus accumbens is nie deur sosiale isolasie beïnvloed nie. Chroniese behandeling met klosapien, maar nie met haloperidol nie, het die tekortkominge in PPI na sosiale isolasie geblokkeer terwyl haloperidol nie NMDA-reseptordigtheid beïnvloed het nie, maar klosapien dit beduidend verhoog het vergeleke met diere wat in isolasie gehou is. Waar klosapien nie D_1-reseptordigtheid vergeleke met dié van geïsoleerde diere beïnvloed het nie, het haloperidol dit beduidend verlaag. Kortikale NMDA-reseptoraffiniteit was egter in albei behandelde groepe beduidend hoër vergeleke met dié van geïsoleerde diere, met D_1-reseptoraffiniteit ook deur haloperidol verhoog terwyl 'n neiging tot hoër D_1-reseptoraffiniteit deur klosapien in geïsoleerde diere aangetoon is. Geen verandering in die eienskappe van NMDA-reseptorbinding in die nucleus accumbens is waargeneem nie.

Gevolgtrekking:

Knaagdiere wat in isolasie grootgemaak is, het beduidende tekortkominge in hul vermoë om sensoriese stimulasie te filtreer, wat met opregulering van NMDA-reseptore in die frontale korteks en afregulering van D_1-reseptore korreleer. Daarby keer chroniese behandeling met klosapien, maar nie met haloperidol nie, tekortkominge in PPI geïnduseer deur SSI om, soortgelyk aan dié waargeneem tydens hipoglutamatergie geïnduseer in die akute MK-801-model wat bevestig dat tekortkominge in PPI, wat óf farmakologies óf deur neuro-ontwikkelende trauma veroorsaak is, meganismes deur glutamaat behels wat selektief op atipiese antipsigotiese middels reageer. Haloperidol verlaag kortikale D_1-reseptordigtheid in

diere wat aan stres vanweë isolasie blootgestel word terwyl klosapien dit net effens doen. Die atipiese werking van klosapien behels ondersteuning van die glutamatergiese roetes terwyl dit streef om kortikale dopaminergiese oordrag vol te hou.

KERNWOORDE: *skisofrenie, NMDA reseptor, sosiale isolasie, disokilpien, klosapien, haloperidol, D1 reseptor, prepulsinhibisie, diermodel, frontale korteks,*

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List of Abbreviations

AMPA	alpha -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
CSF	Cerebrospinal fluid
D_1	Dopamine-1
D_2	Dopamine-2
DSM-IV	Diagnostic and Statical Manual of Mental Disorders
EPS	Extrapyramidal side effects
GABA	γ -amino butyric acid
Nacc	Nucleus accumbens
NMDA	N-methyl-D-aspartate
NMS	Neuroleptic malignant syndrome
PCP	Phencyclidine
PFC	Prefrontal cortex
PPI	Prepulse inhibition
SIS	Social isolation stress
TD	Tardive dyskinesia
VTA	Ventral tegmental area
WHO	World Health organisation

CHAPTER 1

Introduction

1.1 *Problem Statement*

Schizophrenia is a neuropsychiatric disorder affecting approximately 1% of the population worldwide (Weiss & Feldon, 2001). Despite the availability of drug treatment for the disorder since the late 1950's, 20 % of patients on antipsychotic treatment experience a relapse during their lifetime (Fleishhacker et al. 1997). More startling is that between 20-40% of patients on these agents develop restless legs syndrome, 30-60% develop parkinsonism, 10-20% develop dystonia with up to 5% developing tardive dyskinesia (Harvey *et al.*, 1999; Kelly *et al.*, 2005) . The latter figures are very likely responsible for the high incidence of poor adherence among patients using these drugs, with between 40-50% of patients being non-compliant. While the introduction of the atypical group of compounds has led to improvements in treating positive, negative and disorganization symptoms in schizophrenia (Meltzer, 1992), side effects such as extrapyramidal side effects (EPS) (Miyamoto et al., 2002) and Tardive dyskinesia (TD), (Miyamoto et al., 2002) although lower, remains a clinical problem. Moreover, other previously unrecognised adverse effects, such as metabolic side effects, are more often seen (Newcomer, 2007). Lack of overall efficacy and the often intolerable side effect burden severely compromise successful long term treatment outcome of schizophrenia. Clearly, there is an urgent need for new drugs for the treatment of schizophrenia. However, before this can be achieved, new and appropriately validated animal models for preclinical research are imperative. Further, new neuro-biological targets need to be identified that will play a more decisive role in effective management of the illness.

Although the role of dopamine in the neurobiology of schizophrenia is beyond reproach, the above facts concur that there is more to the disorder than is currently

known. It is now recognised that schizophrenia is not a single neurotransmitter disorder, but rather a multifaceted disorder involving genetic, environmental and neurodevelopmental factors (Weiss & Feldon, 2001). With environmental conditions during pregnancy, at birth and in childhood being critical for neuronal development (O'Callaghan *et al.*, 1991; Takei *et al.*, 1996), early-life stress and associated effects on neuronal growth and differentiation (Weiss & Feldon, 2001; Bloom, 1993; Murray, 1994; Roberts, 1990; Weinberger, 1987) has been deemed an important risk factor for the later development of schizophrenia (Lipska & Weinberger, 2000). A hallmark characteristic of schizophrenia is that patients are unable to ignore and discard irrelevant sensory stimuli (Bleural, 1911; Weiss & Feldon, 2001) due to deficits in sensorimotor gating (Braff *et al.*, 1978). This behavioural response can be assessed in both humans (Braff *et al.*, 1999; Grillon *et al.*, 1992) and rodents (Geyer *et al.*, 2001) using the prepulse inhibition (PPI) paradigm. PPI describes the normal attenuation of the startle reflex to an intense acoustic stimulus when the latter is immediately preceded by a weaker stimulus (prepulse) (Graham, 1975; Braff *et al.*, 1999). While the subcortical limbic regions, notably the nucleus accumbens (Nacc), is recognised as a critical brain region involved in the psychotic manifestations of schizophrenia (Carlsson *et al.*, 2004), in recent years the important role of the frontal cortex has been increasingly realised (Weiss & Feldon, 2001), especially with respect to the cognitive manifestations of the illness (Schneider *et al.*, 1994). Indeed, early neurodevelopmental abnormalities of the prefrontal cortex appear to underlie the later development of schizophrenia (Lipska & Weinberger, 2000). Deficits in PPI is an appropriate model to investigate and understand the neural control exerted by cortical and limbic structures on sensorimotor gating processes and the probable dysregulation of these processes in schizophrenia (Weiss & Feldon, 2001). Therefore this may represent an important behavioural trait upon which the development of an animal model can be achieved.

Apart from dopamine, one of the key transmitters involved in the neurocircuitry of schizophrenia is glutamate. "Glutamate plays a key role in recent models of schizophrenia and represent a new approach for pharmacologic treatment" (Javitt & Zukin, 1991; Olney & Farber, 1995). Various tracts in the brain, including corticocortical, thalamocortical and corticocortical association fibers, have been

implicated in schizophrenia, and utilize glutamate as a neurotransmitter (Huntley *et al.*, 1994). Abnormalities in these pathways are known to produce symptoms of schizophrenia (Murase *et al.*, 1993; Castner & Williams, 2007). There is also a significant degree of cross-talk between glutamatergic and dopaminergic systems in especially the cortico-striatal tracts that are deemed critical in schizophrenia and as such can modulate the activity of the other (Grace, 1991).

Glutamatergic inputs are essential for the prefrontal cortex's functioning (Miller, 2000). On the other hand, dopamine has a crucial role in prefrontal cortex (PFC) cognitive functions including reward, working memory and attention. (Schultz, 2002). Several authors have also highlighted the need for dopamine-glutamate co-activation for a number of PFC functions (Gurden *et al.*, 1999; Baldwin *et al.*, 2002; Jay, 2003). Therefore, the interaction between dopamine and glutamate receptors may be a key for proper PFC function. Prefrontal dopamine release is strongly regulated by glutamate via NMDA receptors (Murase *et al.*, 1993). Indeed, the interaction between D_1 and NMDA receptors in this brain region forms a fundamental neural substrate for cognition, particularly in disorders such as schizophrenia, where cognitive function is an important indicator of outcome (Castner & Williams, 2007). Although disturbances in glutamate-dopamine cross talk can contribute to the profile of cognitive dysfunction in schizophrenia (Holcomb *et al.*, 2004), are these neurotransmitters equally involved? Moreover, how do glutamate and dopamine interact in the frontal cortex, and is this interaction important in the response to drug treatment?

1.2 *Project aims*

This study will strive to set up two animal models of schizophrenia in our laboratory. The first will be to establish and validate a pharmacological challenge model based on acute administration of the glutamate NMDA receptor antagonist, dizocilpine, using PPI as behavioural outcome. PPI changes following acute MK-801 challenge, and its dose-dependent reversal by typical (haloperidol) versus atypical (clozapine) neuroleptics, will be studied. Thereafter, we shall use a non-pharmacological (i.e.

non drug-induced) model in an attempt to correlate behavioural and neurochemistry changes. In this neurodevelopmental model of schizophrenia, PPI and cortical NMDA and D_1 receptor binding will be investigated. Using this model, we shall attempt to find a correlation between frontal cortical NMDA as well as D_1 receptor characteristics and sensory motor gating deficits in rats following post natal social isolation stress (SIS). In addition, NMDA binding characteristics in the nucleus accumbens (Nacc) will also be investigated. These data will thus specifically address the face (PPI changes) and construct (NMDA, D_1 receptor changes) validity of the SIS model.

Given the acknowledged differences between the typical and atypical drugs' efficacy in treating schizophrenia (Rang *et al.*, 2003) and by using the most appropriate dosages identified in the MK-801 studies described above, the next aim of the study will be to investigate the predictive validity of the SIS model. Thus, the response of SIS-induced PPI and neuroreceptor changes to chronic treatment with the above-mentioned typical (haloperidol) and atypical (clozapine) neuroleptics will be studied.

Since Sprague-Dawley rats have been found to be the most responsive to PPI changes after social isolation (Weiss *et al.*, 2000; Bakshi *et al.*, 1998; Geyer *et al.*, 1993), both the MK-801 and SIS studies will be performed in these animals.

1.3 *Project Layout*

1.3.1 MK-801 model

The effect of acute treatment with various dosages of the non-competitive NMDA receptor antagonist, MK-801, on PPI will be determined. In order to determine the predictive validity of this procedure, the dose-dependent abilities of haloperidol, a typical agent, and clozapine, an atypical agent, to reverse MK-801-induced PPI deficits, will be studied. Finally, in order to exclude a possible influence of the locomotor effects of the drugs on PPI, the rodents will be routinely tested for drug-

induced catalepsy. The most effective dose of either drug, without cataleptic actions, will be used in the chronic treatment arm of the social isolation studies, described below.

1.3.2 Social isolation model

On postnatal day 21 pups will be randomly assigned to two different housing conditions, namely pups reared in isolation (1 rat per cage) or socially (3 rats per cage). These housing conditions will be maintained for a period of 8 weeks, whereupon PPI testing will take place. In order to confirm the prerequisite of 8 weeks isolation to induce PPI changes, another isolation group will be re-socialised at week 4 and will remain as such for the remainder of the 8 week period. Again PPI assessments will be performed and compared to 8 week isolated and group-housed animals.

In order to assess the response of the model to drug treatment, isolated rodents will be treated chronically with either haloperidol or clozapine or saline, at dosages decided upon from the MK-801 study above, this will be carried out in isolated, group housed and isolated animals receiving drug/ saline-treatment.

Immediately after PPI testing, group-housed, isolated animals and isolated animals receiving drug/ saline treatment will be sacrificed and the frontal cortex and nucleus accumbens (Nacc) removed. These brain regions will be set aside for radioligand receptor binding studies, including D_1 and NMDA receptors in the frontal cortex, and NMDA studies in the Nacc.

1.4 *General points*

This dissertation will be written and submitted in the article format for thesis/dissertation submission, as approved by North-West University. This format includes an introductory chapter, a chapter covering the relevant literature overview, and a chapter containing a full length article for submission to a peer-review, accredited neuroscience journal. Carefully selected, novel and high impact data from the study will be used for this submission. To this end, the article will be prepared according to the house style and author instructions of that particular journal. This house style and the instructions to authors are provided in Appendix 1. All other work performed during this study, including additional validations as well as work performed during the course of the study but not included in the journal article, are also provided in the appendices.

The behavioural (PPI) and neuro-receptor methodology (D_1, NMDA radio-receptor studies), antipsychotic drug-treatment protocols, results and discussion surrounding the initial MK-801 challenge model, as well as the later application of these to the social isolation stress model, and resulting effects on PPI and on cortical D_1 and NMDA receptor binding, form the focus of a full original research paper that will be submitted for publication in *Psychopharmacology (Berl)* (Springer). Additional work performed, including the effects of re-socialisation, assessment of NMDA receptor binding in the Nacc and the effect of handling on the isolation paradigm will be presented in Appendices 2, 3 and 4 respectively.

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CHAPTER 2

Literature Review

2.1 *Introduction*

Schizophrenia is a neuropsychiatric disorder affecting approximately 1% of the population worldwide (Kelly *et al.*, 2005). It is manifested by disruption of thinking, mood, overall behaviour and the inability to filter incoming sensory stimuli (Eisendrath & Lichtmacher, 2005). Consequently, schizophrenia is characterised by positive symptoms, most notably thought disorder, hallucinations and delusions, and negative symptoms which typically includes social withdrawal, anhedonia and flattening of affect. The emergence of the latter significantly impairs cognitive, intellectual and psychomotor performance and impacts significantly on the patient's everyday life (Weiss & Feldon, 2001). Schizophrenia is an extremely devastating illness, presenting with poor long-term prognosis (Harvey *et al.*, 1999).

Schizophrenia is a multifaceted disease, probably involving genetic, environmental and neurodevelopmental factors (Weiss & Feldon, 2001). The genetic component has been demonstrated in twin, family and adoption studies, although the results also demonstrate that genetics is not the only contributing factor (Karayiorgou & Gogos, 1997; McGuffin *et al.*, 1995).

The impact of schizophrenia on health care budgets is considerable, typically contributing between 1 % and 3 % of the total national health care expenditure. There are often significant 'hidden' costs to people with schizophrenia, for example to caregivers and families but also loss of income due to unemployment (Knapp, 2005). Indeed, less than 20 % of schizophrenics are employed (Weiden *et al.*, 1996). Relapse is a significant problem, and approximately 50 % of discharged patients will be rehospitalized within a year (Weiden *et al.*, 1996). Moreover, 10 % of patients with schizophrenia will commit suicide (Weiden *et al.*, 1996). Despite the proven effectiveness of antipsychotics, these agents remain only partially effective, with 20 % of patients on antipsychotic medication experiencing a relapse regardless of the

treatment (Fleischhacker & Hummer, 1997). Further, two thirds of patients on antipsychotic treatment suffer persistent parkinsonism as a side effect of treatment (Harvey *et al.*, 1999), with up 70 % of patients using typical antipsychotics developing acute extrapyramidal side effects (EPS) (Chakos *et al.*, 1994). Tardive dyskinesia (TD) remains one of the most debilitating of these extrapyramidal side-effects (Kane, 2001a; Kane, 2001b) and occurs in approximately 20 % of patients on neuroleptic treatment (Mattay & Casey, 2003). Another side effect of antipsychotic treatment is neuroleptic malignant syndrome (NMS). NMS usually occur during the first 2 weeks of antipsychotic treatment although it can occur at any time (Addonizio *et al.*, 1987). In its most severe form, it has a frequency of 0.02-2.4 %. NMS in a milder forms occur in approximately 12 % of patients treated with typical antipsychotics (Addonizio *et al.*, 1987; Keck, Jr. *et al.*, 1989). Dystonia has an incidence of 10 % among patients with schizophrenia (Keepers *et al.*, 1983). It is also interesting that negative symptoms can be evoked in healthy volunteers with typical antipsychotics (Artaloytia *et al.*, 2006), while in patients with schizophrenia, this leads to what is known as neuroleptic-induced deficit syndrome (Harvey *et al.*, 1999). Despite significant advances over the past few decades with the introduction of the atypical class of antipsychotics, the overall efficacy of these agents, while distinctly improved, amounts to important advantages over the first generation of antipsychotics. It shows superior efficacy in treating positive, negative and disorganization symptoms in schizophrenia (Meltzer, 1992),. Side effects such as EPS (Miyamoto *et al.*, 2002) , TD, (Miyamoto *et al.*, 2002) although lower, remain evident. Moreover, other previously unrecognised adverse events, such as metabolic side effects, are more often seen (Newcomer, 2007). Consequently, schizophrenia remains a disorder that is extremely difficult to treat (Weiden *et al.*, 1996). There is thus an urgency to understand the illness better in order to be able to develop new and improved drug treatments.

2.2 *Signs and symptoms of schizophrenia*

Schizophrenia is characterized by a diversity of symptoms from all domains of mental function, for example language, emotion, reasoning, motor activity and

perception. Moreover, symptom profiles of patients differ. The symptoms are often divided into positive and negative manifestations, where positive symptoms can be described as reflecting an excess of normal function, while negative symptoms are a loss of normal function (Fuller *et al.*, 2003). These symptoms cause an inability to function normally and can interfere with work, social relations, and self-care. As a result, long-term outcomes of schizophrenia include unemployment, social isolation, deteriorated familial relationships, and reduced quality of life (Bondy *et al.*, 1999).

2.2.1 Positive symptoms

2.2.1.1 Delusions and hallucinations

Delusions and hallucinations are sometimes referred to as the psychotic dimension of schizophrenia. Delusions are incorrect convictions that usually involve misinterpreting experience (Bondy *et al.*, 1999), and more recently described as being an inappropriate increase in ascribed salience to environmental cues (Kapur, 2004). Hallucinations are thus an increased awareness of "something" when in fact nothing exists in the perceptual field (Cutting, 2003) and which may occur in any sensory modality, i.e. auditory, visual, olfactory or tactile (Bondy *et al.*, 1999). Hallucinations are defined as a direct result of experiencing the inappropriate environmental stimulus, e.g. auditory, visual, olfactory, gustatory or tactile hallucinations with auditory being the most common (Mueser & McGurk, 2004) ,while delusions are an attempt by the person at making sense of inappropriately significant experiences and associations (the "new awareness") (Cutting, 2003; Kapur *et al.*, 2006). Types of delusions in schizophrenia include delusions of control, e.g. the belief that others can interfere with your thoughts, grandiose delusions, e.g. the person believes that he is Jesus Christ, and somatic delusions, e.g. the person believes that his brain is rotting away (Mueser & McGurk, 2004).

2.2.1.2 Thought disorder

Thought disorder involves unsystematic thinking, characterized primarily by rambling speech that shifts from one topic to another and is not goal-directed (Bondy *et al.*, 1999).

2.2.2 Negative symptoms

2.2.2.1 Social withdrawal

Schizophrenics withdraw themselves from all social contacts (Rang *et al.*, 2003), such that social withdrawal is a core negative symptom in the disorder (Sams-Dodd *et al.*, 1997). People with schizophrenia spend most of their time alone, they do not seek nor do they seem to tolerate the company of others very well (Konigsberg, 2006). To a degree, isolation may also be due to depression (Thornton *et al.*, 1995), which is often co-morbid in the illness (Smulevich, 2003). Isolation may also be due to an intolerance of being in crowds, small gatherings or even being in the company of one person (Thornton *et al.*, 1995).

2.2.2.2 Anhedonia

Anhedonia is described as the loss of pleasure for things that previously or normally would entice an expression of joy, pleasure, satisfaction ,etc. for example. being with friends, seeing a beautiful sunset, or sexual activity (Cutting, 2003). This symptom also draws a significant parallel with major depression.

2.2.2.3 Flattening of affect or blunted affect

Flattening of affect describes a symptoms where the person's face may appear motionless or static with poor eye contact and a lack of expressiveness (Bondy *et al.*, 1999). This too bears a striking analogy with major depression.

2.3 *Diagnosis criteria for schizophrenia*

For the diagnosis of schizophrenia, DSM-IV (Wing & Agrawal, 2003) requires 1 month's duration of characteristic symptoms with at least 6 months of social or occupational dysfunction. At least two symptoms of delusions, disorganized speech, hallucinations, disorganized cationic behavior or negative symptoms must be present. However only one symptom must be present if delusions are bizarre, or if third-person auditory hallucination or running commentary are present. Specific exclusion criteria are schizoaffective or mood disorders, direct result of substance abuse or a general medical condition or persistent developmental disorders (Wing & Agrawal, 2003).

2.4 *Epidemiology of schizophrenia*

Epidemiology is the study of the frequency and determinants of illness in a population. Thus, epidemiologic studies of schizophrenia focus on the incidence rate and factors associated with the prevalence and risk factors with disease onset (Bromet & Fennig, 1999). In this regard, the prevalence in schizophrenia is 1.4- 4.6 persons per 1000 population at risk (Jablensky, 2003).

The biggest single risk factor in etiology is probably genetics (Hunter & Woodruff, 2005). Schizophrenia occurs strongly in families (Akbarian *et al.*, 1995; Jones & Cannon, 1998), with the risk of schizophrenia in first-degree relatives being 5.6 % in the parents of schizophrenics, 12,8 % in children with one schizophrenic parent, and 46,5 % in children with two schizophrenic parents. The overall heritability estimate for the liability to schizophrenia is 60 % to 70 % (Jones & Cannon, 1998) with a 5 to 15 times greater risk in close relatives of schizophrenics compared to the general population (Hunter & Woodruff, 2005).

There is also an inverse relationship between social class and schizophrenia (Dohrenwend *et al.*, 1992), while a sharp decline in social status has been noted to accompany schizophrenia (Cannon & Clarke, 2005). Indeed, unfavorable environmental conditions precipitate the onset of schizophrenia (Jones & Cannon, 1998). Environmental stressors can trigger the occurrence of symptoms in

vulnerable persons, for example stressful life events such as ending a relationship (Bondy *et al.*, 1999). It is thus of interest that the neurodevelopmental hypothesis of schizophrenia (section 2.6.2.3) is based on the notion that early-life environmental factors have significant consequences for processes of brain maturation (Weiss & Feldon, 2001). Indeed, environmental factors in early life development that may play a role in schizophrenia include maternal virus infections, high blood pressure during pregnancy (Harrison, 1997) and low birth weight (Cannon *et al.*, 1989). Moreover, prenatal stressors are also associated risk factors for developing schizophrenia (Cannon & Clarke, 2005), such as maternal influenza, rubella, malnutrition, diabetes mellitus, and smoking during pregnancy and obstetric complications (Susser & Lin, 1992; Takei *et al.*, 1996; Thomas *et al.*, 2001). Other risk factors in relation with schizophrenia include age and gender. Recently it was indicated that predominantly males expressed first-episode schizophrenia (Murray & Van, 1998), particularly in persons under the age of 35 years (Iacono & Beiser, 1992) Also the season of birth, obstetric birth and early childhood complications, substance abuse, stress and geographic location are risk factors associated with schizophrenia (Bromet & Fennig, 1999).

2.5 *Quality of life in schizophrenia*

Schizophrenia has a considerable impact on the patient's quality of life (Hofer *et al.*, 2004). "Quality of life include patients perspectives on what they own, how they are doing and how they feel about their life circumstances" (Lehman, 1996). This entails the measure of the person's material possessions and how the person is doing in terms of functional status (the objective quality of life) and what the person thinks and experiences about his/her situation (the subjective quality of life). Quality of life is therefore an important measurable outcome in patients with schizophrenia (Eack *et al.*, 2007). While the treatment of schizophrenia normally focuses on the positive and the negative symptoms of the illness, improving quality of life must also be an essential therapeutic aim (Naber *et al.*, 2001; Karow & Naber, 2002). Here the choice of antipsychotic is critical as there is substantial evidence that atypical drugs are superior in this regard (see section 2.9) (Voruganti *et al.*, 2000; Csernansky & Schuchart, 2002; Montes *et al.*, 2003; Dossenbach *et al.*, 2005).

2.6 *Pathophysiology of schizophrenia*

2.6.1 Neuroanatomy of schizophrenia

Contrary to earlier thinking, it is now known that psychiatric illnesses such as schizophrenia or depression cannot be explained on the basis of a single neurotransmitter theory, but represent a continuum of environmental, genetic and neurochemical determinants. This all occupy a variable yet distinct role in the etiology, progression and treatment response of disorders as apparently distinct as depression on one end, to psychosis on the other (Harvey, 2007). Thus, while the dopamine hypothesis remains the most robust theory of schizophrenia, there is a disturbing amount of evidence that is not wholly congruent with this theory, foremost among these that dopamine antagonists are not 100% effective in treating the disorder. This has prompted the search for the various neurotransmitters, neuromodulators, environmental and genetic components that pool together to eventually predict the development of schizophrenia. In recent years, much attention has begun to focus on the role of glutamate, GABA and serotonin individually and in cross-interaction with dopamine, in addition to theories that emphasize neurodevelopmental abnormalities.

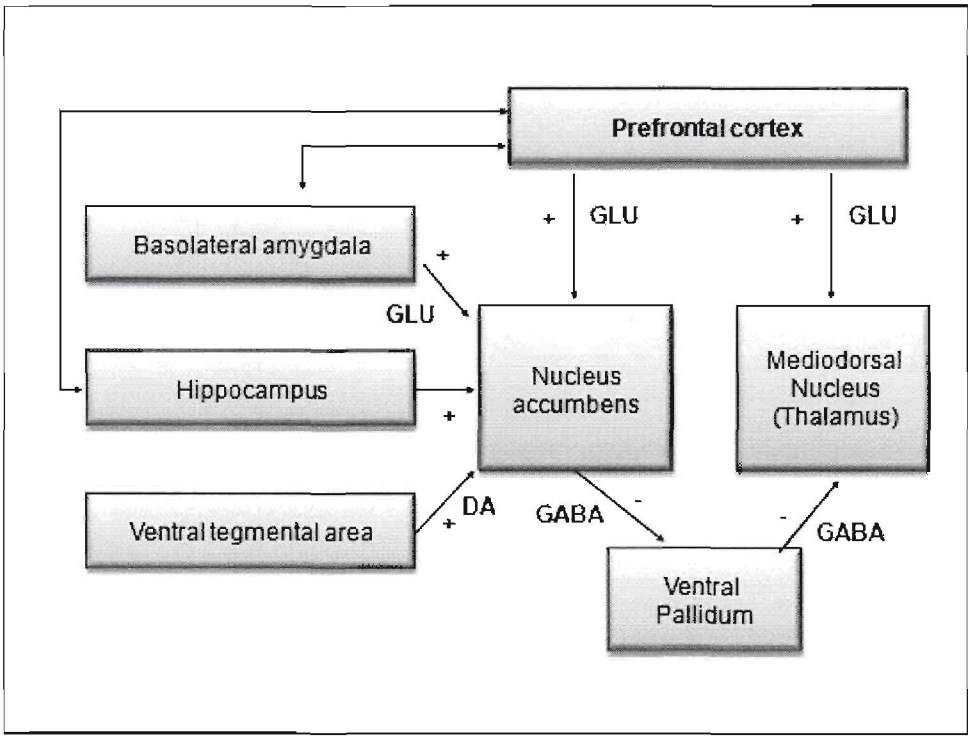


Figure 1: Ventral limbic systems implicated in the positive systems of schizophrenia (Leonard, 2003)

Figure 1 provides a schematic representation of the limbic circuits purported to play a role in mechanisms governing reward, salience and behavioral response to these, and how an aberrant circuit may culminate in psychosis. The ventral tegmental area (VTA) dopamine projection to the nucleus accumbens is a well recognized component of this circuit that, when over active, can precipitate a hallucinatory experience (Leonard, 2003). Conversely, prefrontal dopamine hypofunction is now recognised as a core feature of schizophrenia (Weinberger, 1987; Davis *et al.*, 1991; Svensson, 2000), and has been useful in explaining cognitive decline in these patients (Goldman-Rakic *et al.*, 2004). However, considering Figure 1, cortical hypofunction may also be an indication of reduced glutamatergic activity. Moreover, cortical glutamate pathways also project to the nucleus accumbens (Figure 1). This would suggest that an abnormality in both dopamine and/or glutamate systems in the corticostriatal pallido-thalamic circuit (Figure 1) may underlie the behavior and neurobiology of schizophrenia (Leonard, 2003). Indeed, all these regions are implicated in the illness.(Lipska, 2004) Leonard (2003) has advocated the following

integrative neuroanatomical model of schizophrenia in an attempt to bring together the dopamine-glutamate interactions in the above-mentioned brain circuit (Figure 2).

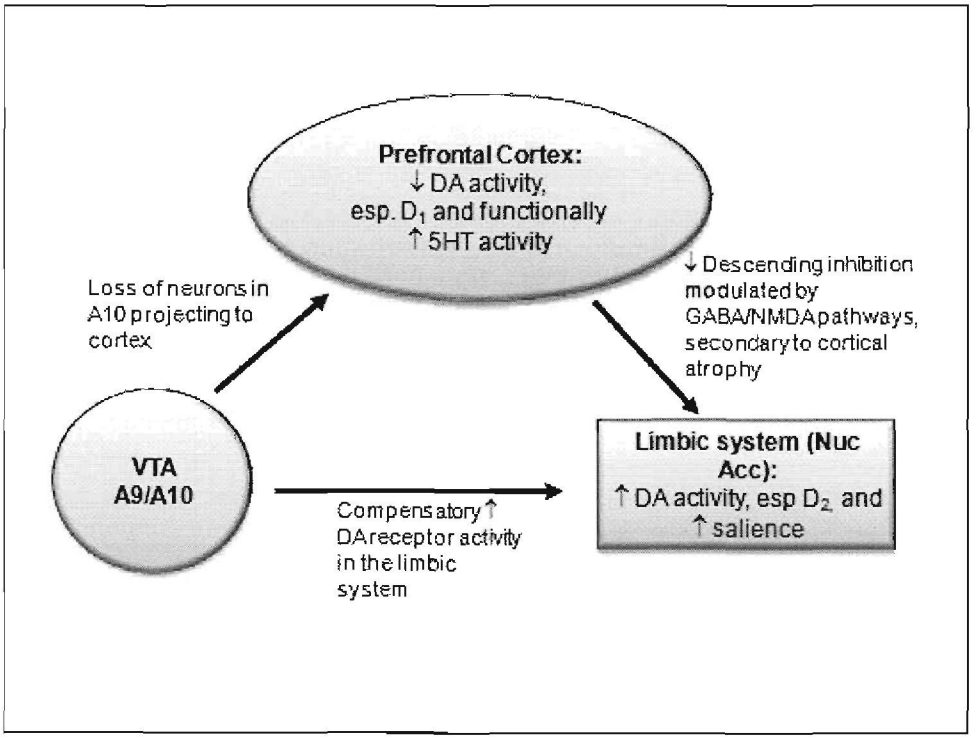


Figure 2: Brain regions involved in schizophrenia (Leonard, 2003)

Important here is to note that, apart from robust evidence in support of frontal hypofunction in schizophrenia, studies have also found that schizophrenia is associated with a loss of subcortical neurons projecting from the VTA to the cortex, either due to neuronal atrophy or degeneration or failed neurogenesis that culminate in a reduced neuronal connectivity of the cortex (Newton & Duman, 2007; Toro & Deakin, 2007). Contrary to theories that schizophrenia begins with dopamine hyperfunction in the limbic brain, evidence would now suggest that dopamine hypofunction in the prefrontal cortex, as a result of reduced D_1 receptor functionality and/or activity, together with reduced dopaminergic VTA-innervation of these frontal areas, engenders a reactive increase in VTA activity to compensate for frontal dopamine hypoactivity. In the face of compromised neuronal connections with the cortex, dopaminergic stimulation now only reaches the limbic brain regions, especially the nucleus accumbens, resulting in uncontrolled positive manifestations. Neuropsychological tests in schizophrenia also suggest that the hippocampus may be the region of origin of positive symptoms. Normally, frontal cortical dopamine

exercises top-down control over sub-cortical structures to dampen excessive salience evoked by dopamine via activation of descending glutamate and GABA projections to the nucleus accumbens (Figure 2). Consequently, as a result of cortical atrophy, this desired response never occurs. Thus, subcortical regions (notably the nucleus accumbens (Nacc) and the hippocampus) and the the frontal cortex together play an interactive and dominant role in the development of schizophrenia (Weiss & Feldon, 2001). This interaction is critical in our understanding of how antipsychotics work, or don't work, and as such in developing new and improved treatments.

2.6.2 Hypotheses

Several hypotheses have been postulated to explain the pathophysiology of schizophrenia. As is now clear from the above neuroanatomical description of the disorder, a role for changes in distinct neurotransmitter systems in the etiology of schizophrenia is especially robust. Particularly in the light of the clinical efficacy of D2 receptor blockers that implicate at least dopamine dysfunction in the illness. However, while we may know or predict which transmitters may be involved, how do these changes occur that initiate the development of schizophrenia? Research to date has demonstrated that such alterations may develop as a result of defects in early neurodevelopmental processes (Jakob & Beckmann, 1986; Tsai *et al.*, 1995).

Current research has begun to focus on especially dopamine and glutamate, especially since these two transmitters play a critical role in sub-cortical-cortical signalling (see Figure 2). The dopamine hypothesis proposes that hyperdopaminergia represents the underlying cause of schizophrenia while the glutamate hypothesis proposes a hypofunctional glutamate system as a possible cause of the illness (Bunney *et al.*, 2000). However, as will be discussed, these two theories are not mutually exclusive.

As the current study will be focusing on the dopamine and glutamate hypotheses of schizophrenia, these will be presented in detail. Other hypotheses include the GABA (Park & Holzman, 1992) and serotonin hypotheses (Baumeister & Hawkins, 2004), but will not be discussed here for the sake of brevity. Moreover, the latter theories

have not been found to be robust, especially since GABA and serotonin modulating agents have not been found effective in treating schizophrenia (Lewis *et al.*, 1999; Meltzer *et al.*, 2003).

2.6.2.1 The Dopamine hypothesis

The dopamine hypothesis postulates that schizophrenia is characterized by an increase in the function of dopamine (Matthysse, 1973) and, as is evident from the anatomical distribution of dopamine within the limbic neurocircuitry involved in the disorder (Figure 2), is responsible for the florid psychotic (positive symptoms) manifestations (Carlsson & Lindqvist, 1963). The hypothesis predicts that antipsychotic effects are directly linked to the known ability of these drugs to block central limbic dopamine D2 receptors (Carlsson, 1978; Creese *et al.*, 1976; Seeman *et al.*, 1976). In agreement with this suggestion, frank psychotic symptoms are induced by dopamine agonists or by dopamine releasing compounds such as amphetamine (Farde, 1997). Given the overwhelming evidence for the efficacy of D_2 antagonists, as well as the lack of efficacy of drugs devoid of D_2 blocking actions (Davis *et al.*, 1991; Meltzer & Stahl, 1976; Duncan *et al.*, 1999), these data have established the dopamine hypothesis as the most robust neurochemical theory of schizophrenia. Despite this evidence, there are noteworthy shortcomings of the dopamine hypothesis, as outlined here:

- Antipsychotics with a high affinity for dopamine D_2 receptors, for example haloperidol, are effective only in improving positive symptoms of schizophrenia and not the negative symptoms (Chavez-Noriega *et al.*, 2002).

- Only 30 % of schizophrenics respond to treatment with typical D_2 receptor antagonists (Chavez-Noriega *et al.*, 2002).

- There is a clear lack of hard evidence for altered dopamine transmission in schizophrenia and it has been proposed by some authors that dopamine transmission might be normal in patients with schizophrenia but elevated in comparison to other systems, such as the glutamatergic or serotonergic systems (Carlsson, 1988; Meltzer, 1989).

- 70 % of patients using typical antipsychotic treatment develop acute extrapyramidal side effects (Chakos *et al.*, 1994). In order to capture this for possible benefit of finding new agents for treating schizophrenia, catalepsy in animals was used as a means of screening for new antispychotic drugs during the early years of neuroleptic development. We now know that this response is linked to striatal motor dysfunction with no relevance for the neurobiology of schizophrenia. This flaw in thinking very likely led to a superficial perception of dopamine involvement in the disorder.

Therefore, it became clear that the dopamine D_2 hypothesis could not fully account for the neurobiological basis of schizophrenia. To realise an improvement in outcome, safer more effective neuroleptics were urgently needed (Chavez-Noriega *et al.*, 2002). However, as the understanding of dopaminergic function in the brain developed over the years, a modification of the dopamine hyperfunction theory began to emerge. Indeed, while hyperdopaminergia is evident in sub-cortical brain regions in schizophrenia (Duncan *et al.*, 1999; Laruelle, 2003), there is distinct evidence for hypodopaminergia in frontal cortical regions (Hietala *et al.*, 1995; Laruelle *et al.*, 1996). Here, cognitive deficits as well as negativism are directly associated with depletion of frontal cortical dopamine (Schneider *et al.*, 1994; Barch *et al.*, 2002; Davis *et al.*, 1991; Goldman-Rakic *et al.*, 2004). Consequently, a new picture of the dopamine hypothesis of schizophrenia became evident, postulating that overactivity in neurotransmission from VTA dopamine cell bodies impacting on select limbic regions results in the development of psychotic symptoms, while in addition, a hypodopaminergic state in the frontal cortical terminal fields of the mesocortical dopamine neurons underlies the negative symptoms of schizophrenia (Duncan *et al.*, 1999). Nevertheless, the dopamine hypothesis today still remains incomplete in its ability to completely explain schizophrenia. Indeed, a much more complex picture emerges. More recently, an exciting and promising new avenue of investigation is the greater focus on the involvement of glutamate in psychosis, and especially its mutual interaction with dopaminergic pathways in the cortico-striatal tracts.

2.6.2.2 The Glutamate hypothesis

Phencyclidine (PCP) and related compounds such as dizocilpine (Mk-801) and ketamine have been found to produce both positive and negative symptoms linked to schizophrenia (Luby *et al.*, 1959; Javitt & Zukin, 1991), (Carlsson & Carlsson, 1990; Olney *et al.*, 1999) while also worsening symptoms in patients suffering from schizophrenia (Luby *et al.*, 1959; Itil *et al.*, 1967). Zukin and Javitt first anticipated that the schizophrenia-like psychosis induced by PCP resulted from non-competitive inhibition of NMDA receptors (Javitt & Zukin, 1991). This was compelling evidence for glutamatergic involvement in schizophrenia and led to the speculation that hypoactive glutamate neurotransmission at the NMDA receptor was involved in the pathophysiology of schizophrenia (Javitt & Zukin, 1991).

A glutamate hypothesis was also suggested by Kim *et al.* (1980) who based their hypothesis on the observations that glutamate levels in the cerebrospinal fluid (CSF) were decreased by as much as 50 % in schizophrenic patients compared to normal individuals. This hypothesis suggested that schizophrenic patients have 1) a deficit in glutamatergic function and/or 2) an increase in dopamine function. These findings were later replicated by some (Macciardi *et al.*, 1990) but not others (Gattaz *et al.*, 1982; Korpi *et al.*, 1987; Perry, 1982). However, it is now known that measurements of glutamate levels in the CSF are not considered an accurate reflection of glutamate-mediated neurotransmission (Moghaddam & Krystal, 2003), which seriously questions the validity of these earlier studies.

"As the primary excitatory neurotransmitters in the brain, glutamate and aspartate, play a key role in recent models of schizophrenia and represent a new approach for pharmacologic treatment" (Javitt & Zukin, 1991; Olney & Farber, 1995). Indeed, apart from their neurotransmitter roles, glutamate and aspartate are of further interest in schizophrenia research because of their involvement in neurodevelopment and neurotoxicity (Goff & Wine, 1997). Considering schizophrenia, excitatory amino acids are involved in four aspects of brain function and development. Firstly, glutamatergic receptors stimulate neurite growth, synaptogenesis and maturation of synapses in the developing brain (McDonald & Johnston, 1990). Secondly, glutamate has a critical role in neurotoxicity, for example in apoptosis (programmed

cell death) that takes place during normal development of the central nervous system (Fiske & Brunjes, 2001). Thirdly, connecting tracts, including corticocortical and thalamocortical association fibers, that have been implicated in schizophrenia, utilize glutamate as a neurotransmitter (Huntley *et al.*, 1994). Any abnormalities in these pathways can produce symptoms of schizophrenia. Schizophrenia also has a strong neurodevelopmental component and the NMDA receptor is crucial in guiding axons to their targets during development (Rakic *et al.*, 1994). Finally there is a significant degree of cross-talk between glutamatergic and dopaminergic systems in especially the cortico-striatal tracts that are deemed critical in schizophrenia and as such can modulate the activity of the other (Grace, 1991) (see Section 2.8).

Glutamate receptors have been divided into a number of subtypes (Seeburg, 1993) ; (Huntley *et al.*, 1994). Glutamate activates two families of receptors: ionotropic receptors, which gate cation channels and metabotropic receptors which are linked to G-proteins (Nakanishi, 1992). The metabotropic glutamate receptors act by means of phospholipase C or by inhibiting adenyl cyclase, thus modulating glutamate transmission in complex ways. The inotropic glutamate receptors are subdivided into the AMPA/kainate receptors and NMDA receptors (Tsai & Coyle, 2002). These receptors can be further subdivided, as outlined in Figure 3. Each glutamate receptor subtype has a unique role in glutamatergic neurotransmission. Further, AMPA, kainate and metabotropic receptors all affect NMDA receptor activity and all these subtypes have a functional inter-relationship (Woodruff & Kleinman, 2002). Thus disturbances in any of the glutamate receptors can result in NMDA receptor dysfunction, as has been postulated in schizophrenia (Woodruff & Kleinman, 2002).

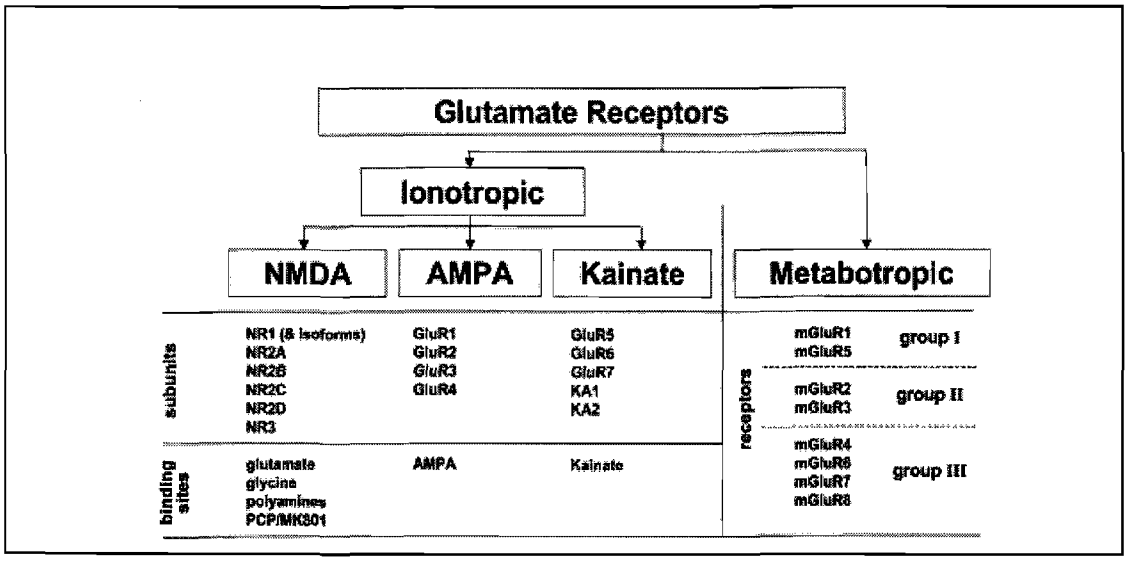


Figure 3: Subtypes of glutamate receptors (Woodruff & Kleinman, 2002)

The NMDA receptor has features that distinguish them from other glutamate ionotropic receptors (Figure 3). Firstly they are voltage-dependent and therefore are susceptible to block by magnesium under normal resting membrane potential. This blockade can be alleviated by depolarization. Secondly the NMDA receptor possesses a glycine modulatory binding site, which must be occupied by a co-agonist, for example glycine or D-serine or D-alanine, in order for glutamate to open the ion channel (Sucher *et al.*, 1995). Thirdly, the ion channel has increased permeability to calcium influx (Mayer *et al.*, 1984; Nowak *et al.*, 1984).

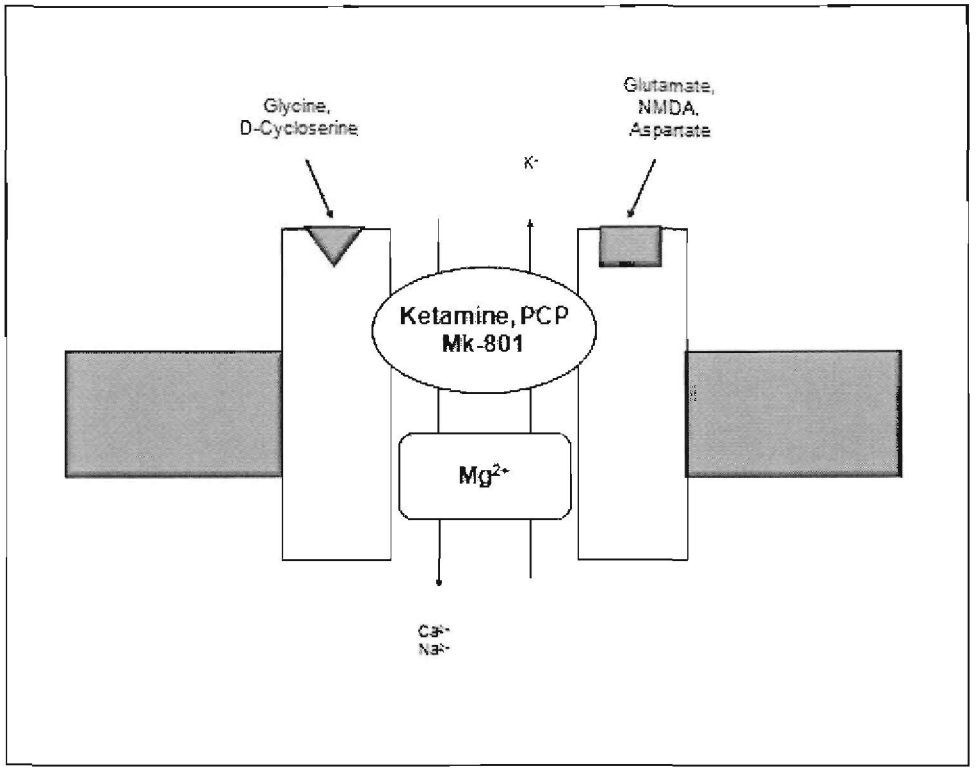


Figure 4: NMDA receptor (postsynaptic neuron) Depolarization of the presynaptic membrane causes glutamate to be released in the synaptic cleft and glutamate binds the NMDA receptor site. Together with the binding of glycine at the glycine modulatory site glutamate causes openinig of the cation channel. Ketamine, MK-801 and PCP (NMDA receptor antagonists) can block this channel by binding on the PCP receptor site within the channel. (Goff & Wine, 1997)

Dissociative anaestetics, such as ketamine and PCP, act as non-competitive antagonists at the NMDA receptor by binding to a site within the ion channel (Figure 4) (Tsai & Coyle, 2002) ,thus preventing glutamate from adequately stimulating the receptor. The means by which NMDA antagonists produce schizophrenia-like symptoms are still a mystery. Olney & Farber (1995) highlighted the inhibitory role of NMDA receptors in the frontal cortex by their activation of inhibitory GABAergic interneurons and synapses in cortical pyramidal neurons. Thus PCP and related compounds cause a decrease in activation of GABAergic neurons resulting in a reactive increase (disinhibition) in excitatory transmission and an enhancement of neuronal glutamate release (Svensson, 2000; Tsai & Coyle, 2002; Benes *et al.*, 2001). While these disturbances in glutamate levels have major implications of their own, e.g, excitotoxicity, disturbances in glutamate also impact on dopamine function in both the cortex and ventral tegmentum, as will be discussed below. (Section 2.8)

In conclusion the excitatory amino acids, particularly glutamate, are involved in the pathophysiology of schizophrenia and are therefore relevant to most models of schizophrenia (Goff & Wine, 1997). With respect to glutamate's role in neurodevelopment and neurotoxicity (Goff & Wine, 1997), and the fact that imaging studies in schizophrenics have highlighted diverse neuroanatomical changes in select brain regions (Feinberg, 1990; Turner *et al.*, 1997; Kovelman & Scheibel, 1984; Akbarian *et al.*, 1993; Heimer, 2000), a neurodevelopmental hypothesis of the illness has subsequently been developed.

2.6.2.3 Neurodevelopmental hypothesis

This hypothesis of schizophrenia is based on the concept that environmental factors taking place in early life can have significant consequences for processes of brain maturation (Weiss & Feldon, 2001). Such adverse environmental events in early periods of neuronal growth may profoundly affect the way nerve cells are laid down, differentiated, selectively picked by apoptosis and remodeled by expansion and retraction of dendrites and synaptic connections (Bloom, 1993; Murray, 1994; Roberts, 1990, Weinberger, 1987). These changes begin around birth while their functional correlates are only expressed in early adulthood (Arnold & Trojanowski, 1996; Bunney *et al.*, 1995; Harrison, 1999). It is of major interest then, that schizophrenics show little to no behavioural changes in childhood, while the full-blown manifestations of schizophrenia most often begin to occur in early adulthood around the age of 16-30 years (Almeida *et al.*, 1995), at about the time the recruitment of new neurons and synaptic connections are initiated.

Periods of fast growth and synaptogenesis during brain development are known as growth spurts. In rodents growth spurts occurs in the first 3 postnatal weeks (Dobbing & Sands, 1979) while in humans this extends from the 3rd trimester to several years after birth. During this developmental period the brain undergoes neuronal overproduction, elimination and modification and about half of the neurons are eliminated by apoptosis. The reason for this is to increase the efficiency of synaptic transmission (Landmesser, 1980). Synapse and receptor densities continue to increase during the developmental period and synaptic pruning slows down when

adulthood is reached (du Bois & Huang, 2007). This outline of synapse/receptor development has been observed in humans (Giedd *et al.*, 1999) and rats (Andersen *et al.*, 2000). During this period of development and synaptic remodeling, the development of the processes of maturation of motor behavior, cognition and affect (Andersen, 2003) is also included. Several authors have hypothesized that an error in synaptic pruning may contribute to the development of schizophrenia (Feinberg, 1982; Hoffman & Dobscha, 1989; Keshavan *et al.*, 1994).

In support of the neurodevelopmental hypothesis of schizophrenia, there are consistent findings of persistent structural changes in certain brain regions that undergo maturation during development and are then present at the onset of schizophrenia (Akbarian *et al.*, 1993; Akbarian *et al.*, 1995). Thus, there are important examples of diffuse neuropathology in schizophrenia (Feinberg, 1990; Turner *et al.*, 1997), including neuropathological and cytoarchitectural abnormalities in the prefrontal cortex, basal ganglia, thalamus, hippocampus and medial temporal lobe (Kovelman & Scheibel, 1984; Akbarian *et al.*, 1993; Heimer, 2000), decreases in brain volume, and increases in ventricular size and surface abnormalities (Andreasen *et al.*, 1994). The expression of NMDA receptors and brain developmental synaptogenesis takes place in the first 2 weeks after birth in rats. During this time, the brain is susceptible to NMDA receptor hypofuntion-induced apoptosis in the cortex (du Bois & Huang, 2007). When NMDA receptor antagonists are given during the first 2 postnatal weeks correlating with the brain growth spurt period of synaptogenesis mentioned above, it triggers apoptotic neurodegeneration (Hansen *et al.,* 2004). Therefore the neurodevelopmental hypothesis suggests that brain development can be adversely affected at a crucial time of life, particularly through early life events, that may provoke the development of schizophrenic symptoms in later adulthood (Weiss & Feldon, 2001). Moreover, this hypothesis brings together the glutamate and dopaminergic hypotheses of schizophrenia into a single descriptive entity, and is outlined in more detail below (Section 2.6.2.1 & 2.6.2.2).

2.7 *Neurochemistry of schizophrenia*

2.7.1 Dopamine

The most important dopamine projections in the pathophysiology of schizophrenia are the mesocortical tract originating in the VTA and projecting to the prefrontal cortex (Fallon *et al.*, 1978; Bannon *et al.*, 1983), and the mesolimbic tract which also arises from the VTA, and projecting to the limbic structures such as the nucleus accumbens, amygdala and hippocampus (Figure 6). Dopamine receptors show a distinctive distribution within these brain regions, with D_1 receptors predominating in the cortex and D_2 receptors in the limbic regions (Camps *et al.*, 1989; Cortes *et al.*, 1989) where they occupy separate roles. Cognition and frontal lobe function involves D_1 receptor function (Goldman-Rakic, 1992), while striatal D_2 receptors are involved in reward and affect (Strafella *et al.*, 2003). As have been discussed earlier, schizophrenia is characterized by dopamine dysfunction at D_1 and D_2 receptors within these two pathways (Laruelle, 1998), namely cortical D_1 receptor associated cognitive dysfunction and increased salience to environmental cues mediated by limbic D_2 receptors (Goldman-Rakic *et al.*, 2004; Kapur *et al.*, 2006). Post mortem findings have indeed shown an increase in D_2 receptors in patients with schizophrenia (Zakzanis & Hansen, 1998), as well as alterations in PFC D_1 dopamine receptors (Goldman-Rakic *et al.*, 2004; Okubo *et al.*, 1997), specifically a down-regulation of D_1 binding. This has propogated the idea that schizophrenia is associated with a disturbed relationship between D_1 and D_2 receptors (Seeman *et al.*, 1989).

2.7.2 Glutamate

Glutamate is the most widely distributed transmitter system in the brain, and is well represented in the cortico-striatal and limbic brain regions (Tsai & Coyle, 2002; Coyle, 1996). Regional-specific changes in glutamate receptor binding has been implicated in schizophrenia (Iraide Aparicio-Legarza *et al.*, 1998). While there is strong evidence supporting NMDA receptor binding changes in various brain regions in schizophrenics, viz. putamen, frontal cortex, hippocampus, nucleus accumbens and amygdala, the direction of these changes have been inconsistent (Kornhuber *et*

al., 1989; Noga *et al.*, 1997; Iraide Aparicio-Legarza *et al.*, 1998; Kerwin *et al.*, 1990; Ishimaru *et al.*, 1994). Increases in kainate receptor binding in the prefrontal cortex and decreased AMPA and kainate receptor binding in the hippocampus, with no changes in NMDA receptor density, have been reported (Breese *et al.*, 1995), (Kerwin *et al.*, 1988). Thus dysfunction of glutamatergic neurotransmission may ths play an important role in schizophrenia (Goff & Coyle, 2001).

2.8 *Glutamate-dopamine crosstalk*

The prefrontal cortex receives several inputs from cortical and subcortical areas implicated in sensorimotor and limbic functions (Tseng & O'Donnell, 2004). Moreover, several authors have highlighted the need for dopamine-glutamate co-activation for the functioning of the prefrontal cortex (Gurden *et al.*, 1999; Baldwin *et al.*, 2002; Jay, 2003).

The significant role of dopamine in prefrontal cognitive functions is widely recognized. Dopamine and glutamate transmission modulates working memory in the prefrontal cortex (Goldman-Rakic, 1996). While sub-cortical dopamine activity is well recognized in positive symptom manifestations, as has been discussed earlier, this monoamine also plays a significant role in the frontal cortex (Murase *et al.*, 1993; Castner & Williams, 2007). Prefrontal dopamine release, however, is strongly regulated by glutamate via NMDA receptors (Murase *et al.*, 1993). Indeed, the interaction between D_1 and NMDA receptors in this brain region forms a fundamental neural substrate for cognition, particularly in disorders such as schizophrenia where cognitive function is an important indicator of outcome (Castner & Williams, 2007). Glutamate-NMDA receptors can increase discharge of dopamine neurons in the VTA (Kalivas *et al.*, 1989) and indirectly modulate cortical dopaminergic activity (Murase *et al.*, 1993; Kalivas *et al.*, 1989). Subsequently, NMDA receptor blockade has been shown to increase dopamine release in the prefrontal cortex (Hondo *et al.*, 1994; Hertel *et al.*, 1995). Dopamine D_1 signaling in turn also modulates glutamate release (Castner & Williams, 2007), with a decrease in medial prefrontal cortex glutamate suggested to be mediated by D_1 receptor activation of local GABAergic inhibition (Harte & O'Connor, 2004).

In conclusion, the interaction between dopamine and glutamate receptors is crucial for the functioning of the prefrontal cortex (Tseng & O'Donnell, 2004) and, as such, disturbances in the glutamate-dopamine cross talk can contribute to the profile of cognitive dysfunction in schizophrenia (Holcomb *et al.*, 2004).

2.9 *Drug treatment in schizophrenia*

It has earlier been emphasised how the side effect profile of the elected drug treatment can adversely affect compliance and thus long-term outcome (Section 2.1). Indefinite pharmacological treatment is required in almost all patients diagnosed with schizophrenia, and it can as such be described as a life-long disorder that is also progressive over time (Mueser & McGurk, 2004; Peuskens, 2004). Consequently, prevention of recurring psychotic episodes are increasingly important for improving the quality of life for patients with schizophrenia (Csernansky, 1995). Goals of antipsychotic drug treatment are to decrease the severity of psychotic symptoms, prevent recurrence of symptoms and associated deterioration of functioning (Bondy *et al.*, 1999). Drug treatment in schizophrenia is divided into acute drug treatment and maintenance drug treatment. Acute drug treatment concerns the introduction or reintroduction of medication to lessen an exacerbation of psychosis. These episodes are characterized by an increase in positive symptoms and sometimes also an increase in negative symptoms. An episode may be rapid in onset, while the form and content may change from one episode to the next (William *et al.*, 2007). The goal of maintenance drug treatment is then the prevention of relapse as well as improving the quality of life for patients with schizophrenia (Csernansky, 1995). It is especially during the latter that side effect profile and compliance plays a major role in achieving a successful outcome.

Antipsychotic drugs, also referred to as neuroleptics, have been developed over a long period of time. There are two different groups of antipsychotic drugs, namely typical and atypical. Typical antipsychotics comprise those drugs that were originally developed for the treatment of schizophrenia over 50 years ago, and include agents such as chlorpromazine and haloperidol. Atypical drugs, on the other hand, are a more recently developed group of compounds that have been so named due to their

penchant for a reduced tendency to cause motor side effects and also an improved negative symptom efficacy, for example clozapine and olanzapine (Rang *et al.*, 2003). Several of the earlier typical compounds were an accidental finding as opposed to being a product of rational research with concrete objectives. Thus Laborit & Huguenard (1951) administered the aliphatic phenothiazine, chlorpromazine, to patients to test its potential anesthetic effects during surgery. Only later it was discovered that these drugs had notable antipsychotic properties (Hamon *et al.*, 1952; Delay *et al.*, 1952). Chlorpromazine remained the first choice antipsychotic throughout the 1960s and early 1970s (Shen, 1999). Between 1954 and 1975 around 40 antipsychotic drugs were introduced throughout the world (Shen, 1999), with the introduction of haloperidol in 1958 being the most significant. Despite its age and limitations with regard to its side effects, haloperidol remains a gold standard in many drug treatment trials as a positive control, especially for the control of frank positive manifestations, and is also on the World Health organisation (WHO) list of essential drugs (Ozdemir *et al.*, 2006).

The development of these drugs was based on the hypothesis of hyperdopaminergic activity in schizophrenia (see Section 2.6.2.1) with the D2 receptor blocking actions of these drugs most robustly associated with their antipsychotic properties (Seeman, 1992). The wide use of typical antipsychotic drugs, such as chlorpromazine and haloperidol, was bolstered by their unquestioned efficacy in the treatment of positive symptoms of schizophrenia (Kane, 1989). However, it soon became apparent that about 25 to 60 % of patients treated with typical antipsychotics do not respond, or are only partially responsive, to such treatment (Miyamoto *et al.*, 2000). This was later attributed to their poor efficacy with regard to the negative symptoms of the illness (Fleischhacker, 1995; Hawkins *et al.*, 1999). However, this was set to change.

Ongoing work in the 1960s on the synthesis of novel antipsychotic compounds led to the discovery of clozapine, an antipsychotic with minimal to no extrapyramidal side effects (Shen, 1999; Hippius, 1989). Clozapine was briefly marketed in certain regions of the world, including South Africa, in the early 1970's but was quickly withdrawn due to its association with a potentially fatal agranulocytosis (Hippius, 1989). However, clozapine was reintroduced on the market in 1990 after extensive clinical studies with obligatory white blood cell monitoring (Lieberman *et al.*, 1989).

This reintroduction was based on several advantages that clozapine presented over other antipsychotics, which as originally noted, was its low motor side effect potential. However, it soon became apparent that clozapine was also more effective than typical antipsychotic drugs in treatment-resistant schizophrenia (Kane *et al.*, 1988) and also in addressing both positive and negative symptoms of schizophrenia (Lieberman, 1993). Clozapine also more effectively reduced relapse and suicidal behavior (Lieberman *et al.*, 1989). It's completely opposite clinical response profile to earlier drugs led to the coining of the term "atypical" to describe the unique features of this drug. Clozapine's reintroduction was heralded a breakthrough in the treatment of schizophrenia. This seminal discovery led to the development of other atypical antipsychotics, most notably risperidone (approved in 1994) (Marder & Meibach, 1994)), olanzapine (1996) (Beasley, Jr. *et al.*, 1996), sertindole (1997) (van Kammen *et al.*, 1996) and quetiapine (1997) (Arvanitis & Miller, 1997). Still newer atypicals are in development or have already been launched, including ziprasidone and aripiprazole. More importantly, however, is that clozapine stimulated a world-wide rush by clinical and basic scientists to unravel the secrets of clozapine's unique mode of action. This has not only benefited the development of new and improved drugs for the treatment of schizophrenia, but has lead to a significant improvement in our understanding of the underlying neurobiology of the illness.

Given the vast amount of typical and atypical drugs available on the market today, this discussion will henceforth only focus on two examples that have direct bearing on this study, namely haloperidol and clozapine.

2.9.1 Typical antipsychotics: Haloperidol

Typical antipsychotics can be classified as high, intermediate, or low potency based on their affinity for dopamine D_2 receptors (Baldessarini *et al.*, 1984) with haloperidol regarded as a high-potency typical antipsychotic (Seeman, 1992).

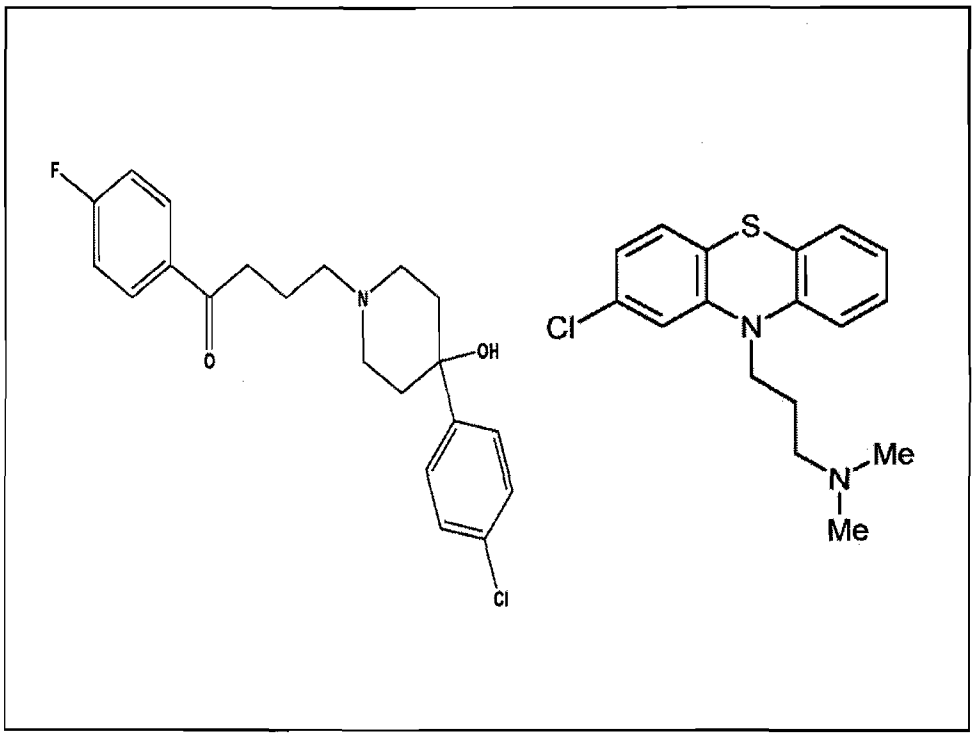


Figure 5: Structure of haloperidol (a) and chlorpromazine (b)

Their pharmacological action has been linked to their blockade of sub-cortical dopamine D2 receptors (Rung *et al.*, 2005) which, as mentioned earlier, was instrumental in the development of the dopamine hypothesis of schizophrenia (see Section 2.6.2.1). This hypothesis has since been accredited with significant construct validity given that all currently effective antipsychotics are effective D2 receptor antagonists. Because of their potent antidopaminergic activity, typical antipsychotics often evoke non-specific blockade of dopamine sites in a number of areas in the brain, but more notably in the hippocampus, nucleus accumbens, striatum, cortex and pituitary gland.

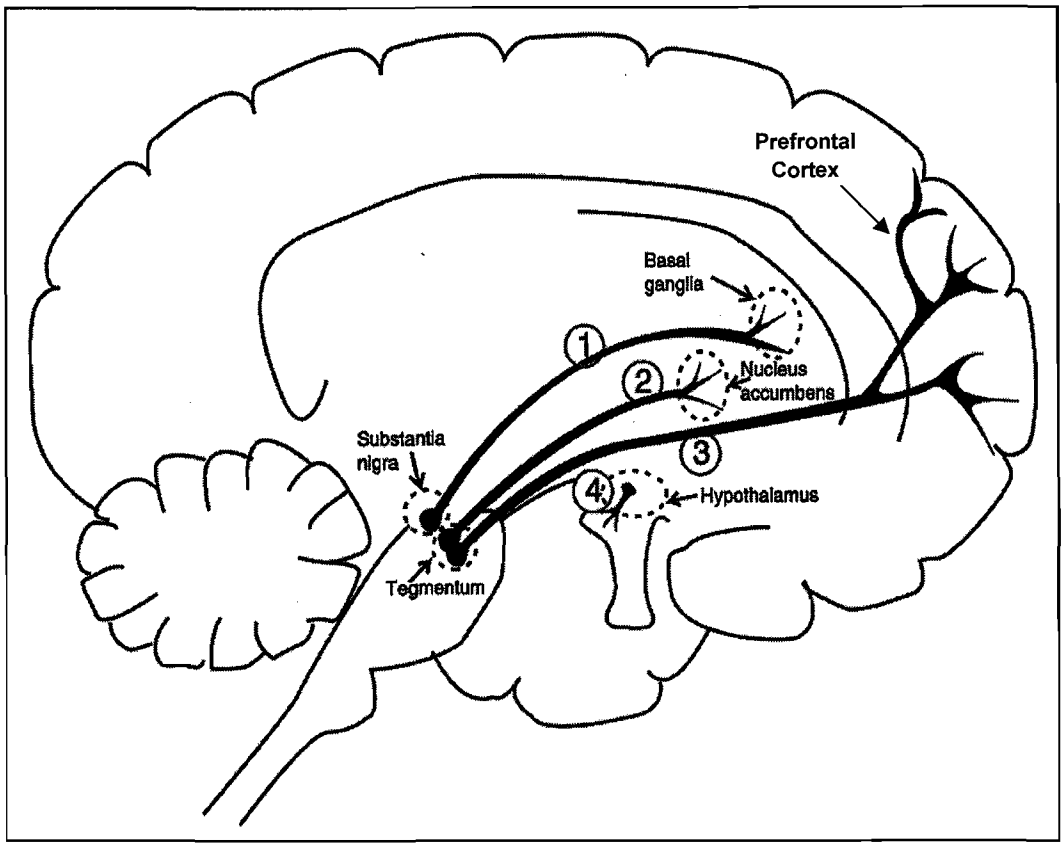


Figure 6: The four principle dopaminergic projections of the brain

Pathway 1: nigostriatal projection, Pathway 2: mesolimbic projection, Pathway 3 mesocortical projection, Pathway 4: tuberoinfundibular (Leonard, 2003)

Dopaminergic cell bodies located in the ventral tegmentum and substantia nigra in the brain stem region project to various cortical and sub-cortical regions (Figure 6). Two projections from the ventral tegmentum can be identified, namely the mesocortical pathway (pathway 3, Figure 6) innervating the frontal cortical regions, the mesolimbic pathway (pathway 2, Figure 6 innervating the limbic structures, in particular the nucleus accumbens. The nigostriatal pathway (pathway 1, Figure 6) projects from the substantia nigra of the brain stem to the basal ganglia, while the tuberoinfundibular pathway (pathway 4, Figure 6) projects from cell bodies in the hypothalamus, eg. median emenence, to innervate the pituitary gland (Leonard, 2003).

The nigrostriatal pathway is involved in the control of posture and motor behaviour (Janhunen & Ahtee, 2007), with disturbances in this projection associated with disorders of motor function, e.g. Parkinson's disease, Huntington's disease, as well

as drug-induced motor disturbances, such as TD and parkinsonism. The mesolimbic/mesocortical pathway links the ventral tegmental area with limbic structures and the limbic cortex (Ungerstedt, 1971). The mesolimbic pathway is involved in the control of emotion, reward and motivation. The mesocortical pathway on the other hand regulates higher cognitive functions such as working memory, learning and reward (Janhunen & Ahtee, 2007) and is associated with the neurobiology of major depression as well as disorders characterised by disorders of memory, such as Alzheimer's diease. The tuberoinfundibular pathway connects the hypothalamus to the pituitary gland where its most important role is the regulation of pituitary hormone secretion, particularly growth hormone and prolactin (Dubuc, 2002).

Hyperactivity in the subcortical dopaminergic areas, especially in the nucleus accumbens, has been strongly correlated with the positive symptoms of schizophrenia (Carlsson *et al.*, 2004), while negative symptoms and associated cognitive impairment, on the other hand, is thought to be driven by reduced dopaminergic function in the frontal cortical areas (Knable & Weinberger, 1997). Classical antipsychotics induce extrapyramidal side effects by blocking dopamine D2 receptors in the nigrostriatal pathway (Janhunen & Ahtee, 2007), resulting in symptoms of parkinsonism in humans and catalepsy in rodents (Mandhane *et al.*, 1997). Other basal ganglia-related side effects include dystonic reactions.

As has been alluded to earlier, many patients do not respond to typical antipsychotic medication (Nordstrom *et al.*, 1993). In fact, 30 % of patients with psychotic symptoms do not respond to typical neuroleptics and in the region of 50 % show only a partial response to medication (Kane, 1989; Fleischhacker, 1995). Primary negative symptoms are very resistant to treatment with typical neuroleptics (Fleischhacker, 1995; Hawkins *et al.*, 1999) which invariably leads to poor social functioning and a poor long-term outcome (Green, 1996; Keefe *et al.*, 1999). Moreover, there is evidence that these agents may even worsen negative symptoms (Stahl, 1996; Harvey *et al.*, 1999), possibly due to inappropriate suppression of frontal cortical dopamine activity (Harvey *et al.*, 1999). This is particularly worrisome, especially since it is now accepted that schizophrenia on its own is a progressive illness. If the medication also worsens cognitive performance, then the battle is surely lost.

In conclusion, due to notable deficits in overall efficacy and issues with tolerability and compliance necessary for long-term treatment, typical antipsychotics may in effect result in lower medical and quality-of-life outcomes than atypical antipsychotics (Meltzer, 1999) ,which will significantly compromise a favorable prognosis.

2.9.2 Atypical antipsychotics: Clozapine

Despite many years of ongoing research on atypical agents, their exact pharmacological properties and the basis for their improved therapeutic response in schizophrenia remains poorly understood (Miyamoto *et al.*, 2002). Nevertheless, significant advances have been made.

Clozapine is a dibenzazepine tricyclic and is related chemically to loxapine, a neuroleptic that was first tested in the 1960's in Europe. Clozapine's preclinical profile is consistent with a drug that is an effective antipsychotic without extrapyramidal side effects (EPS), for example catalepsy (Meltzer, 1991). The distinguishing pharmacological feature of clozapine compared to typical antipsychotics is its high affinity for 5-HT_{2A} receptors. Evidence now has affirmed that combined 5-HT_{2A}/D_2 antagonistic actions, with higher affinity for 5-HT_{2A} receptors, represents the key to clozapine's enhanced efficacy and fewer EPS (Meltzer, 1989). Clozapine also has affinity for D_1, alpha-1, muscarinic and histamine-H_1 receptors, and is thus referred to as a multi-receptor antagonist (Nutt, 1994).

One troublesome side effect of clozapine is agranulocytosis (Krupp & Barnes, 1989), which has been ascribed to an altered immune response (Kane, 1993). Because of clozapine's low potency (dosage 200-600 mg/ day), higher doses are needed for therapeutic efficacy (as say compared to the potent atypical agent, olanzapine). This is associated with high binding to other receptors leading to side effects such as sedation and postural hypotension (Gerlach & Peacock, 1995; Harvey *et al.*, 1999). In addition, high affinity for histamine H_1 receptors, together with $5HT_2$ antagonism is associated with weight gain, insulin-resistance and other metabolic disturbances (Leadbetter *et al.*, 1992; Wirshing *et al.*, 1998). Clozapine is also a muscarinic acetylcholine M_4 receptor agonist (Zorn *et al.*, 1994) and an a_2-adrenergic antagonist

(Nutt, 1994) which has been linked to symptoms of hypersalivation (Richelson, 1996).

Because of their equal efficacy in addressing positive symptoms and greater effectiveness in treating negative symptoms (Gerlach & Peacock, 1995), and coupled with markedly less side effects, essential long-term maintenance therapy of schizophrenia with atypical antipsychotic drugs now provides a much improved long-term prognosis for the illness, lengthening the symptom-free period and improving overall quality of life (Fitton & Benfield, 1993). Nevertheless, the possible implications of iatrogenic metabolic disturbances and resultant endocrine-related disorders, particularly obesity, diabetes mellitus type II, polycystic ovary syndrome, infertility, hypercholesterolemia and cardiovascular disorders, is cause for concern (Wirshing *et al.*, 1998; Wetterling, 2003; Lindenmayer *et al.*, 2003; Ried *et al.*, 2006).

2.10 *Animal models of relevance for schizophrenia*

2.10.1 Introduction

Animal models allow examination of brain-behavior relations to gain more insight into human behavior and underlying processes. Although the most applicable information is derived from the study of humans, it is not always possible to do. Animal models therefore are an important tool for studying human illness, the neurobiological processes involved and their response to drug treatment (van der Staay, 2006). The definition of an animal model is that "animal models are living experimental systems used to analyze brain behavior relationships under controlled conditions" (van der Staay, 2006). The main purpose of an animal model is to improve the understanding of the human occurrence (Geyer & Markou, 1995).

An animal model can represent a disease on three different levels

- It can replicate an inducing factor, for example pathological processes underlying the disease.
- It may reflect certain symptoms of the disease.

- Lastly it can predict sensitivity to current available treatments for the disorder, for example antipsychotics (Lipska & Weinberger, 2003).

An animal model must be reproducable and experiments must be done under controlled conditions. Thus, standardized housing and testing conditions must be employed, any possible variable(s) that can influence the outcome of the study must be excluded (Hossain *et al.*, 2004; van der Staay & Steckler, 2002) and the model must be reproducable across species and strains (van der Staay, 2006).

2.10.2 Validity of animal models

Validity of a certain model refers to the degree to which the model is useful for a given purpose (Geyer & Markou, 1995). To this end, an animal model must have face validity, construct validity and predictive validity (Willner, 1984). These different types of validity help to identify possible strengths/weaknesses of a specific animal model and to compare models with each other (Willner, 1991). These three tiers of validity are discussed below.

2.10.2.1 Face validity

Face validity refers to the behavior evident in the animal model and the specific symptoms of the human condition as well as the degree to which they are similar (Geyer & Markou, 1995). Thus the closer an animal model resembles the human condition it is modeling, the more robust (valid) the model is. Although useful, face validity is of limited value and is generally regarded as the weakest of the validation criteria (van der Staay, 2006). The reason for this is that animal models have their own species-specific behavior and thus there are very often little resemblance between the specific behavior and that of humans (van der Staay, 2006).

2.10.2.2 Construct validity

Construct validity is defined as the accuracy with which the model measures what it is proposed to measure (Cronbach & Meehl, 1955) and is considered the most important aspect of validity. This is, however, difficult to establish (Geyer & Markou, 1995) and therefore construct validity most often refers to the resemblances in the underlying neurobiological mechanisms between the animal model and humans in the expression of certain behaviors (Van den Buuse *et al.,* 2004), for example the involvement of altered serotonin levels in an animal model of depression and in the human equivalent.

2.10.2.3 Predictive validity

Predictive validity is defined as the ability of a model to accurately predict the criterion that are under investigation (Cronbach & Meehl, 1955). Therefore an animal model that can make predictions of the human condition based on the performance of the animal model has predictive validity. Predictive validity also refers to the model's ability to identify drugs that have therapeutic significance in humans (Matthysse, 1986; Willner, 1984). Thus, drug treatment modalities that are selectively effective in the human condition should show equal selectivity in the model, and is regarded as an essential criterion for the validity of an animal model (Geyer & Markou, 1995). Thus, according to the analogy described above, using an antidepressant in both an animal model of depression and in humans with depression, should show equal ability to attenuate a given behaviour.

2.10.3 Animal models of schizophrenia

Since schizophrenia presents with a very specific group of behavioural manifestations, involves a very distinct array of neuro-anatomical substrates and demonstrates response to a select group of psychotropic drugs, it is possible to develop animal models of the human condition, which are discussed below.

2.10.3.1 Prepulse inhibition (PPI) models

One of the many diverse symptoms of schizophrenia is the patient's inability to ignore and discard irrelevant stimuli, a symptom first described by Bleural in 1911 (Bleural, 1911). This inability to filter incoming sensory information can lead to a stimulus overflow (Weiss & Feldon, 2001). Therefore patients with schizophrenia exhibit a deficit in what is referred to as sensorimotor gating (Geyer *et al.*, 2001).

This deficit in sensorimotor gating can be assessed in humans using the prepulse inhibition (PPI) paradigm (Braff *et al.*, 1978; Braff *et al.*, 1999; Grillon *et al.*, 1992). Similar deficits in PPI can be replicated in rodents by means of pharmacological and developmental interventions (Geyer *et al.*, 2001). PPI can be defined as the normal reduction of the startle reflex to an intense acoustic stimulus (pulse) when this stimulus is immediately preceded by a weaker stimulus (prepulse) (Graham, 1975; Braff *et al.*, 1978). PPI is expressed as the percentage of inhibition of the prepulse + pulse trial's startle amplitude compared to the amplitude of the pulse-alone trial (Weiss & Feldon, 2001). The startle response is controlled by brain structures at the level of the brain stem, while the mechanism of its inhibition by the prepulse requires forebrain structures, particularly the nucleus accumbens, hippocampus, amygdala or prefrontal cortex (Swerdlow *et al.*, 1997). These findings strongly suggest that PPI deficits are an enduring feature of schizophrenia and that they reflect an abnormal information processing (Weiss & Feldon, 2001).

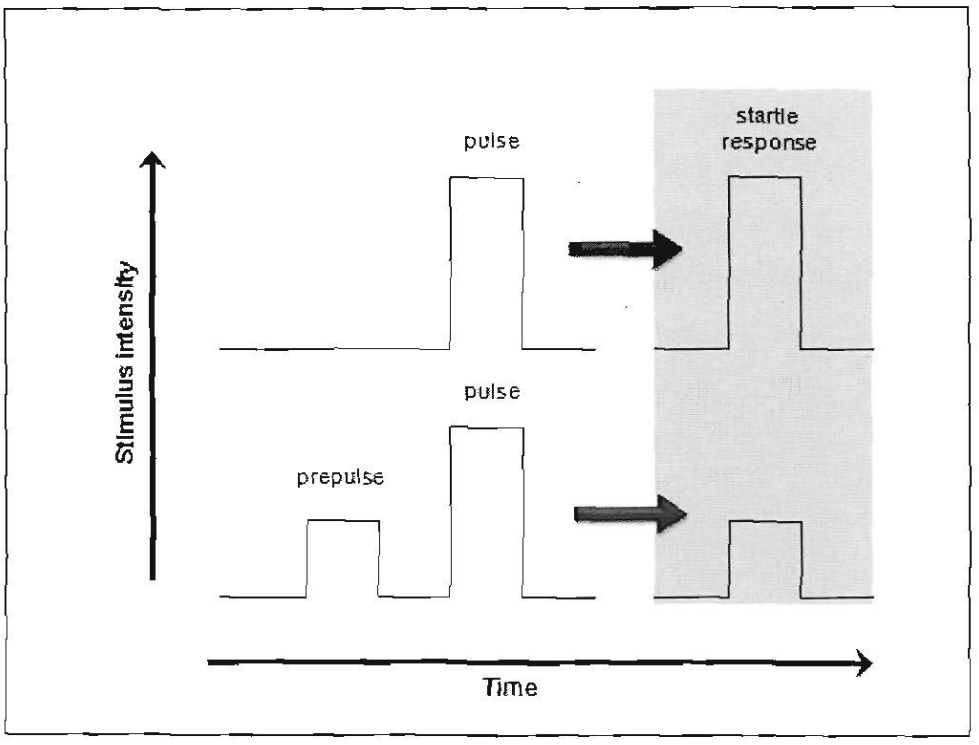


Figure 7: Paradigm of prepulse inhibition

The literature describes four PPI models applicable to schizophrenia and that are associated with a disruption in PPI, namely the dopamine agonist model, the glutamate antagonist model and the environmental animal model (Geyer *et al.*, 2001). Referring to the foregoing sections on the symptoms, treatment and neurobiology of schizophrenia, it is clear that these four different PPI models of schizophrenia show important face (sensory motor gating deficits), predictive (antipsychotic treatment response) and construct (glutamate and dopamine involvement) validity and will be separately discussed for each PPI model (Weiss & Feldon, 2001).

2.10.3.2 Dopamine agonist PPI model

The earliest PPI model was the dopaminergic model, and was valuable for its modelling of the dopamine hypothesis of schizophrenia (Geyer *et al.*, 2001). The administration of a direct or indirect acting dopamine agonist, such as apomorphine or d-amphetamine, causes a deficit in PPI comparable to that seen in schizophrenia

(Mansbach *et al.*, 1988; Swerdlow *et al.*, 1986). This disruption of PPI by dopamine agonists is due to an increased stimulation of dopamine D2 receptors in the nucleus accumbens (Koch, 1999; Swerdlow *et al.*, 1994), and is supported by evidence that haloperidol, a D_2 antagonist, reverses deficits in PPI in this model (Bast *et al.*, 2000). Therefore, this model has been used to test the ability of potential antipsychotic drugs to reverse the PPI disruptive effects of dopamine agonists (Swerdlow *et al.*, 1991; Swerdlow *et al.*, 1994). In this regard, typical and atypical antipsychotics have been found to reverse dopamine agonist-induced PPI disruptive effects (Keith *et al.*, 1991; Geyer & Ellenbroek, 2003; Geyer *et al.*, 2001). The dopamine agonist PPI model in rats therefore has strong predictive validity (Geyer & Markou, 1995) and can be regarded as a valid animal model of schizophrenia.

2.10.3.3 Glutamatergic antagonist PPI model

Noncompetitive NMDA antagonists like PCP, ketamine and MK-801 mimic characteristics of schizophrenia in humans (Mansbach, 1991; Moghaddam, 1994) by binding to a site located on the NMDA receptor complex and inhibiting cation flow through the NMDA channel (Rung *et al.*, 2005). Mansbach and Geyer were the first to establish that noncompetitive NMDA antagonists cause PPI deficits in rats (Mansbach & Geyer, 1989). Moreover, PCP and other related NMDA antagonists cause hyperlocomotion (Hoffman, 1992), stereotypy (Tiedtke *et al.*, 1990), neurotoxicity (Farber *et al.*, 1993) and cognitive deficits (Jentsch *et al.*, 1997) which contributes to the face validity of the model. However, of interest is that the disruption in PPI caused by NMDA antagonists is not reversed by typical antipsychotics such as haloperidol, but by atypical antipsychotics like clozapine (Cornell *et al.*, 1985). The above-mentioned hyperlocomotion, stereotypy, neurotoxicity and cognitive impairment are strongly reminiscent of schizophrenia, and with only clozapine (an atypical antipsychotic) found to be effective, is analogous to the preferential efficacy of this group of compounds in humans (Malhotra *et al.*, 1997). This response has also been replicated using other atypicals like olazapine, quetiapine, remoxipride and risperidone (Geyer *et al.*, 2001). Therefore the NMDA-antagonist PPI model has great value in identifying potential new atypical drugs

(Geyer & Ellenbroek, 2003). However, this interaction between atypical antipsychotics and NMDA antagonists appears not to be through interactions at a single receptor or brain region but is probably dependent on a complex circuit of interactions (Bakshi & Geyer, 1998). In contrast, however, the typical antipsychotic chlorpromazine has been found to also block the PPI deficits caused by ketamine (Swerdlow *et al.*, 1996a), with a similar result reported for PCP (Yamada *et al.*, 1999). How the PPI deficits caused by an NMDA antagonist are reversed by this typical agent is not known, although it may involve several other receptors, e.g. 5-HT_{2A} and a_1-adrenergic receptors (Geyer *et al.*, 2001).

In conclusion the NMDA antagonist model presents with strong face and predictive validity for schizophrenia (Javitt & Zukin, 1991; Sams-Dodd, 1996) and also has strong construct validity for investigating the cognitive and attentional deficits evident to the illness (Geyer & Markou, 1995).

The serotonin agonist model, while also representative of one of the four PPI models, is beyond the scope of the present work, although the reader is encouraged to consult papers by Geyer (Mansbach *et al.*, 1989; Geyer *et al.*, 2001). The serotonin PPI model presents with predictive validity due to the efficacy of antipsychotics in the model and some abnormalities demonstrated at the onset stages of schizophrenia (Geyer, 1998). The model also demonstrates construct validity in its ability to model exaggerated responses to cognitive stimuli, resulting in a deficit in sensorimotor gating and PPI, a manifestation noted in both the symptoms of schizophrenia and in the serotonin agonist model (Geyer & Braff, 1987). However, the predictive validity of this model is questionable as result of recent research in human subjects (Bakshi *et al.*, 1998; Braff *et al.*, 2001) More specifically, the aforementioned authors described that the serotonin releasing agent, MDMA, has the opposite effect in humans compared to rodents in that it increases PPI rather than causing a deficit in PPI (Vollenweider *et al.*, 1999). Clearly, the predictive validity of the serotonergic model of PPI disruption is compromised by these findings.

2.10.4 Environmental animal models of schizophrenia

There is a general agreement that the environment during pregnancy, at birth and in childhood is critical for brain development (O'Callaghan *et al.*, 1991; Takei *et al.*, 1996). The neurodevelopmental hypothesis of schizophrenia is based on the notion that early-life environmental factors can have significant consequences for processes of brain maturation (Weiss & Feldon, 2001). It is therefore possible to manipulate the environment of an animal in order to produce a disturbance in the expression of normal behavior, thus conferring construct validity. Social withdrawal during specific phases of development can be carried out during either the pre-weaning period, from weaning to adulthood or during adulthood. There are three different environmental manipulations with effects on PPI that have been studied, namely early handling, maternal separation, and social isolation (Weiss & Feldon, 2001).

2.10.4.1 Social isolation

An environmental manipulation with marked consequences for the manifestation of behavioural responses in animals is that of post weaning social isolation (Domeney & Feldon, 1998). Isolation rearing refers to the paradigm in which animals are placed into an isolated housing condition (no cage mates) from the age of weaning (typically from post-natal day 21-28) and into adulthood, at which time comparisons are made with socially reared counterparts (Bakshi *et al.*, 1998). Altered behaviors under these conditions include spontaneous locomotor activity in open field environments, disrupted PPI (Reboucas & Schmidek, 1997; (Weiss *et al.*, 2000), altered responsiveness to psychostimulants (Phillips *et al.*, 1994), neophobia (Hall *et al.*, 1997), increased timidity (Einon & Morgan, 1977) increased anxiety (Wright *et al.*, 1991) and preservative behaviours (Morgan & Einon, 1975), and deficits in cognitive learning tasks (Weiss & Feldon, 2001). These are all suggestive of strong face validity for schizophrenia.

A number of brain regions have been implicated in these responses, including the prefrontal cortex, hippocampal formation and the NAcc (Van den Buuse M. *et al.*,

2003). Since isolation rearing constitutes a chronic stressor (Holson *et al.*, 1991) this again confers significant face validity to the model (Weiss & Feldon, 2001). Isolation-rearing-deficits can also be reversed by treatment with either typical (Varty & Higgins, 1995) or atypical antipsychotic drugs such as clozapine (Geyer *et al.*, 1993) and olanzapine (Bakshi *et al.*, 1998), thereby conferring predictive validity to the model.

Rats isolated when they are immature (21 days post natal) show PPI impairment in adulthood, thereby mimicking abnormal sensorimotor gating in schizophrenic patients. These deficits are reversed by a range of antipsychotic drugs suggesting that a social isolation-induced PPI deficit has predictive validity for schizophrenia (Weiss & Feldon, 2001). Of great importance is that the effect of isolation is developmentally-specific in that similar isolation during adulthood does not affect PPI, such that the deficit in PPI emerges only after puberty (four weeks; (Krebs-Thomson *et al.*, 2001). The model also has construct validity, as PPI dysfunction has been associated with dopaminergic hyperactivity in the brains of isolation reared rats (particularly in the NAcc (Jones *et al.*, 1992). There is also evidence that the effect of isolation rearing on PPI is dependent on a variety of other factors, including gender, strain of rats and caging conditions during the social isolation period (Weiss & Feldon, 2001). Consequently, the isolation–rearing paradigm provides a non-pharmacological and developmentally-specific method of inducing schizophrenia-like behavioural deficits in rodents (Geyer & Ellenbroek, 2003). This not only has significant value for the screening of novel antipsychotic drugs, but also towards understanding the underlying neurobiological dysfunctions of schizophrenia (Weiss & Feldon, 2001).

2.10.4.2 Maternal separation

When rat pups are newborn, their social environment is described by interactions with the mother and the other pups of that specific litter. The mother is therefore a distinctive link between the pups and the external world (Weiss & Feldon, 2001). When pups are maternally deprived, they show a deficit in PPI in adulthood (Husum *et al.*, 2002; Ellenbroek *et al.*, 1998). This has lead to maternal separation being

advocated as a developmental manipulation that produces schizophrenia-like deficits in rodents later in life (Ellenbroek *et al.*, 1998; Ellenbroek & Cools, 1998), and thus of possible value as an analogous animal model.

Maternal separation consists of separating the pups from their mothers for a certain time period (1-24 hours) during the pre-weaning period (Weiss & Feldon, 2001). The frequency of this separation, the time period of separation and on what point the pups are deprived in the pre weaning period, plays an important role in the behavioural response (Lehmann & Feldon, 2000). Ellenbroek (1998) found that maternal deprivation on post natal day nine for eight hours causes the most robust deficit in PPI. It has also been reported that certain rat strains are more sensitive to early life manipulations such as maternal separation (Ellenbroek & Cools, 2000). Importantly, deficits in PPI caused by maternal separation is only manifested after puberty and can be blocked by treatment with typical (haloperidol) and atypical (quetiapine) neuroleptics (Ellenbroek *et al.*, 1998), thus giving the model noteworthy predictive validity. Some studies, however, have reported that malnutrition that is invariably associated with maternal separation may underlie the observed PPI deficits caused by this model and not the maternal separation as such, and that developmental malnutrition on its own causes a deficit in PPI (Geyer *et al.*, 2001). A study by Pryce and Feldon (2000) also found no deficit in PPI using a 24 hr maternal separation paradigm with unrelated male and female pups (Lehmann *et al.*, 2000). While evidence would indicate that early-life events, for example maternal deprivation, can increase the risk of developing schizophrenia in later life (Ellenbroek & Cools, 1998; Walker & Diforio, 1997), there are some doubts as to the robustness of this paradigm in rodents, despite some evidence for predicitve validity.

2.10.4.3 Early handling

Early handling consists of daily separation of the pups from their mother for time periods of between 3-15 minutes, but does not exceed the period of time when the mother normally leaves the nest for the purpose of foraging etc (Weiss & Feldon, 2001). This postnatal manipulation is considered to stimulate maternal care and is not a social deprivation (Liu *et al.*, 1997).

Neonatal handling is considered an early mild stress procedure that can cause permanent changes in the rat brain, particularly in improving the rat's ability to cope with stressful stimuli in adulthood (Meaney *et al.*, 1985; Tejedor-Real *et al.*, 1998). When rats are exposed to early handling stress, they show a decrease in emotional responses, reduced hypothalamic-pituitary-adrenal (HPA) axis responsiveness and a rapid post stress endocrine recovery compared to undisturbed rats (Levine, 1967; Meaney *et al.*, 1989; Meaney *et al.*, 1996).

Early handling affects many aspects of behaviour, including reduced anxiety when tested in the elevated plus maze (Vallee *et al.*, 1997), an increase in spontaneous locomotion in an open field while their locomotor activity is also increased when d-amphetamine is administered compared to non-handled pups (Weinberg *et al.*, 1978; Feldon & Weiner, 1988). Latent inhibition, a delayed conditioning to a stimulus that has previously been presented without any significant consequences, is also not influenced in early handling, but is disrupted in non-handled rats (Weiner *et al.*, 1985). In the context of this work, latent inhibition is also deficient in schizophrenia (Feldon & Weiner, 1992b) (Section 2.10.7). In addition, early handled rats are less resistant to becoming helpless in stressful circumstances and less emotionally reactive (Costela *et al.*, 1995; Tejedor-Real *et al.*, 1998). Early handling does not cause any significant disruption in PPI (Pryce *et al.*, 2001), with early handled rats having normal sensorimotor gating abilities compared to non-handled rats (Pryce *et al.*, 2001). Therefore, early handling may not be a suitable model to investigate the underlying cause of deficient sensorimotor gating in schizophrenia (Weiss & Feldon, 2001).

2.10.5 Genetic animal models

Schizophrenia is a highly inherited disorder which involves multiple genes (Kendler *et al.*, 1996). Advances in human genomics and also in the manipulation of the mouse genome, has allowed for exceptional advancements in modeling schizophrenia (Powell & Miyakawa, 2006). The development of genetic animal models of schizophrenia (Smithies, 1993) have greatly enhanced existing models. Behaviours in schizophrenia might be based on certain genes that have overlapping

functions in mice and humans. Another approach is transgenic overexpression in an animal model. With this approach certain genes that are suspected to be up-regulated in post-mortem brains in schizophrenia (Powell & Miyakawa, 2006) may be studied in genetically manipulated animals. Some recent examples of genetic approaches in schizophrenia include genetic mouse models based on the susceptibility genes and candidate gene genetic models (Powell & Miyakawa, 2006). Mouse models based on the hypoglutamatergic hypothesis (Duncan *et al.*, 2004; Fradley *et al.*, 2005; Stefansson *et al.*, 2004), models based on the hyperdopaminergic hypothesis (Barr *et al.*, 2004; Gainetdinov *et al.*, 2001) and neurodevelopmental genetic models (Chen *et al.*, 2006) are examples of models in this group that have been developed. Genetic mouse models based on genetic susceptibility, however, are still in development, but may hold great promise in shedding light on the pathophysiology of schizophrenia regarding cognitive processes, neuronal function and molecular mechanisms underlying this illness (Chen *et al.*, 2006). However, since these topics fall out of the immediate scope of this dissertation, they will not be discussed further. However, the reader is encouraged to peruse the above-mentioned references for more detailed information on this exciting field.

2.10.6 Habituation

Habituation of the startle response is a form of plasticity that has been connected with cognitive function. Habituation is defined as the reduction in responding to an initial stimulus when it is presented continuously at rates too slow to produce sensory adaptation (Geyer & Braff, 1982). Habituation is considered to be the simplest form of learning (Petrinovich & Peeke, 1973). In schizophrenia, a decreased habituation of startle response has been reported by various investigators (Braff *et al.*, 1978; Grillon *et al.*, 1992; Bolino *et al.*, 1994). Habituation of startle in schizophrenia shows significant deficits (Geyer & Braff, 1982), although on the other hand some studies failed to detect a deficit (Bolino *et al.*, 1994; Braff *et al.*, 1999). Therefore, the data available at present suggests that habituation is not as accurate a measure of cognitive functioning in schizophrenia as is the PPI paradigm (Ludewig *et al.*, 2002).

2.10.7 Latent inhibition

Latent inhibition, which has been briefly mentioned earlier, is a model that is used to assess certain aspects of schizophrenia (Feldon & Weiner, 1992a). Latent inhibition is a model for understanding cognitive deficits in schizophrenia (Vaitl *et al.*, 2002) This refers to the retarding effects to a stimulus that has previously been repeatedly offered without significant consequences (Weiss & Feldon, 2001). The disruption of latent inhibition is the outcome of an inability to ignore irrelevant external stimuli (Vaitl *et al.*, 2002) In some studies it has been reported that latent inhibition is diminished in patients with schizophrenia (Baruch *et al.*, 1988; Gray *et al.*, 1995), although this has not been reproducible (Swerdlow *et al.*, 1996b). Latent inhibition presents with noteworthy face and construct validity and to a lesser extent predictive validity (Moser *et al.*, 2000). Latent inhibition can be disrupted by a variety of drugs for example amphetamine, nicotine, DOI (5-$HT_{2A/2C}$ agonist) and other compounds (Moser *et al.*, 2000). Amphetamine-induced disruption in latent inhibition can be reversed by a small number of antipsychotics, e.g. haloperidol, clozapine and sertindole (Ellenbroek *et al.*, 1999). However, social isolation, that causes a deficit in PPI, was not found to affect latent inhibition (Wilkinson *et al.*, 1994). Therefore interest in this model is now waning and not relevant for this study.

2.11 *Conclusion*

Schizophrenia is a neuropsychiatric disorder affecting in the region of 1% of the people worldwide (Kelly *et al.*, 2005). Schizophrenia is a complex, multifactorial disease probably involving genetic, environmental and neurodevelopmental factors (Weiss & Feldon, 2001). Research has implicated dysfunction of a number of neurotransmitter systems in the pathophysiology of schizophrenia, with the dopaminergic hyperfunction hypothesis the most well known and best supported (Goff & Coyle, 2001). However, the glutamatergic hypothesis has begun to gain increasing recognition, and suggests that glutamatergic hypofunction in frontal-cortical areas is the initiating factor evoking a reactive increase in dopaminergic function in limbic brain regions (Olney & Farber, 1995; Olney *et al.*, 1999). Indeed, it has been speculated that glutamate N-methyl-D-aspartate (NMDA) receptor

hypofunction might offer a more compelling pharmacological model of the illness than models selectively mimicking dopaminergic hyperfunction (Becker & Grecksch, 2004) The genetic component has been demonstrated in twin, family and adoption studies, although the results also demonstrate that genetics is not the only contributing factor (Karayiorgou & Gogos, 1997; McGuffin *et al.*, 1995). Several hypotheses have emerged around the implication of environmental risk factors in the development of schizophrenia ((Weiss & Feldon, 2001; Lipska & Weinberger, 2000). There is a general agreement that the environment during pregnancy, at birth and in childhood is critical (O'Callaghan *et al.*, 1991; Takei *et al.*, 1996). The neurodevelopmental hypothesis of schizophrenia is based on the notion that early-life environmental factors can have significant consequences for processes of brain maturation (Weiss & Feldon, 2001)

Schizophrenia remains a disorder that is extremely difficult to treat (Weiden *et al.*, 1996). There is thus an urgency to better understand the illness and so to better be able to develop new and improved drug treatments. Validated animal models must however be developed before new novel pharmacotherapies can be investigated.

Animal models are useful to investigate psychiatric disorders like schizophrenia. These models can be used to investigate potential novel pharmacotherapies as well as to identify novel therapeutic targets. Indeed, the main purpose of an animal model is to improve our understanding of the human disorder in a manner that is ethically responsible yet scientifically sound (Geyer & Markou, 1995).

2.12 *Summary of aims and objectives*

As has been outlined in detail in Section 1.2 (Aims and Objectives), this study will first set up and validate a pharmacological challenge model based on the glutamate hypofunction model of schizophrenia using the NMDA antagonist, MK-801 and exploiting PPI of startle as the primary behavioral parameter. After the dose-dependency of MK-801 have been determined, dose-dependent reversal of PPI deficits induced by MK-801 will be studied using haloperidol (typical antipsychotic)

and clozapine (atypical antipsychotic). This validation will be essential to standardise the PPI behavioral paradigm in our laboratory and to determine the most effective dose of the two neuroleptics for later use in the social isolation stress paradigm. In the latter, a non-pharmacological model of schizophrenia, based on an early life social isolation model, will be developed and validated with respect to face (PPI) and predictive (antipsychotic treatment response) validity for schizophrenia. In addition, in order to extend the possible construct validity of the model, PPI and chronic drug treatment response will be coupled with dopamine and glutamate neuro-receptor studies in the frontal cortex of socially isolated rats. This study will not only assist in the novel validation of SIS after chronic drug treatment, but will address the different roles of cortical glutamate and dopamine following SIS and its relevance for SIS associated effects on sensory motor gating.

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CHAPTER 3

Article

Article for submission for publication in *Psychopharmacology (Berl)*

Introduction

This chapter presents the full-length manuscript for submission to *Psychopharmacology (Berl)*, published by Springer. The manuscript is presented in the required format prescribed by *Instructions to the Authors*, as per the journal website:

http://www.springerlink.com/content/100390/ under "About this journal".

The complete Instructions to the Authors have been reproduced and included in the dissertation under Appendix 1. Thus, the manuscript will begin with the title, contributing authors and affiliations on a separate page, followed by an Abstract, also on a single page. Thereafter will follow the main body of the manuscript, including Introduction, Materials and Methods, Results, Discussion, Acknowledgements, Bibliography and Legends to Figures. As per the journal submission format, all figures are inserted into the text.

Social isolation effects on sensory motor gating in rats: Involvement of frontal cortical NMDA-D_1 receptors and modulation by chronic clozapine versus haloperidol

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Abstract

Rationale:

Early life stress may be a prodromal event pre-dating the later development of schizophrenia, with both glutamate and dopamine implicated in frontal cortical dysfunction. Post natal social isolation stress (SIS) represents a neurodevelopmental model of schizophrenia, although the role of glutamate and dopamine, as well as the differential response to typical/atypical antipsychotics, has not been studied.

Objectives:

To study the effects of SIS on sensorimotor gating in rats, its association with frontal cortical N-methyl-D-asparate (NMDA) and D_1 receptors, and their response to haloperidol and clozapine treatment.

Methods:

To confirm dosages, the dose-dependent reversal of dizolcilpine (MK-801; 0.25mg/kg i.p) induced suppression of prepulse inhibition (PPI) by acute haloperidol (0,1 ;0,2 and 0,5 mg/kg i.p) and clozapine (5 and 10mg/kg i.p) was determined, with routine catalepsy assessments. Thereafter, SIS (post natal day 21-61) effects on PPI and frontal cortical NMDA and D_1 receptor binding characteristics were studied with(out) chronic haloperidol (0,2 mg/kg i.p; day 49-60) or clozapine (5 mg/kg i.p, day 49-60) treatment.

Results:

Clozapine, but not haloperidol, reversed MK-801-evoked PPI deficits. SIS evoked significant PPI deficits together with increased NMDA-, but reduced D_1 receptor density in the frontal cortex, with PPI deficits reversed by chronic clozapine, but not by haloperidol. Clozapine, but not haloperidol, significantly bolstered NMDA B_{max} following isolation. SIS reduced D_1 receptor density, which was exacerbated by haloperidol, but unaltered by clozapine. SIS decreased NMDA, but increased D_1 affinity with only haloperidol exacerbating the latter.

Conclusion:

SIS-induced PPI deficits involve opposite effects on frontal cortical NMDA and D_1 receptor binding. Clozapine, but not haloperidol, bolsters glutamatergic and stabilises dopaminergic pathways.

Keywords: *social isolation; NMDA receptor; D_1 receptor; clozapine; haloperidol; dizocilpine; frontal cortex; prepulse inhibition; receptor binding; rats*

1 Introduction

Schizophrenia affects approximately 1% of the population worldwide (Kelly, Conley et al. 2005), while its causation is linked to genetic, environmental and neurodevelopmental factors (Weiss and Feldon 2001). Early-life environmental conditions are critical for neuronal development (O'Callaghan, Sham et al. 1991;O'Callaghan, Sham et al. 1991). Indeed, early-life stress affects neuronal growth and differentiation (Bloom 1993; Weiss and Feldon 2001) and is deemed an important risk factor for the later development of schizophrenia (Lipska and Weinberger 2000).

Schizophrenia is characterized by deficits in sensorimotor gating (Geyer, Krebs-Thomson et al. 2001;Geyer, Krebs-Thomson et al. 2001), such that patients are unable to ignore/discard irrelevant sensory stimuli (Bleural 1911; Weiss and Feldon 2001). Its assessment in both humans (Braff, Stone et al. 1978; Braff, Swerdlow et al. 1999; Braff, Swerdlow et al. 1999; Grillon, Ameli et al. 1992) and rodents (Geyer, Krebs-Thomson et al. 2001) is achieved by determining prepulse inhibition (PPI); the normal attenuation of the startle reflex to an intense acoustic stimulus when the latter is immediately preceded by a weaker stimulus (prepulse) (Graham 1975; Braff, Stone et al. 1978). Interestingly, deficits in PPI have been related to both negative (Braff, Swerdlow et al. 1999; Weiss and Feldon 2001) and positive symptoms of schizophrenia (Weiss and Feldon 2001).

The frontal cortex controls behaviour and adaptation to environmental change and decision-making by mediating selection and perception of relevant information and working memory (Javitt, Shelley et al. 2000), and is disrupted in schizophrenia (Chudasama and Robbins 2006). Dopamine and glutamate transmission modulates working memory in the prefrontal cortex (Goldman-Rakic 1996), whereas subcortical dopamine activity is well recognised in positive symptom manifestations, this monoamine also plays a significant role in the frontal cortex (Murase, Grenhoff et al. 1993; Castner and Williams 2007). While effective in positive symptoms management, traditional dopamine-2 (D_2) receptor antagonists are ineffective for

negative symptom schizophrenia (Kirkpatrick, Buchanan et al. 2001). However dopamine-1 (D_1) receptors are especially concentrated in the frontal cortex (Steele, Hodges et al. 1997), outnumbering D_2 receptors by 20 to 1 (Lidow, Goldman-Rakic et al. 1991). Although, neuroimaging studies in this brain region have reported either reduced (Okubo, Suhara et al. 1997), no change (Karlsson, Farde et al. 2002), or increased (bi-Dargham, Mawlawi et al. 2002) D_1 binding, prefrontal cognitive impairment follows dopamine depletion (Schneider, Sun et al. 1994) suggesting deficient prefrontal D_1 signalling in negative symptom schizophrenia (Barch, Csernansky et al. 2002; Davis, Kahn et al. 1991; Goldman-Rakic, Castner et al. 2004).

Prefrontal dopamine release, however, is strongly regulated by glutamate via N-methyl-D-aspartate (NMDA) receptors (Murase, Grenhoff et al. 1993), with D_1-NMDA receptor interactions forming a fundamental neural substrate for cognition, particularly in schizophrenia (Castner and Williams, 2007). NMDA receptors can increase discharge of dopamine neurons in the ventral tegmental area (Kalivas, Duffy et al. 1989) and indirectly modulate cortical dopaminergic activity (Murase, Grenhoff et al. 1993; Kalivas, Duffy et al. 1989). Subsequently, NMDA receptor blockade has been found to increase prefrontal cortex dopamine release (Hondo, Yonezawa et al. 1994; Hertel, Mathe et al. 1995). Dopamine D_1 signaling in turn also modulates glutamate release (Castner and Williams, 2007), decreasing medial prefrontal cortex glutamate release via GABAergic inhibition (Harte and O'Connor 2004). Given the critical involvement of NMDA receptors in dopaminergic activity as well as in frontal cortical function, it is not surprising that non-competitive NMDA antagonists like phencyclidine (PCP), ketamine and dizoclipine (MK-801) mimic the positive and negative characteristics of schizophrenia (Javitt and Zukin 1991; Steinpresis 1996; Mansbach 1991; Moghaddam 1994; Holcomb, Rowland et al. 2004). NMDA antagonist-evoked PPI deficits in rats (Mansbach, Geyer et al. 1988) that are also selectively reversed by atypical antipsychotics (Cornell, Milden et al. 1985), correlating with their improved negative symptom efficacy over typical agents (Lidow 2000).

In rats, NMDA receptor expression in the brain is critical for developmental synaptogenesis in the first 2 weeks after birth (Ikonomidou, Bosch et al. 1999). Interestingly, early life stress associated PPI deficits in rats also only become evident 4 weeks post-natal (Weiss and Feldon 2001), while such events are suggested to increase the susceptibility of the cortex to neurodevelopmental disturbances, such as NMDA receptor hypofunction-induced apoptosis (du Bois and Huang 2007; du Bois and Huang 2007). While frontal cortical dopamine and glutamate dysfunction underlies the neurobiology of schizophrenia, the preferential role, if any, of cortical D_1 and NMDA receptors following neurodevelopmental trauma, as well as which are targeted following successful drug treatment, is unknown. We have studied the dose response of acute clozapine or haloperidol in reversing hypoglutamatergic-associated PPI deficits in rats following MK-801 administration. Subsequently, the correlation between frontal cortical NMDA and D_1 receptor binding and PPI deficits following post natal social isolation stress (SIS) was studied. Using doses selected from the MK-801 study, the ability of chronic haloperidol or clozapine treatment to modify PPI deficits evoked by early life neurodevelopmental trauma was determined.

2 Materials and methods

2.1 *Animals*

Male Sprague-Dawley rats, weighing 250-300 grams and provided by the Animal Research Centre of North-West University, were either group (three rats/cage) or isolation-housed (one rat/cage) in identical cages. The rats were reared in sawdust cages (Weiss and Feldon 2001c) and kept at constant conditions of temperature (21± 5 °C), relative hurnidity (50±5%) with free access to food and water. Full spectrum cold white light (350-400 lux) was provided over a 12 hour light/dark cycle. Positive air pressure was maintained in the facility with air filtration 99,7 % effective for a particle size of 2 micron and 99,9% for a particle size of 5 micron. All animals were handled according to the code of ethics in research, training and testing of drugs as laid down by the Animal Ethics Committee of the North-West University (Ethics approval number 06D08).

2.2 *Drugs*

(+)Mk-801 (dizocilpine), haloperidol and clozapine were purchased from Sigma–Aldrich, Johannesburg, South Africa. The latter two drugs were first dissolved in a 50 ul glacial acetic acid and buffered with sodium hydroxide (NaOH) (pH=6.0) before being administered intraperitoneally. In both cases, an injection volume of 0,5 ml was used. Drugs were freshly prepared each day before experimental testing and stored in glass bottles covered with aluminium foil. Dizocilpine was dissolved in saline. All drugs were administered via intraperitoneal injection to the rodents.

2.3 *Experimental design*

MK-801 model: Control animals received either saline (i.p) pre-treatment 30 min prior to PPI testing, followed by either a second saline injection (control), or MK-801 (0.25 mg/kg i.p.) 15 min prior to PPI testing. Further groups were pre-treated with increasing dosages of either clozapine (5 and 10 mg/kg i.p.) or haloperidol (0,1 ;0,2 and 0,5 mg/kg i.p.) 30 min before PPI, followed 15 min later by either saline or 0,25 mg/kg MK-801 (Hoffman, Donovan et al. 1993; Bast, Zhang et al. 2000; Keith, Mansbach et al. 1991).

Social isolation model: Social isolation stress (SIS) was essentially performed as previously described (Weiss, Di et al. 2000; Varty, Braff et al. 1999). Briefly, on post-natal day 21, rat pups were randomly assimilated into two groups (n=28/group), viz. one group-housed (control+saline) and the second isolation-housed (test) in cages as described above. The pup's room temperature was elevated to mimic the effect of the litter and to prevent mortalities due to hypothermia. Group or isolation housing was maintained for 8 weeks whereupon the animals were subjected to behavioral testing to assess deficits in PPI. Isolation groups were injected with either clozapine (n=15), haloperidol (n=17) or saline (n=15) in the last 11 days of isolation rearing.

2.4 *Behavioural testing*

2.4.1 Catalepsy:

In the MK-801 pilot study, the possible cataleptic actions of the various doses of haloperidol and clozapine were investigated. Catalepsy was routinely assessed using a catalepsy box (Turski, Schwarz et al. 1984). Both forepaws of the animal were placed on the horizontal bar, 7.5 cm above the floor, repeated at 30 min intervals for 240 min after neuroleptic administration. A maximum score of 300 s was given to an animal which remained on the bar for 5 min or more. This was aimed at ensuring that the dose selected for the SIS study would not induce catalepsy and as such confound the PPI studies.

2.4.2 Prepulse inhibition (PPI)

PPI was assessed using an illuminated and ventilated sound–attenuated startle chamber (SR-LAB, San Diego Instruments, San Diego, USA). The startle chamber consists of a stabilimeter system composed of a transparent Plexiglas cylinder (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame. A speaker mounted 24 cm above the cylinder provides the acoustic noise bursts. The startle responses of the rat within the cylinder is detected and transduced by a piezoelectric accelerometer mounted below the frame. Startle amplitudes are defined as the average of one hundred 1-ms stabilimeter readings collected from the stimulus onset.

The startle session was initiated with a 5 min acclimatization period, with a 68 dB background noise level that continues throughout the test session. To evaluate the basal startle response, five 120 dB startle pulses of 40 ms duration were then presented to the rat. Thereafter, the rat received 10 blocks of 7 trials to measure PPI, each block consisting of four different trial types presented throughout the session, namely a pulse alone (two trials), prepulse alone, prepulse and pulse (1 trial for each prepulse intensity), or no stimulus (1 trial). The four different prepulses had an intensity of either 72, 76, 80 or 84 dB and a duration of 20 ms with an inter-trial interval of 15 seconds. The time interval between the prepulse offset and the pulse onset was 100 ms. The percentage of PPI-induced by each prepulse intensity was

calculated as [100- (100tartle amplitude on prepulse trial)/(startle amplitude on pulse alone trial)] (Weiss, Di et al. 2000) (modified). All PPI procedures were performed between 09h00-13h00.

2.5 *NMDA receptor density in the frontal cortex of SIS rats*

Group-housed rats and SIS rats receiving chronic drug treatments (i.e. haloperidol, clozapine or saline) were sacrificed by decapitation and the frontal cortex rapidly dissected on ice. The tissue was then snap frozen in liquid nitrogen and stored at -80°C until neuroreceptor analysis. Tissues were pooled for the determination of NMDA receptor density (2-3 cortices were pooled per measurement). The tissue was homogenized in 50 volumes of cold HTS buffer (5 mM HEPES/4.5 mM Tris buffer, pH 7.4) with a Brinkman polytron (setting 6, 10 seconds) and the suspension centrifuged (48 000×g for 15 minutes at 4°C). The resulting supernatant was decanted, 30 ml of buffer was added and the centrifugation step repeated as above. The pellet was re-suspended in 60 volumes of HTS buffer (1 g tissue in 60 ml) and the homogenate kept on ice.

The following was added to the test tubes to define total binding: 300 µl homogenate, 50 µl 300 µM glycine, 50 µl 100 µM *l*-glutamate, 50 µl HTS-buffer, 50 µl [^{3}H]-Mk 801 in a concentration range of 0.71- 25.21 nM. To determine non-specific binding, the following was added to the test tubes: 300 µl homogenate, 50 µl 300 µM glycine, 50 µl 100 µM *l*-glutamate, 50 µl 300 µM MK-801, 50 µl [^{3}H]-Mk801 in each test tube of the concentration range. Each test tube was then vortexed and thereafter incubated in a shaking water bath for 90 min at 25°C. The contents of each tube was transferred to a Whatman GF/B filter pre-soaked with ice-cold buffer on the Hoeffler apparatus and the labeled membranes were harvested by rapid vacuum filtration. The filters were rinsed with 4 ml ice-cold buffer and placed in scintillation vials containing 3 ml Filter Count® scintillation cocktail and radioactivity determined by scintillation counting using a Packard Tri-carb 4660 scintillation spectrometer. To determine each standard of the radioligand concentration series, 10 µl of each radioligand concentration was placed on a clean filter and 3 ml scintillation cocktail added to generate a standard curve. The radioactivity on the filters was then

determined by scintillation counting. NMDA receptor density was expressed in fmol/mg protein, with protein routinely assayed using the Bradford assay.

2.6 *Dopamine D_1 receptor density in the frontal cortex of SIS rats*

Group-housed rats and SIS rats receiving chronic drug treatments (i.e. haloperidol, clozapine or saline) were sacrificed by decapitation and the frontal cortex rapidly dissected on ice. The tissue was snap frozen in liquid nitrogen and stored at -86°C until neuroreceptor assays were performed. Tissues were pooled for the determination of D_1 receptor density (2-3 cortices were pooled per measurement). The tissue was homogenized in 50 volumes of cold Tris buffer (Tris HCL (50 mM), NaCl (120 mM), KCl_2 (5 mM), $CaCl_2$ (2 mM), $MgCl_2$ (1 mM), ascorbic acid (0.1%)pH 7.4) with a Brinkman polytron (setting 6, 10 seconds) and thereafter the suspension was centrifuged (48 000×g for 15 minutes at 4°C). The supernatant was decanted, 30 ml buffer added and again centrifuged as above. The pellet was then re-suspended in 60 volumes of HTS buffer (1 g tissue in 60 ml) and the homogenate kept on ice.

The following was added to the test tubes to define total binding: 300 µl homogenate, 50 µl buffer, 100 µl 0.5 µM ketanserin, 50 µl of [^{3}H]-SCH 23390. Thereafter, to determine non-specific binding, the following was added to each test tube in the concentration range, 300 µl homogenate, 100 µl 0.5 µM ketanserin, 50 µl 10 µM SCH23390, 50 µl [^{3}H]-SCH 23390. Each tube was then vortexed and incubated in a shaking water bath (15 min at 37°C). The contents of each tube was then transferred to a Whatman GF/B filter pre-soaked with ice-cold buffer on the Hoeffler apparatus. Thereafter the filters were rinsed with 4 ml ice-cold buffer and placed in scintillation vials containing 3 ml Filter Count® scintillation cocktail. Radioactivity was determined by scintillation counting using a Packard Tri-carb 4660 scintillation spectrometer. 10 µl of each radioligand concentration was placed on a clean filter together with 3 ml scintillation cocktail to generate a standard curve and radioactivity determined by scintillation counting. D_1 receptor density were expressed in fmol/mg protein, with protein routinely assayed using the Bradford assay.

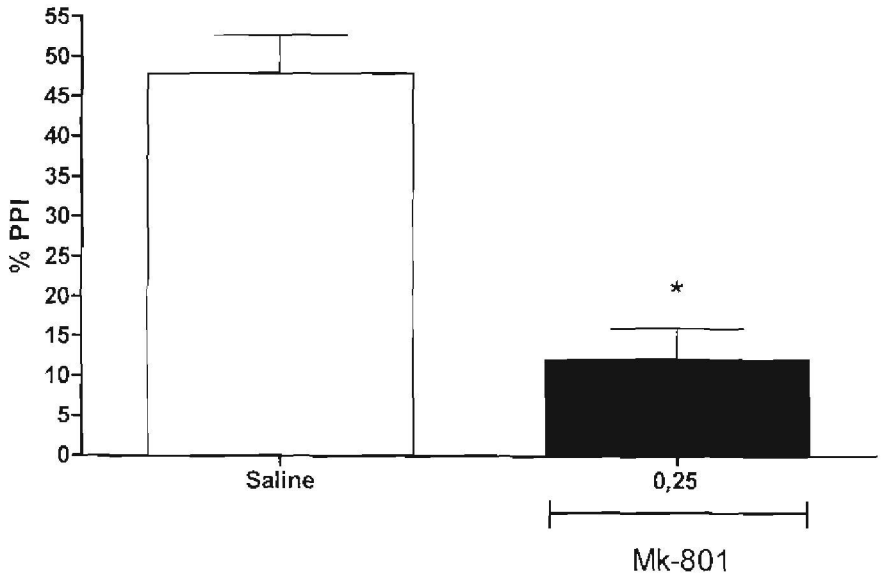
2.7 *Statistical analysis*

Graphpad Prism version 4 for windows (Graphpad software, San Diego, USA) was used for the statistical analysis and graphical presentations. For the validation of PPI with MK-801 with/without varying doses of neuroleptic, control vs MK-801 was analysed using an unpaired Student's T test, while MK-801 with/without drug treatments were analysed using one-way analysis of variance (ANOVA) followed by Dunnetts test. In the SIS study, in all cases SIS vs. group-housed data were analysed using an unpaired Student's T-test, with SIS with/without neuroleptic treatment analysed using one-way ANOVA followed by Dunnett's test, as were the catalepsy studies. In all instances, statistical significance was defined as $p<0.05$.

3 Results

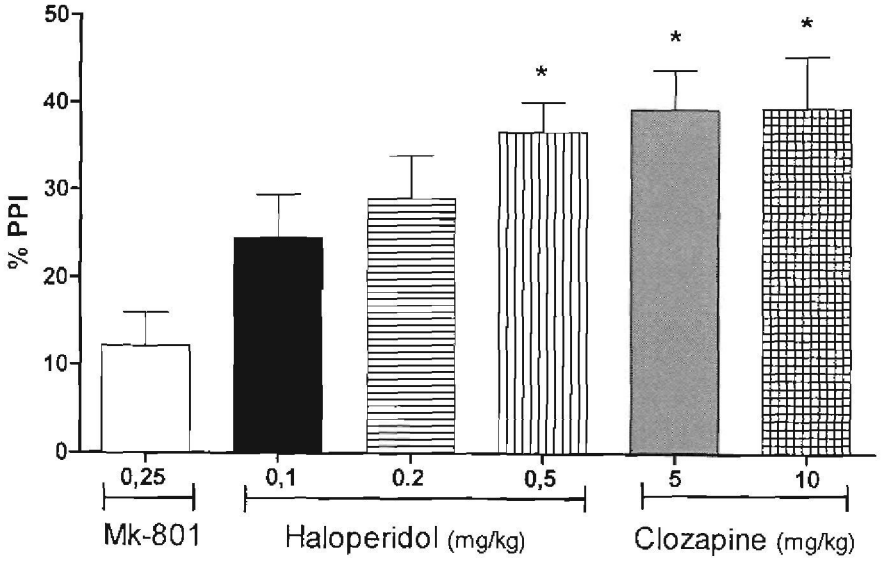
3.1 *MK-80- induced sensory motor gating and response to drug treatment*

MK-801 evoked a significant reduction in % PPI compared to saline-treated animals ($p<0.0001$; Figure 1, Student's t test). One-way ANOVA of the MK-801 compared to MK-801 plus the various drug treatment groups revealed significant differences across the groups [$F(6,63)$; 4.52, $p=0.0007$]. Subsequent post hoc analysis revealed MK-801 associated deficits in PPI to be significantly reversed by 0,5 mg/kg haloperidol ($p<0,01$, Figure 2, Dunnetts), with 0,1 mg/kg and 0,2 mg/kg haloperidol failing in this regard. Both dosages of clozapine (5 mg/kg and 10 mg/kg) significantly reversed the disruption in PPI caused by MK-801 ($p<0.01$, Figure 2, Dunnetts).



Statistics: * p<0.001 vs. Mk-801 (Unpaired student t test)

Figure 1



Statistics: * p<0.01 vs. Mk-801 (Dunnetts post test)

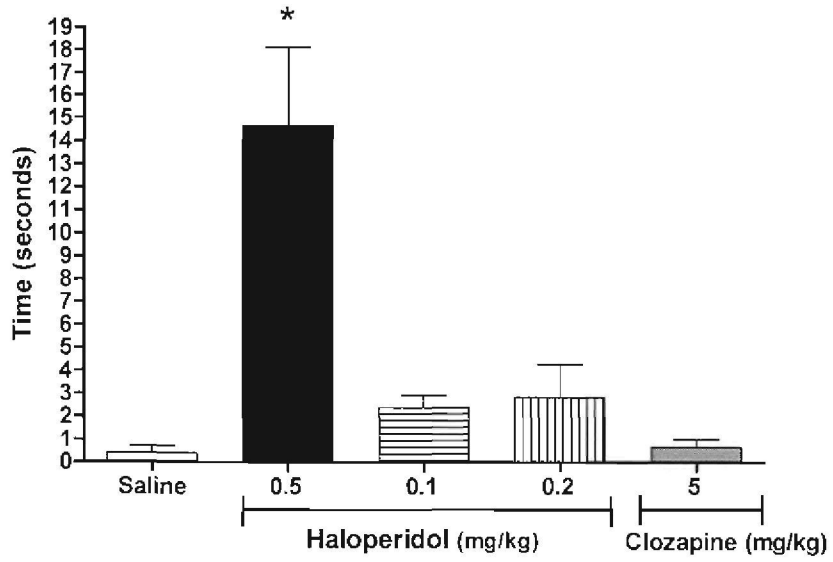
Figure 2

However, the apparent reversal of MK-801-induced PPI deficit by haloperidol (0.5mg/kg) and clozapine (5 and 10mg/kg) may be confounded by the known

cataleptic effects of neuroleptic drugs. Consequently, the cataleptic actions of these two drugs were tested and related to their possible effects on PPI.

3.2 *Catalepsy associated with haloperidol and clozapine*

Drug induced catalepsy 30 minutes post injection of different doses of haloperidol, clozapine and saline control was characterised by significant group differences (one-way ANOVA [F(4,34); 7.666, p<0.0002]). Haloperidol, at a dose of 0,5 mg/kg, but not at the lower dosages (0.1 and 0.2mg/kg), resulted in a significant longer period of time spent gripping the cross bar compared to rodents injected with saline control (p<0.01; Figure 3; Dunnetts), thus demonstrating significant cataleptic actions at this dosage. Clozapine, at a dose of 5mg/kg, resulted in a similar behaviour to control, thus not demonstrating any cataleptic actions (p>0,05; Figure 3; Dunnetts).



After 30 minutes

Statistics: * p<0.01 vs. Saline (Dunnetts post test)

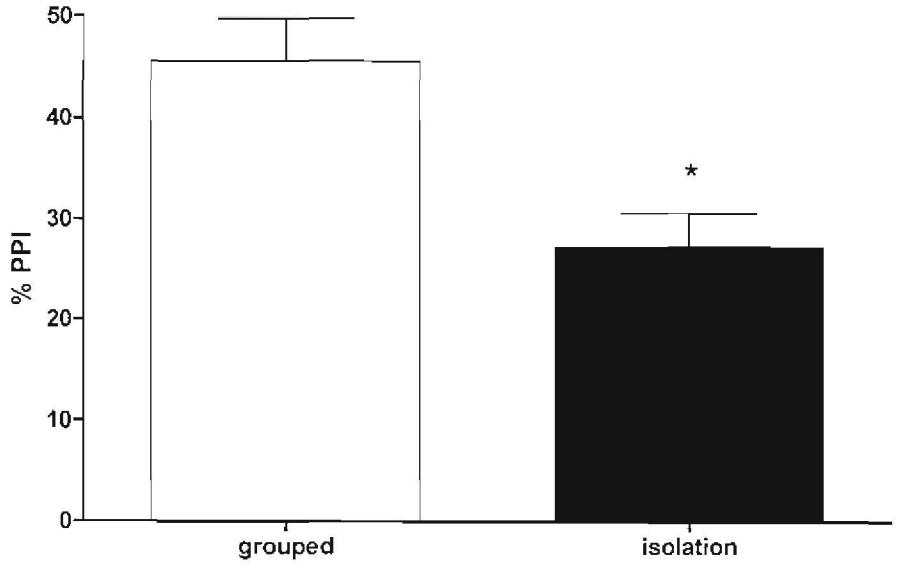
Figure 3

Based on these data, it could not be assumed that haloperidol 0.5mg/kg was effective in reversing MK-801-induced PPI deficits described earlier, given the confounding effects of this drug on locomotor response. The drug induced catalepsy effect was, however, not shared by clozapine. With drug treatment studies e.g.

(Varty and Higgins 1995; Schwabe, Brosda et al. 2005) using haloperidol or clozapine at dosages of 5 mg/kg and 10 mg/kg clozapine or 0,1 mg/kg haloperidol, respectively, a dose of 0.1 mg/kg haloperidol and 5 mg/kg clozapine was selected for use in the chronic drug treatment arm of the SIS study. These are described below.

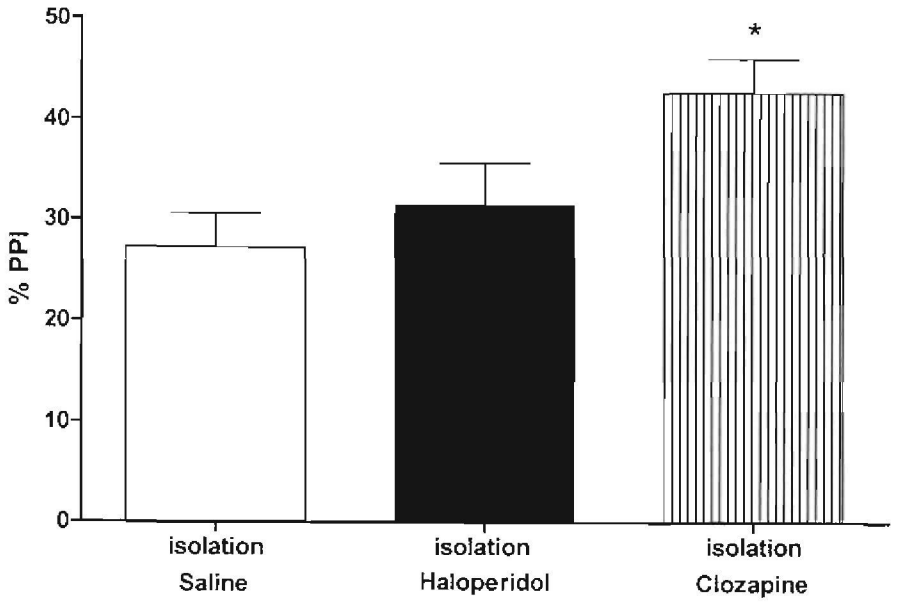
3.3 *Effect of social isolation stress (SIS) on PPI and response to atypical/typical antipsychotics*

SIS evoked a significant reduction in % PPI compared to group-housed saline-treated animals ($p=0.0013$; Figure 4, Student's t test), confirming a marked deficit in sensorimotor gating induced by SIS. For isolation housed saline-treated animals versus isolation housed receiving the various chronic drug treatments, one-way ANOVA of the data revealed significant group differences [$F(2,50)$; 4.259, $p=0.02$]. Chronic treatment of SIS animals with haloperidol failed to modify the attenuation of PPI compared to saline-treated SIS animals ($p>0.05$; Figure 5; Dunnetts). In contrast, SIS animals treated chronically with clozapine demonstrated a significant reversal in PPI deficits compared to saline-treated SIS animals ($p<0.05$; Figure 5; Dunnetts).



Statistics: * p< 0,01 vs. Grouped saline (Unpaired student t test)

Figure 4



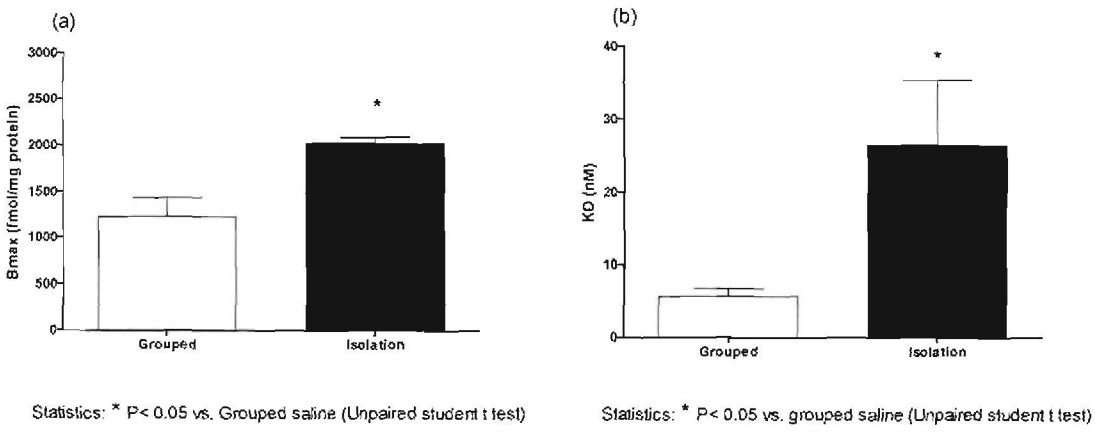
Statistics: * p< 0,05 vs. Isolation saline (Dunnetts post test)

Figure 5

Importantly, in a separate study to exclude the possible confounding effects of injection stress on SIS-associated PPI changes, the effects of SIS in group-housed naive rats and group-housed rats treated with saline, and in naive isolation rats compared to saline-treated isolation rats, were studied. No differences in PPI were found between these comparisons thereby confirming that injection stress is unlikely to alter SIS-associated effects on PPI.

3.4 *Social isolation stress effects on frontal cortex glutamate NMDA receptor binding and response to drug treatment*

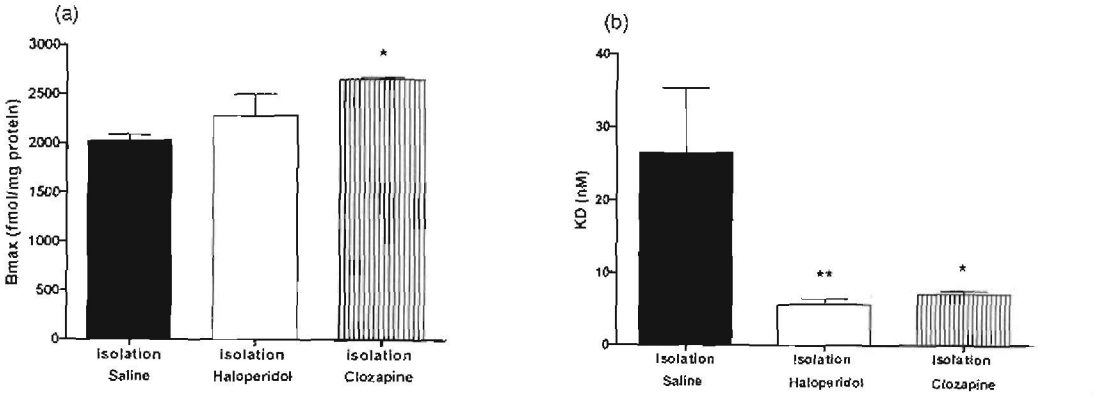
SIS significantly increased B_{max} -($p=0.02$; Figure 6a, Student's t test), as well as a significantly increased K_D –values ($p=0.03$; Figure 6b, Student's t test) compared to saline-treated isolation animals, thereby confirming a marked increase in NMDA receptor density, and an associated decrease in receptor affinity, following SIS. For SIS animals receiving the various chronic drug treatments, one-way ANOVA of the NMDA receptor B_{max} data revealed significant group differences [$F(2,6)$; 5.793, $p=0.04$]. Similarly, one-way ANOVA of the NMDA receptor K_D data indicated significant differences across these groups [$F(2,6)$; 10.22, $p<0.012$]. Chronic treatment with haloperidol failed to reverse the isolation-induced increase in NMDA receptor density compared to the saline treated isolation group ($p>0.05$; Figure 7a, Dunnetts). Chronic treatment with clozapine, on the other hand, resulted in a sustained and further increase in isolation stress induced NMDA receptor density compared to saline treated isolation animals ($p<0.05$; Figure 7b; Dunnetts). Considering K_D –values in isolation groups receiving either haloperidol ($p<0.01$) or clozapine ($p<0.05$), these values were significantly reduced compared to saline treated isolation animals (Figure 7b; Dunnetts).



Statistics: * P< 0.05 vs. Grouped saline (Unpaired student t test)

Statistics: * P< 0.05 vs. grouped saline (Unpaired student t test)

Figure 6



Statistics: * P< 0 05 vs. isolation saline (Dunnetts post test)

Statistics: ** P< 0.01 vs. isolation saline (Dunnetts post test)

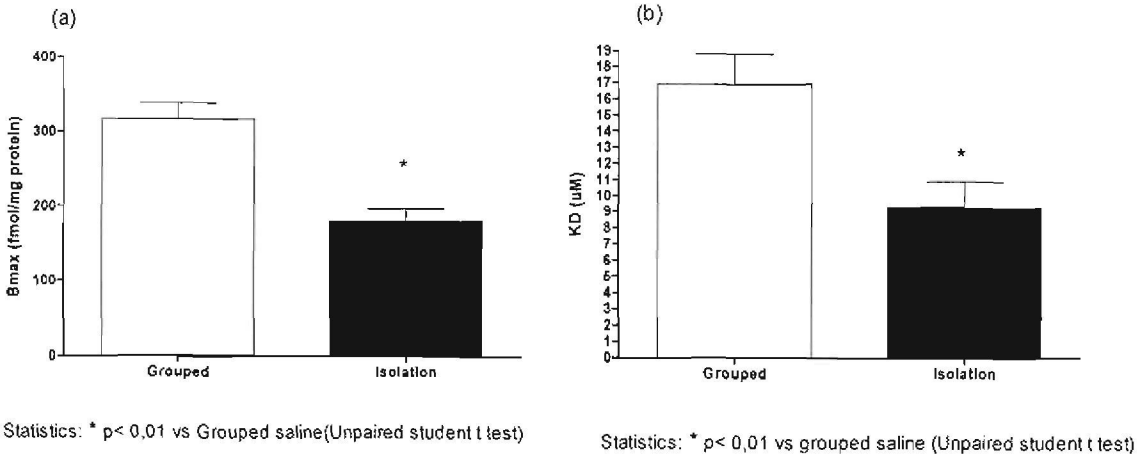
* P< 0.05 vs. isolation saline (Dunnetts post test)

Figure 7

3.5 *Social isolation stress effects on frontal cortex dopamine D_1 receptor binding and response to drug treatment*

SIS evoked a significant reduction in B_{max} for the D_1 receptor compared to saline-treated group-housed animals (p=0.007; Figure 8a, Student's t test), as well as engendering a significant reduction in the K_D – value, (p=0.037; Figure 8b, Students T test), thereby confirming a marked decrease in D_1 receptor density, and an associated increase in receptor affinity, following SIS. One-way ANOVA of the D_1 receptor B_{max} data in isolated rats receiving the various chronic drug treatments indicated significant group differences, viz. [F(2,8); 4.968, p=0.04], with similar

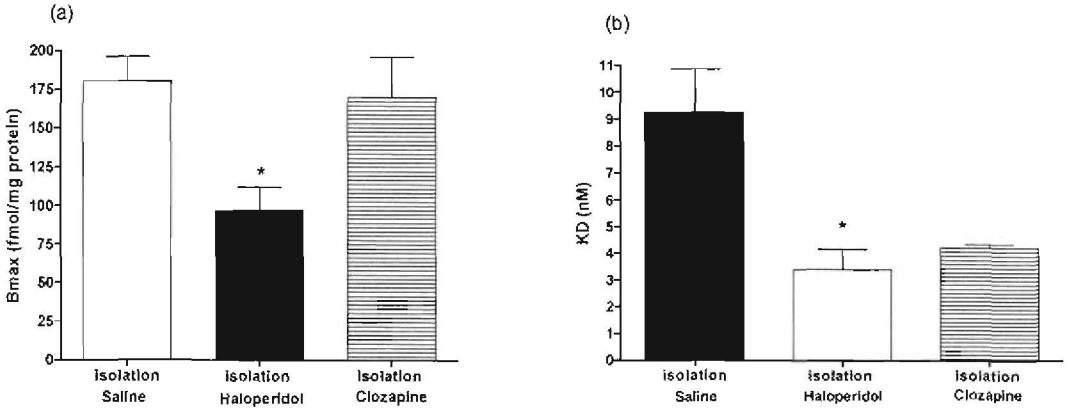
significance observed in K_D vales across the groups [F(2,7); 5.958, p=0.03]. Chronic treatment of socially isolated animals with haloperidol caused a significant down-regulation of cortical dopamine D_1 receptor density compared to saline treated isolation animals (p<0.05; Figure 9a; Dunnetts). In contrast, however, chronic treatment with clozapine did not significantly alter cortical D_1 receptor density compared to that in the saline treated isolation housed animals (p>0.05; Figure 9A; Dunnetts). Interestingly, haloperidol treatment (p<0.5; Figure 9b, Dunnetts) but not clozapine treatment increased D_1 receptor affinity (Figure 9b).



Statistics: * p< 0,01 vs Grouped saline(Unpaired student t test)

Statistics: * p< 0,01 vs grouped saline (Unpaired student t test)

Figure 8



Statistics: * p< 0,05 vs Isolation saline (Dunnetts post test)

Statistics: * p< 0,05 vs Isolation saline (Dunnetts post test)

Figure 9

4 Discussion

In the present study we describe the effects of 8 weeks post-natal social isolation stress (SIS) on sensory motor gating deficits in rats, as determined by PPI of startle, and its associated effects on glutamate NMDA and dopamine D_1 receptors in the frontal cortex. In addition, we report on the response to 11 days treatment with either clozapine or haloperidol immediately prior to closure of the isolation period on the above-mentioned neuro-behavioral markers. Dosages used in the chronic drug study and effects on SIS-associated PPI changes were confirmed by prior pilot studies in a glutamate deficit model of schizophrenia using MK-801. Critical observations of the SIS study were that rodents reared in isolation immediately after weaning (post natal day 21) showed a significant deficit in PPI compared to group housed controls, that was correlated with a significant up-regulation of frontal cortex NMDA receptors. However, D_1 receptors were down-regulated, suggesting that SIS induced PPI deficits involve diagonally opposed effects on frontal cortical glutamatergic and dopaminergic transmission. Concomitant clozapine treatment further increased NMDA receptor density but did not affect cortical D_1 receptors in isolated rats. Chronic clozapine but not haloperidol treatment completely reversed SIS-induced PPI deficits, similar to that observed in the MK-801 model. However, haloperidol significantly reduced D_1 receptor density in SIS animals. SIS also significantly decreased NMDA receptor affinity, which was fully reversed by both clozapine and haloperidol. On the other hand SIS significantly increased D_1 receptor affinity which was exacerbated by haloperidol treatment. Clozapine only showed a trend towards a similar response. These data indicate that isolation rearing impacts both on glutamatergic and dopaminergic pathways in the frontal cortex, presenting with different responses to atypical and typical antipsychotics.

With its emphasis on the adverse effect of early life stress on neurodevelopmental processes in the brain resembling that associated with schizophrenia (Weiss and Feldon 2001), social isolation rearing has robust construct validity. Schizophrenic patients have difficulties in ignoring external sensory stimulation (Weiss and Feldon 2001), presenting with sensory gating deficits (Grillon, Ameli et al. 1992; Bolino, Di, V et al. 1994). The latter has also been reported in isolation reared rats (Geyer, Wilkinson et al. 1993), and can be blocked by various classes of antipsychotic agents (Geyer, Krebs-Thomson et al. 2001), thus asserting the face and predictive

validity of the model. While earlier drug studies on the effects of SIS on PPI have employed acute treatment protocols (Varty and Higgins 1995) the current study has not only confirmed the negative effects of SIS on sensory motor gating in rats, but has extended current data by attesting to the selective efficacy of an atypical antipsychotic, clozapine, over a typical agent, haloperidol, in reversing SIS-induced PPI deficits over a *sub-chronic* treatment period. While deficits in PPI have more been associated with cognitive disturbances (Braff, Swerdlow et al. 1999; Grillon, Ameli et al. 1992), some investigators also suggest a contribution towards positive symptoms of schizophrenia (Kane, Honigfeld et al. 1988). With atypical agents effective for both positive and negative symptoms (Perry and Braff 1994), and typical agents only targeting positive symptoms, these data provide new evidence for the predictive validity of the SIS model.

The improved clinical efficacy of the new generation atypical agents over their earlier counterparts has been a matter of intense investigation for over two decades, but especially after the availability of clozapine. The development of typical agents was based exclusively on their cataleptic actions, now known to be a D_2-mediated locomotor response, but which serendipitously also addressed hyper-functional limbic D_2 receptors responsible for the psychotic features of schizophrenia. Clozapine, however, is relatively devoid of cataleptic actions with a markedly lower affinity for striatal D_2 receptors (Harvey et al, 1999). This superiority over typical agents was again illustrated in the current study using the rat catalepsy box test. Yet clozapine remains highly effective for florid psychosis (Buchanan 1995) without inducing severe motor disturbances (Marder 1996) while also providing effective pharmacological management of negative symptoms (Gerlach and Peacock 1995). It is now apparent that its multi-targeted affinity for various other neuro-receptors (Nutt 1994), but especially its higher 5-HT_{2A} : D_2 receptor binding ratio may be the key to its atypical actions (Harvey, Stein et al. 1999; Lidow, Williams et al. 1998). Recently, however, pre-clinical studies have revealed new actions of clozapine on glutamatergic transmission (Heresco-Levy 2003; Chen and Yang 2002; Abekawa, Ito et al. 2006) which appear to be selective for clozapine and related atypical agents but not haloperidol (Heresco-Levy 2003; Ninan, Jardemark et al. 2003). With the important role of glutamate in the frontal cortex and in determining cognitive

performance (Tseng and O'Donnell 2004; Castner and Williams 2007) this action is an important consideration.

Prefrontal cognitive impairment has been found to follow dopamine depletion (Schneider, Sun et al. 1994) such that deficits in prefrontal D_1-mediated signalling clearly underlies the negative symptom manifestations of schizophrenia (Barch, Csernansky et al. 2002; Davis, Kahn et al. 1991; Goldman-Rakic, Castner et al. 2004). However, NMDA receptor blockade in the brain also results in cognitive deficits akin to schizophrenia (Javitt and Zukin 1991). Moreover, NMDA receptor antagonism exerts important modulatory effects on dopamine function in cognition (Castner and Williams 2007), while there is substantial evidence for NMDA-D_1 interactions in schizophrenia (McGurk and Meltzer 2000; Liddle 2000). Prefrontal dopamine release is strongly regulated by NMDA receptors (Murase, Grenhoff et al. 1993), while dopamine and glutamate systems are interconnected in the prefrontal cortex (Goff and Wine 1997), with diverse effects on working memory (Goldman-Rakic 1996). However, there is limited data on which pathway is more important in regulating cognitive deficits and negative symptoms in schizophrenia and which are preferentially targeted by antipsychotic medication. Atypical drugs like clozapine increase extra-cellular dopamine in the medial prefrontal cortex whereas only small increments are evident with haloperidol (Gessa, Devoto et al. 2000). With deficits in working memory involving dopamine transmission (Davis, Kahn et al. 1991) and underlying much of the cognitive impairment seen in schizophrenia (Goldman-Rakic 1991), the above-mentioned actions of clozapine very likely underlie its superiority in addressing negative symptoms (Moghaddam and Bunney 1990; Nomikos, Iurlo et al. 1994; Pehek and Yamamoto 1994;Volonte, Monferini et al. 1997).

Nevertheless, as highlighted earlier, glutamatergic neurotransmission also plays an important role in atypical antipsychotic action (Heresco-Levy 2003). Indeed, in agreement with earlier studies (Bast, Zhang et al. 2000; Bakshi, Swerdlow et al. 1994; Geyer, Krebs-Thomson et al. 2001), we noted a profound worsening of PPI following acute MK-801 administration. This response has been linked to increased glutamate release in the frontal cortex that is driven by extra-cortical NMDA receptors, possibly in GABAergic neurons that tonically inhibit glutamatergic inputs to

the frontal cortex (Lopez-Gil, Babot et al. 2007). In both the acute MK-801 model and the SIS study, we have demonstrated that clozapine, but not haloperidol, effectively reverses MK-801-induced PPI deficits. Moreover, isolation-induced PPI deficit are closely associated with frontal cortical NMDA receptor up-regulation, suggesting altered (possibly reduced) glutamatergic transmission and a compensatory increase in NMDA receptor density. The fact that chronic clozapine treatment further increased frontal cortical NMDA receptor density emphasises a marked effect on glutamatergic pathways in this brain area. Indeed, glutamate release and reuptake have both been found to be significantly altered by clozapine treatment (Schneider, Wade et al. 1998; Lopez-Gil, Babot et al. 2007). Moreover, the marked decrease in NMDA receptor affinity following SIS, and the complete reversal thereof by both clozapine *and* haloperidol further corroborates a role for altered glutamatergic transmission in the frontal cortex following SIS, and in the therapeutic efficacy of antipsychotic drugs in targeting frontal cortical dysfunction (Lidow, Williams et al. 1998). However, the fact that haloperidol also succeeded in reversing the effects of SIS on NMDA receptor K_D is of interest, given its penchant for not adequately addressing frontal lobe dysfunction in schizophrenia and indeed, possibly worsening negative symptoms (Harvey, Stein et al. 1999; Artaloytia, Arango et al. 2006). However, this again reiterates the error in considering schizophrenia as a single neurotransmitter disorder, and that both glutamate and dopamine need to be considered.

Indeed, when considering receptor B_{max} data for cortical D_1 receptors, there is an obvious difference in how these two drugs performed, with clozapine maintaining (but not reversing) cortical D_1 receptor density changes following SIS, but haloperidol significantly reducing receptor density. Haloperidol also more severely bolstered SIS-associated effects on D_1 receptor K_D than did clozapine. SIS significantly decreased cortical D_1 receptor density (with an associated increase in affinity), thus illustrating that clozapine probably does not immediately target pathologically altered dopaminergic signalling in the cortex, but rather glutamatergic signalling. However, the ability of haloperidol to suppress D_1 receptor density, yet at the same not being unable to reverse SIS associated PPI deficits, indicates less of a therapeutic role for D_1 receptors in maintaining cognitive performance following neurodevelopmental

trauma which, as alluded to above, more prominently involves NMDA receptors. Untoward effects of haloperidol on D_1 cortical pathways more likely explains why this agent is less likely to benefit frontal lobe function, possibly exacerbating it. Chronic exposure to D_2 antagonists increases D_2 receptors in the cerebral cortex as well as down regulates D_1 receptors (Lidow, Williams et al. 1998). The possible worsening of negative symptoms may be as a result of inappropriate changes in frontal cortical dopamine activity. Moreover, this property may also play a role in destabilising striatal D_1-D_2 balance resulting in the development of motor disturbances (Harvey et al, 1999), as illustrated by the catalepsy box tests performed in this study. The current model therefore indicates that while frontal cortical D_1 receptors are indeed involved in SIS-associated PPI deficits, typical and atypical agents may differ in how they selectively target frontal cortical dopamine and glutamate function. The property of clozapine to address SIS-induced PPI deficits is dependent on its differential actions on NMDA and D_1 signalling, thereby underlying its favourable affect on cognition that is not shared by haloperidol.

The increase in NMDA receptor density in clozapine-treated isolation-reared rats may represent a means whereby clozapine promotes frontal cortical glutamatergic function. Thus, acute and chronic clozapine treatment increases glutamate release in prefrontal cortex (Daly and Moghaddam 1993). Further evidence, albeit indirect, for this comes from studies in animal models of tardive dyskinesia where chronic haloperidol treatment decreases striatal nitric oxide synthase activity, a down-stream messenger of NMDA receptor function, but which is reversed by the clozapine analogue, olanzapine (Nel and Harvey 2003), supportive of the conclusion that atypical agents promote NMDA receptor signalling while typical agents like haloperidol inhibit this function. Taking this further, frontal cortical glutamatergic activity stimulates ventral tegmental dopamine neurons to bolster dopaminergic activity in the cortex (Murase, Grenhoff et al. 1993). This may explain how clozapine increases dopamine levels in the medial prefrontal cortex (Koyama, Matsubara et al. 1994), enhances D_1 receptor mediated neurotransmission (Ahlenius 1999) and ultimately conserves frontal dopaminergic function. Chronic clozapine treatment has indeed been noted to increase cortical dopamine concentrations (Yamamoto and Cooperman 1994). This comes down to clozapine essentially addressing frontal

cortical hypodopaminergia through bolstering glutamate activity and thus reversing a hypoglutamatergic state, which has been suggested as a basis for the development of schizophrenia (Javitt and Zukin 1991). With SIS up-regulating NMDA receptors due to hypoglutamatergia, clozapine further bolsters up-regulation of NMDA receptors with beneficial, albeit indirect, effects on dopamine transmission in this brain region (Hall, Ghaed et al. 2002). Thus, SIS-induced PPI deficits involve changes in both glutamatergic and dopamine transmission in the frontal cortex, although effective treatment primarily involves correcting glutamate dysfunction, with subsequent effects on dopamine.

5 Conclusions

Rodents reared in isolation have significant deficits in sensory motor gating that are correlated with up-regulation of frontal cortex NMDA receptors and down-regulation of D_1 receptors. Moreover, chronic treatment with clozapine but not haloperidol reverses SIS induced PPI deficits, similar to that observed during hypoglutamatergia induced in the acute MK-801 model, confirming that PPI deficits evoked by either pharmacological means or by neurodevelopmental trauma, represent glutamate-driven mechanisms that responds selectively to atypical antipsychotic agents. Haloperidol abrogates cortical D_1 receptor density in animals subjected to isolation stress, while clozapine is conservative in this action. The atypical action of clozapine involves bolstering of glutamatergic pathways, while striving to maintain cortical dopaminergic transmission.

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Figure legends:

Figure 1:

The effects of acute saline or MK-801 (0.25 mg/kg ip) administration on % prepulse inhibition of startle in rats.

Figure 2:

The effects of acute MK-801 (0.25 mg/kg ip) with prior saline administration, on % prepulse inhibition of startle in rats, and response to prior acute administration of haloperidol (0.1-0.5 mg/kg ip) or clozapine (5-10 mg/kg ip).

Figure 3:

The effect of saline, haloperidol (0.2-0.5 mg/kg ip) and clozapine (5 mg/kg ip) administration on catalepsy, as tested in the rat catalepsy box.

Figure 4:

Effect of 8 weeks social isolation rearing in rats compared to group-housed controls, and its effect on % prepulse inhibition of startle. Both groups received saline injection.

Figure 5:

The effects of 8 weeks social isolation rearing in rats, receiving daily saline injection, on % prepulse inhibition of startle, and response to sub-chronic treatment with haloperidol (0.2 mg/kg ip) or clozapine (5 mg/kg ip).

Figure 6:

The effects of 8 weeks social isolation rearing in rats compared to group-housed controls, on frontal cortex NMDA receptor density (B_{max}) (a) and affinity (K_D) (b). Both groups received saline injection.

Figure 7:

The effects of 8 weeks social isolation rearing in rats, receiving daily saline injection, on frontal cortex NMDA receptor density (B_{max}) (a) and affinity (K_D) (b), and response to sub-chronic haloperidol (0.2 mg/kg ip) or clozapine (5 mg/kg ip) treatment.

Figure 8:

The effects of 8 weeks social isolation rearing in rats compared to group-housed controls, on frontal cortex D_1 receptor density (B_{max}) (a) and affinity (K_D) (a). Both groups received saline injection.

Figure 9:

The effects of 8 weeks social isolation rearing in rats, receiving daily saline injection, on frontal cortex D_1 receptor density (B_{max}) (a) and affinity (K_D) (b), and response to sub-chronic haloperidol (0.2 mg/kg ip) or clozapine (5 mg/kg ip) treatment.

CHAPTER 4

Conclusion

Schizophrenia is a major mental disorder with devastating effects on the individual and his/her family, and is most often associated with a poor long term prognosis (Harvey *et al.*, 1999). Indeed, schizophrenics occupy approximately 25 % of hospital beds in psychiatric hospitals (Terkelsen & Menikoff, 1995). Schizophrenia is a life-long disorder requiring extensive care (Mueser & McGurk, 2004), with up to 20 % of patients experiencing a relapse in their lifetime while on an antipsychotic (Fleischhacker & Hummer, 1997). A better understanding of the disorder is needed, as are new and improved drug treatments. Important to attaining this goal is establishing highly specific and validated animal models for schizophrenia that will allow preclinical research to more closely address the underlying neurobiology of the disorder.

This study has aimed to establish two separate animal models of schizophrenia under local laboratory conditions. Both were required to be standardised according to robust levels of face, predictive and construct validity. The first model set up was a pharmacological model using the NMDA receptor antagonist, MK-801. This model has great value and relevance for schizophrenia due to the strong parallels drawn between the clinical presentation of schizophrenia and the behavioural manifestations evident in this model (Snyder, 1988).The second model represents an attempt at setting up a non-pharmacological model of schizophrenia. This latter model holds great attraction due to the fact that it represents a natural progression to the development of schizophrenia-like symptoms that is based on the neurodevelopmental hypothesis of schizophrenia (Domeney & Feldon, 1998). Indeed, adverse early life environmental conditions, in this case post-natal social isolation, have been causally linked to the later development of the illness (Weiss & Feldon, 2001). One of the hallmark traits of schizophrenia is deficits in sensory motor

gating (Geyer *et al.*, 2001), and can be assessed both clinically and in rodents using the PPI paradigm. PPI was therefore used as a behavioral measure in both models.

As alluded to earlier, while current treatment options for schizophrenia have shown remarkable advancement over the past 5 decades with respect to efficacy and side effect profile, these agents continue to show shortfalls in efficacy, as well as having troublesome side effects (Harvey *et al.*, 1999) (Chakos *et al.*, 1994). While their clinical efficacy can be ascribed to their ability to block limbic D_2 receptors, particularly in the Nacc (Rung *et al.*, 2005), new experimental evidence has begun to reveal an important role for the glutamatergic system in schizophrenia. Thus, glutamatergic hypofunction in frontal-cortical areas has been suggested to be the initiating factor evoking a reactive increase in dopaminergic function in limbic brain regions (Olney *et al.*, 1999). Sub-cortical dopamine activity is responsible for positive symptoms in schizophrenia and also plays a significant role in the frontal cortex (Murase *et al.*, 1993; Castner & Williams, 2007). However prefrontal dopamine release is strongly regulated by glutamate via NMDA receptors (Murase *et al.*, 1993). Without a doubt the interaction between D_1 and NMDA receptors in the prefrontal cortex forms a fundamental neural substrate for cognition, particularly in diseases such as schizophrenia where cognitive function is a vital marker of outcome (Castner & Williams, 2007).

The social isolation model was therefore investigated with respect to PPI changes and the co-involvement of frontal cortical NMDA and D_1 receptors, as well as NMDA receptors in the Nacc, with respect to these behavioural changes. In addition, both models were evaluated with respect to their response to atypical and typical antipsychotic drugs at both the behavioural and neurochemical levels. Thus, both the MK-801 model and the SIS model were standardised with respect to face validity, namely PPI changes, with respect to construct validity, namely glutamate, DA and neuroanatomical involvement (frontal cortex and Nacc), and finally with respect to predictive validity, namely differential response to clozapine and haloperidol.

Primary outcomes of this study:

MK-801 study:

- The NMDA receptor antagonist, MK-801, causes marked deficits in sensorimotor gating and an altered PPI response.
- PPI disruption by MK-801 is not reversed by the typical antipsychotic, haloperidol, but is reversed by the atypical antipsychotic clozapine.
- MK-801 associated PPI deficits are correlated with a state of hypoglutamatergia in the rodent.

SIS study:

- Rats reared under conditions of social isolation stress (SIS) show marked deficits in sensorimotor gating and altered PPI response.
- PPI disruption by SIS is not reversed by the typical antipsychotic, haloperidol, but is reversed by the atypical antipsychotic clozapine.
- SIS associated PPI deficts is correlated with upregulation of NMDA receptor density in the frontal cortex.
- SIS associated PPI deficts is correlated with down-regulation of D_1 receptors in the frontal cortex.
- Haloperidol does not reverse SIS associated effects on NMDA receptor density in frontal cortex.
- Clozapine up-regulates frontal cortical NMDA receptors.
- Haloperidol further down-regulates frontal cortical D_1 receptors rats exposed to SIS, while clozapine conserves dopaminergic function in this brain region.

Secondary outcomes of this study:

- Four weeks of social isolation can be considered a viable alternative to 8 weeks social isolation, both presenting with similar robust deficits in PPI.
- No significant changes in PPI are discernable with respect to injection stress in isolation housed or group-housed rodents.

- SIS associated PPI deficits are not associated with NMDA receptor density changes in the Nacc, although SIS does increase NMDA receptor affinity.
- Neither haloperidol nor clozapine was able to reverse the effects of SIS-associated NMDA receptor affinity values in the Nacc.

Future studies

- With the SIS (non-pharmacological) model of schizophrenia established, further drug studies testing the therapeutic potential of novel antipsychotics can now be tested.
- Further investigations employing the 4 weeks social isolation model needs to be undertaken to further develop the validity of this variation of the model.
- Further receptor studies in the Nacc should be conducted in the social isolation paradigm, particularly with regard to the role of the D_2 receptor, and also the NMDA receptor in order to confirm data obtained in the current study.
- Further receptor (particularly serotonergic) studies in both the frontal cortex and Nacc should be conducted in the social isolation paradigm, as well as establishing their role in antipsychotic response.

In conclusion, rats treated with MK-801, as well as rats reared in isolation from post natal day 21, demonstrate significant deficits in sensorimotor gating, thereby attesting to the face validity of these models. The up-regulation of frontal cortical NMDA receptors and down-regulation of D_1 receptors following SIS not only is a unique and important finding of value in the understanding of schizophrenia, but also confers important construct validity upon the SIS model. We also conclude that PPI deficits associated with both the MK-801 and social isolation model show preferential reversal by clozapine and not haloperidol, thereby demonstrating the robust predictive validity of these models for schizophrenia. In both instances, a causal role for disrupted glutamatergic function in the frontal cortex is indisputable, congruent with the hypoglutamatergia model of schizophrenia. Furthermore it is evident that glutamatergic pathways represent an important target for antipsychotic drug response, particularly in the frontal cortex where cognitive deficits such as abrogated sensory motor gating, represents a cardinal symptom in the illness.

Of note in this study, was that PPI deficits evoked by either pharmacological means or by neurodevelopmental trauma, represent glutamate-driven mechanisms that respond selectively to atypical antipsychotic agents. Moreover, that haloperidol further compromises cortical D_1 receptor density in animals subjected to isolation stress, while clozapine is conservative in this action, provides an important explanation for the superiority that the atypical drugs have over the older agents with respect to frontal lobe function, such as cognitive disturbances, flattened affect etc. That clozapine and not haloperidol, is able to bolster cortical glutamatergic pathways, further exemplifies its ability to rather maintain cortical excitatory transmission and in this way sustain dopaminergic function as opposed to attenuating it, as is the case with typical agents. The atypical action of clozapine therefore involves bolstering of glutamatergic pathways, while striving to maintain cortical dopaminergic transmission, attributes that are notably absent in typical agents, such as haloperidol. These unique properties of clozapine serve as potential neurobiological targets for understanding antipsychotic action, and provide a rationale for future drug development.

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Appendices

APPENDIX 1

Instructions to the authors

1 Instructions for Authors

Psychopharmacology

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- information that cannot be printed: animations, video clips, sound recordings.

- information that is more convenient in electronic form: sequences, spectral data, etc.
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1.8 *Proofreading*

Authors should make their proof corrections on a printout of the pdf file supplied, checking that the text is complete and that all figures and tables are included. After online publication, no more changes are possible. In exceptional cases, an Erratum has to be prepared, which will be hyperlinked to the article. The author is entitled to formal corrections only. Substantial changes in content, e.g. new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case please contact the appropriate Principal Editor before returning the proofs to the publisher.

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Two complimentary copies of the respective issue are supplied. Orders for offprints can be placed by returning the order form with the corrected proofs.

APPENDIX 2

Effect of re-socialization on PPI deficits induced by social isolation stress (SIS)

2.1 *Introduction*

As has been described in more detail in Chapter 2 (Section 2.10.3.1), schizophrenic patients are unable to ignore and discard irrelevant sensory stimuli (Bleural, 1911;Weiss & Feldon, 2001) due to deficits in sensorimotor gating (Geyer *et al.*, 2001). This behavioral response can be assessed in rodents using the prepulse inhibition (PPI) paradigm (Geyer *et al.*, 2001) (see Section 2.10.3.1 for detailed description). Deficits in PPI have been related to both negative (Braff *et al.*, 1999; Weiss & Feldon, 2001) and positive symptoms of schizophrenia (Weiss & Feldon, 2001) and hence can be exploited for the development of a useful animal model of schizophrenia.

Adverse environmental factors have been proposed to play a causal role in the development of schizophrenia (Weiss & Feldon, 2001; Lipska & Weinberger, 2000). There is general agreement that conditions during pregnancy, at birth and in childhood are critical (O'Callaghan *et al.*, 1991; (Takei *et al.*, 1996) for neuronal development. The neurodevelopmental hypothesis of schizophrenia is based on the notion that early-life environmental factors can have significant consequences for processes of brain maturation (Weiss & Feldon, 2001). Thus, it is possible to manipulate the environment of an animal in order to produce a disturbance in the expression of normal behaviour, thus conferring construct validity to the model. This dissertation has focussed on one such environmental manipulation, namely post weaning social isolation, or otherwise referred to as social isolation stress (SIS).

The SIS procedure, and its behavioural and neurochemical effects in rodents, have been described in detail in Chapter 3. This paradigm requires animals to be placed into isolated housing conditions (no cage mates) from the age of weaning (typically 21-28 days) and into adulthood, at which time comparisons are made with socially

reared counterparts (Bakshi *et al.*, 1998). Isolation–rearing provides a valuable developmentally specific method of inducing schizophrenia-like behavioral deficits in rodents (Geyer & Ellenbroek, 2003), but requires a number of important provisos. Social isolation associated PPI changes in rodents are age specific, only becoming noticeable in early adulthood (immediately after puberty) (Bakshi & Geyer, 1999; Varty *et al.*, 1999) but not evident in adulthood (Wilkinson *et al.*, 1994), Moreover, the duration of isolation also appears critical, with rats isolated from weaning not showing noticeable behavioral changes after 2 weeks of isolation, with intermediate changes after 4 weeks, but significant changes after 6-8 weeks of isolation (Geyer *et al.*, 1993). On the other hand, some studies have found that rats isolated for only 4 weeks after weaning demonstrate robust PPI deficits (Bakshi & Geyer, 1999), leading us to question whether an 8 week period of isolation is not excessive and unnecessary. Nevertheless, the developmental timing and the window of isolation are crucial factors in successfully inducing a PPI deficit (Varty *et al.*, 1999).

After having already extensively validated both the MK-801 as well as the SIS models with respect to face validity (PPI changes), predictive validity (clozapine versus haloperidol treatment) as well as construct validity (cortical D1 and NMDA receptor changes), and using a 8 week isolation period for the SIS model (Chapter 3), the above-mentioned evidence prompted us to further validate the paradigm of social isolation by investigating whether the behavioral effects evoked by 8 weeks of social isolation on PPI, could be prevented if a 4 week period of re-socialization was introduced into the 8 week SIS period. This study would also provide information as to whether a restricted period of isolation (4 weeks as opposed to 8 weeks), would be sufficient to induce PPI changes in rodents, and thirdly whether SIS-induced PPI changes are sustained or expressed for a protracted period of time subsequent to re-socialization of the animals. This addendum therefore describes the methodology, set-up and results of the effect of 4 weeks of re-socialization introduced in the last 4 weeks of the 8 weeks of social isolation, and how this manipulation impacts on sensory motor gating compared to SIS and group-housed animals.

2.2 *Materials and methods*

2.2.1 Animals

Male Sprague-Dawley rats, weighing 250-300 grams, were provided by the Animal Research Centre of North-West University. The isolation and group-housed rats were housed one or 3 rats per cage, respectively, according to procedures and conditions described in Chapter 3, Section 2.1. All animals were maintained according to the code of ethics in research, training, diagnosis and testing of drugs in South Africa, and approved by the Ethics Committee for Research on Animals at the NWU (Ethics approval number: 06D08).

2.2.2 Behavioral paradigm: Prepulse inhibition

2.2.2.1 Apparatus

PPI was assessed using sound–attenuated startle chambers (SR-LAB, San Diego Instruments, San Diego, USA) as described in Chapter 3, Section 2.4.2.

2.2.2.2 Testing of PPI

The set-up and design of the protocol for the determination of PPI was as previously described in Chapter 3, Section 2.4.2. As before, all PPI procedures took place between 09h00-13h00.

2.2.3 Experimental design

Two groups of animals (n=8/group) were randomly selected, for introduction to the SIS paradigm on post-natal day 21, as described in Chapter 3, Section 2.3. While one group of SIS animals were reared under social isolation conditions for the nominal period of 8 weeks, the second group were re-introduced to their litter mates and dam at the end of the 4th week of the SIS period (see Figure 8) and maintained

as such for the remainder of the designated 8 week period. A group-housed control was run concurrently over the same period and used for comparison. At the end of week 8, all groups were subjected to sensory motor gating assessment using the PPI method described above.

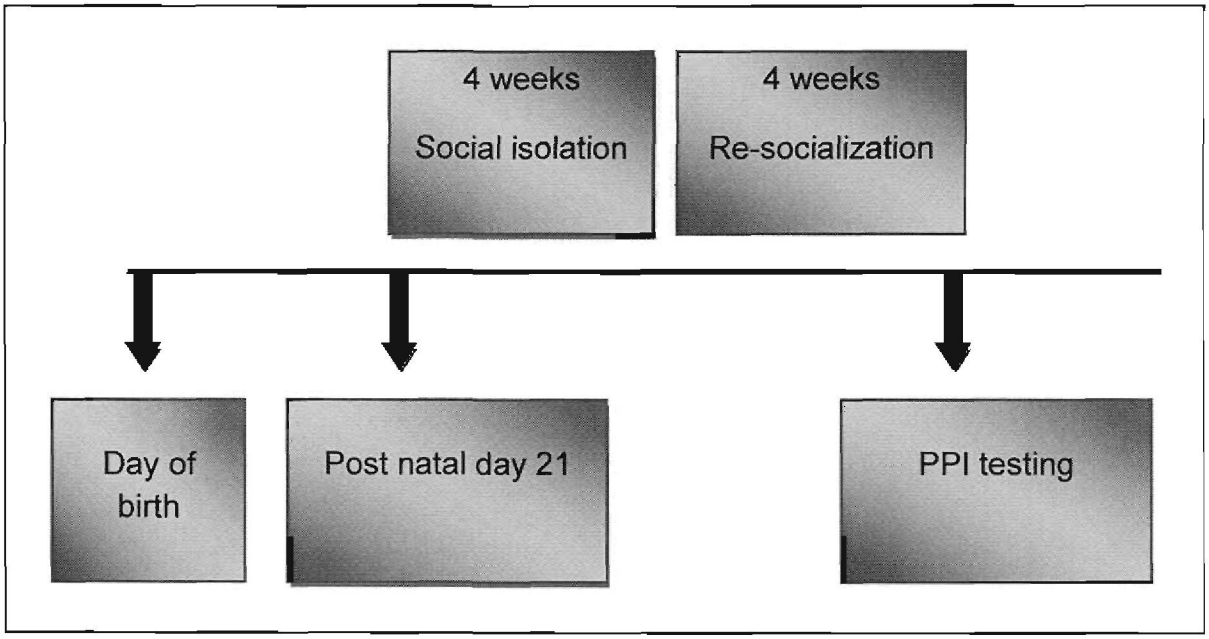


Figure 8: Study layout of the re-socialization study

2.2.3.1 Statistical analysis

Graphpad Prism version 4 for windows (Graphpad software, San Diego, USA) was used for the statistical analysis and graphical presentations. Group-housed rats, re-socialized rats and SIS rats were compared using a one way analysis of variance (ANOVA) followed by post hoc Tukey tests. In all instances, statistical significance was defined as $p<0.05$.

2.3 *Results*

2.3.1 PPI in group reared rats, SIS rats and re-socialized rats

One-way ANOVA of the data revealed significant group differences [F(2,37); 7.972, p<0.0013]. Social isolation-reared rats showed a significant attenuation of normal PPI compared to group-housed rats (p<0.001; Figure 9; Tukey), confirming a marked deficit in sensorimotor gating induced by SIS. Moreover, animals that were re-socialized on week 4 also displayed significant deficits in PPI (p<0.05; Tukey, Figure 9) compared to group-housed animals, with no difference compared to animals that were in isolation for the full 8 weeks. This confirms a marked deficit in sensorimotor gating in both the SIS and re-socialized animals.

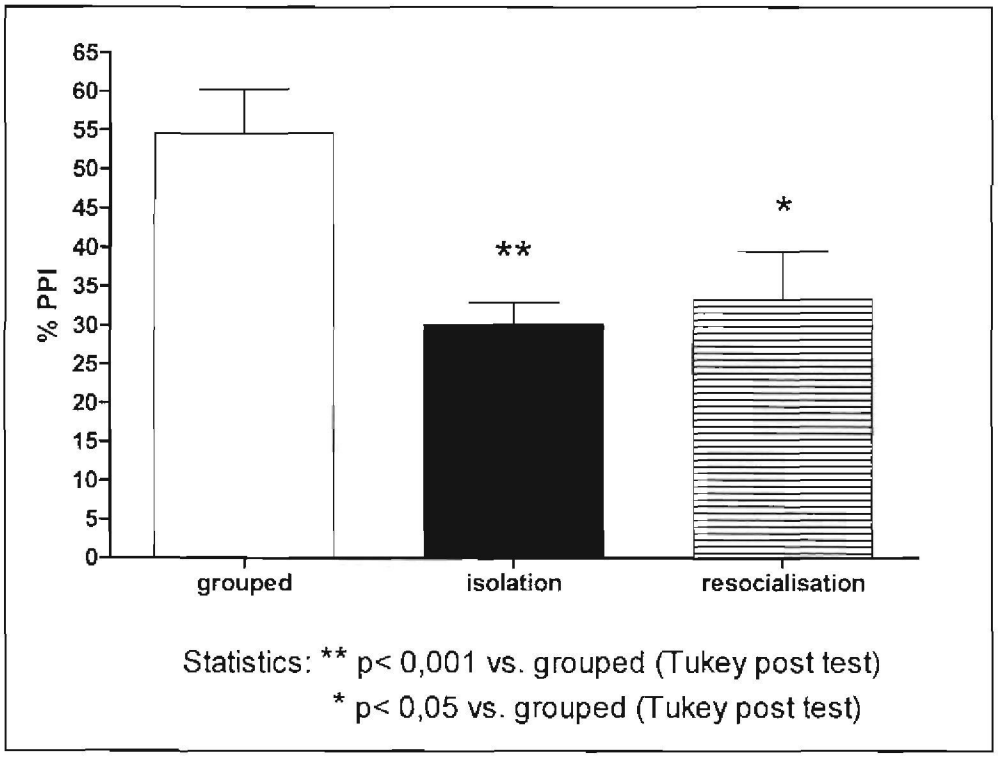


Figure 9: Sensory motor gating in rats exposed to social isolation, re-socialized rats and group-housed controls.

2.4 *Discussion*

In the present study we report that rodents reared in social isolation for a period of 8 weeks, or for 4 weeks and thereafter re-socialized for another 4 weeks, all show marked deficits in PPI compared to group housed rats. Therefore, deficits in sensorimotor gating is apparent in SIS animals and animals that are resocialized 4 weeks after initiation of SIS, providing conclusive evidence that 4 weeks of re-socialization on week 4 of the SIS protocol remains effective in evoking deficits in PPI. Moreover, these data suggest firstly that a 4 week period of SIS is adequate under our laboratory conditions, to induce PPI changes in Sprague Dawley rats, and in agreement with an earlier study (Bakshi *et al.*, 1998) although Bakshi & Geyer tested the rats after 4 weeks of isolation and did not include resocialising the rodent for another 4 weeks before testing in their study (Bakshi *et al.*, 1998). Secondly, it indicates that the deficit caused in PPI by the social isolation paradigm is sustainable for up to 4 weeks after re-introducing the SIS animals to their litter mates and dam. Varty *et al.* (1999) found that 4 weeks of isolation was not sufficient for inducing lasting PPI deficits after 8 weeks. Thus this result is contradictory to the findings in our laboratory. While most studies have advocated an 8 week social isolation period (Bakshi *et al.*, 1998; Geyer *et al.*, 1993), our data would suggest that future SIS-PPI studies, at least under our laboratory conditions, could well be conducted with confidence using a 4 week social isolation period.

In conclusion, 4 weeks of social isolation can be considered a viable non-pharmacological animal model of schizophrenia, presenting with similar robust changes in PPI to that of 8 weeks SIS. This approach has distinct ethical benefits when considering the use of animals, while it also is markedly less time consuming. Studies investigating the usefulness of this abbreviated version of the SIS model when testing the efficacy of established and novel antipsychotics should be carried out to further establish the validity of this method.

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APPENDIX 3

NMDA receptor binding in the nucleus accumbens of rats subjected to social isolation rearing, and response to antipsychotic treatment

3.1 *Introduction*

Environmental manipulation, in particular post weaning social isolation, is reported to have consequences for the expression of behavioural responses in animal species later in life (Domeney & Feldon, 1998). Isolation rearing refers to the paradigm in which animals are placed into isolated housing conditions (no cage mates) from the age of weaning (typically 21-28 days) and into adulthood, at which time comparisons are made with socially reared counterparts (Bakshi *et al.*, 1998).

The nucleus accumbens (Nacc) is involved in the pathophysiology of schizophrenia and has received substantial attention over the years (Gray, 1995; O'Donnell & Grace, 1998). This brain region is involved in numerous behavioral processes, for example motor activity (Di *et al.*, 2001; Koob, 1992), motivation and reward (Koob, 1992; Salamone, 1994) as well as certain cognitive functions (Annett *et al.*, 1989; Davidson & Irwin, 1999). The Nacc receives dopaminergic projections from the ventral tegmental area and glutamatergic projections from the cortico-limbic system (Koob, 1992; Smith-Roe & Kelley, 2000; Baldwin *et al.*, 2002), emphasizing its dual regulation by these two important neurotransmitter systems. This neuroanatomical interaction is depicted in Figure 1 and Figure 2 and currently forms an important focus in our understanding of the neurobiology of schizophrenia and its treatment. Indeed, schizophrenia may be conceived equally as a disorder of diminished glutamatergic drive as it is a disorder of overactive dopaminergic drive (Macciardi *et al.*, 1990).

NMDA receptor antagonists, such MK-801, PCP and ketamine are known to induce behavioural manifestations in humans that are in most respects indistinguishable from schizophrenia, including both positive and negative symptoms (Javitt & Zukin, 1991; Carlsson & Carlsson, 1990; Olney et al., 1999). Importantly, marked increases

in dopamine and glutamate release occur in the Nacc and medial prefrontal cortex after systemic administration of an NMDA receptor antagonist (Adams & Moghaddam, 1998; Moghaddam et al., 1997). Glutamatergic abnormalities have been assigned a significant role in explaining the various structural and functional abnormalities in the prefrontal cortex and anterior hippocampal formation in schizophrenia, especially the ventral striatum containing the Nacc. Such early developmental abnormalities have been proposed to underlie the later development of schizophrenia in early adulthood (Lipska & Weinberger, 2000).

The neurodevelopmental hypothesis of schizophrenia (Chapter 2, Section 2.6.2.3) postulates that perinatal alterations in hippocampal function may alter glutamatergic and dopaminergic inputs to the Nacc, resulting in the cardinal symptoms of schizophrenia (Lipska & Weinberger, 2000). Thus, it has been suggested that these changes drive hypoglutamatergia in the PFC manifesting as attrition of cognitive functioning, with hyperdopaminergia in the Nacc resulting in psychotic symptoms (see Chapter 2 for detailed discussion). When considering the design and application of a suitable animal model of schizophrenia, disturbances in glutamatergic and dopaminergic function in key neuroanatomical regions of the brain involved in the disorder form an important consideration in the construct validity of the model (see Chapter 2, Section 2.10.2 for discussion on validity criteria). Both dopamine and glutamate signaling pathways are recognized as being closely involved in the regulation and expression of cognitive function and indeed, also in cognitive disturbances during schizophrenia (Castner & Williams, 2007).

In Chapter 3, behavioral and neuro-receptor data were presented in favor of the face and predictive validity of the SIS model for schizophrenia. A marked up-regulation of NMDA receptors, as well as a reduction in D_1 receptors, was found in the frontal cortex following SIS and which accompanied SIS-associated PPI deficits. SIS induced sensory motor deficits are thus associated with profound frontal cortical glutamate and dopamine receptor changes that may underlie deficits in sensory motor gating. Moreover, we described how clozapine, but not haloperidol, reverses PPI deficits, with clozapine seemingly acting to conserve cortical D_1 receptor density while haloperidol further reduced cortical D_1 receptor density. Further, clozapine treatment of isolation reared animals bolstered frontal cortex glutamatergic NMDA receptor density, while haloperidol did not alter this compared to the untreated

isolation reared animals. Thus, not only does SIS evoke PPI deficits in rodents, akin to that in schizophrenia (face validity), but we were able to demonstrate that these changes could be reversed by atypical but not typical antipsychotic drugs (predictive validity). Moreover, these behavioral changes were accompanied by marked disturbances in frontal cortical NMDA and D_1 receptors, supporting the construct validity of the model, at least at the level of the frontal cortex. These receptor changes also showed a distinctive response to clozapine and haloperidol treatment.

In this appendix, additional neuro-receptor data are presented with respect to NMDA receptor binding properties in the Nacc that may further distinguish the construct validity of this animal model. Consequently, NMDA receptor binding characteristics, namely density (B_{max}) and affinity (K_D), were determined in the Nacc of socially-reared vs. isolation-reared rats. In addition, the response of these parameters to chronic administration of the antipsychotics, haloperidol and clozapine, was also studied. These studies should be seen as complimenting the data described in Chapter 3 and to further establish the construct and predictive validation of the SIS model.

3.2 *Materials & Methods*

3.2.1 Animals

Male Sprague-Dawley rats, weighing 250-300 grams, were provided by the Animal Research Centre of North-West university. The rats were housed three rats per cage (group-housed) or one rat per cage (isolation housed), under conditions described in Chapter 3, Section 2.3. All animals were maintained according to the code of ethics in research, training, diagnosis and testing of drugs in South Africa, and approved by the Ethics Commitee for Research in Animals at the North-West University (Ethics approval number 06D08).

3.2.2 Experimental design

Social isolation stress (SIS) and drug treatments were carried out as described previously (Chapter 3, Section 2.3; Figure 10). Briefly, on post-natal day 21, rat pups were randomly assimilated into two groups (n=28/group), viz. one group-housed

(control) and the second isolation-housed (test) in cages as described above. The pup's room temperature were elevated to mimic the effect of the litter and to prevent mortalities due to hypothermia. Group or isolation housing was maintained for 8 weeks whereupon the animals were sacrificed, brains removed and the Nacc dissected for NMDA receptor binding studies. An additional 2 isolation groups were selected for the drug treatment studies. These groups received either clozapine (n=15), haloperidol (n=17) the last 11 days of isolation rearing at a dose of 0.1 mg/kg and 5 mg/kg for haloperidol and clozapine, respectively (Figure 11). these groups were compared to socially housed animals. These dosages were chosen based on the aforegoing MK-801 study (Chapter 2).

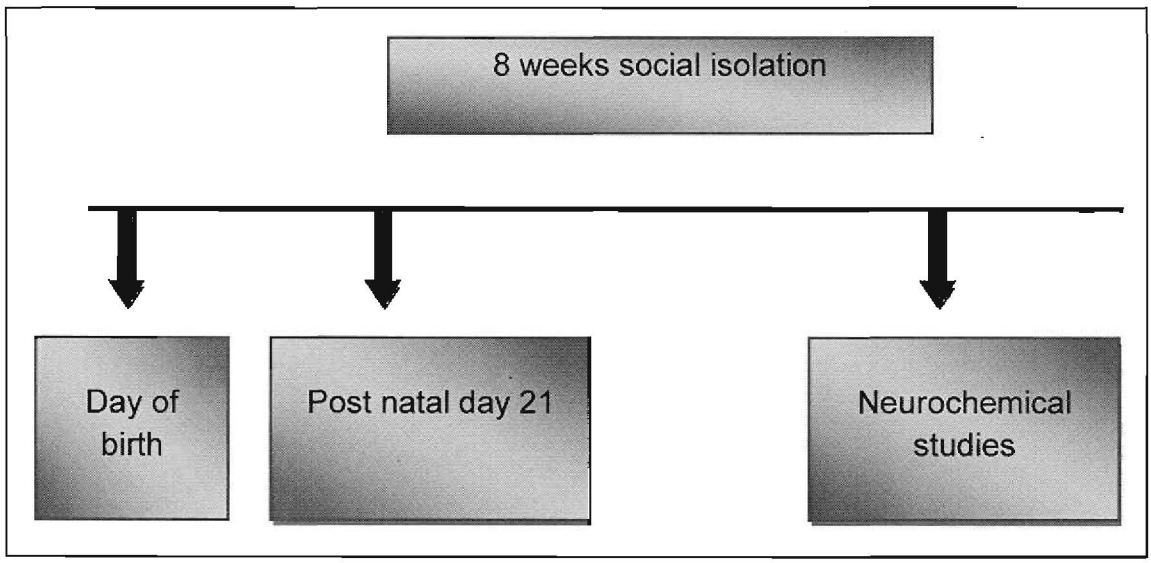


Figure 10: Social isolation experimental layout: Pups were reared in isolation from post natal day 21 for a period of 8 weeks.

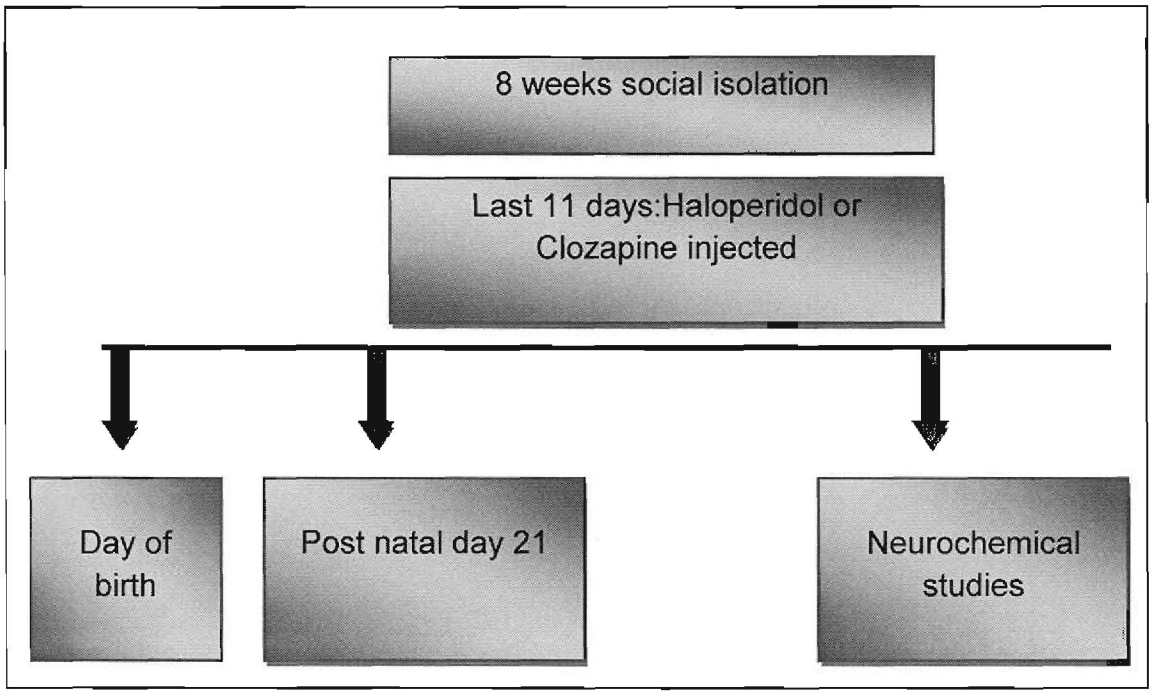


Figure 11: Social isolation treatment groups: Pups were reared in isolation from post natal day 21 for a period of 8 weeks. Haloperidol, clozapine or saline were administered in the penultimate 11 days of isolation rearing.

3.2.4 Determination of NMDA receptor binding characteristics in the nucleus accumbens

Isolation reared rats, social rats, and isolated rats receiving the various chronic drug treatments (i.e. haloperidol or clozapine) were sacrificed and the Nacc rapidly dissected on ice. The tissue were immediately snap frozen in liquid nitrogen and kept in the -86°C freezer until assays were performed. Tissues were pooled for the determination of NMDA receptor density (B_{max}) and affinity (K_D) as described previously (Chapter 3, Section 2.5).

3.3 *Results*

SIS evoked no significant reduction in B_{max} for the NMDA receptor density compared to group-housed animals in the nucleus accumbens (p=0.9225; Figure 12, Student's T test), but showed a significant reduction in K_D (p=0.0177; Figure 13, Students T test). One-way ANOVA of the NMDA receptor B_{max} data in isolated rats receiving the various chronic drug treatments indicated no significant group differences, viz. [F(2,8); 1.012, p=0.4057], and also no significance observed in K_D values across the groups F(2,8); 1.432, p=0.2941]. Chronic treatment of socially isolated animals with haloperidol or clozapine caused no significant effect on NMDA receptor binding characteristics in the Nacc compared to isolation reared animals (p>0.05; Figure 12; Dunnetts).

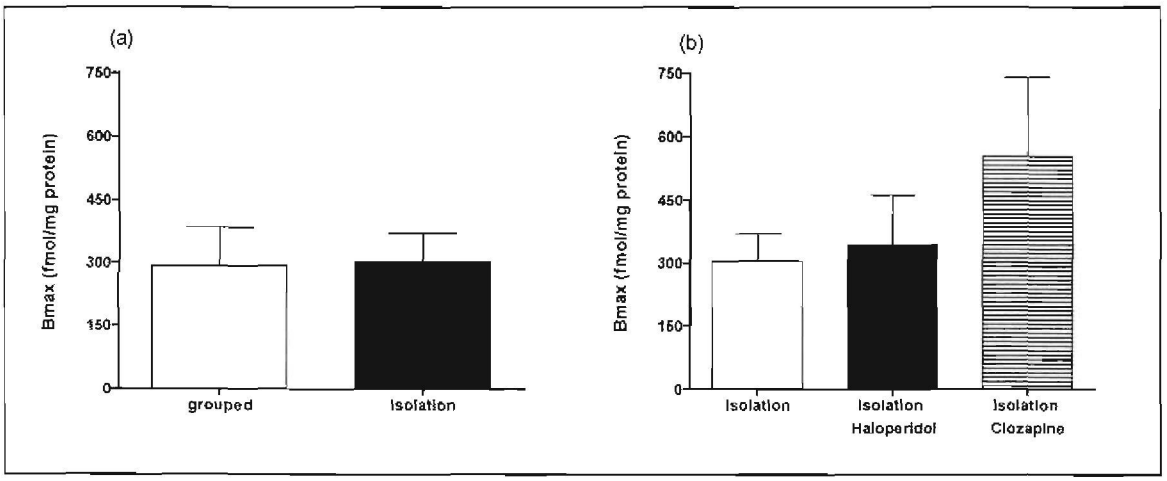


Figure 12: NMDA receptor Bmax in group-housed vs isolation reared rodents (a) and in isolation reared animals receiving chronic drug treatments, as indicated (b)

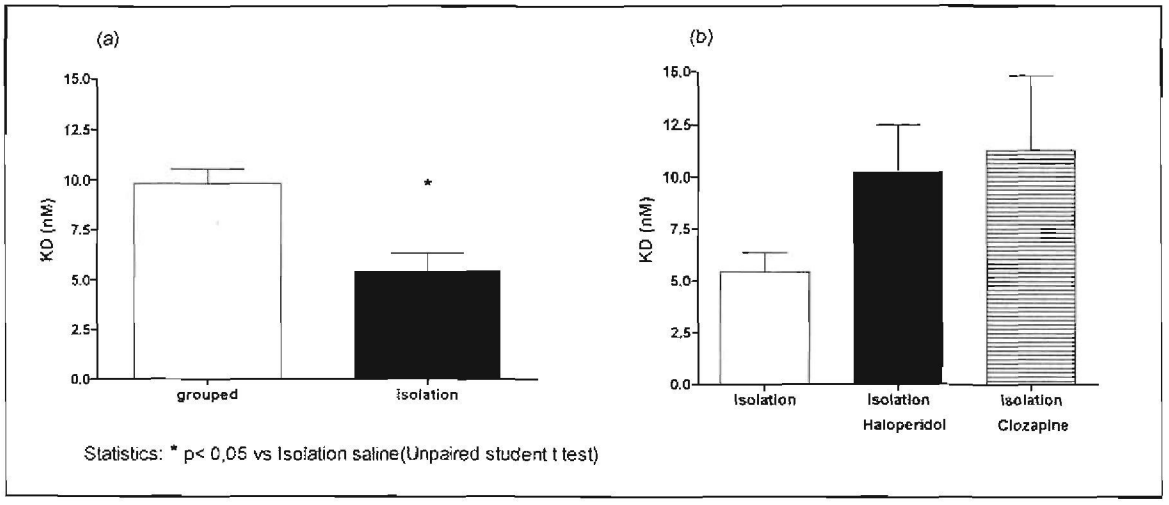


Figure 13: NMDA receptor K_D in group-housed vs isolation reared rodents (a) and in isolation reared animals receiving chronic drug treatments, as indicated (b)

3.4 *Discussion*

In the present study, the effects of 8 weeks post-natal SIS in rats on NMDA receptor binding characteristics in the Nacc were determined. Contrary to the noteworthy effects of SIS on frontal cortical NMDA receptor density (described in Chapter 3), no notable effects on NMDA receptor density were discernable in the Nacc. However, SIS did significantly increase NMDA receptor affinity.

That said, difficulties were experienced during the tissue dissection procedure and during the NMDA receptor binding assay that preclude a definitive conclusion being made as to the role of the Nacc following SIS. Firstly, being a very small and often poorly defined anatomical region of the brain, difficulty was experienced in accurately and reliably dissecting the Nacc, especially with our initial attempts at separating the core and a shell area (Zaborsky 1985) (Figure 14). The shell plays a role in motivational and emotional processes in the brain whereas the core plays predominantly a role in motor functions (Deutch 1993). Consequently, these two regions have distinctly different roles in the functioning of the Nacc and indeed, in the possible neurobiology of schizophrenia (see Chapter 2, Section 2.6.1 for further discussion).

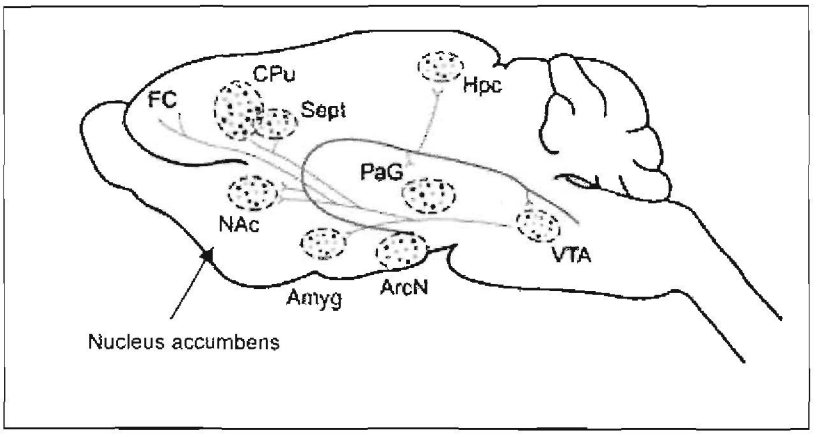


Figure 14: Anatomical location and size of the nucleus accumbens in the rat brain (Gianoulakis, 1998)

Moreover, a critical mass of tissue was necessary for the NMDA assay, which eventually necessitated the pooling of both core and shell for receptor analysis. Difficulties in accurately dissecting the Nacc may have been a reason for the fluctuation in the data points and the resulting large error bars, which may have obscured the presence of any changes that may have occurred. Thus, the fact that the radio-ligand receptor binding assay may not be sensitive enough to distinguish differences between the different groups tested, especially when receptor densities are low, or with the limited amount of tissue available, suggests that further explorative studies are needed before a conclusion can be made, possibly using other more sensitive assay of receptor density, e.g. quantitative autoradiography. Nevertheless, based on the data presented here, it can be inferred that SIS does not involve Nacc NMDA receptors to a significant degree, or that pharmacological treatment does not involve notable effects on these receptors in this brain region. Further, NMDA receptor changes may not be related to the observed decrease in PPI following this procedure described in Chapter 3, Section 3.3. Indeed, the current work has demonstrated emphatically that such behavioral changes, and their response to antipsychotic treatment, involve frontal cortical NMDA and D_1 receptors predominantly (see Chapter 3).

With regard to response to drug treatment, it has earlier been described in Chapter 3 how SIS-associated effects on frontal cortical NMDA receptors are affected by 11 days treatment with either clozapine or haloperidol administered immediately prior to

closure of the isolation period. While SIS alone did not affect NMDA density in the Nacc, it did evoke a significant increase in NMDA receptor affinity. Both clozapine and haloperidol treatment tended to reverse these receptor changes back to values of group-housed animals, at least suggesting a treatment associated reversal of isolation rearing effects on NMDA receptor affinity. More robust differences may have been evident had there not been the above-mentioned laboratory difficulties highlighted earlier.

A study by (paricio-Legarza *et al.*, 1997) showed a significant decrease in the number of glutamate uptake sites in the Nacc in patients with schizophrenia. The authors speculate that this indicates impaired glutamatergic innervation of the subcortical regions in the disorder (paricio-Legarza *et al.*, 1997). Therefore, reduced uptake or reduced innervation by implication suggests altered synaptic availability of glutamate, which will impact on receptor binding properties, possibly related to the increased NMDA receptor affinity observed in our study.

Several prominent hypotheses have associated abnormal neural processing in the Nacc with the pathophysiology of schizophrenia (Gray, 1998; O'Donnell and Grace, 1998). Marked increases in neurotransmitter release, particularly dopamine and glutamate, occur in the NAcc and medial prefrontal cortex after systemic administration of an NMDA receptor antagonist (Adams and Moghaddam, 1998; Moghaddam et al, 1997). The decreases in [^{3}H]D -aspartate binding observed in caudate nucleus, Nacc and putamen from schizophrenic patients has suggested deficits in cortico-striatal innervation in schizophrenia (Fonnum *et al.*, 1981). Radioligand postmortem studies have described an increase in MK-801 ligandbinding in putamen (Kornhuber *et al.*, 1989) and in temporal cortex (Slater *et al.*, 1992). However, similar MK-801 ligand binding studies in rats have, to the best of our knowledge, not been performed. Similarly, MK-801 ligand binding studies in the social isolation paradigm has not been studied previously. Our data are thus of great interest and further studies on the SIS model are undoubtedly needed.

While glutamate is critically important in the Nacc (Klein *et al.*, 2004), it is predominantly D_2 receptors that are involved in the regulation of salience (see Chapter 2). Therefore, for complete validation of the SIS model, assessment of D_2 receptors in the Nacc would be a valuable extension of this work in order to assess

Glu-DA inter-play in this brain region and, indeed, in the SIS-PPI model as a whole. In conclusion, further studies must be done to determine the role of NMDA and dopamine D_2 receptors in the Nacc to more discretely ascertain the role of glutamate and dopamine in the social isolation model of schizophrenia.

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APPENDIX 4

Post-weaning handling as an additional variable in the effects of isolation rearing on the prepulse inhibition paradigm in rats

4.1 *Introduction*

Prepulse inhibition (PPI) is an operational measure of sensorimotor gating (Braff & Geyer, 1990) and forms an integral behavioural assessment in the current project. While isolation rearing represents a non-pharmacological model with noteworthy effects on PPI in rats, in this study we have introduced chronic drug treatment schedules where daily injection stress may adversely affect PPI and as such, confound the interpretation of the various drug treatments on isolation stress associated PPI changes.

The outcome of an isolation paradigm on PPI can be influenced by a variety of independent variables, such as rat strain, gender, age of rats, onset of the isolation paradigm, caging conditions during isolation rearing as well as parameters of the PPI paradigm (Weiss & Feldon, 2001). Importantly, handling of rodents before behavioural testing may also influence the results obtained (Reboucas & Schmidek, 1997). Thus with this part of the study we investigated the effect of handling, particularly with respect to injection stress, on the operational measure of PPI in *isolation reared vs. group-housed rats*. Those data were then used to support the use of saline injected group-housed animals and saline-injected isolation reared animals as the point of reference for the PPI study described in Chapter 3.

4.2 *Materials and Methods*

4.2.1 Animals

Male Sprague-Dawley rats, weighing 250-300 grams, were provided by the Animal Research Centre of North-West University. The isolation and group-housed rats were housed one or 3 rats per cage, respectively, according to procedures and conditions described in Chapter 3, Section 2.1. All animals were maintained according to the code of ethics in research, training, diagnosis and testing of drugs in South Africa, and approved by the Ethics Committee for Research on Animals at the NWU (Ethics approval number: 06D08).

4.2.2 Behavioral paradigm: Prepulse inhibition

4.2.2.1 Apparatus

PPI was assessed using sound–attenuated startle chambers (SR-LAB, San Diego Instruments, San Diego, USA) as described in Chapter 3, Section 2.4.2.

4.2.2.2 Testing of PPI

The set-up and design of the protocol for the determination of PPI was as previously described in Chapter 3, Section 2.4.2. As before, all PPI procedures took place between 09h00-13h00.

4.2.3 Experimental design

Control animals ± Saline injection: Two groups were randomly selected (n=15/group). One group was left with no interference for 8 weeks (naïve rats) while the second group received a daily saline injection over the last 11 days of the 8 week period. At the end of week 8, both groups were subjected to sensory motor gating assessment using the PPI method described above.

SIS animals ± Saline injection: Two groups of animals (n=15/group) were randomly selected, for introduction to the 8 week period of isolation (SIS paradigm) from post-

natal day 21, as described in Chapter 3, Section 2.3. One group of isolation reared animals did not receive any injections (naïve rats), while the second group received daily saline injections for the last 11 of the isolation paradigm. At the end of the isolation period, both groups were analysed for PPI changes.

4.3 *Results*

Control animals ± Saline injection:

An unpaired Student's t-test revealed no significant difference between naïve group-housed rats and saline injected group-housed rats ($p>0.05$, Figure 15, Student's t test).

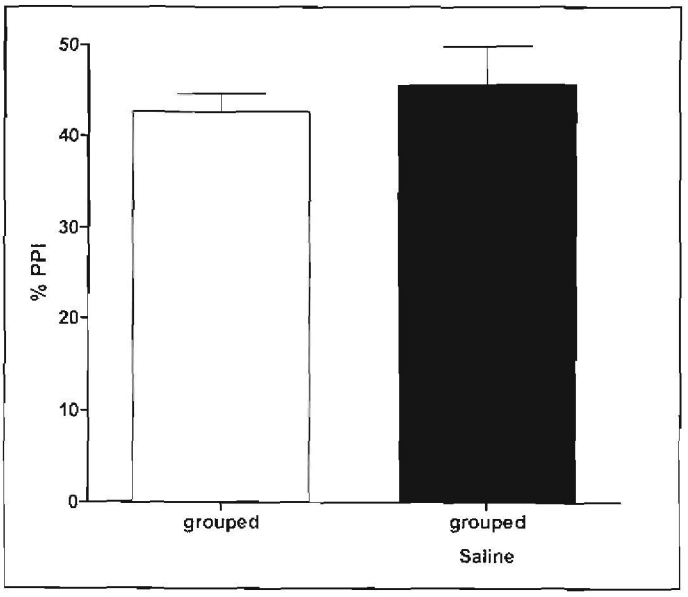


Figure 15: Sensory motor gating in naïve group-housed rats and saline injected group-housed rats.

SIS animals ± Saline injection: An unpaired Student's t-test revealed no significant difference between the naïve isolation-housed rats and the saline injected isolation-housed rats ($p>0.05$, Figure 16, Student's t test).

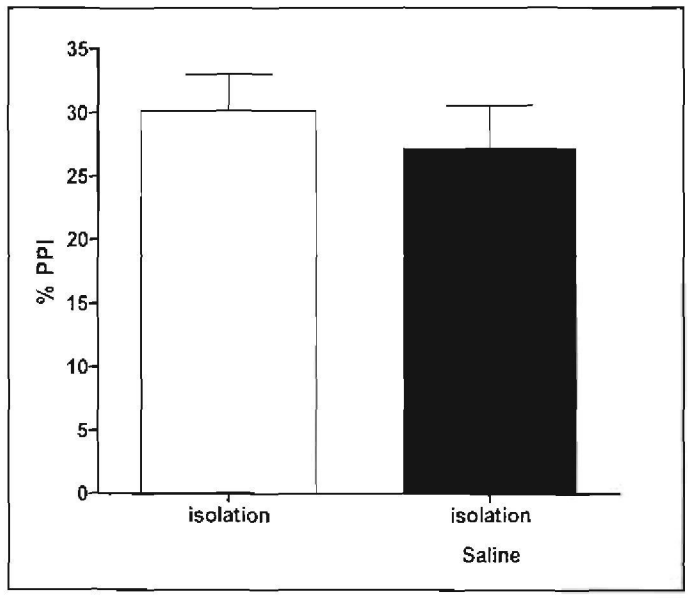


Figure 16: Sensory motor gating in naïve vs saline injected isolation reared rats.

4.4 *Discussion*

Using both control unstressed animals, as well as animals subjected to isolation rearing stress, we are able to confirm a negligible effect of daily injection stress over 11 days on PPI. Thus, in control naive and saline injected animals, as well as in SIS injection free and saline injected animals, no significant perturbations on PPI were observed.

Several factors may influence the PPI results evoked in the isolation paradigm (Krebs-Thomson *et al.*, 2001). For example Weiss and colleagues (Weiss *et al.*, 1999) investigated the effect of caging conditions on PPI in the isolation paradigm and found that PPI deficits was present in rats reared on sawdust, while PPI deficits were absent in isolated rats reared on a grid floor cage. Moreover, these same authors found that animals reared on grid-floor cages, isolation reared and the social animals (3 rats per cage) showed similar PPI changes (Weiss & Feldon, 2001). The authors suggest that the grid-floor represents a type of chronic stressor which can

weaken the sensorimotor gating abilities of the adult rat (Weiss & Feldon, 2001). The age of the rats at the beginning of the social isolation paradigm is also an important factor. PPI deficits are only present when the animal is socially isolated from weaning onwards and not when isolated from adulthood (Wilkinson *et al.*, 1994). This implies that SIS-associated PPI deficits are developmental specific (Weiss & Feldon, 2001). Other factors that have been identified as important co-variables that can interfere with the reliability of the PPI changes, are the strain of rat, parameters used to express PPI and the window of the isolation period (Weiss & Feldon, 2001). These factors are described in more detail in Chapter 2 and Appendix 2.

It is widely acknowledged that the manner in which animals are handled before behavioural testing can have a significant effect on results obtained in a behavioural experiment (Reboucas & Schmidek, 1997). Indeed, daily handling produces significant deficits in PPI in daily handled group-housed rats, such that the authors conclude that daily handling is a chronic, mild stressor with disruptive effects on PPI (Krebs-Thomson *et al.*, 2001) Under our experimental conditions, however, we were unable to identify a noticeable impact of 11 days injection stress.

In conclusion, no significant changes were discernable with respect to injection stress in any of the groups tested in terms of their effect on PPI. We could therefore confidently utilise either saline-injected group-housed or isolation reared animals as reference in the chronic drug treatment arms of the SIS study described in Chapter 3. This work also extends the validation of the SIS model under our experimental conditions.

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