

**TOBACCO ADDICTION IN INDIVIDUALS  
WITH SCHIZOPHRENIA:  
A STUDY IN RATS**

**BY**

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## ABSTRACT

Schizophrenia as with most mental disorders develops due to an interaction of multiple genetic and environmental factors. Prenatal exposure to a maternal immune activation (MIA) is an environmental risk factor that can predispose offspring to develop schizophrenia later in life. The neurodevelopmental theory suggests that an immune challenge during gestation can lead to long-lasting impairments such as in learning, memory, attention, or language (Brown & Patterson, 2012). Based on findings in human studies, prenatal exposure to a MIA has been utilized in preclinical research. Thus, the first aim of this study was to establish an animal model that generates subjects with schizophrenia-like cognitive impairments. To this end, a bacterial endotoxin, lipopolysaccharide (LPS) was used, which like most infectious agents, cannot cross the blood-placenta-barrier, yet reliably mimics an infection and initiates a MIA. Pregnant rats were subcutaneously (sc) injected with LPS (0.5 mg/kg) at one of three important neurodevelopmental time periods, gestation days (GD) 10/11, 15/16 or 18/19 (Fortier, Luheshi, & Boksa, 2007; Graciarena, Depino, & Pitossi, 2010). As individuals with schizophrenia commonly show deficits in multiple domains, three assessment paradigms were used to examine sensory and cognitive abilities in early and late adulthood. Tasks included prepulse inhibition to assess sensorimotor gating, latent inhibition to measure selective attention, and delayed non-matching to sample to evaluate working memory (WM).

Several theories have been suggested to explain high smoking incidence in schizophrenic patients (75-90%) compared to the general population (23%). The self-medication theory suggests high smoking rates amongst patients because nicotine, the primary addictive constituent in tobacco smoke, ameliorates some of the symptoms of the disorder such as cognitive deficits (D'Souza & Markou, 2012). Thus, the second aim of this study was to determine whether repeated experimenter and self-administered nicotine ameliorates or reduces schizophrenia-like cognitive deficits. Finally, the third aim was to investigate the common substrate theory, which suggests that shared underlying biological pathways may lead to increased susceptibility for an individual to develop both schizophrenia and tobacco addiction (Chambers, Krystal, & Self, 2001).

In conclusion, the findings of this study were coherently consistent and revealed that firstly, prenatal exposure to MIA early during foetal development led to long-lasting deficits in cognitive domains such as selective attention and WM. Secondly, supporting the self-medication theory, nicotine reversed MIA-induced cognitive impairments independent of the

administration paradigm. Thirdly, increased responding rates for nicotine during self-administration acquisition in animals prenatally exposed to MIA were observed, yet there was no effect of prenatal treatment in dose response or progressive ratio testing. Thus, these findings only offer weak support for the common substrate theory.

Importantly, the findings of this study revealed that animals can be repeatedly assessed in these paradigms to examine the therapeutic efficacy of drugs and other treatments. This is of particular importance considering the lack of effective pharmacological treatments for cognitive deficits in schizophrenic patients.

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## ABBREVIATIONS

Acetylcholine	ACh
$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	AMPA
Animal Ethics Committee	AEC
Analysis of variance	ANOVA
Apomorphine-susceptible	APO-SUS
Average startle response	ASR
Cannabinoid-1	CB1
Central nervous system	CNS
Conditioned place preference	CPP
Copy number variation	CNV
Catechol-O-methyl transferase	COMT
Dopamine	DA
Diagnostic and Statistical Manual of Mental Disorders	DSM
Disrupted in schizophrenia 1	DISC 1
Deoxyribonucleic acid	DNA
Delayed non-matching to sample	DNMTS
Dose response	DR
Fixed ratio	FR
Functional magnetic resonance imaging	fMRI
Gestation day	GD
Genome-wide association study	GWAS
Interleukin	IL
Intraperitoneally	ip
Inter-trial-interval	ITI
Intravenous	iv
Intelligent quotient	IQ
Latent inhibition	LI
Lithium Chloride	LiCl
Lipopolysaccharide	LPS
Magnetic resonance imaging	MRI

Maternal immune activation	MIA
Mecamylamine	MEC
Nucleus accumbens	NAcc
Sodiumhydroxid	NaOH
Nicotinic acetylcholine receptor	nAChRs
Nicotine	NIC
Nicotine replacement therapy	NRT
N-methyl-D-aspartate	NMDA
Non pre-exposed	NPE
Neonatal ventral hippocampal lesion	NVHL
New Zealand Dollar	NZD
Phencyclidine	PCP
Pre-exposed	PE
Prefrontal cortex	PFC
Positron emission tomography	PET
Postnatal day	PND
Polyinosinic polycytidylic acid	PolyI:C
Prepulse inhibition	PPI
Progressive ratio	PR
Radial arm maze	RAM
Roll your own	RYO
Saline	SAL
Self-administration	SA
Subcutaneous	sc
Substantia nigra	SN
Transforming growth factor	TGF
Tobacco particulate matter	TPM
Tyrosine hydroxylase	TH
Substance use disorder	SUD
Ventral tegmental area	VTA
Wisconsin Card Sorting Test	WCST
Working memory	WM
World Health Organization	WHO

## FORMAT OF THIS THESIS

This thesis is composed of general introductory chapters (Chapters 1 to 4 and Chapter 6), an article published (Chapter 5), an article under review for publication (Chapter 7), and a general discussion chapter (Chapter 8). Repetition may occur between the introductory and general discussion chapters and the introductions and discussions of the empirical studies. The referencing style of the empirical studies (Chapters 5 and 7) has been adjusted to APA reference, except for the outline. In addition, spelling has been changed to British English as opposed to American English.

*Incorporated in Chapter 5*

**Waterhouse, U., Roper, V. E., Brennan, K. A., & Ellenbroek, B. A. (2016).** Nicotine ameliorates cognitive deficits induced by maternal LPS exposure: A study in rats. *Disease Models & Mechanisms*. Published online 11 May. DOI: 10.1242/dmm.025072

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## **GENERAL INTRODUCTION**

### **PURPOSE**

The purpose of this PhD research was to investigate the phenomenon of high smoking incidence amongst individuals with schizophrenia. This high smoking incidence could relate to the effect of nicotine (in the smoke) in alleviating schizophrenia-associated cognitive deficits and/or could be an inherent tendency within these individuals towards nicotine addiction. Each of these specific possibilities was explored using an animal model of schizophrenia and behavioural tests.

### **AIM**

Schizophrenia, as with most mental disorders, develops due to an interaction of genetic and environmental factors (Carr & McNulty, 2006). One environmental factor that predisposes an individual to develop schizophrenia later in life is a prenatal immune challenge. The neurodevelopmental theory of schizophrenia suggests that disruptions early in brain development can lead to long-lasting cognitive deficits in domains such as learning, memory, attention, or language (Brown & Patterson, 2012; Meyer, 2014). More recent research suggests that components of the maternal immune activation (MIA), rather than the infection itself, lead to disruptions in brain development of the foetus (Meyer, Feldon, & Yee, 2009). The first aim of this study was to establish an animal model that produces general neurocognitive deficits comparable to those found in individuals with schizophrenia. Therefore, pregnant rats were subcutaneously (sc) injected with a bacterial endotoxin, lipopolysaccharide (LPS), which mimics a bacterial infection and reliably activates the maternal immune reaction (Graciarena et al., 2010). Assessment paradigms used in animal studies to evaluate schizophrenia-like cognitive impairments should be comparable to those used in human studies to create the most representative model. To this end, the test paradigms utilized in this PhD research included prepulse inhibition (PPI), latent inhibition (LI) and a delayed non-matching to sample (DNMTS) task to assess working memory (WM) deficits.

High smoking rates (75 – 90%) amongst patients with schizophrenia compared to individuals with other mental disorders (50%) and the general population (23%) have been well established. One theory to explain the high smoking incidence in this group of patients suggests that nicotine, the primary addictive constituent in tobacco smoke, may ameliorate

some of the symptoms such as the cognitive deficits (self-medication hypothesis). Alternatively, common underlying biological pathways may lead to increased susceptibility for an individual to develop both schizophrenia and substance use disorder (SUD). It is important to note that these theories are not necessarily mutually exclusive. Thus, the second aim of this PhD research was to determine whether repeated experimenter (Chapter 5) and self-administered (Chapter 7) nicotine ameliorates schizophrenia-like cognitive deficits. Finally, the third aim was to investigate whether animals prenatally exposed to MIA show higher responding rates in nicotine self-administration compared to controls as would be expected if common underlying neurobiological pathways increase the risk to develop both schizophrenia and substance use disorder. These are novel studies that provide insight as to why individuals with schizophrenia exhibit high smoking incidence.

The first chapter provides the context for the thesis by discussing the definition of schizophrenia, schizophrenia symptoms and the aetiology of the disorder.

### 1.0 Schizophrenia definition

Schizophrenia is a complex disorder characterized by three clusters of symptoms: positive symptoms such as delusions (false beliefs) and hallucinations (false perceptions), negative symptoms like anhedonia (inability to experience pleasure), and cognitive deficits in domains such as learning, memory, attention, or executive functioning. The Diagnostic and Statistical Manual of Mental Disorders (DSM, fifth edition, p. 99) requires the following criteria to be met to diagnose an individual with this disorder:

- A. Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least one of these must be (1), (2), or (3):
  - 1. Delusions.
  - 2. Hallucinations.
  - 3. Disorganized speech (e.g., frequent derailment or incoherence).
  - 4. Grossly disorganized or catatonic behavior.
  - 5. Negative symptoms (i.e., diminished emotional expression or avolition).
- B. For a significant portion of the time since the onset of the disturbance, level of functioning in one or more major areas, such as work, interpersonal relations, or self-care, is markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, there is failure to achieve expected level of interpersonal, academic, or occupational functioning).
- C. Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs,

unusual perceptual experiences).

- D. Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive or manic episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.
- E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.
- F. If there is a history of autism spectrum disorder or a communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations, in addition to the other required symptoms of schizophrenia, are also present for at least 1 month (or less if successfully treated).

Schizophrenia has a prevalence rate of approximately one percent in the general population and the onset of the disorder is usually in early adulthood (American Psychiatric Association, 2013; Carr & McNulty, 2006). The symptom-clusters will be explained in more detail below, however, as this PhD project is mainly concerned with cognitive impairments, positive and negative symptoms will only be discussed briefly.

## **1.1 Schizophrenia symptoms**

### **1.1.1 Positive symptoms**

Positive symptoms are commonly referred to as psychosis and include “thoughts, sensory experiences, and behaviors that are present in persons with the disorder, but are ordinarily absent in persons without the illness” (Hersen & Beidel, 2012, p. 263). For example, an individual might falsely believe (delusion) that others are following and intend to harm the person. Hallucinations are false perceptions and can be, for example, auditory or visual in nature where auditory hallucinations such as hearing sounds or voices are the most common. Further positive symptoms are disorganized or catatonic behaviour such as posturing or waxy flexibility (Carr & McNulty, 2006).

The underlying mechanisms as to how positive symptoms develop in schizophrenia are not fully understood. Kapur, Mizrahi, and Li (2005) suggest an accumulation of aberrant sense of novelty and stimuli salience over time, which may influence internal representations (distortions). Thus, the formation of delusions and hallucinations could be an attempt to make sense of overstimulation and extreme salient experiences. On a cellular basis, this is consistent with the “dopamine theory”, as imbalances of this neurotransmitter in particular brain areas might be, at least in part, responsible for symptoms in schizophrenia (Gray et al., 1995; Scarr, Gibbons, Neo, Udawela, & Dean, 2013). Gray and colleagues (1995) and Kapur et al. argue that prolonged dopamine (DA) hyperactivity in subcortical regions, as evident in many individuals with schizophrenia, may lead to distortions and over-salience of perceptual experiences. Supporting evidence for this theory derives from imaging studies, which indicate an increase in presynaptic activity of dopaminergic neurons projecting to the striatum from the ventral tegmental area (VTA) in individuals with schizophrenia (for review E Scarr et al., 2013).

Additionally, an increase in DA release in brain areas such as the ventral and dorsal striatum as well as in the prefrontal cortex (PFC) have been identified due to exposure to stress. Relevant to stress-induced alterations, a core feature in individuals with schizophrenia is a deficit in gating incoming stimuli, more precisely, diminished capacity in distinguishing between relevant and irrelevant information. This phenomenon is referred to as latent inhibition (LI) and is commonly impaired in schizophrenic patients, especially in the early stages of the disorder. Diminished LI is associated with dysfunctions in selective attention and it has been suggested that such inability to “tune out irrelevant information” might lead to overstimulation and consequently to distortions and psychosis. The importance of the neurotransmitter DA in central information processing such as LI (for review Weiner, 2003) and sensorimotor gating (for review Heinz & Schlagenhauf, 2010) has been well established. Further support for the implication of DA in positive symptoms in schizophrenia originates from pharmacological studies. Most antipsychotics act as DA antagonists and therefore reduce DA neurotransmission, while at the same time reducing positive symptoms (Moritz, Andreou, Klingberg, Thoering, & Peters, 2013).

### **1.1.2 Negative symptoms**

Negative symptoms are referred to as an “absence of feelings, or behaviors that are ordinarily present in persons without the illness” (Hersen & Beidel, 2012, p. 263). Buchanan (2007) highlights the importance to distinguish between two categories, primary and



secondary negative symptoms. Primary negative symptoms are persistent and are independent of other aspects of schizophrenia. They include, but are not limited to, the inability to experience pleasure (anhedonia), poverty of speech such as diminished or incoherent verbal communication, withdrawal or significant decrease of social interactions, loss of interest, lack of motivation, or aimlessness (American Psychiatric Association, 2013). These negative symptoms are different from positive symptoms in that they are stable and persistent. Positive symptoms fluctuate and may go into remission between episodes, primary negative symptoms, however, are stable over time (Hersen & Beidel, 2012). The more severe the primary negative symptoms, the worse the prognosis for treatment success, in particular with regards to treatment compliance (Carr & McNulty, 2006; Hersen & Beidel, 2012). Secondary negative symptoms or deficit symptoms, on the other hand, develop as a consequence of positive symptoms or are due to medication side effects. Secondary symptoms vary with the severity of positive symptoms and usually disappear when psychosis has been successfully treated (Buchanan, 2007).

On a cellular level, a DA hypoactivity in mesocortical projections to the PFC has been implicated in negative as well as cognitive symptoms (Meyer & Feldon, 2009). As the mesocortical pathway is associated with cognitive functioning and emotional responses, it is not surprising that an imbalance in activity levels of the primary neurotransmitter DA in this area leads to behavioural dysfunctions (Guillin, Abi-Dargham, & Laruelle, 2007; Scarr et al., 2013). Neuroimaging studies (positron emission tomography, PET) have repeatedly shown decreased DA D1 receptor binding in the PFC in schizophrenia patients (Okubo et al., 1997). However, the theory of DA hypoactivity as the underlying mechanism of negative and cognitive symptoms is more controversial compared to the role for DA hyperactivity in positive symptoms. Not all studies show a reduction in DA D1 receptor levels, where some have found no change (Karlsson, Farde, Halldin, & Sedvall, 2002) or an increase in receptor density (Abi-Dargham et al., 2002).

### **1.1.3 Cognitive deficits**

The severity of cognitive impairment varies greatly amongst patients, yet approximately 75% of all individuals with schizophrenia show some kind of cognitive anomaly. For these reasons, cognitive deficits are described as reliable and distinguishing features in the diagnosis of the disorder. Cognitive dysfunctions are particularly debilitating as they usually do not respond to antipsychotics, yet significantly impact on daily functioning, long-term outcome of the illness, and mortality (D'Souza & Markou, 2012; Lett,

Voineskos, Kennedy, Levine, & Daskalakis, 2013; O'Carroll, 2000). A recent meta-analysis showed generalised impairment in schizophrenic patients in several cognitive domains such as learning, memory, attention, language, as well as in aspects of executive functioning (Fioravanti, Bianchi, & Cinti, 2012).

The neurodevelopmental theory suggests that cognitive deficits in schizophrenia develop due to disruptions in the early stages of brain development (Meyer et al., 2009). Support for this theory derives from birth cohort studies as individuals diagnosed with schizophrenia in adulthood often display significant impairment in cognitive functioning during childhood. For example, Jones and colleagues (1994) examined data from a British birth cohort study (n=5362, born 1946), where participants were repeatedly assessed over an extended period of time across four domains (socio-demographics, early milestones, educational achievement, socio behavioural). Findings revealed delayed milestones in these individuals evident as early as age two. Educational assessments in non-verbal, verbal, mathematics, vocabulary and reading at ages 8, 11 and 15 showed consistently lower mean scores for individuals later diagnosed with schizophrenia compared to controls (Jones, Rodgers, Murray, & Marmot, 1994).

Cannon and colleagues (2002) analysed data from a New Zealand birth cohort (1972-1973), the Dunedin Multidisciplinary Health and Development Study (n=1037). Data collection occurred twice a year (from 3 – 11 years of age) and most individuals (n=1000) were followed up until age 26. Of this population sample, 36 individuals (3.7%) were diagnosed with schizophreniform disorder (includes schizophrenia, schizoaffective disorder and others). Cannon et al. focused on developmental precursors of adulthood schizophreniform disorders and found impairments in three specific domains evident at an early age: deficits in neuro-motor activities, for example delayed milestones in motor skills and walking in particular; impairments in language and especially in verbal comprehension; and a significantly lower intelligent quotient (IQ) (Cannon et al., 2002). Although some research indicates that there is progression of cognitive deficits throughout the illness (Meier et al., 2014), others suggest that cognitive performance does not decline with the onset of psychosis or over time, other than age-related deterioration (Bora & Murray, 2014).

The underlying pathology of cognitive deficits in schizophrenia is complex. A number of structural and functional anomalies contributing to cognitive impairment have been observed. For example, data from meta-analyses indicate morphological differences such as volume reduction in grey matter as well as in temporal lobe structures such as the hippocampus and amygdala. These findings have led to the “dysconnectivity hypothesis” of

schizophrenia, which suggests alterations in connectivity may be related to core symptoms in the disorder, especially between the frontal lobe and other brain regions. Connections involved are: frontal lobe, thalamus, and the cingulate gyrus as well as between the frontal lobe, insula, hippocampus and temporal lobe (for review Falkai et al., 2015; Pettersson-Yeo, Allen, Benetti, McGuire, & Mechelli, 2011).

Alterations in neurotransmitter levels in schizophrenia have been researched for decades. For example, findings suggest increased levels of the neurotransmitter glutamate in the hippocampus and PFC (van Elst et al., 2005). Glutamate is the most predominant excitatory neurotransmitter in the central nervous system (CNS) and interacts with two receptor types: ionotropic receptors (N-methyl-D-aspartate, NMDA;  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA; kainate) and metabotropic receptors (mGluR1-8). Exposure to NMDA antagonists (phencyclidine, PCP or ketamine) has led to schizophrenia-relevant deficits in motor activity (hyper-locomotion), memory and attention (for review E Scarr et al., 2013). For example, ketamine treatment (0.3 mg/kg) can lead to schizophrenia-like psychotic symptoms in normally developed individuals (n=18) and intensifies symptoms in schizophrenic patients (n=17) (Lahti, Weiler, Tamara Michaelidis, Parwani, & Tamminga, 2001). It has been suggested that ketamine increases glutamate release in cortical regions (for example cingulate cortex) through inhibition of GABAergic neurons (presumably in the thalamus) (Stone et al., 2012).

Additionally, widespread alterations in GABA levels, the main inhibitory neurotransmitter in the brain, have also been observed. GABAergic receptors are most abundant in cortical, hippocampal, thalamic, and basal ganglia regions. Reduced inhibitory modulation has been found in the corticolimbic regions and the dorsolateral PFC in this subgroup (Blum & Mann, 2002). GABA is responsible for the balance between excitation and inhibition in the brain, which is essential for normal cognitive functioning. Decreased GABA activity levels in particular brain areas has been linked to cognitive dysfunctions such as in working memory deficits (Lewis et al., 2008).

The neurotransmitter acetylcholine (ACh) has two receptor types, the muscarinic and nicotinic receptors. Acetylcholine is essential in several functions in the CNS, including cognition, motor control and sensory processes. For these reasons, pharmacological treatments for cognitive enhancement usually focus on increasing cholinergic neurotransmission by targeting these receptors. Several anomalies have been identified in ACh receptor functioning and density in schizophrenia. For example, a general reduction of

muscarinic receptors, but in particular in M1 receptors, has been well established (Scarr et al., 2009). The most predominant nicotinic acetylcholine receptors (nAChRs) subtypes are  $\alpha 7$  and  $\alpha 4\beta 2$ , which are widely distributed throughout the CNS. Amongst their many functions, presynaptic nAChRs modulate the release of several neurotransmitters essential to normal cognitive functioning such as glutamate, GABA, serotonin and DA. A decrease in  $\alpha 7$  receptor densities has been observed in patients with schizophrenia in the hippocampal area, which has been linked to core features of the disorder such as deficits in sensorimotor gating and working memory (for review Gibbons et al., 2013; Scarr et al., 2013). This receptor has recently received attention as a specific target for pharmacological interventions to enhance cognitive functioning in individuals with impairments. Findings suggest that  $\alpha 7$  receptor-stimulation could potentially balance abnormal cholinergic and glutamatergic cell signalling (for review Freedman, 2014; Wallace & Porter, 2011).

The role of DA in cognitive symptoms in schizophrenia is less clear, mainly due to inconsistent findings. However, large numbers of DA receptors have been located in the PFC and for this reason, DA has been implicated in high-level cognitive functions such as working memory and cognitive control processes such as reasoning, planning, and spatial processing. Evidence for a mediating role for DA in cognitive processes derives mainly from pharmacological studies that have utilized DA receptor agonists and antagonists. However, the effect of DA receptor stimulation seems to be conflicting, as it leads to improvements in some tasks, yet impairments in others (Cools, 2011; Mouri, Nagai, Ibi, & Yamada, 2013). It has been suggested that baseline DA levels play a significant role in determining the effects of dopamine agonists and antagonists. Specifically, Cools and D'Esposito (2011) suggest that different levels of DA are required depending on the cognitive process and that there is a fine line between cognitive stability and flexibility.

In sum, numerous neurotransmitter systems are affected in schizophrenia, where most are complexly interrelated. Overall, cognitive deficits in schizophrenia may be described as a dysfunction in central neurotransmission across multiple brain regions.

## **1.2 Schizophrenia aetiology: Genetic and environmental factors and interactions**

Schizophrenia is a very complex disorder due to the variation in symptomology. Progress has been made in identifying specific dysfunctional neurobiological systems, yet there is a high degree of heterogeneity amongst patients making it difficult to isolate common impairments. It is known that schizophrenia develops due to an interaction of multiple

genetic and environmental factors (Clarke, Tanskanen, Huttunen, Whittaker, & Cannon, 2009; Mulle, 2012). It is difficult to model a genetic contribution in animal studies due to the involvement of multiple, currently unidentified, genetic components in schizophrenia. For these reasons, the present PhD research utilized an environmental factor: prenatal exposure to infectious agents, to produce subjects with schizophrenia-relevant cognitive deficits. Yet for the sake of completeness, genetic factors as well as gene and environment interactions are discussed briefly.

### **1.2.1 Genetic factors**

Support for a genetic or heritability component in the development of schizophrenia arises mainly from family, adoption, and twin studies and, more recently, from genome-wide association studies (GWAS). GWAS identify common genetic variants in individuals suffering from the disorder compared to the general population (Mulle, 2012). Adoption and cross-fostering studies have examined the occurrence of schizophrenia based on genetic versus shared environmental factors. Cross-fostering studies refer to comparisons between offspring raised by their biological parents versus foster parents and ideally include identical twins (Brown & Patterson, 2012). With the main focus of analysis lying on first-degree relatives, findings suggest a significant association between prevalence for schizophrenia in biological relatives (0 to 5.8%) versus control groups (0.2 to 0.6%) (Fleming & Martin, 2011). However, it is important to note that adoption studies do not separate the prenatal environment. Twin studies indicate a concordance rate in identical (monozygotic) twins of up to 40 to 60% versus fraternal (dizygotic) twins of 6 to 14%. Though, early studies have been criticised for several methodological problems such as the absence of clear diagnostic criteria for schizophrenia and comparison groups. More recent twin studies suggest a concordance rate in the range of 22.4% for identical and 4.6% for fraternal twins (Fleming & Martin, 2011).

A large number of genetic variants have been implicated in schizophrenia. For example, a recent GWAS compared patients with schizophrenia (n=36,989) and healthy controls (n=113,075) and found 108 schizophrenia-associated variants (Schizophrenia Working Group of the Psychiatric Genomics, 2014). The main question arising from this large variety is how these diverse genes with individual contributions and functions may contribute to schizophrenia (Stachowiak et al., 2013). These large numbers of genes indicate small contributions of each genetic mutation leading to the complexity of the disorder. For example, Disrupted in Schizophrenia 1 (DISC1) and the Neuregulin 1 gene are plausible

candidates for a genetic predisposition. Specifically DISC1 has been suggested as a prime candidate as families with this rare genetic mutation represent higher prevalence rates. Further, these genes play a role in normal brain development such as cell proliferation or migration, thus it is possible that mutations may lead to abnormalities (Patterson, 2011). However, it is important to note that most GWAS have not identified DISC1 as a susceptibility gene for the disorder (Giusti-Rodriguez & Sullivan, 2013).

A genetic variation that has been linked to an increased risk for schizophrenia is called “copy number variation” (CNV). CNV refers to structural variations in deoxyribonucleic acid (DNA) such as deviations in the number of copies of a particular section during transcription. Consequently, this leads to either depletion (fewer copies) or duplication (more than normal numbers) of a number of genes on a chromosome. These DNA variations are widespread amongst the general population and are responsible for normal human variation in genomic DNA (Bassett & Chow, 2008; Lupski, 1998). However, some CNV’s have been associated with the development of psychopathology. Findings indicate a 20 times elevated risk to develop schizophrenia in the presence of a 22q11.2 deletion. Additionally, brain imaging studies have identified structural abnormalities such as grey matter volume reduction in particular brain regions in individuals with this particular CNV (for review Bassett, Scherer, & Brzustowicz, 2010; Chow et al., 2011).

Another approach to investigate genetic differences is using a candidate gene approach. This is based on the idea that specific genes are ‘causally’ associated with the disorders (such as genes involved in dopamine, glutamate or neurodevelopment) and thus specific genotypical variations may be more common in individuals with the disorder than in healthy controls. Such allelic variations (usually in the form of single nucleotide polymorphisms or SNPs) refer to alternative forms of one and the same gene. Some specific allelic variations such as polymorphism of the serotonin transporter SERT have been identified as plausible predispositions for mental disorders. Serotonin is a neurotransmitter involved in mood, sleep, or appetite regulation. SERT is responsible for the re-uptake or recycling of serotonin from the synaptic cleft, which terminates and regulates neurotransmitter activity (Kellendonk, Simpson, & Kandel, 2009). Polymorphic differences in the length of the gene-promoter (part of the DNA that initiates transcription or the copying of a DNA segment) lead to shorter or longer alleles in carriers. Research suggests that the shorter version might be involved in schizophrenia-related symptoms such as abnormalities in auditory and visual sensorimotor gating (Goldman, Gleib, Lin, & Weinstein, 2010).

### **1.2.2 Environmental factors**

Support for the importance of an environmental factor in the development of psychopathology is, in first instance, based on twin studies. The concordance rate in identical twins to develop schizophrenia is around 50%, which highlights the importance of environmental factors in the development of the disorder. A large variety of pre- and postnatal environmental factors have been identified as possible contributors to psychopathology such as toxins, complications during birth, trauma or early childhood stress (Carr, 2006; Cicchetti & Walker, 2003).

An environmental factor shown to interfere with the neurodevelopment of the foetus leading to an elevated risk to develop schizophrenia later in life is a prenatal immune challenge due to maternal exposure to infectious agents. In line with this, Brown and Derkits (2010) found that approximately 30 percent of all individuals with schizophrenia were prenatally exposed to some form of maternal infection. In particular, Brown and colleagues (2004) found a 3-fold increased risk to develop schizophrenia later in life in individuals prenatally exposed to maternal influenza infection during early to mid stages of pregnancy. Interestingly, the risk to develop the disorder was increased 7-fold for offspring exposed to maternal infection during the first trimester (Brown et al., 2004). The maternal immune activation (MIA) is an innate response (first defence mechanism), also called inflammation, in response to an infection initiated by various infectious agents (Brown, 2012; Meyer, 2013). Examples for common infections during pregnancy in humans include, but are not limited to, influenza, measles, rubella and pneumonia (Meyer & Feldon, 2009). Maternal infection as a possible predisposition for schizophrenia in offspring has been researched for decades. The most compelling evidence derives from birth cohort and longitudinal data. These studies allow researchers to draw from prospective rather than retrospective information due to the documentation of existing biomarkers for infections such as antibodies during pregnancy (Brown & Patterson, 2012). The exact underlying mechanisms as to how a maternal exposure to infectious agents leads to disruptions are not fully understood. Very few infectious agents have the ability to cross the placenta directly and therefore, components of the immune response of the mother have been suggested as the underlying cause for neurodevelopmental disturbances (Altamura, Pozzoli, Fiorentini, & Delloso, 2013; Ashdown et al., 2006). The cytokine hypothesis suggests that elevated inflammatory cytokine levels may affect foetal neurodevelopment and contribute to psychopathology. Cytokines are chemical messengers involved in the eradication of foreign substrates during

infection, but are equally important in neurodevelopmental processes such as neurogenesis and synaptogenesis. Thus, intense elevation subsequent to maternal infection might disrupt early processes, which would also affect later maturational processes as the brain, at least in part, develops sequentially (Miller, Culpepper, Rapaport, & Buckley, 2013; Monji, Kato, & Kanba, 2009; Workman, Charvet, Clancy, Darlington, & Finlay, 2013). Evidence for this hypothesis derives from studies (meta analysis, N=62 studies, schizophrenia patients n=2298, healthy volunteers n=1858) that found increased serum levels of pro-inflammatory cytokines such as Interleukin (IL) 6 and 1RA in schizophrenic patients compared to controls (for review Potvin et al., 2008). Further, animal studies have demonstrated that prenatal exposure to maternal infection leads to alterations in cytokine levels in the placenta, amniotic fluid, as well as the brain of the developing foetus (Ashdown et al., 2006; Boksa, 2010).

Neurodevelopmental disturbances can lead to cognitive deficits in many domains. For example, Ellman and colleagues (2009) compared cognitive functioning in individuals later diagnosed with schizophrenia or schizoaffective disorder (n=111) and matched controls (n=333) in a longitudinal study. Prenatal sera were collected during gestation, which allowed the identification of a prenatal exposure to an immune challenge due to maternal infection. First cognitive assessment (Wechsler Intelligence Scale for Children) at age seven revealed significantly lower scores in verbal intelligent quotient (IQ) and performance IQ in those individuals that were prenatally exposed to maternal influenza infection who later developed schizophrenia/schizoaffective disorder. On the other hand, a prenatal immune challenge had no effect on cognitive functioning in controls, suggesting a gene environment interaction in the development of cognitive deficits. These findings also show that cognitive deficits are often already evident in the prodromal phase of the illness (Ellman, Yolken, Buka, Torrey, & Cannon, 2009). Further, Brown and colleagues (2009) examined executive functioning in individuals with schizophrenia compared to controls based on prenatal exposure to maternal infection. Patients with schizophrenia made significantly more errors in all tasks compared to controls. More importantly, those individuals with schizophrenia that were exposed to MIA performed significantly worse when compared to schizophrenic patients not exposed (see Chapter 4, working memory, for a detailed description of this study) (Brown et al., 2009).

A number of factors appear to influence the effects of prenatal exposure to infectious agents on neurodevelopment such as the nature of the infectious agent, the severity and duration of the infection, as well as the timing during pregnancy (Brown & Derkits, 2010; Khandaker, Zimbron, Lewis, & Jones, 2013). Preclinical studies have been valuable



contributors to the existing knowledge in this field as they allow for a controlled examination of these factors in isolation. To model the human condition of a prenatal immune challenge in animals, pregnant rats have been injected with molecular immunogens such as lipopolysaccharide (LPS), a bacterial endotoxin, or the viral mimic polyinosinic polycytidylic acid (polyI:C). These substrates mimic a bacterial or viral infection and reliably initiate a maternal immune response (Meyer & Feldon, 2012; Raetz & Whitfield, 2002). A number of behavioural changes in offspring prenatally exposed to MIA have been demonstrated in animals despite methodological differences. For example, deficits in sensorimotor gating (Borrell, Vela, Arevalo-Martin, Molina-Holgado, & Guaza, 2002; Romero, Guaza, Castellano, & Borrell, 2010), impairments in selective attention (latent inhibition) (Zuckerman & Weiner, 2003), and memory (Graciarena et al., 2010; Meyer, Feldon, Schedlowski, & Yee, 2006) have been revealed. Findings in these cognitive domains are further discussed in Chapter 4.

However, the timing of the infection seems crucial. Although to date only very few studies have systematically investigated this, it has been suggested that early insults are more damaging compared to later disturbances. For example, the aforementioned study by Brown and colleagues (2004) showed that although MIA leads to an overall increased risk to develop schizophrenia later in life, susceptibility to psychopathology was significantly more pronounced when the insult occurred in the first trimester of human pregnancy. This is further supported by animal studies. For example, MIA induced by polyI:C led to diminished latent inhibition when administered on gestation days (GD) 12.5 (Smith, Li, Garbett, Mirnics, & Patterson, 2007), but not when induced at a later stage (GD 17) (Bitanhirwe, Peleg-Raibstein, Mouttet, Feldon, & Meyer, 2010). Animal studies allow for a controlled initiation of infections during ‘neurodevelopmental vulnerability windows’ as well as the assessment of long-lasting structural and functional abnormalities at different time points due to these insults (Fortier et al., 2007; Meyer, Yee, & Feldon, 2007). For example, Fortier and colleagues (2007) compared the effect of MIA induced by different doses of either LPS or polyI:C administered at one of three significant neurodevelopmental time periods (GD 10/11, 15/16, 18/19) on sensorimotor gating (PPI). PPI was significantly disrupted in male offspring in adulthood after prenatal LPS-induced MIA at GD 15/16 and 18/19, yet there was no effect at GD 10/11 or when MIA was induced by polyI:C.

A number of long-term structural and functional anomalies due to exposure to prenatal immune challenges have been identified in rodents that are relevant for schizophrenia. At the neurochemical level, alterations in neurotransmitter functioning have

been observed in DA, serotonin, glutamate, and GABA systems (for review Boksa, 2010). For example, the theory of increased glutamate release in individuals with schizophrenia in the PFC suggests that NMDA receptor hypofunction leads to decreased GABAergic activity. The decreased stimulation of GABAergic neurons in the PFC results in increased glutamate activity in this area (for review Marsman et al., 2013). Increased glutamate levels have been identified after MIA in animal studies. For example, Roenker and colleagues (2011) utilized polyI:C (8 mg/kg, ip, GD14) to induce MIA in rats. Microdialysis showed increased basal extracellular glutamate in the PFC in animals prenatally exposed to MIA compared to saline exposed controls. Further, treatment with antipsychotic medication (paliperidone) reduced basal glutamate to levels observed in controls, thus antipsychotic medication normalised glutamate release in these animals (Roenker et al., 2011). As has already been outlined, alterations in DA levels have been implicated in all three clusters of symptoms in schizophrenia (Mouri et al., 2013; Romero et al., 2007; Scarr et al., 2013). A causal link has been established between prenatal exposure to MIA and disruptions in the dopaminergic system in animal studies. For example, Kirsten et al. (2012) showed that prenatal exposure to MIA induced by LPS (0.1 mg/kg, ip, GD 9.5) leads to a decrease in dopamine in the striatum. Further, the authors suggest that reduced levels of DA activity in this area is due to MIA-induced disruptions in DA synthesis as a reduction in striatal tyrosine hydroxylase (TH) expression was observed (TH is the first enzyme in the synthesis of DA) (Kirsten et al., 2012). However, not all studies report DA hypoactivity after MIA. For example, Luchicchi and colleagues (2016) found increased extracellular DA levels in the NAcc in animals exposed to maternal polyI:C treatment (4.0 mg/kg, iv, GD 15) compared to saline controls. It has been suggested that differences in findings may be due to variations in the nature or the dose of the pathogens used or the timing of the MIA during gestation (Boksa, 2010).

Morphological abnormalities in individuals with schizophrenia have been identified in post-mortem and neuroimaging studies. These anomalies include, but are not limited to, reduced grey matter volume (Harms et al., 2010) and lower cell spine density in the frontal cortex (Glausier & Lewis, 2013). Morphological anomalies have been directly linked to prenatal exposure to infectious agents in human as well as animal studies. Ellman et al. (2010) examined the effect of prenatal exposure to elevated maternal cytokine levels (IL-8, archived prenatal sera) in individuals diagnosed with schizophrenia/schizoaffective disorder (n=17) compared to non-exposed matched controls (n=25) using magnetic resonance imaging (MRI). Significant brain volume reductions were evident in those individuals exposed to elevated maternal cytokine levels in the left entorhinal cortex as well as the right posterior

cingulate compared to controls (Ellman et al., 2010). Further, MRI studies revealed reduced hippocampal volume in individuals with schizophrenia compared to healthy controls (Rossi et al., 1994), presumably due to reduced neurogenesis. Neurogenesis includes several steps such as cell proliferation (multiplication of cells), cell migration (transport of neurons to final destination), and cell differentiation (specialisation). The site of neurogenesis is predominately the hippocampus (Bear, Connors, & Paradiso, 2007). Evidence has accumulated that the exposure to infectious agents can lead to long-lasting alterations in the release of microglia and cytokines and, subsequently, to inhibited neurogenesis and increased neuronal apoptosis (for review Na, Jung, & Kim, 2014). In addition to a decrease in neurogenesis, findings suggest an increase in neuronal apoptosis (programmed cell death) in individuals with schizophrenia. A consequence of an increased rate of apoptosis is brain volume reduction as the tissue in affected areas decreases gradually (Altamura et al., 2013; Christie & Cameron, 2006). These morphological changes relevant to schizophrenia have also been examined in animal models using prenatal infectious agent exposure (for review Boksa, 2010). For example, Baharnoori and colleagues (2009) examined the effect of maternal LPS treatment (0.1 mg/kg, ip, GD 15 and 16) in rats. A significant reduction in dendritic arborisation (branching) was observed at all postnatal ages investigated in the medial PFC. A reduction in dendritic spine length was observed on postnatal days (PND) 10 and 35, yet there was not difference compared to controls on PND 60. However, reduced dendritic spine length was only observed on PND 60 in the CA1 region of the hippocampus (Baharnoori, Brake, & Srivastava, 2009). Furthermore, MIA induced by LPS exposure (0.5 mg/kg, sc, GD 14 to 20, every second day) in rats impaired adult (PND 67) neurogenesis in the hippocampus (Graciarena et al., 2010). Increased apoptosis in the striatum was evident after maternal LPS treatment (0.3 mg/kg, ip, GD 19 and 20) in rats (Rousset et al., 2006).

Overall, these studies show that MIA can be viewed as a reliable animal model that produces subjects with schizophrenia-like anomalies and deficits with high construct validity, although the precise details on the vulnerability windows are yet to be determined. Thus, one of the aims of the present thesis is to investigate whether the long-term effects of prenatal exposure to LPS on cognitive performance depend on the time of LPS injection.

### **1.2.3 Gene and environment interaction**

Most mental disorders, including schizophrenia, seem to develop due to an interaction of multiple genetic and environmental factors (Carr & McNulty, 2006). Birth cohort studies have been valuable contributors in this field as they allow the evaluation of

data recorded from a very early age and can lead to the identification of possible influencing factors predisposing an individual to develop psychopathology later in life.

For example, Caspi et al. (2005) used data from the aforementioned New Zealand birth cohort study, the Dunedin Multidisciplinary Health and Development Study, to evaluate an interaction between genetic and environmental factors in schizophrenia. More specifically, they examined the association between a polymorphism in the catechol-O-methyltransferase (*COMT*) gene and cannabis use (environmental factor). *COMT* refers to an enzyme involved in the breakdown or inactivation of several neurotransmitters such as DA. A polymorphism in this gene, called *COMT* Val 158 MET, leads to two different allele forms, *COMT* Val allele versus *COMT* Met allele. The Met-version is known to produce less enzyme activity and consequently leads to slower enzymatic degradation of DA. The Val version is associated with high rates of enzyme activity, thus increases neurotransmitter metabolism. Increased breakdown-rates result in reduced levels of dopamine in the PFC. Due to the importance of DA in the disorder, it is not surprising that the *COMT* Val allele has been associated with an increased risk to develop schizophrenia (Brown & Patterson, 2012).

Cannabis is one of the most commonly used illegal drugs of abuse. Chronic cannabis use can cause dose and duration dependent psychotic episodes in some individuals (Brown & Patterson, 2012). Caspi and colleagues found a link between chronic cannabis exposure and the *COMT* Val allele in individuals who started cannabis use during adolescence, a time of great neurodevelopmental vulnerability. The risk to be diagnosed with schizophreniform disorder was five times greater for individuals with the genetic predisposition who started to use cannabis by age 15, even when controlling for influencing variables such as self-reported childhood psychosis compared to controls (OR = 4.5, 95% CI = 1.11 to 18.21).

Another example is the analysis of data from a birth cohort study from Finland (n=9596 treatment group versus n=13808 controls, born between 1947 and 1990). Clarke et al. (2009) evaluated family history (genetic factor) of psychiatric disorders and prenatal exposure to maternal infectious agents (environmental factor). Individuals with a genetic vulnerability as well as prenatal exposure to maternal infectious agents had a five times greater risk to develop schizophrenia later in life.

## **CHAPTER 2            TOBACCO/NICOTINE ADDICTION**

The second chapter provides a detailed description of tobacco addiction including definitions of who is classified as a regular and current smoker. Additionally, it is discussed how nicotine, the main constituent in tobacco smoke responsible for its addictive properties, may affect the brain.

### **2.0 Tobacco/nicotine addiction**

Tobacco refers to the dried leaves of the tobacco plant, a member of the nightshade family, where the leaves can either be chewed or smoked. The most common use is to smoke tobacco in the form of cigarettes. It has only been known since the early 1960's that regular tobacco use has dangerous, health-threatening side effects (Eriksen, Mackay, & Ross, 2012; Hammond, 2009; The Smokefree Coalition, 2014; World Health Organization, 2012). In the Ministry of Health (New Zealand) Tobacco Use Survey 2009, a current smoker is defined as someone who smokes more than 100 cigarettes in their lifetime and at the time of the survey was smoking at least once a month (Ministry of Health, 2010, p. 12).

Smoking tobacco has been identified as the main preventable cause of death in the 21<sup>st</sup> century. Examples for common diseases related to regular tobacco use are cardiovascular and pulmonary diseases. Other smoking-related health risks include osteoporosis, blindness or hearing loss. Most importantly, smoking has been associated with the development of cancer, especially of the lung and breast (Cancer Society of New Zealand, 2006; Eriksen et al., 2012; World Health Organization, 2012). The World Health Organization (WHO) highlights that smoking, including second-hand-smoking, could potentially lead to the death of one billion individuals this century if no effective preventative measures are taken (World Health Organization, 2012). For these reasons, many countries have initiated “stop smoking campaigns”, such as offering free or cost reduced nicotine replacement therapy (NRT) such as nicotine gums or patches. Additionally, some countries such as New Zealand have introduced high taxes for tobacco products in an attempt to control or minimise tobacco consumption (Ministry of Health, 2011b).

Increased taxes exacerbate the financial burden of already expensive tobacco products. Potentially, this could lead to socio economic deprivation and poverty in individuals addicted to tobacco use. Money spent on tobacco products limits resources for other needs such as nutrition, housing, or medication and treatment (Winterer, 2010; World

Health Organization, 2012). Additionally, there are costs to society as valuable resources are also attributed to treatments of smoking-related illnesses campaigns such as the “quit line”. For example, in 2004 the New Zealand government spent approximately NZD 200 million on health care required by individuals with tobacco-related diseases (Eriksen et al., 2012; Ministry of Health, 2011b; World Health Organization, 2012). More recent statistics show that there is a small, but steady decline in smoking prevalence rates in many developed countries (for example United Kingdom, approximately 0.5% per annum). However, it has been observed that some subgroups of the general population are not following the declining trend. Individuals with mental disorders, for example, show a significantly lower cessation rate compared to the general population (Szatkowski & McNeill, 2015). This phenomenon will be further discussed in the next chapter.

Despite declining smoking trends in some countries, a significant number of individuals still engage in tobacco use. Approximately 20% of the world’s population smokes tobacco. The latest tobacco related health statistics in New Zealand show that 18% of the population engage in smoking, with a considerably higher prevalence rate (44%) amongst the Maori population (Ministry of Health, 2010, 2011a). Tobacco is the most commonly used legal drug of abuse (Eriksen et al., 2012). A recent study by Gowing and colleagues (2015) evaluated global data on addictive behaviour and found that tobacco and alcohol are the most widely used legal drugs. An estimated 22.5% of the adult world population regularly smoke tobacco products (Gowing et al., 2015) because smoking is highly addictive and associated with positive and highly rewarding properties (self-reports). Examples are calming, stress- and anxiety-reducing effects, but also stimulating or energizing experiences. Similarly common are pro-cognitive (cognitive enhancing) effects, such as an increased ability to sustain attention over an extended period of time or faster reaction times (Hughes & Hatsukami, 1986).

There is still disagreement amongst scholars regarding the definition of dependence and addiction. Whereas addiction refers to drug seeking behaviour, dependence can be defined as the physiological response or physical dependence to substances. Historically, The Diagnostic and Statistical Manual of Mental Disorders has utilized the terminology ‘dependence’ to describe both, firstly, processes of adaptations in the brain to a wide range of substances, including substances of abuse, and secondly, compulsive and involuntary drug taking behaviour. It has been argued that such a simplification in terminology could lead to confusion amongst professionals and patients because tolerance and withdrawal to medication could occur without evidence of abuse (O'Brien, 2011). Thus, the latest edition,

The Diagnostic and Statistical Manual of Mental Disorders, fifth edition, (American Psychiatric Association, 2013) has opted for the term ‘addiction’ in the context of substance use disorders. For these reasons, the term ‘addiction’ is also predominately used in this thesis. Tobacco use disorder is defined as a “problematic pattern of tobacco use leading to clinically significant impairment or distress” (American Psychiatric Association, 2013, p. 571). Symptoms of tobacco addiction include, but are not limited to, cravings (strong desire to use tobacco), tolerance (the need to consume larger amounts to experience the same effect), or withdrawal symptoms (feelings such as being irritable, restless, or anxious) during abstinence from the drug (American Psychiatric Association, 2013).

## **2.1 Nicotine**

The chemical compound identified as mainly responsible for the highly addictive properties of tobacco smoke is a naturally occurring alkaloid called nicotine. Nicotine can be found in tobacco plants, but also in smaller quantities in potato, tomato, and eggplants. However, in addition to nicotine, tobacco comprises approximately 4,000 other chemicals. On average, a cigarette contains 10 to 14 mg of nicotine (Benowitz, 2009; Brennan, Putt, Roper, Waterhouse, & Truman, 2015; Dome, Lazary, Kalapos, & Rihmer, 2010). Smoking tobacco in the form of cigarettes is considered the most effective means of nicotine delivery due to enhanced bioavailability of nicotine following inhalation. Bioavailability refers to the ability of a compound to cross biological membranes (absorption) and depends, (amongst other factors as well) upon a solution’s acidity or alkalinity, also referred to as the pH value (Benowitz, Hukkanen, & Jacob, 2009). For optimal functioning, the human body requires a pH value between 7.35 and 7.46 (Bear et al., 2007). Nicotine itself has a high pH and is more alkaline, yet tobacco smoke due to the large variety of compounds is often acidic with a pH between 5.5 and 6. Therefore, many tobacco companies insert additives that adjust the pH value presumably to facilitate absorption (Benowitz et al., 2009; Dome et al., 2010).

When tobacco smoke is inhaled and reaches the lungs, the large inner respiratory surface allows nicotine to be extracted and rapidly absorbed into the blood circulation to be distributed throughout the body. It is absorbed with highest affinity (binding capacity) in organs such as the liver, kidneys, spleen and lungs. Most importantly, nicotine crosses the blood-brain-barrier within 10 to 20 seconds after smoke inhalation (Benowitz, 2009; Benowitz et al., 2009; Dome et al., 2010).

## 2.2 Central nicotinic acetylcholine receptors (nAChRs)

In the body and the brain nicotine binds to nicotinic acetylcholine receptors (nAChRs), which are widely distributed throughout the CNS. Nicotine was one of the first identified acetylcholine (ACh) agonists, hence the receptor was named after it (Leslie, Mojica, & Reynaga, 2012). ACh is a neurotransmitter with a wide range of functions in the somatic, central and autonomous nervous systems. ACh is involved in the activation of skeletal muscles in the somatic nervous system and regulation of heart rate, digestion, or breathing in the autonomous nervous system. In the CNS, ACh is mainly responsible for arousal, reward and cognitive functioning such as learning and memory (Dome et al., 2010; Scarr et al., 2013).

The nAChRs are ligand-gated ion channels, comprising of subunits categorised into alpha ( $\alpha$ ) and beta ( $\beta$ ) units. These different subunits combine and form a number of ligand-gated pentameric nAChR sub-types. In general, nAChRs operate or exist in three stages: closed, open and in the refractory stage. After receptor binding, nicotine produces receptor structural changes. Subsequent to the structural changes, the channel opens and cations enter the cell. Then the channel closes and becomes desensitised, also referred to as the refractory stage. For a short period of time, the receptor cannot respond to agonists, thus, the refractory stage can be seen as a “recovery period” (Bear et al., 2007; Benowitz et al., 2009; Dome et al., 2010; Hurst, Rollema, & Bertrand, 2013). High concentrations of agonists such as nicotine over a prolonged period of time can, however, result in a more pronounced and long-lasting form of desensitisation. This “protection or compensation” mechanism (Bear et al., 2007) leads to a decreased biological response to the substance (Brennan et al., 2010, p. 794), in other words a reduction in receptor functioning to avoid over-excitation. The phenomenon of desensitisation and its consequences play an important role in the reinforcing effects of chronic nicotine exposure and are further discussed below.

Receptor subtypes can vary significantly in their pharmacological properties and differ in their reaction to nicotine binding (Dani & Bertrand, 2007; Hurst et al., 2013; McGehee & Role, 1995). Nicotine binds with especially high affinity to the  $\alpha 7$  and  $\alpha 4\beta 2$  receptor subtypes (Benowitz, 2009; Brennan, Lea, Fitzmaurice, & Truman, 2010; Dani & Bertrand, 2007; Dome et al., 2010). The  $\alpha 4\beta 2$  receptor subtype is densely populated in areas such as the cortex, the striatum, amygdala, and the hippocampus. The  $\alpha 7$  receptor subtype can be found predominately in the cortex, hippocampus, and the limbic regions (Dani &



Balfour, 2011; Leslie et al., 2012). These brain regions are relevant for memory, learning and emotions and play a prominent role in the addictive effects of nicotine.

### **2.3 Nicotine addiction and the “reward pathway”**

The underlying mechanisms whereby nicotine leads to tobacco/nicotine addiction are still not well understood. However, there is a wealth of evidence that suggests that the pharmacological effects of chronic nicotine result in extensive neurological changes in structure and function (Benowitz, 1988; Brennan et al., 2010), where some of these are discussed below.

The dopaminergic system and its four major pathways: the mesolimbic, the mesocortical, the nigrostriatal, and the tubero-infundibular pathway are of particular interest as they play a key role in the motivating and reward-related effects of all drugs of abuse, including nicotine (Scarr et al., 2013). In short, the nigrostriatal pathway is mainly involved in motor coordination, while the tubero-infundibular pathway originates in the hypothalamus and is implicated in the inhibition of hormone secretion. In the interaction with nicotine, the mesolimbic, the mesocortical, and the nigrostriatal pathways are most relevant and are discussed further below. The mesolimbic pathway consists of the ventral tegmental area (VTA) in the brain stem and parts of the limbic structures such as the nucleus accumbens (NAcc), the hippocampus, and the amygdala. The mesocortical pathway connects the VTA with the cerebral cortex and is involved in cognitive functioning such as memory and attention, but also motivation, and emotional responses. These two pathways are collectively referred to as the mesocorticolimbic pathway or the “reward pathway” (Albanese, Altavista, & Rossi, 1986; Noback, Ruggerio, Demarest, & Strominger, 2005). Findings show that all drugs of abuse, including nicotine, stimulate the mesolimbic system to unfold their rewarding properties (Brennan et al., 2010; Dani & Balfour, 2011; Di Chiara, 2000; Leslie et al., 2012; Mansvelder & McGehee, 2002).

Nicotine addiction develops over time and on a biological level seems to involve three main stages: (1) acute nicotine exposure and initial effects; (2) chronic nicotine exposure and neuro-adaptation leading to an increase in the reinforcing effect of nicotine; and (3) the withdrawal stage with, for example, drug cravings during periods of abstinence from nicotine (Brennan et al., 2010). These stages and some of the associated biological and psychological effects are briefly discussed below.

### 2.3.1 Acute nicotine exposure

The first or initial phase of nicotine exposure varies between individuals and can produce positive and/or aversive effects. The initial phase is important, as individuals experiencing mainly positive effects are at higher risk for developing tobacco addiction. Examples of initial positive experiences are relaxation or euphoria that are likely mediated via central nAChR activation, whereas aversive symptoms include nausea or increased heart rates (Brennan et al., 2010; Spring, Pingitore, & McChargue, 2003). Negative effects may be mediated by the release of hormones such as epinephrine, where elevated levels have been associated with increased heart rates, blood pressure or changes in the respiratory rate (Brennan et al., 2010; Fasanmade & Oyebola, 1993; Haass & Kuebler, 1997). In addition, nicotine could affect peripheral nAChRs to produce involuntary muscle movements such as hand tremors during the initial stage of tobacco/nicotine use (Brennan et al., 2010; Perkins et al., 1990).

The positive effects likely relate to nicotine binding to central nAChRs that facilitate dopaminergic neurotransmission in the mesolimbic system, or the “reward pathway”. The nAChRs are densely populated in these areas where the  $\alpha 4\beta 2$  and  $\alpha 7$  subtypes present high nicotine-binding capacity (Dome et al., 2010; Mansvelder & McGehee, 2002). In brief, an increase in VTA activity results in enhanced dopaminergic neurotransmission in the nucleus accumbens (Pidoplichko, M, Williams, & Dani, 1997; Pontieri, Tanda, Orzi, & Di Chiara, 1996). This has been associated with reinforcing effects such as feelings of wellbeing, pleasure, and reward.

The impact of nicotine on this pathway is dependent on where nicotine interacts with nAChRs. For example, the administration of antagonists (mecamylamine, MEC) in the VTA leads to a blocking of the receptors and a subsequent nicotine exposure does not induce the same rewarding effects, presumably as nicotine-induced increase of extracellular dopamine in the NAcc is inhibited. However, if the nAChRs antagonist is administered in the NAcc directly, instead of the VTA, nicotine exposure still leads to an increase in dopamine levels (Nisell, Nomilos, & Svensson, 1994). These findings are confirmed by animal studies using nicotine self-administration methods. Self-administration of nicotine is reduced if nAChRs are blocked in the VTA, but is not affected by receptor-blocking in the NAcc (Corrigall, Coen, & Adamson, 1994; Mansvelder & McGehee, 2002; Nisell et al., 1994). These studies indicate that (1) nAChRs in the cell body region of the mesolimbic pathway mediate the

reinforcing effects of nicotine and (2) that receptor populations in distinctive regions produce differential effects on dopaminergic transmission and behaviour.

The NAcc has been divided into a core and shell, based on structural and functional differences. The NAcc shell has been purported to play an especially important role in mediating the initial reinforcing properties of drugs of abuse, including nicotine. The stimulation of dopamine transmission in this area has an impact on the amygdala, the hypothalamus, and the central grey matter, thus affecting the release of hormones and emotional experiences (Dome et al., 2010; Pidoplichko et al., 1997). Pontieri and colleagues (1996) compared the effect of a nicotine-induced increased dopamine release in the core versus the NAcc shell using dialysis. There was a dose dependent increase in dopamine activity in the NAcc shell, but not in the core, which was attributed to variations in receptor subunits (Pontieri et al., 1996).

However, because the central nAChRs are so widespread, nicotine does not act solely on dopaminergic neurons in the VTA. The nAChRs on GABA neurons comprise mainly  $\alpha 4\beta 2$  and  $\alpha 7$  receptor subtypes, thus nicotine binds to, and activates these neurons with high affinity (Mansvelder & McGehee, 2002). GABA is an inhibitory neurotransmitter, which generally decreases neuronal activity. Acute nicotine exposure leads to an initial increase in inhibitory GABA levels in the VTA, which subsequently results in reduced dopamine activity (Laviolette & van der Kooy, 2001; Mansvelder & McGehee, 2002). However, these receptors desensitise rapidly leading to a long-lasting increase of the excitatory effect of nicotine on dopamine neurons in this area (Laviolette & van der Kooy, 2001; Mansvelder & McGehee, 2002; Steffensen, Lee, Stobbs, & Henriksen, 2001). Equally important in nicotine addiction is the effect of nicotine on glutamate release. Upon binding to nAChRs such as  $\alpha 7$  subtype located on glutamate neurons, nicotine increases glutamate transmission. Compared to GABA, nAChRs located on glutamate neurons show slower desensitisation, which could contribute to increased excitation of the dopaminergic system after repeated nicotine exposure (Koukouli & Maskos, 2015; Mansvelder, Keath, & McGehee, 2002).

### **2.3.2 Chronic nicotine exposure**

Prolonged nicotine exposure may ameliorate the initial aversive effects and increase positive subjective experiences. Evidence for adaptation to nicotine comes, for example, from studies that have compared the effect of nicotine in smokers and non-smokers. Findings suggest that non-smokers experience fewer positive and more aversive effects compared to

smokers (Brennan et al., 2010; Foulds et al., 1997). Upon first encounter with nicotine, the brain attempts to maintain normal functioning or homeostasis in the presence of a foreign substance. Over time and with prolonged exposure, adaptations lead to a reduction of initial aversive effects and an enhancement of the reinforcing effects (De Biasi & Dani, 2011; Koob & Le Moal, 2001).

On a cellular level, adaptation processes include receptor desensitisation or up-regulation. Receptor desensitisation can be described as a process to avoid over-stimulation and has been observed in the mesocorticolimbic system. Consequently, tolerance to the substance of abuse develops, where an increased intake of the psychostimulant is required to experience effects that are similar to the initial experience (Belluzzi, Lee, Oliff, & Leslie, 2004; Brennan et al., 2010; Pidoplichko et al., 1997). However, not all nAChRs are equally susceptible to desensitisation. Receptor-subtype-dependent differences can be found, as for example, the  $\alpha 4\beta 2$  receptor subtype in the VTA undergo desensitisation within seconds after nicotine binding. Conversely, other nAChRs remain activated over an extended period of time, thus contributing to a prolonged signalling of neurons in the VTA (Leslie et al., 2012).

Up-regulation may follow the desensitisation of receptors due to long-term agonist exposure. Receptor up-regulation can be described as a measure of compensation for the reduced availability of receptors and comprises a change in functional capacity, evident in an increase of responsiveness and sensitivity to nicotine (Brennan et al., 2010; Leslie et al., 2012). The up-regulation of nicotinic nAChRs is subtype and brain region specific (De Biasi & Dani, 2011; Rezvani, Teng, Shim, & De Biasi, 2007). The  $\alpha 4\beta 2$  receptor subtype shows compensatory up-regulation after lower nicotine concentrations and shorter exposure times compared to the  $\alpha 7$  receptor subtype. It has also been suggested that nicotine concentration following cigarette smoke exposure might not be high enough in the human brain to result in the up-regulation of all receptor subtypes. Thus chronic exposure to nicotine differentially affects the functional statuses of receptor populations throughout the brain, resulting in overall changes in neurotransmission.

Supporting evidence for these overall changes derives from animal studies that examined the effects of acute versus chronic nicotine exposure on locomotor sensitisation in rodents. In general, drugs of abuse produce sensitisations in locomotor activity sensitisation, which has been associated with neuroadaptations. For example, Brennan and colleagues (2013) compared the effects of acute and chronic nicotine treatments (0.0, 0.2, 0.4 mg/kg, sc) in rats. Acute nicotine administration had no effect on locomotor activity, yet there were

significant enhancements in baseline activity levels observed after chronic exposure. These findings are consistent with previous research (Harris, Stepanov, & Pentel, 2012), where it has been hypothesised that drug-induced changes of DA levels could contribute to differences in activity levels. Imaging technology utilizing micro-PET and functional magnetic resonance imaging (fMRI) scans in rats before and after locomotor activity testing have been used to assess general brain activation patterns. Results revealed significant changes in neural activity after repeated nicotine exposure in the hippocampus, NAcc, PFC, ventral pallidum and ventral tegmentum (Li, DiFranza, Wellman, Kulkarni, & King, 2008) as well as structural changes in the NAcc and PFC (Calderan et al., 2005). These findings suggest that chronic nicotine exposure leads to widespread changes in behaviour that are associated with altered brain activation patterns and morphology.

### **2.3.3 Nicotine withdrawal**

The third and last phase of nicotine addiction is the stage of withdrawal. As aforementioned, chronic exposure to nicotine leads to brain region and receptor subtype specific neuroadaptations such as the facilitation of DA neurotransmission, a decrease of GABA levels due to receptor desensitisation (predominately  $\alpha 4\beta 2$ ), and an increase in glutamate levels due to ionotropic glutamine receptor binding (Pistillo, Clementi, Zoli, & Gotti, 2015). Thus, nicotine withdrawal could be associated with decreased DA neurotransmission due to the absence of nAChR stimulation and subsequently alterations in brain reward functioning (Zhang, Dong, Doyon, & Dani, 2012). Additionally, it has been proposed that nicotine-induced desensitized nAChRs such as the  $\alpha 4\beta 2$  receptor subtype might regain their full functional ability in the absence of the drug, leading to an increase in inhibitory GABA levels during withdrawal (Nashmi et al., 2007). Furthermore, glutamate and DA levels may also decrease due to a lack of receptor stimulation. Hence, it is possible that hypofunctional DA and glutamate combined with hyperfunctional GABA activity initiate the withdrawal symptoms observed after nicotine cessation (Pistillo et al., 2016). Withdrawal symptoms are evident on a psychological and physiological level and include, but are not limited to, increased anxiety, irritability, anhedonia (diminished pleasure), hand tremors, headaches as well as fatigue. These physical symptoms may contribute to a state of dysphoria (dissatisfaction), which is likely to encourage drug-taking behaviour in an attempt to reduce unpleasant bodily sensations and feelings (Benowitz et al., 2009), and hence could initiate the cycle of addictive behaviours.

#### **2.3.4 The role of conditioned reinforcers in nicotine addiction (nigrostriatal pathway)**

Everitt and Robbins (2005) have suggested that the transition from occasional to compulsive drug taking is strongly influenced by an interaction “between Pavlovian and instrumental learning processes” (p. 1481). This hypothesis stresses the importance of conditioned reinforcers in drug addiction. An initially neutral stimulus can become a conditioned reinforcer through repeated pairing with the originally reinforcing stimulus (for example a drug of abuse). This is particularly relevant to nicotine, as compared to abused drugs such as cocaine, nicotine is only weakly reinforcing (Risner & Goldberg, 1983). Consequently, a major role for conditioned reinforcers in the development of nicotine addiction has been demonstrated. For example, Sorge et al. (2009) compared the reinforcing effects of nicotine in a self-administration paradigm (0.015 mg/kg) with and without exposure to non-pharmacological stimuli (light, white noise). Reinforcing effects of these non-pharmacological stimuli without nicotine exposure were only marginal (light cue) or showed no effect (white noise). Nicotine infusions alone were weakly reinforcing, yet nicotine self-administration was significantly facilitated when presented with drug-paired conditioned stimuli (Sorge, Pierre, & Clarke, 2009).

Consistent with the increase in DA activity reported in the NAcc shell only (Pontieri et al., 1996), Everitt and Robbins suggest that the shell predominately mediates the psychomotor stimulant effects of the drug. More specifically, the DA projections from the ventral tegmental area to the NAcc shell mediate the increase in responding for a drug during the stages of acquisition, or initial drug taking. An increase in the response rate increases exposure to the drug, thus heightens the incentive salience of the conditioned reinforcer. The NAcc core is mainly involved in the effects of the conditioned reinforcer (for example a cue light) such as maintaining instrumental behaviours over time delays. The authors suggest that as the effects of the conditioned reinforcers become significantly more salient and pervasive with prolonged drug taking, a shift of control over drug taking related behaviour occurs within the NAcc from the shell to the core (for review Everitt & Robbins, 2005).

Additionally, there could be a progression of control from the ventral striatum to the dorsal regions of the striatum. Evidence for this theory arises from microdialysis in vivo studies showing DA release in the dorsal striatum due to exposure to drug-paired conditioned stimuli (Ito, Dalley, Robbins, & Everitt, 2002), yet no effect was observed in the NAcc core or shell (Neisewander, O'Dell, Tran-Nguyen, Castaneda, & Fuchs, 1996). In support of this idea of a progression of control, Vanderschuren et al. (2005) showed that the administration of a DA receptor antagonist  $\alpha$ -flupenthixol into the NAcc core had no effect in subjects that

demonstrated cue-controlled cocaine self-administration, yet an infusion of the antagonist into the dorsal striatum resulted in significantly reduced responding to cocaine (Vanderschuren, Di Ciano, & Everitt, 2005). The dorsal striatum is associated with habit learning and Everitt and Robbins speculate that prolonged exposure to drugs of abuse could potentially lead to a “maladaptive stimuli response habit” (p. 1485). Neuroimaging studies support this theory as it has been established that the ventral striatum is implicated in Pavlovian conditioning, whereas the dorsal striatum is concerned with instrumental learning.

Importantly, the role of the striatum in drug addiction is influenced by other structures in the limbic cortical network such as the basolateral amygdala, the hippocampus and the medial PFC. The role of all these structures in drug seeking through their projections to the NAcc has been well established (for review Everitt & Robbins, 2005). For example, lesions to the medial PFC leads to an increase in response rates in cocaine self-administration in rats, possibly due to impairments in executive functioning and inhibitory control (Dalley, Cardinal, & Robbins, 2004). Reductions in PFC grey matter density has been reported in long-term drug users (for example, cocaine, heroin, nicotine), leading to significant deficits in executive functioning (for review Goldstein & Volkow, 2011). For these reasons, Everitt and Robbins describe the transition from social drug taking to addiction as a “ventral to dorsal unidirectional cascade of information processing mediated by the corticolimbic loop circuitry” (p. 1485), which on a neural level includes a shift of control from the PFC to the dorsal striatum.

## **CHAPTER 3            TOBACCO ADDICTION IN SCHIZOPHRENIA, EXPLANATORY THEORIES**

The third chapter provides the context for high smoking incidence in individuals with schizophrenia and discusses three explanatory theories, which are not necessarily mutually exclusive.

### **3.0 Tobacco addiction in schizophrenia**

Influenced and encouraged by worldwide antismoking campaigns, smoking prevalence rates in the general population are declining in most countries (World Health Organization, 2015). Some population subgroups, however, are less likely to stop smoking, such as individuals with mental disorders, who show significantly higher smoking rates (50%) compared to the general population (23%) (Szatkowski & McNeill, 2015). Intriguingly, high smoking incidence is particularly common in schizophrenic patients (75 to 90%), where approximately 50% of these individuals are heavy smokers (25+ cigarettes per day) (de Leon & Diaz, 2005; Hughes, Hatsukami, Mitchell, & Dahlgren, 1986; Lasser et al., 2000). Thus there is great concern that individuals with schizophrenia are at greater risk to develop chronic smoking related illnesses, as cessation rates are also exceptionally low (Ziedonis et al., 2008).

Schizophrenia is a debilitating disorder and life expectancy is significantly lower in this subgroup (by approximately 20 years) (Laursen, Nordentoft, & Mortensen, 2013). Factors that influence early mortality amongst patients include predispositions towards higher blood cholesterol levels, hypertension and obesity leading to an increased risk of cardiovascular diseases (Hennekens, Hennekens, Hollar, & Casey, 2005). These underlying susceptibilities to illness could be further exacerbated in patients by smoking. Schizophrenic patients purportedly smoke differently, or more ‘efficiently’ compared to heavy smokers in the general population. Increased cotinine plasma/urinary levels (nicotine metabolite) have been identified, presumably, due to these individuals extracting more nicotine per cigarette by taking more or longer puffs (Olincy, Young, & Freedman, 1997).

In addition to increased health risks, heavy smoking leads to a significant financial burden. Schizophrenic patients spend approximately 30% of their available budget to finance their smoking habit (Steinberg, Williams, & Ziedonis, 2004). This significantly limits available resources required for basic needs such as nutrition, housing, or treatment and medication (Lasser et al., 2000). Despite awareness of smoking related disadvantages, many



schizophrenic patients fail to reduce or stop smoking. As aforementioned, cessation rates are particularly low (9%) compared to the general population, but more importantly, also when compared to individuals with other mental disorders (38%) (Williams & Hughes, 2003). One explanation could be that individuals with schizophrenia have a subjectively more rewarding experience when smoking compared to others.

Spring and colleagues (2003) examined the reward value of smoking cigarettes (compared to other pleasant activities) in individuals with schizophrenia (n=26), patients with major depression (n=26), as well as healthy controls (n=26). All participants were addicted to smoking and there were no differences between groups in the number of cigarettes smoked on a daily basis. Interestingly, their findings show that participants did not differ in their perception of negative consequences attached to smoking. However, both patient groups differed significantly from controls in their evaluation of positive smoking-related effects. Compared to non-psychiatric individuals, schizophrenic and depressed patients are twice as likely to choose smoking cigarettes over other pleasant activities (Spring et al., 2003). These findings revealed that individuals with mental disorders recognized smoking related disadvantages to the same extent as the general population. Smoking-induced benefits, however, seem to be valued considerably higher, outweighing known disadvantages. These findings indicate that higher smoking rates in individuals with mental disorders compared to the general population are mediated by reward related experiences, yet the results do not explain why schizophrenic patients are twice as likely to be heavy smokers compared to individuals with major depression.

Several explanatory theories have been proposed to explain high smoking incidence in schizophrenic patients. Firstly, nicotine is thought to reduce aversive side effects of antipsychotic medication (D'Souza & Markou, 2012; Levin & Rezvani, 2007). Secondly, nicotine could improve symptoms of the disorder such as negative or cognitive impairments, which are largely unaffected by pharmacological treatment (Fioravanti, Carlone, Vitale, Cinti, & Clare, 2005). Thirdly, shared neurobiological abnormalities underlying schizophrenia and SUD may increase an individual's susceptibility to both disorders (Chambers et al., 2001; Dome et al., 2010). It is important to note that these theories are not necessarily mutually exclusive. The present PhD research project examined two of these theories: (1) the potentially cognitive enhancing effects of nicotine and (2) possible neurological substrates in schizophrenia predisposing towards addiction.

### **3.1 Explanatory theories**

#### **3.1.1 Nicotine reduces side effects of antipsychotic medication**

Antipsychotic drugs were developed in the 1950s and remain the first line of treatment for individuals suffering from schizophrenia. Overall, antipsychotics reduce positive symptoms such as delusions or hallucinations, yet negative and cognitive symptoms remain largely unaffected (Fusar-Poli et al., 2015). Although it is still not completely understood how antipsychotics affect the brain and influence behaviour, all antipsychotics currently available act on the dopaminergic system by blocking DA receptors of the D2-type family (Ellenbroek, 2012).

Medication side effects are severe and differ depending on the medication type. For example, subjective ratings suggest that antipsychotic medications dampen experiences of novelty or salience and reduce emotions (Kapur et al., 2005). Such medication-induced effects are also referred to as neuroleptic dysphoria and complex alterations in neurotransmitter levels (such as in the dopaminergic system) have been suggested as possible underlying mechanisms (Kapur et al., 2005; Moritz et al., 2013). However, since anhedonia is common amongst individuals with schizophrenia, it is difficult to distinguish the effects of the medication from the symptoms of the disorder. Therefore, it is possible that the reported subjective reduction in emotional affect is unrelated to medication intake.

Antipsychotics can be categorized into first and second generation drugs and are also commonly referred to as typical or atypical antipsychotics. Scholars are still in disagreement as to whether the second generation drugs offer improvement in schizophrenic symptoms compared to the first generation antipsychotics. Whilst many studies indicate that both generations reduce positive symptoms, there are minimal improvements of negative symptoms or cognitive deficits. For example, a recent meta-analysis by Fusar-Poli and colleagues (2015) shows that neither first nor second generation drugs lead to a substantial improvement in negative symptoms (Ellenbroek, 2012; Fusar-Poli et al., 2015). The main difference between both types of antipsychotics lies in the accompanying medication side effects. First generation drugs are known to cause extrapyramidal psychomotor abnormalities (Parkinson's like motor dysfunctions) or tardive dyskinesia in many patients, which refers to involuntary and repetitive body movements. Second generation antipsychotics do not lead to these severe abnormalities in motor movements. However, debilitating side effects such as excessive weight gain or resistance to insulin leading to diabetes have been observed. Further, increased risk to develop dyslipidemia, an increase in

blood lipids (i.e. fats) has been found, which could lead to cardiovascular or pulmonary diseases (Ellenbroek, 2012; Kishimoto et al., 2013; Navari & Dazzan, 2009). Thus, non-compliance rates in schizophrenic patients are very high, ranging between 50 to 70% (Carr & McNulty, 2006). These findings are supported by a recent meta-analysis (K=212 studies, 43,049 patients, 15 antipsychotic drugs), which highlights the importance of drug efficacy and tolerability (side effects) in treatment continuation (Leucht et al., 2013).

Differences in relapse prevention rates between first and second generation drugs have been investigated and findings are mixed. While some meta analyses (for example Kishimoto et al., 2013) suggest that second generation drugs lead to significantly less relapse (29%) compared to first generation antipsychotics (37.5%), others imply that the majority of second generation drugs do not provide any improvement compared to first generation antipsychotics (Davis, Chen, & Glick, 2003). Speculatively, differences in findings might be due to grouping of medications and generalization as great variations have been identified within both drug types. Additionally, there is great heterogeneity within the group of schizophrenic patients. As aforementioned, all antipsychotics act on the dopaminergic system, predominately by blocking receptors of the D2-type receptor family (except aripiprazole, which acts as a partial agonist) (Ellenbroek, 2012). It has been observed that individuals with schizophrenia have increased basal levels of DA activity (Abi-Dargham, 2002), and antipsychotic medication might affect schizophrenic patients differently depending on their individual baseline levels (de Haan et al., 2004; Rissanen et al., 2012). Such differences in baseline levels might also play a role in tobacco addiction in this subgroup.

The effect of antipsychotic drug types on relapse rates and motivation for smoking was examined by Barr and colleagues (2008). The authors examined these aspects in patients diagnosed with schizophrenia or schizoaffective disorder (n=61) and healthy controls (n=33), where both groups were matched on the number of cigarettes smoked per day (self-report measure of dependence). Interestingly and consistent with studies that found differences in relapse prevention rates between first and second generation drugs, Barr and colleagues found a strong association between daily medication dose (for example chlorpromazine) and the motivation to smoke. These findings indicate a dose dependent effect on smoking behaviour. Additionally, individuals using first generation antipsychotics showed significantly higher smoking rates (smoked more cigarettes per day) compared to those treated with second generation drugs. Thus, variations in medication side effects between

both drug types may affect smoking behaviour differently (Barr, Procyshyn, Hui, Johnson, & Honer, 2008).

These findings are consistent with a more recent study that compared socio-demographic and clinical characteristics of heavy versus non-heavy smoking Chinese individuals with schizophrenia. Heavily smoking patients with schizophrenia start smoking at a younger age and take larger daily doses of antipsychotic drugs compared to non-heavy smokers. Consistent with Barr and colleagues (2008), findings revealed that patients exposed to first generation drugs smoked more cigarettes on a daily basis compared to patients who took second generation antipsychotics (Zhang et al., 2013). These results indicate an association between antipsychotic drug-type and smoking rate, which is potentially related to medication side effects. Indeed, there is evidence that nicotine can alleviate both physical side effects, such as restlessness or involuntary motor movements, as well as cognitive side effects, such as deficits in memory, attention, or psychomotor processing speed (Barr et al., 2008; Levin et al., 1990).

For example, the aforementioned study by Barr and colleagues (2008) further revealed that patients with schizophrenia/schizoaffective disorder were less motivated to smoke cigarettes for ‘sociability’ factors compared to the control group. The most prominent factors leading to higher smoking incidence amongst patients were the need for ‘sensorimotor manipulation’ (for example the physical handling of cigarettes and lighters) and ‘increased stimulation’. As these factors represent aspects of physical activities such as handling cigarettes, the authors suggest that the need for sensorimotor stimulation might be related to the “loss of energy and cognitive slowing” commonly found in these individuals (Ritsner, Gibel, & Ratner, 2006). Speculatively, smoking and the accompanying physical aspects of handling cigarettes might be an attempt to counterbalance antipsychotic medication-induced impairments in the control of motor movements. However, it is important to note that the majority of individuals with schizophrenia show cognitive deficits such as impairments in psychomotor processing speed long before the onset of psychosis. Thus, it remains debatable whether the observed deficits in these cognitive domains existed prior to the onset of the illness or whether deficits developed subsequent to antipsychotic medication intake.

### 3.1.2 Nicotine improves symptoms such as cognitive deficits

The so called ‘self-medication hypothesis’ suggests an increased smoking rate in individuals with schizophrenia due to nicotine’s ameliorating effect on psychiatric symptoms (Kumari & Postma, 2005). For example, improvements in negative symptoms such as anhedonia (reduced experience of pleasure) and alogia (poverty of speech) have been found in trials using partial nAChR  $\alpha 7$  agonists (Freedman et al., 2008). The effect of acute and chronic nicotine on schizophrenia-relevant cognitive impairments has been studied across species for decades, particularly in areas such as working memory, sensorimotor gating, and attention (for review dos Santos Coura & Granon, 2012; Wallace & Porter, 2011). Deficits in these domains are considered to be core features of the disorder and are also referred to as endophenotypes (characteristics/traits) due to the common appearance in schizophrenia.

Although findings are mixed, a number of studies have reported pro-cognitive effects of nicotine in these domains in individuals with cognitive impairments as well as in healthy controls. For example, Barr and colleagues (2008) examined the effect of transdermal nicotine (14 mg nicotine patches) and placebo in non-smoking individuals with schizophrenia (n=28) and healthy controls (n=32) in a within subject study. The cognitive assessment battery included measures of attention, processing speed, working memory, and psychomotor speed. Nicotine enhanced cognitive performance in both groups in attention, however, schizophrenic patients showed greater improvement in inhibition and impulse control compared to non-psychiatric individuals (Barr et al., 2008). In a similar experiment, the authors investigated the effect of transdermal nicotine on episodic memory performance in non-smoking individuals with schizophrenia (n=10) and controls (n=12). Compared to placebo control conditions, both groups increased in processing speed and accuracy in recognising novel objects. Additionally, there was a trend for a stronger nicotine-induced effect in schizophrenic patients in the reduction of false alarms (Jubelt et al., 2008). Similarly, nicotine exposure (14 mg and 7 mg nicotine patch) enhanced performance in an antisaccade task (sensorimotor gating) in smokers and non-smokers with schizophrenia and healthy controls (Petrovsky et al., 2013).

Methodological differences in animal studies, similar to human research, in particular in outcome measures and nicotine doses, make comparisons between studies difficult. This might, at least in part, explain inconsistent results. However, in spite of this, there are numerous studies that show pro-cognitive effects of nicotine in preclinical research. For example, Levin and colleagues (1992) examined the effect of chronic nicotine (subcutaneous

nicotine pellets, 40 mg/kg which deliver approximately 3.4 mg nicotine/day for 3 weeks before testing commenced) and placebo in rats in a working memory task (eight arm radial arm maze). Persistent nicotine-induced enhancements in accuracy were observed for at least four weeks after nicotine withdrawal (Levin, Briggs, Christopher, & Rose, 1992). Consistent with findings in human studies, cognitive enhancing effects of nicotine in attention have been found. For example, Stoleran et al. (2000) utilized the five-choice serial reaction time task to assess the effect of nicotine (1 ml/kg, sc) in rats, where a nicotine-induced increase in accuracy was observed (Stoleran, Mirza, Hahn, & Shoaib, 2000). Using the same outcome measure, Semenova and colleagues (2007) found a cognitive enhancing effect (accuracy) of chronic nicotine (minipump, 3.16 mg/kg/day or 9 mg/kg/day) in rats during testing days four to six, after an initially observed nicotine induced increase in impulsivity diminished (Semenova, Stoleran, & Markou, 2007). Further, Acri and colleagues (1994) showed a dose dependent effect of chronic nicotine exposure on prepulse inhibition (PPI). Lower doses (0.001 – 0.01 mg/kg, sc) increased the ability to inhibit startle response, whereas higher doses of nicotine (0.5 to 5.0 mg/kg, sc) decreased performance (Acri, Morse, Popke, & Grunberg, 1994).

In sum, these findings in human and animal studies show that nicotine from cigarette smoke can have cognitive enhancing effects. Thus high smoking incidence in schizophrenic patients can be, at least in part, to ameliorate existing cognitive deficits or antipsychotic medication induced side effects.

### **3.1.3 Shared neurobiological pathways underlying schizophrenia and SUD**

A third explanation for high comorbidity suggests common or partly overlapping neurobiological pathways might predispose individuals to develop both schizophrenia and substance use disorder (SUD) (Chambers et al., 2001).

McEvoy and Brown (1999) argue that if high smoking incidence in individuals with schizophrenia was solely for self-medicating purposes or to reduce antipsychotic medication side effects, individuals with chronic schizophrenia would show significantly higher smoking prevalence rates compared to first-episode patients. However, this was not the case as increased smoking rates similar to those found in chronic patients with schizophrenia have been identified in first-episode patients (n=22, schizophrenia or schizophreniform disorder) (McEvoy & Brown, 1999). Additionally, comparable smoking rates to those found in individuals with schizophrenia have been identified in first-degree relatives. First-degree relatives of individuals with schizophrenia potentially share genetic and developmental risk

factors for schizophrenia. Subclinical manifestations of symptoms found in schizophrenia are commonly referred to as schizotypy. These dimensional traits exist in the general population, yet are significantly more likely in biological relatives of individuals with schizophrenia, presumably due to aforementioned underlying genetic predispositions. Esterberg and colleagues (2007) examined smoking status in association with level of schizotypy symptoms in unmedicated healthy first-degree relatives of individuals with a diagnosis of schizophrenia (n=42) and controls (n=50). Their findings suggest that individuals who scored higher on schizotypal features were more likely to be heavy smokers; however, this was only significant in relatives of schizophrenic patients, not in controls (Esterberg, Jones, Compton, & Walker, 2007). Ferchiou and colleagues (2012) confirmed Esterberg's findings that smoking status was higher in first-degree relatives of individuals diagnosed with schizophrenia, yet they found no evidence of an association between nicotine addiction and magnitude of schizotypal features (Ferchiou et al., 2012). Similar results have been obtained in studies that examined a range of substances of abuse. Individuals with higher levels of schizotypal features engage significantly more in tobacco smoking, alcohol as well as cannabis use (Esterberg, Goulding, McClure-Tone, & Compton, 2009). These studies show that addictions to a number of substances are not necessarily related to the onset of psychosis, thus this eliminates antipsychotic medication side effects as the main driver for smoking. Yet, it is possible that first-degree relatives show high smoking incidence to ameliorate existing cognitive deficits, as these might be present in the absence of a psychotic symptomatology.

The most compelling evidence for the theory of shared underlying substrates in schizophrenia and SUD arises from studies showing that schizophrenic patients, in general, engage in poly substance use. Although some substances such as nicotine can have cognitive enhancing effects, other drugs of abuse frequently used by patients such as cocaine or cannabis can aggravate aversive symptoms (Lundqvist, 2005). Poly substance use in these individuals includes, but is not limited to, legal drugs of abuse such as tobacco and alcohol as well as illegal drugs such as cannabis, cocaine, heroin or amphetamines (Fowler, Carr, Carter, & Lewin, 1998). Interestingly, illicit drug use in this population subgroup has increased significantly over time. Prevalence rates of 30% were observed in the 1970s, yet in 2006 numbers ranged between 70 and 80%. It has been suggested that factors such as deinstitutionalization and access to financial means (government benefit payments for example) substantially influenced this development (for review Westermeyer, 2006). Poly substance use in individuals with psychosis is associated with a number of negative

consequences such as increased hospitalization, higher relapse rates and treatment as well as medication noncompliance (for review Cantor-Graae, Nordstrom, & McNeil, 2001; Donoghue & Doody, 2012).

More importantly, substances of abuse such as cocaine or cannabis have been linked to cognitive dysfunctions. For example, Vonmoos and colleagues (2014) compared cognitive performance in cocaine users (n=57) and controls (n=48) in a longitudinal design (baseline and after one year). Cognitive assessment included performance measures in attention, working memory, declarative memory, and executive control. At baseline, cocaine users showed significant deficits compared to controls in working and declarative memory as well as in executive functioning. Although not statistically significant, a trend for deficits in attention was also evident. Cognitive assessment one year later showed that those individuals who increased cocaine use during this time displayed cognitive decline compared to baseline, yet those individuals who decreased cocaine intake showed small improvements in functioning in all four domains measured. These findings suggest that cocaine use can lead to impairments in some domains of cognitive functioning, yet these are partially reversible after abstinence from the drug (Vonmoos et al., 2014).

Similar observations have been made in chronic cannabis users. Solowij and Michie (2007) reviewed previous research regarding cannabis use and cognitive dysfunctions focusing on endophenotypes commonly found in individuals with schizophrenia. In particular, cannabis-induced deficits in domains such as sensorimotor gating (P50 suppression), inhibition (for example, selective attention) as well as aspects of WM such as goal maintenance over time were examined. The authors suggest that cognitive deficits commonly observed in individuals with schizophrenia are similar in nature to those dysfunctions frequently found in chronic cannabis users. Interestingly, studies also show comparable negative effects on cognitive functioning in individuals exposed to long-term maintenance treatment such as methadone, an opiate substitution. Methadone-induced impairments have been observed in cognitive domains such as attention, decision-making, psychomotor speed as well as lower IQs compared to controls (King & Best, 2011; Mintzer & Stitzer, 2002).

In sum, these findings suggest that high smoking incidence in schizophrenic patients might be partially motivated by self-medicating purposes due to nicotine's cognitive enhancing properties. However, as evident from the above, substance use in these individuals is multifaceted and includes substances that exacerbate cognitive impairments. Thus it is highly likely that the explanatory theories are not mutually exclusive. In particular,



as similar structures are involved in cognitive processes as well as in the reinforcing effects of substances of abuse such as the hippocampus, the basal ganglia, and the cortical regions. The relevance of these structures in cognition and substance use as well as commonly found anomalies in schizophrenia such as dysregulations in the cholinergic system are briefly discussed below.

### **3.1.4 Underlying mechanisms relevant to the theories on comorbidity**

It is still not fully understood how nicotine affects cognition, yet findings suggest that nicotine binds with high affinity to nAChRs, predominantly to the  $\alpha 7$  and  $\alpha 4\beta 2$  subtypes. These receptors are densely populated in brain areas associated with cognitive functioning such as the cerebral cortex, the medial temporal lobe and the hippocampal area (Newhouse, Potter, Dumas, & Thiel, 2011) and are particularly important in cognitive processes such as learning, memory formation, and attention (Bear et al., 2007; Boess et al., 2007; Sarter, Lustig, & Taylor, 2012). Nicotine as an ACh agonist mimics the neurotransmitter and stimulates cell activity upon binding, which can lead to a wide range of effects including neurotransmitter release such as dopamine, GABA, and glutamate. The effect of nicotine on cognition is further discussed in Chapters 5 and 8.

The importance of dysregulations in the cholinergic system in the psychopathology of schizophrenia has been well established. In the CNS, the cholinergic system controls crucial functions such as sleep, arousal, motor control, sensory processes, and cognitive functioning through interactions between ACh and two receptor family types, muscarinic receptors and nAChRs. Thus it is not surprising that dysregulations within this system as commonly found in individuals with schizophrenia can affect both cognitive functioning and reinforcing effects of psychostimulant drugs. As discussed in Chapter 2, nicotine binds with high affinity to nAChRs, thus muscarinic receptors will not be addressed here.

Cholinergic dysfunctions in schizophrenia have been observed in, for example, elevated choline acetyltransferase levels, an enzyme responsible for ACh synthesis, in brain areas such as the hippocampus, caudate, putamen, and thalamus (McGeer & McGeer, 1977). Contrary, reduced levels of this enzyme have been found in areas such as the NAcc and pons (Bird et al., 1977). Abnormal levels of this enzyme would significantly influence ACh activity levels leading to widespread functional disruptions in these brain areas.

In addition to anomalies in ACh synthesis, receptor abnormalities have been observed in patients in post mortem studies. More specifically, a reduction in nAChR  $\alpha 7$  expression

has been shown in brain areas such as the hippocampus, thalamus as well as the PFC in schizophrenic patients (Freedman, Hall, Adler, & Leonard, 1995). Decreased  $\alpha 4\beta 2$  binding has also been found in the hippocampus, cortex, and striatum (Breese et al., 2000). However, it is important to highlight that findings from post mortem studies are potentially influenced by confounding factors such as the medication history or smoking status of the individual (Hyde & Crook, 2001). More recent studies, utilizing neuroimaging techniques, have examined receptor availability in schizophrenic patients. For example, D'Souza and colleagues (2012) compared nAChR subunit  $\beta 2$  availability in the frontal cortex, parietal cortex, and thalamus in smokers with schizophrenia (n=12) compared to matched controls (n=12). Consistent with post mortem results (Breese et al., 2000), their findings showed significantly reduced (21 to 26%) receptor availability in individuals with schizophrenia. In addition, findings show alterations in structure and function of the remaining receptors, which may lead to dysfunctions in neurotransmitter release and gene expression (Leonard et al., 2000; Leonard, Mexal, & Freedman, 2007).

These receptor and ACh alterations may also critically impact on other neurotransmitter levels, which are crucial in cognitive functioning and play a significant role in the effect of drugs of abuse in the brain, as many nicotinic receptors are presynaptically located as discussed above. For example, cholinergic interneurons are crucial in mediating, GABA activity, the main inhibitory neurotransmitter, in the striatum. A significant decrease in cholinergic interneuron density in individuals with schizophrenia compared to controls has been observed overall in the striatum and in particular in the ventral striatum. It has been suggested that a decrease in density/function of these interneurons would disrupt the frontostriatal pathway, thus impacting on cognitive processes such as executive functioning (Holt et al., 2005; Holt et al., 1999). The importance of this pathway in the reinforcing effect of drugs of abuse has also been discussed (see Chapter 2). Schizophrenia is associated with decreased GABA neurotransmission (Blum & Mann, 2002), which might contribute to cognitive deficits such as working memory impairment (Lewis et al., 2008) as well as increased susceptibility to tobacco addiction as reduced GABA levels have been associated with deficits in ignoring smoking-related cues (Janes et al., 2013).

Another structural alteration commonly found in schizophrenia relevant to cognitive deficits and SUD is hippocampal atrophy due to reduced neurogenesis. Abnormalities in hippocampal neuronal activities, for example due to prenatal exposure to maternal infection, are evident in a range of psychiatric disorders such as post-traumatic stress disorder, major

depression, schizophrenia, as well as SUD (for review Chambers, 2013). In addition to a significant reduction in generating new neurons, functional abnormalities have been identified in existing neurons (Belarbi, Arellano, Ferguson, Jopson, & Rosi, 2012; Monje, Toda, & Palmer, 2003; Seguin, Brennan, Mangano, & Hayley, 2009). Although there is still debate amongst scholars to what extent adult neurogenesis occurs and whether this process is limited to the hippocampus (Bonfanti, 2016), alterations in hippocampal functioning would greatly impact on other brain regions such as the PFC and striatal circuits due to projections. For example, Moran and colleagues (2013) examined the functional connectivity of the neuronal circuit between the dorsal anterior cingulate and limbic regions (ventral striatum, amygdala, para-hippocampal areas) in an fMRI study to determine whether abnormalities are evident in both patients with schizophrenia as well as individuals suffering from SUD. To this end, functional connectivity was examined in individuals with schizophrenia, smokers (n=36) and non-smokers (n=18), and matched controls, smokers (n=37) and non-smokers (n=28), as well as first-degree relatives of individuals with schizophrenia, smokers (n=8) and non-smokers (n=16). Their findings show reduced activity in this circuitry in non-smoking schizophrenic patients as well as first-degree relatives, confirming circuit abnormalities in schizophrenia regardless of smoking status. Importantly, matched healthy controls that were heavy smokers also showed reduced functioning, confirming the impact of chronic drug use to circuit strength. Decreased functioning was most severe in individuals with schizophrenia who were also heavy smokers (Moran, Sampath, Kochunov, & Hong, 2013).

Relevant to substance use disorders, it has been suggested that hippocampal dysfunctions may significantly influence an individual's susceptibility to addiction. Evidence for this theory arises from preclinical studies that show increased self-administration in animals with hippocampal lesions (Berg, Sentir, Cooley, Engleman, & Chambers, 2013). Theoretically, maladaptive learning and memory formation associated with the reinforcing effects of drugs of abuse may play a significant role as they potentially influence the motivation to continuous drug taking despite experiencing drug-related negative consequences. Hippocampal abnormalities may change an individual's ability to develop more adaptive behaviour strategies, which could lead to increased addiction vulnerability (Chambers, 2013).

## **CHAPTER 4                      COGNITIVE ASSESSMENT**

The first aim of this PhD research was to establish an animal model utilizing prenatal exposure to maternal infection to produce subjects with schizophrenic-like cognitive deficits. To this end, pregnant female rats were injected with lipopolysaccharide (LPS) once daily at one of three gestational time points representing early (gestation days, GD 10/11), middle (GD 15/16) and late (GD 18/10) stages of pregnancy, which are considered to be important neurodevelopmental vulnerability windows (Fortier et al., 2007). However, it is important to note that it is not possible to directly compare human and rat gestation times, as different developmental processes within each these species run at different speeds. To examine whether prenatal exposure to MIA leads to long-lasting schizophrenic-like cognitive deficits, animals were assessed in early adulthood (commencing postnatal day, PND 60).

Prenatal immune challenges have been implicated in all aspects of schizophrenia, yet the most compelling evidence derives from studies that have examined the impact of MIA on cognitive functioning (Buka et al., 2001; Khandaker et al., 2013). Cognitive impairments commonly found in schizophrenic patients include deficits in WM, sensorimotor gating as well as in selective attention. The paradigms used in this research project to assess aspects of these cognitive domains are further discussed below, however, for a detailed description of the methodology, please refer to the method and materials sections of Chapters 5 and 7. It is beyond the scope of this PhD project to discuss all studies that ever used these paradigms, thus examples given will focus on research that utilized neurodevelopmental models to induce cognitive deficits.

### **4.1 Working memory**

Working memory (WM) can be defined as a short-term system with limited capacity to temporarily maintain and manipulate mental representations and is strongly influenced by attention (Bear et al., 2007; Park & Gooding, 2014). WM deficits are commonly observed in individuals with schizophrenia and other schizophrenia-spectrum disorders. For example, a recent study examined executive functioning and WM performance in schizophrenic patients (n=125) and healthy controls (n=64). Assessment tasks included a number of cognitive tests such as the Stroop Colour Word Test (name colour of the word and inhibit reading the word), the N-Back task (repeat sequence of stimuli n-steps earlier in sequence), and the Wisconsin Card Sorting Test (WCST, matching cards by colour, shape or numbers). Findings revealed that patients with schizophrenia required significantly more trials and had longer response

times across tests, thus confirming significant deficits in WM and executive functioning. Additionally, lower intelligent quotients were observed in this subgroup (Wongupparaj, Kumari, & Morris, 2015).

More importantly, prenatal exposure to maternal infection has been directly linked to various deficits in high level functioning. For example, Brown and colleagues (2009) examined cognitive performance in the WCST and the Trail Making Test (Trails B). The Trail Making Test version B refers to a task where participants are required to draw lines to connect circles in an ascending pattern while alternating between numbers and letters (i.e. 1-A-2-B-3-C etc.). Participants were individuals diagnosed with either schizophrenia, schizoaffective disorder or with other schizophrenia spectrum disorders (n=26) and matched controls (n=24). These individuals were participants of the Child Health and Development Study (1959 to 1966), California, USA. As part of this research, serological data collection during pregnancy allowed the detection of prenatal exposure to MIA induced by various infectious agents such as influenza or toxoplasmosis. Firstly, Brown and colleagues compared executive functioning between individuals with schizophrenia (and related disorders) and matched healthy controls. As expected, schizophrenic patients performed significantly worse on all tasks. Secondly, performance within patients was assessed by comparing those prenatally exposed to MIA (n=8) versus non-exposed (n=16). The results revealed that, in spite of the small number of subjects in each group, prenatal exposure to an immune challenge led to impaired performance in both aforementioned tasks, and particularly in the ability to shift attention compared to schizophrenic patients not exposed to MIA. More precisely, patients prenatally exposed to an immune challenge made significantly more total errors in the WCST and needed significantly more time to complete the Trail Making Test (Brown et al., 2009).

Assessment tasks utilized in pre-clinical studies to examine memory performance include, but are not limited to, the Morris water maze (more spatial memory), object recognition task (known versus novel objects, more episodic memory), and the radial arm maze (more spatial memory) (Yee & Singer, 2013). A number of animal studies used the model of MIA induced by, for example, LPS, as used in the present study to examine the effect of prenatal infections on aspects of memory. For example, Graciarena and colleagues (2010) found deficits in novel-object-recognition in male offspring rats in adulthood (PND 60) after an LPS-induced prenatal immune challenge (0.5 mg/kg, sc, GDs 14-20). In addition, findings showed a reduction of cell proliferation and neurogenesis. Likewise, Hao and colleagues (2010) looked at the impact of prenatal exposure to maternal LPS treatment

on performance in the Morris water maze. A deterioration of memory due to ageing (PND 10 months versus 20 months) was more pronounced in animals exposed to the immune challenge (GD 8, 10, 12). Variations in MIA inducing agents as well as outcome measures make comparisons of these results difficult, yet despite methodological differences, there was consistently impaired memory performance in subjects pre-exposed to MIA (for review Meyer, 2014). At this point it is important to note that these paradigms do not assess WM per se, however, as WM is an integral part of executive functioning it is difficult to tease functions apart.

There is still debate regarding the validity of paradigms used in animal studies to assess schizophrenia-relevant impairments in memory (Neuroscience and Biobehavioral Reviews (Editorial), 2013). However, patients with schizophrenia commonly show deficits in particular aspects of WM such as in goal maintenance and interference control (Barch & Smith, 2008). Thus animal models evaluating schizophrenia-related WM deficits in rodents should ideally incorporate these aspects. Performance measures such as the delayed alternation task (for example in a T-maze) require the animal to flexibly adapt to a rule and maintain information (goal maintenance) over a time delay while controlling for interference (Dudchenko, Talpos, Young, & Baxter, 2014). For these reasons, the paradigm called delayed non-matching to sample (DNMTS) was utilized in the present study to assess whether prenatal exposure to MIA leads to deficits in working memory. In this task, animals learn during training to retrieve a food reward by making a forced arm visit in trial one (in a T-maze with one arm blocked off). After a variable time delay (inter-trial interval) the animal has to visit the opposite arm in trial two (compared to trial one) to obtain a second food reward. Thus, the animal has to maintain a goal actively in mind, visit the opposite arm, while controlling for interference during the time delay between the two trials. The percentage correct second arm visits is then assessed.

#### **4.2 Sensorimotor gating measured in prepulse inhibition (PPI)**

Another core element of schizophrenia is impairment in information processing such as in sensorimotor gating. Normally developed individuals have the innate ability to inhibit a startle reflex when the startle-initiating stimulus is preceded by a weaker stimulus (prepulse). This process can be conceptualized as a temporal adaptation of the nervous system to strong incoming sensory stimuli to prevent overstimulation, and has been observed across species. A paradigm that assesses this adaptation process is called prepulse inhibition (PPI). Various types of stimuli can induce a startle response, however, a burst of white noise is most

commonly used. The startle reflex is generally measured by recording muscular reactions. In humans, movements of the oculomotor muscles, also called the eye-blink reflex, are analysed in response to prepulses at various intensities preceding the acoustic startle stimulus. It has been repeatedly shown that individuals with schizophrenia show reduced ability to inhibit the startle reflex after prepulse and therefore, prepulse inhibition is considered as a reliable biomarker for the disorder (Braff, Geyer, Light, et al., 2001; Braff, Geyer, & Swerdlow, 2001). More recently, studies found impairments in sensorimotor gating in individuals identified as high risk for developing psychosis (De Koning et al., 2014). Another study examined prepulse inhibition in individuals suffering from disorders along the schizophrenia-spectrum and found abnormalities were most severe in schizophrenic patients, yet significant deficits were also observed in individuals with schizotypal personality disorder (Hazlett et al., 2015). These findings suggest that anomalies in sensorimotor gating are evident before the onset of psychosis. Additionally, deficits in sensorimotor gating have been observed in other disorders with a neuropathological aetiology such as obsessive compulsive disorder, Tourette's syndrome or posttraumatic stress disorder (Braff, Geyer, & Swerdlow, 2001).

Prepulse inhibition (PPI) paradigms utilized in animal research are comparable to those used in human studies with the exception that whole body motor movements in response to startle reflex in animals are recorded and measured in startle chambers (for review Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001). A number of studies have used prenatal exposure to MIA to induce sensorimotor gating abnormalities in rodents. Findings show that PPI is a robust effect following prenatal immune challenges, in spite of considerable methodological differences between studies. For example, Borrell and colleagues (2002) administered LPS on alternate days throughout pregnancy (1 mg/kg, sc) and found gender as well as age-dependent disruptions in PPI in offspring during adulthood (PND 60, 100, 300). Male rats prenatally exposed to MIA showed reduced inhibition at all three assessment ages, yet PPI disruptions were only observed on PND 100 and 300 in females. Romero and colleagues (2012) administered LPS daily throughout pregnancy (2 mg/kg, sc). PPI testing at five different age points starting in adolescence (PND 28, 35, 70, 170, and 400) showed age-dependent effects. The ability to inhibit startle reflex was significantly weaker during adolescence (PND 28, 35) compared to adulthood measurements. Consistent with Borrell et al., Romero and colleagues also found that male rats exposed to MIA showed more pronounced deficits in prepulse inhibition.

In sum, impairments in sensorimotor gating are commonly found deficits in patients with schizophrenia and prenatal exposure to MIA in animals has been identified as a significant contributing factor. A significant advantage of this paradigm in animal studies is the comparability to human assessment. Thus virtually identical techniques can be used in humans and rodents. This explains the popularity of the PPI test for schizophrenia. However, PPI is also disturbed in many other psychiatric disorders as, for example, in obsessive compulsive disorder or post-traumatic stress disorder (Braff, Geyer, & Swerdlow, 2001; Kohl, Heekeren, Klosterkötter, & Kuhn, 2013). It is therefore important not to limit the analysis to PPI only. For this reason, the present set of experiments also included the analysis of WM and selective attention.

#### **4.3. Selective attention in latent inhibition (LI)**

Deficits in attention and the impact on learning and memory are commonly observed impairments in schizophrenic patients. Dysfunctions in selective attention can be assessed in a variety of paradigms including latent inhibition (LI). The underlying concept of LI is the ability in normally developed individuals to “tune out irrelevant information” to optimise limited attentional resources. Repeated pre-exposure to a stimulus without any consequences leads to reduced attention allocated to this particular stimulus (Lubow & Gewirtz, 1995; Weiner, 2003). Due to extensive research in this domain, specifically in the relationship between schizophrenia and LI, this paradigm is viewed as a task with high face validity. Although the procedures to induce LI in humans and animals vary, the underlying processes remain the same. However, different theories have been suggested to explain LI. For example, as highlighted above, Lubow and colleagues propose LI is an “acquisition deficit” which occurs due to a decline of attention directed towards a particular stimulus after repeated pre-exposure without consequences (Lubow, 2005). Weiner’s “two-headed” LI model, on the other hand, describes LI as “an expression deficit resulting from a competition between the stimulus-no event and the stimulus-reinforcement association” after conditioning, thus suggesting LI occurs in the “retrieval memory” stages (Weiner, 2003, p. 259).

A number of studies have evaluated LI in patients with schizophrenia (Baruch, Hemsley, & Gray, 1988). Although, Swerdlow and colleagues (1996) did not identify any deficits in LI, neither in acute nor in chronic patients, there is a wealth of evidence that shows diminished LI in individuals with schizophrenia. For example, Rascle and colleagues (2001) examined LI in patients with schizophrenia (n=65) and healthy controls (n=40) and



found deficits in patients with acute schizophrenia. Consistent with these findings, Kaplan and Lubow (2011) compared LI in individuals with schizotypal traits, more specifically individuals with a low tendency for psychosis, and healthy controls. Interestingly, they found a gender and schizotypy interaction. Male, but not female participants, who presented with schizotypal features showed diminished LI.

Studies examining LI in animals are numerous, yet few studies looked at the impact of MIA on this phenomenon. Zuckerman and colleagues (2003) induced cognitive deficits in rats by mimicking a viral infection (polyI:C on GD 15) and found significant disruptions in LI in offspring in adulthood (around PND 90), however, no impairment was observed when animals were tested during adolescence (PND 35). These findings indicate that MIA in animals can lead to long-lasting neurodevelopmental disturbances in attention that emerge after puberty and are therefore suggested to be similar to those found in individuals with schizophrenia. Further, the authors suggest that the late onset might be related to processes of brain maturation after puberty. However, it should be recognised that, as discussed in previous sections, although schizophrenia indeed develops after puberty, cognitive deficits are likely to be present during the prodromal phase, and are even apparent in young children who later on develop schizophrenia. Thus, it may well be that deficits in LI may also already be present before puberty.

Overall, LI to assess impairments in selective attention in individuals with schizophrenia is considered as a task with good validity and impaired selective attention is considered a core element of the disorder. Further, as the same underlying mechanisms are involved in LI in humans and in animals, LI can be seen as a task with high construct validity to evaluate schizophrenia-like selective attention deficits across species (Lubow, 2005).

## **CHAPTER 5            NICOTINE AMELIORATES COGNITIVE DEFICITS INDUCED BY MATERNAL LPS EXPOSURE: A STUDY IN RATS<sup>1</sup>**

In Chapter 5 the effect of prenatal exposure to MIA compared to saline (LPS, 0.5 mg/kg, sc) was evaluated at three different gestational time periods: Gestation days 10/11, 15/16 and 18/19 as a neurodevelopmental animal model of cognitive deficits similar to those commonly found in individuals with schizophrenia. Cognitive testing in adulthood (postnatal day, PND 60) assessed sensorimotor gating (PPI), selective attention (LI), and WM (DNMTS). To determine whether repeated nicotine leads to pro-cognitive effects as suggested by the self-medication theory, nicotine was experimenter-administered once daily for ten days (0.6 mg/kg, sc) before animals were re-tested on the same tasks whilst nicotine injections continued throughout the experiment.

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<sup>1</sup> This chapter is composed of a manuscript with the following bibliographic detail:  
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## **Nicotine ameliorates cognitive deficits induced by maternal LPS exposure: A study in rats**

### **ABSTRACT**

**Rationale** Maternal exposure to infectious agents is a predisposing factor for schizophrenia with associated cognitive deficits in offspring. A high incidence of smoking in these individuals in adulthood might be, at least in part, due to nicotine's cognitive enhancing effects.

**Objectives** Here we have used prenatal exposure to maternal lipopolysaccharide (LPS, bacterial endotoxin) treatment at different time points as a model for cognitive deficits in schizophrenia to determine whether nicotine reverses any associated impairments.

**Materials and methods** Pregnant rats were treated subcutaneously (sc) with LPS (0.5 mg/kg) at one of three neurodevelopmental time periods (gestation days, GD 10/11, 15/16, 18/19). Cognitive assessment in male offspring commenced in early adulthood (postnatal day, PND, 60) and included: prepulse inhibition (PPI), latent inhibition (LI), and delayed non-matching to sample (DNMTS). Following PND 100, daily nicotine injections (0.6 mg/kg, sc) were administered and animals were re-tested in the same tasks (PND 110).

**Results** Only maternal LPS exposure early during foetal neurodevelopment (GD 10/11) resulted in deficits in all tests compared to animals prenatally exposed to saline at the same gestational time point. Repeated nicotine treatment led to global (PPI) and selective (LI) improvements in performance.

**Conclusion** Early but not later prenatal LPS exposure induced consistent deficits in cognitive tests with relevance for schizophrenia. Nicotine reversed the LPS-induced deficits in selective attention (LI) and induced a global enhancement of sensorimotor gating (PPI).

## INTRODUCTION

Schizophrenia develops due to an interaction of multiple factors of genetic and environmental origin (Caspi et al., 2005; Clarke et al., 2009; Fleming & Martin, 2011; Modinos et al., 2013; Mulle, 2012). Early pre- and postnatal environmental factors are central to the neurodevelopmental theory, which argues that disturbances early during neuronal development predispose an individual to schizophrenia (for review Khandaker, Barnett, White, & Jones, 2011; Khandaker et al., 2013; Murray & Lewis, 1987). Maternal infection (for example influenza, rubella, measles) has been identified as a prenatal risk factor and the subsequent process of inflammation is thought to interfere with early foetal brain development, increasing the susceptibility for the offspring to later develop schizophrenia (Brown, 2012; Ellman et al., 2008; Meyer & Feldon, 2009; Miller et al., 2013).

Based on findings in human research, studies have successfully utilized the model of prenatal exposure to maternal infectious agents in animals. These preclinical studies have revealed important behavioural, neurophysiological and neurochemical alterations relevant to those found in individuals with schizophrenia (for review Boksa, 2010; Brown & Derkits, 2010). However, a large variety of protocols to expose animals to infectious agents exist and many different behavioural effects have been reported. For example, several different agents such as lipopolysaccharide (LPS, a bacterial endotoxin), polyinosinic:polycytidylic acid (polyI:C, a viral mimetic), or turpentine (induces local inflammation) have been used to induce maternal immune activation (MIA). Likewise, these agents have been administered at various time points during gestation using either a single injection or repeated administrations. Finally, relatively large differences in the doses have been reported. For instance, while some injected pregnant rats on two consecutive gestational days (GD) with 0.025 – 0.1 mg/kg intraperitoneally (ip) with LPS (Fortier et al., 2007), others have injected 0.5 mg/kg sc on GD 14, 16, 18 and 20 (Graciarena et al., 2010) or even 1 mg/kg sc every second day from GD 7 until delivery (Basta-Kaim et al., 2012).

These differences in protocol have led to varying behavioural and neurobiological effects, yet there is nonetheless significant face validity (for example phenotypical similarity) for schizophrenia in these models. However, much less is known with respect to the predictive validity of these models. Indeed, very few researchers have examined whether pharmacological manipulations affect alterations produced by maternal infectious agent exposure. Basta-Kaim and colleagues (2012), for example, have looked at the effect of antipsychotic medication on cognitive deficits induced by prenatal exposure to LPS (1 mg/kg, sc injections every second day from GD 7). Their findings revealed that clozapine,

but not chlorpromazine, reversed deficits in sensorimotor gating (Basta-Kaim et al., 2012). Whether these data have clinical relevance is currently unknown. In general, cognitive symptoms in patients are relatively unresponsive to antipsychotic medication (Ellenbroek, 2012). However, there is some evidence that sensorimotor gating may respond to (second generation) antipsychotics (for review Kumari & Sharma, 2002), yet longitudinal studies have provided contradictory results (Mackeprang, Kristiansen, & Glenthøj, 2002).

Cognitive symptoms occur in approximately 75% of all individuals with schizophrenia. Deficits can manifest in domains such as impairments in language, attention, memory, information processing, verbal memory, and executive functioning (Altamura et al., 2013; Brown et al., 2009; Khandaker et al., 2013). Cognitive dysfunctions are amongst the most debilitating and problematic symptoms in patients as they are, as aforementioned, not very responsive to antipsychotic medication (Ellenbroek, 2012), yet significantly impact on daily functioning (D'Souza & Markou, 2012; Green, 1996). Although the treatment of cognitive deficits in schizophrenia has proven particularly difficult, there is some evidence that nicotine can reverse some of the cognitive impairments (for review Heishman, Kleykamp, & Singleton, 2010). However, studies investigating the effect of nicotine in schizophrenia are confounded by the fact that the vast majority of patients are (heavy) smokers (de Leon & Diaz, 2005). Animal models, however, allow the assessment of potentially beneficial effects of nicotine in drug-naïve subjects.

The aim of the present study was to determine whether repeated nicotine exposure could reverse cognitive deficits induced by prenatal exposure to maternal LPS treatment. Given the many varying existing procedures for maternal infectious agent exposure, we first examined three different protocols to test whether these produce cognitive deficits and impairments in sensorimotor gating (similar to those found in individuals with schizophrenia) compared to controls prenatally exposed to maternal saline treatment at the same time points. To this end, LPS (0.5 mg/kg, sc) was administered to pregnant rats at one of three critical neurodevelopmental time points in foetal brain development (gestation days 10/11, 15/16, or 18/19) to model insults during early, middle or late stages of rat pregnancy (Fortier et al., 2007).

To assess cognitive performance in offspring in adulthood, three specific paradigms were used that measure aspects in domains that are commonly impaired in schizophrenia and utilize comparable parameters to those used in human assessment. Firstly, deficits in selective attention (ability to “tune out irrelevant information”) were assessed in latent inhibition (LI) (Gray, Pilowsky, Gray, & Kerwin, 1995; Lubow & Gewirtz, 1995; Weiner,

2003; Weiner & Feldon, 1997). Patients with schizophrenia often show a lack of LI, especially during the early stages of the illness (Lubow & Gewirtz, 1995; Weiner, 2003). Secondly, deficits in working memory such as goal maintenance and interference control are also frequently found in schizophrenia (for review Park & Gooding, 2014). Thirdly, impaired sensorimotor gating assessed in prepulse inhibition (PPI) is commonly seen in patients with schizophrenia (Braff, Geyer, Light, et al., 2001; Braff, Geyer, & Swerdlow, 2001; De Koning et al., 2014).

To our knowledge, this is the first study to examine the effect of repeated nicotine treatment on multiple cognitive impairments induced by prenatal infectious agent exposure in rats.

## **MATERIALS AND METHODS**

### **Subjects**

Subjects were male Sprague Dawley rats that were exposed to either prenatal maternal infectious agent exposure (N=29, 8 litters) or saline (N=35, 8 litters). The pregnant females used to breed these subjects were randomly assigned to 1 of the 6 different treatment conditions (either LPS or Saline injections at 1 of 3 gestational periods). All animals, (pregnant dams and their male offspring) were bred in the vivarium at Victoria University of Wellington. Animals were weaned at PND 21 and housed in groups of 2 to 4 with unlimited access to food and water in housing facilities on a 12-hour light/dark cycle (lights on at 7:00, lights off at 19:00). Rooms were humidity (77%) and temperature (21°C) controlled. From PND 50 onwards and for the duration of the experiment, the animals were housed in facilities with a reversed light dark cycle (light: 19:00-7:00) and placed on a food restriction (85 – 90% of their normal body weight). Animals were fed approximately 20 g of food pellets (Diet 86, Sharpes Stockfeeds, Carterton/New Zealand) per day following testing. This mild food restriction maintains a healthy gradual weight gain over time and has been previously successfully utilized in our laboratory and others to facilitate learning (Brennan et al., 2015). No statistically significant differences between treatment groups were observed in litter size and birth weight or in adult weight throughout the experiment. Cognitive and behavioural testing commenced around PND 60. For a timeline of the experimental design refer to supplementary Table S1.

### **Drug treatments**

#### Prenatal treatment

Pregnant female rats were administered a once daily injection on two consecutive days with either LPS (0.5 mg/kg, sc) or saline at one of the following gestational periods: GD 10/11, 15/16, or 18/19. This moderate LPS dose was chosen as it has previously led to a) persistent microglial activation and b) a down-regulation of transforming growth factor (TGF beta 1) especially in the hippocampus, leading to a long-lasting reduction in cell proliferation and neurogenesis (Graciarena et al., 2010). Subjects for the present study were male offspring born to these dams. This group will also be referred to as “LPS group” for simplification purposes, although offspring have not been directly exposed to the endotoxin LPS (prenatal exposure to maternal LPS treatment). For group size information refer to supplementary Table S2.

### Nicotine treatment

Around PND 100 all animals received experimenter-administered injections of nicotine (0.6 mg/kg, sc, and dose refers to the base weight) once daily for 10 consecutive days before re-testing commenced, where daily nicotine injections continued during the re-testing phase and were administered 30 minutes prior to testing. This nicotine dose was based on the observation that rats readily self-administered 0.5 to 1 mg/kg in a 2-hour session (Brennan, Putt, Roper, Waterhouse, & Truman, 2013; Brennan et al., 2015) in our laboratory. In addition, this dose had an ameliorating or decreasing effect on cognitive deficits in a dose response study within our laboratory (unpublished data).

### **Apparatus/Procedure**

#### Cognitive and behavioural tests

As some of the cognitive/behavioural tests required days/weeks of training/testing, paradigms were administered in a random and counter-balanced order during re-testing (commencing PND 110) to account for differences in nicotine levels and age differences. The minimum average time difference between test and re-test was 40 days. Testing occurred between 09:00 and 17:00, Monday to Sunday. Each test session began with a general habituation period to the experimental room of 15 minutes.

#### PPI

Average startle response (ASR) and PPI of acoustic startle were assessed using 4 startle chambers (San Diego® Instruments, San Diego, USA), for more details refer to (Ellenbroek, Liegeois, Bruhwyler, & Cools, 2001). Startle trials (P120) consisted of a single acoustic burst of white noise (120 dB, 20 ms). Pre-pulse trials included prepulses with different intensities (72 dB, 74 dB, 78 dB, or 86 dB) of white noise for a duration of 20 ms followed by a startle trial after a latency of 100 ms. Each session started with 5 startle trials, followed by 10 blocks of startle and prepulse inhibition trials and finished with another block of 5 startle trials. Percent Prepulse inhibition (% PPI) was calculated as follows:  $(1 - [\text{startle amplitude on prepulse trial} / \text{mean startle amplitude on startle trial}]) \times 100$ .

#### LI

LI was assessed using the conditioned taste aversion paradigm. Within groups, animals were randomly assigned to a pre-exposure (PE) or non-pre-exposure (NPE) group and water deprived 23.5 hours before the start of the experiment (Ellenbroek, Budde, &



Cools, 1996). Animals assigned to the pre-exposure group had once daily (3 days) access for 30 minutes to a drinking bottle with 100 ml of a 5% sucrose solution; the non-pre-exposure animals had access to a drinking bottle containing plain water. During conditioning, all animals had access to a bottle filled with the sucrose solution for 30 minutes, following an injection of lithium chloride (LiCl) (75 mg/kg; in 10 ml/kg, ip) to induce conditioned taste aversion. The following day (testing day) all animals had access to both, a bottle with 5% sucrose solution and plain water. The ratio of sucrose water consumption was calculated as follows: Day 5 sucrose consumption / (day 5 sucrose + water consumption) (Ellenbroek et al., 1996).

### DNMTS

DNMTS was assessed using a T-maze with the dimensions: arm length 30 cm, width 9 cm and wall height 10 cm. Animals were habituated to the maze and familiarized with retrieving sugar pellets from both arms before training commenced. Once the animal had reached an accuracy of 75% correct second arm visits on 3 consecutive days during training, working memory testing commenced. During working memory assessment, 4 sessions with 2 trials each for 3 consecutive days were performed. The inter-trial intervals varied (5, 30, 60 and 120 seconds) and the sequence was randomly assigned over the 4 sessions per day with the restriction that every interval was selected once per day per animal. Variable of interest was percent (%) correct of initial arm visits in trial 2.

### **Drugs**

(-)-Nicotine hydrogen tartrate salt and LPS (*Escherichia coli* 0111:B4) were obtained from Sigma-Aldrich® (Dorset, UK) and dissolved in 0.9% sterile saline. The nicotine solution was adjusted to pH 7.2 - 7.4 with NaOH. Lithium chloride was obtained from SciChem (Bilston, West Midlands, UK) and dissolved in 0.9% sterile saline.

All methods and procedures used in this study are in accordance with the guidelines and have been approved by the Victoria University of Wellington Animal Ethics Committee (reference number AEC2013-R7).

**Statistical analysis**

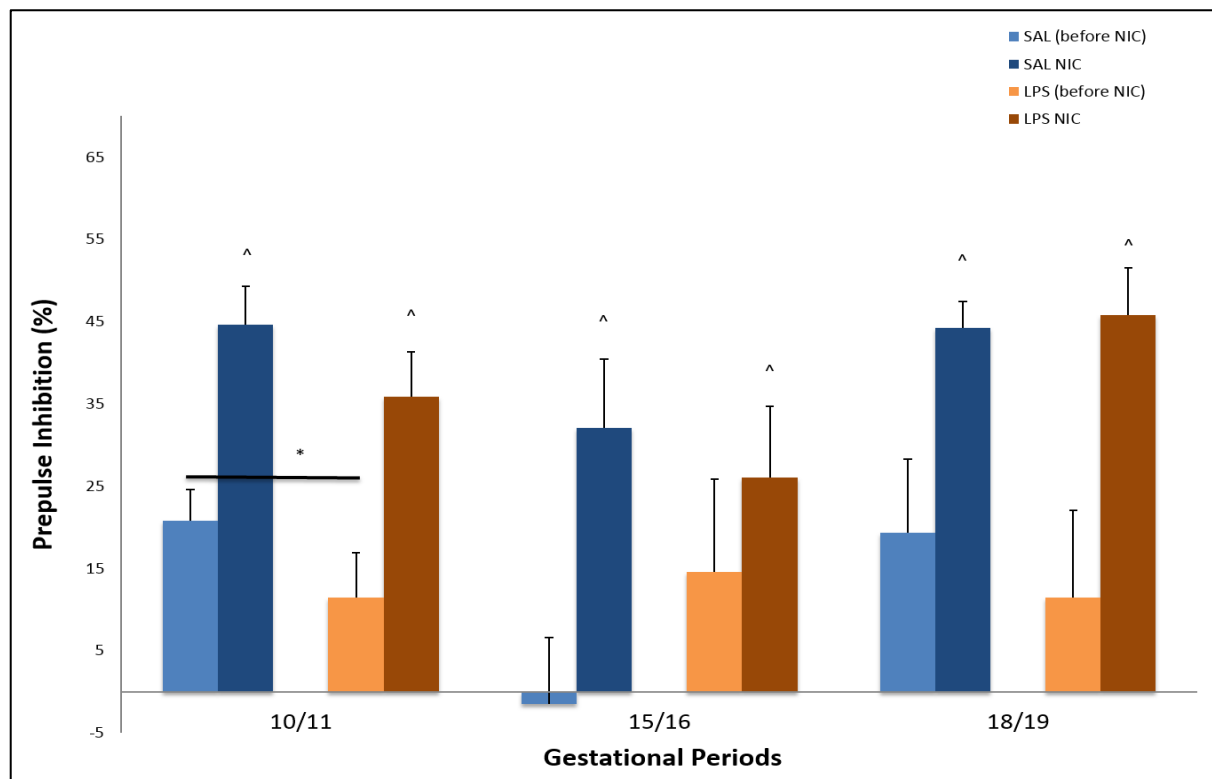
Data analysis was performed using mixed analysis of variance (ANOVA) where gestational periods were considered separately, with p values  $< 0.05$  considered to be statistically significant. For a detailed explanation of the within- and between-subjects factors per paradigm please refer to the individual tests in the result section.

## RESULTS

The results of the present study are displayed in Figures 1 to 3.

### Prepulse Inhibition (PPI)

Two outliers were detected ( $>3$  SD from the mean) and these were removed from the analysis. There was a significant effect of prepulse intensity (with larger prepulses leading to stronger inhibition), but none of the interactions (with either prenatal or adult treatment) were significant. All prepulses were therefore collapsed into a single mean PPI value per experimental group (see Figure 1). A mixed ANOVA was performed with the within-subjects factor Time (before versus after nicotine treatment) and the between-subjects factor Prenatal treatment (LPS versus Saline).



*Figure 1* Prepulse inhibition (%) as group average for animals prenatally exposed to maternal lipopolysaccharide treatment (LPS) or saline control (SAL) at 3 gestational periods measured at 2 time points, pre and post nicotine treatment (mean  $\pm$  s.e.m.). Group sizes before and after nicotine are equal: GD 10/11 SAL  $n=12$ , LPS  $n=11$ ; GD 15/16 SAL  $n=11$ , LPS  $n=7$ ; GD 18/19 SAL  $n=12$ , LPS  $n=11$ . The asterisk (\*) indicates a significant difference for prenatal treatment (between subject factor) at gestational days 10/11 (prenatally exposed to maternal LPS versus saline) ( $P < 0.05$ ). The accent (^) indicates a significant difference for time (within subject factor, before versus after nicotine treatment) evident at all gestational periods (GD 10/11:  $P < 0.001$ ; GD 15/16:  $P = 0.003$ ; GD 18/19:  $P = 0.001$ , mixed ANOVA).

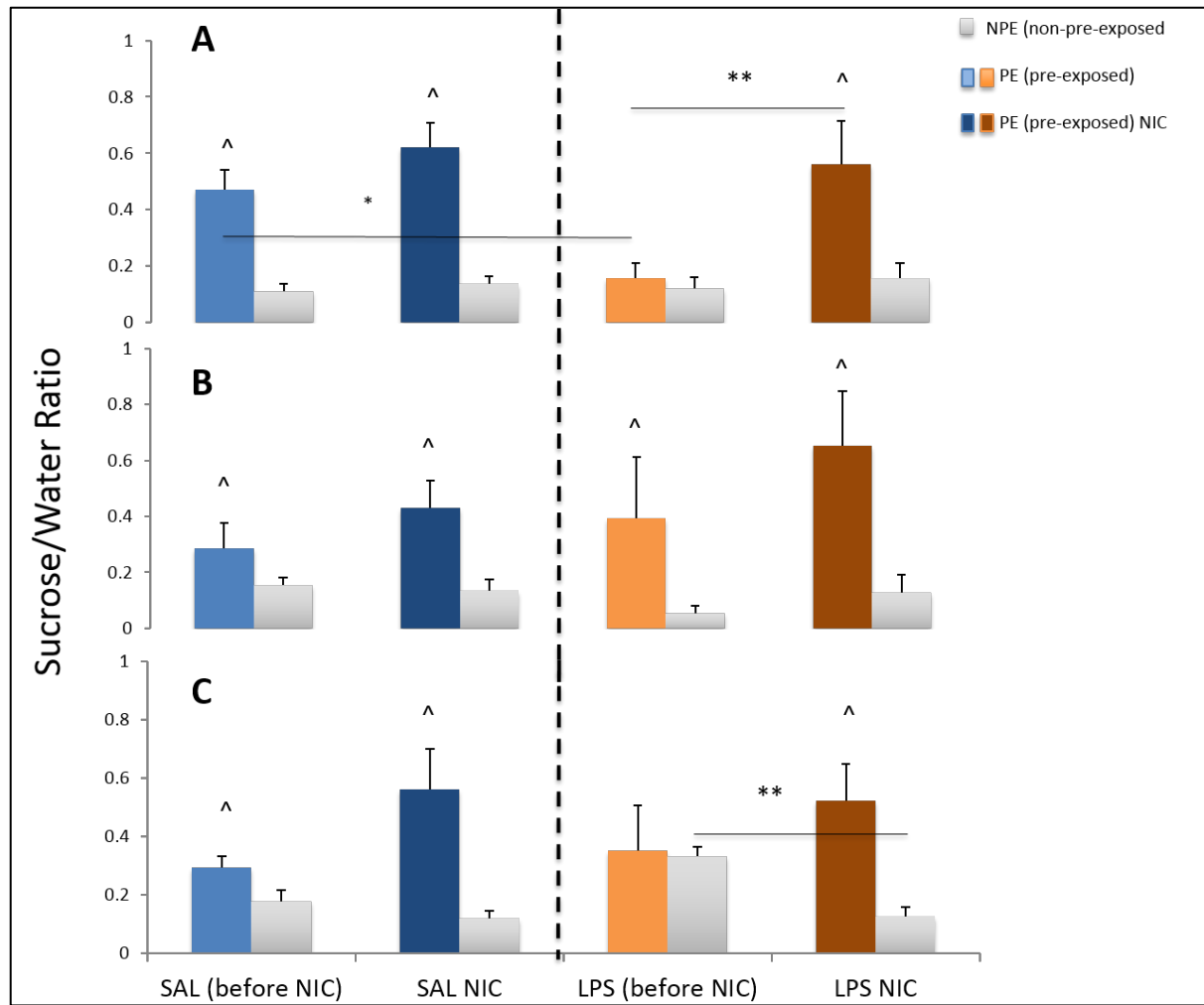
Statistical analysis showed that there was a significant main effect of prenatal treatment on GD 10/11 on PPI: ( $F(1,19) = 5.780, P < 0.05$ ). Figure 1 shows that rats prenatally exposed to LPS had significantly reduced PPI. There was no main effect of prenatal treatment on days 15/16 or 18/19.

Nicotine treatment significantly increased PPI in all pretreatment groups: GD 10/11:  $F(1,19) = 18.125, P < 0.001$ ; GD 15/16:  $F(1,15) = 12.185, P = 0.003$ ; GD 18/19:  $F(1,20) = 16.244, P = 0.001$ . As there were no interactions between prenatal and adult treatment, the results indicate that nicotine improved PPI independent of prenatal treatment. This is also evident from Figure 1, where all the groups (saline and LPS prenatal treatments) increased PPI.

There was a statistically significant difference in basal startle amplitude (see Table S3) between treatment groups before nicotine treatment for GD 10/11  $F(1,20) = 21.082, P < 0.001$ , but not for the other prenatal treatment periods.

### **Latent Inhibition (LI)**

The results of the LI experiments are shown in Figure 2 A-C. A mixed ANOVA was performed. The within-subjects factor was Time (before versus after nicotine treatment) and the between-subjects factors were 1) Prenatal treatment (LPS versus Saline) and 2) LI Pre-exposure (Sucrose versus Water).



**Figure 2** Latent inhibition as group average (consumption ratio, defined as sucrose consumption/sucrose + water consumption on testing day) for animals prenatally exposed to maternal lipopolysaccharide treatment (LPS) or saline (SAL): (A) GD 10/11, (B) GD 15/16, and (C) GD 18/19 at 2 time points (before versus after nicotine exposure) (mean  $\pm$  s.e.m.). Group sizes before and after nicotine are equal: GD 10/11 SAL/PE  $n=6$ , SAL/NPE  $n=6$ , LPS/PE  $n=6$ , LPS/NPE  $n=5$ ; GD 15/16 SAL/PE  $n=6$ , SAL/NPE  $n=5$ , LPS/PE  $n=3$ , LPS/NPE  $n=4$ ; GD 18/19 SAL/PE  $n=7$ , SAL/NPE  $n=5$ , LPS/PE  $n=5$ , LPS/NPE  $n=6$ . The accent (^) indicates a significant difference for LI pre-exposure at all gestational time points (between subject factor, pre-exposure/sucrose versus non-pre-exposure/water) (Mixed ANOVA, GD 10/11:  $P < 0.001$ , GD 15/16:  $P < 0.005$ , GD 18/11:  $P < 0.001$ ). The asterisk (\*) indicates a significant interaction between prenatal treatment (between subject factor, maternal LPS treatment versus saline) at GD 10/11 (A) in LI pre-exposure (Mixed ANOVA,  $P < 0.02$ ). LI occurred in SAL/PE, but not in LPS/PE. At GD 10/11 (A), two asterisks (\*\*) indicate a significant effect of Time (before versus after nicotine, mixed ANOVA,  $P < 0.005$ ) where nicotine normalized LI in the LPS/PE, but had no effect on LPS/NPE. At GD 18/19 (C) two asterisks (\*\*) indicate a significant interaction between LI non-pre-exposure and time (mixed ANOVA,  $P < 0.03$ ). Nicotine normalized failed conditioning in LPS/NPE.

Statistical analysis revealed that for all prenatal periods there was a significant effect of pre-exposure: GD 10/11:  $F(1,19) = 26.370$ ,  $P < 0.001$ ; GD 15/16:  $F(1,13) = 15.345$ ,  $P < 0.005$ ; GD 18/19:  $F(1,19) = 19.441$ ,  $P < 0.001$ . This shows that LI occurred where the pre-exposed animals drank significantly more sucrose on the test day than non-pre-exposed rats. However, in the GD 10/11 group there was a significant interaction between prenatal treatment and pre-exposure:  $F(1,19) = 7.317$ ,  $P < 0.02$ , but there was no three way interaction. Inspection of Figure 2A shows that latent inhibition occurred in the prenatal saline group but not in the prenatal LPS group (maternal LPS exposure), due to a significantly diminished effect in the LI pre-exposed group.

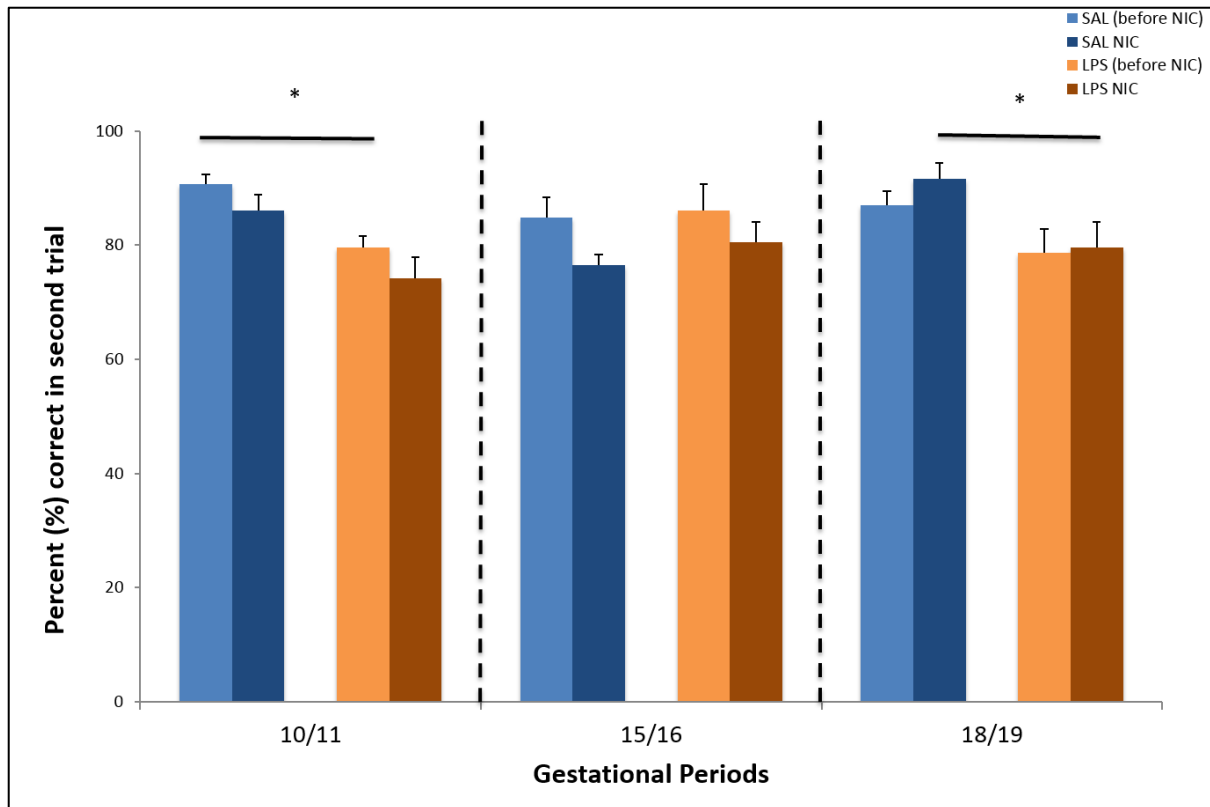
There was no significant interaction between prenatal treatment and pre-exposure in the GD 15/16 group (see Figure 2B). Whereas, for the GD 18/19 group there was again a significant interaction:  $F(1,19) = 5.634$ ,  $P < 0.03$ . Interestingly, inspection of Figure 2C shows that in contrast to prenatal LPS on GD 10/11, exposure on GD 18/19 increased sucrose intake in the non-pre-exposed group, suggesting a reduction in conditioned taste aversion.

On GD 10/11 there was a main effect of nicotine treatment:  $F(1, 19) = 11.501$ ,  $P < 0.005$ . Nicotine treatment restored LI in the prenatal LPS group by increasing the sucrose consumption in the pre-exposed group, while not affecting the non-pre-exposed group.

Nicotine did not alter sucrose intake in any of the groups on GD 15/16, yet there was a significant interaction on GD 18/19 between nicotine treatment and pre-exposure  $F(1,19) = 5.634$ ,  $P < 0.03$ , but no three-way interaction. Sucrose consumption decreased in the non-pre-exposed animals in the prenatal LPS group after nicotine exposure, thus nicotine ameliorated the deficit in conditioning induced by exposure to maternal LPS treatment.

### **Delayed non-matching to sample (DNMTS)**

A mixed ANOVA was performed with the within-subjects factor Time (before versus after nicotine treatment) and the between-subjects factor was Prenatal treatment (LPS versus Saline). There was a significant effect of pretreatment on accuracy for GD 10/11:  $F(1,18) = 14.749$ ,  $P < 0.005$ . Inspection of Figure 3 revealed that the LPS group GD 10/11 made significantly more errors than the saline controls. No statistically significant effect was found in any of the other pretreatment groups.



*Figure 3* Delayed non-matching to sample for 3 gestational periods. All data are given as mean values (mean  $\pm$  s.e.m.) in percent (%) correct second arm visits over 4 trials/day on 3 consecutive days. Group sizes before and after nicotine are equal: GD 10/11 SAL  $n=12$ , LPS  $n=11$ ; GD 15/16 SAL  $n=11$ , LPS  $n=7$ ; GD 18/19 SAL  $n=12$ , LPS  $n=11$ . The asterisk (\*) indicates a significant difference for prenatal treatment (between subject factor, maternal LPS treatment versus saline) at GD 10/11 before nicotine treatment (Mixed ANOVA,  $P < 0.005$ ). At GD 18/19 the asterisk (\*) indicates a significant difference for prenatal treatment after nicotine treatment ( $P < 0.01$ ).

In contrast to the previous two tests, there was no significant effect of nicotine in any of the pretreatment groups (Figure 3). However, a statistically significant effect of pretreatment on accuracy was evident for GD 18/19 after nicotine exposure:  $F(1,16) = 9.309$ ,  $P < 0.01$ . Inspection of Figure 3 shows that animals prenatally exposed to LPS made more errors compared to the saline animals in accuracy after nicotine exposure.

## DISCUSSION

The present study led to several main findings. First of all, prenatal exposure to maternal LPS treatment on GD 10/11 led to significant deficits in all tests compared to saline controls: reduced PPI, LI and DNMTS. Maternal LPS administration later in development was ineffective in this respect. Secondly, specific cognitive deficits (LI) induced by maternal LPS exposure were ameliorated by repeated nicotine. Finally, a general cognitive enhancing effect by nicotine was observed in PPI in all treatment groups.

### **The effects of prenatal exposure to maternal LPS treatment**

The present findings were inherently consistent with previous studies as compared to saline controls, deficits were repeatedly found in the early intervention group (GD 10/11). Similarly to patients with schizophrenia, animals prenatally exposed to maternal LPS treatment at GD 10/11 showed impairment in PPI. These findings are also consistent with previous studies that have shown impairments in sensorimotor gating after a prenatal immune activation (Borrell et al., 2002; Romero et al., 2007; Romero et al., 2010), although some differences have been found in relation to timing (Fortier et al., 2007). Using a similar two-day LPS protocol, Fortier and colleagues (2007) found a significant reduction in PPI after LPS exposure on GD 15/16 and 18/19, but not GD 10/11. Methodological differences such as the dose of LPS and the route of administration might explain the discrepancies. The present study injected 0.5 mg/kg subcutaneously, whereas Fortier et al. administered 0.025, 0.05 and 0.1 mg/kg intraperitoneally. Fortier et al. reported that all pups in the GD 10/11 (but not GD 15/16) group died at the dose of 0.1 mg/kg, indicating that the GD 10/11 pups are very sensitive to maternal LPS exposure. Moreover, considering that we found no evidence of reduced litter size, the subcutaneous-injection protocol might have induced more subtle deficits. Compared to prenatal saline controls, maternal LPS exposure on later times during development did not lead to significantly reduced PPI, although the controls in the GD 15/16 group showed a relatively low prepulse inhibition.

Consistent with the PPI findings, a reduction in LI after maternal LPS exposure on GD 10/11 was found compared to saline controls GD 10/11. These results could relate to patients with schizophrenia, as this deficit in LI was specifically found in the pre-exposure (PE) group. Interestingly, maternal LPS exposure on GD 18/19 also reduced the difference between the PE and NPE group, yet in this case it was due to a selective alteration in the non-pre-exposed (NPE) group. This suggests a reduced conditioned taste aversion, although as is usual for latent inhibition experiments, we did not include a LiCl-free condition. Compared



to PPI, fewer studies have investigated the effects of maternal infectious agent exposure on LI. However, the rodent studies published thus far are consistent with our finding that early (Meyer, Feldon, Schedlowski, & Yee, 2005; Smith et al., 2007) but not late (Bitanhirwe et al., 2010) exposure reduced LI.

Finally, our results showed that prenatal exposure to maternal LPS treatment led to a small, but significant increase in errors in DNMTS, the effects again limited to the GD 10/11 group and could relate to several studies showing deficits in working memory in patients with schizophrenia (Park & Gooding, 2014). Several studies have found deficits in cognitive performance after maternal immune challenge such as deficits in the novel object recognition test (Graciarena et al., 2010) or spatial learning (Hao et al., 2010), but to our knowledge this is the first rat study investigating working memory deficits.

### **Mechanisms underlying the effects of prenatal exposure to maternal LPS treatment**

Our results show that prenatal exposure to maternal LPS treatment induced long-lasting cognitive deficits similar to those seen in schizophrenia. These results are largely congruent with previous studies using LPS or other immune activating agents such as polyI:C, or turpentine. Since these different agents appear to induce comparable deficits in offspring (Boksa, 2010; Meyer, 2014) it seems likely that they all affect a common process. The most parsimonious common process is general activation of the maternal immune system, especially as most agents, including LPS, cannot cross the blood-placental barrier (Ashdown et al., 2006; Oskvig, Elkahoul, Johnson, Phillips, & Herkenham, 2012). The maternal immune response involves the production of several different pro-inflammatory cytokines in both the mother and the foetus (Boksa, 2010; Meyer et al., 2009). Prenatal exposure to the cytokine interleukin-6 (IL-6, GD 12.5), for example, resulted in behavioural (PPI, LI) as well as transcriptional deficits in offspring in mice (Smith et al., 2007). Likewise, the effects of polyI:C are significantly reduced in IL-6 knock out mice (Smith et al., 2007).

Cytokines not only play a vital role in the immunological response to infection as they subsequently lead to the eradication of foreign, infectious agents (Curfs, Meis, & Hoogkamp-Korstanje, 1997), but are also involved in many aspects of normal brain development including neurogenesis and synaptogenesis (Howard, 2013; Meyer et al., 2009). Thus the “cytokine hypothesis” states that elevated cytokine levels induced by an immune challenge during gestation interfere with foetal brain development. Consistent with changes in brain morphology (Cui, Ashdown, Luheshi, & Boksa, 2009; Graciarena et al., 2010; Li et al.,

2009), alterations in neurotransmitter transmission (Boksa, 2010) have been reported as well as alterations in cell migration and synapse maturation (Cui et al., 2009). For example, maternal immune activation early during gestation (GD 9.5) led to reductions in dopamine and dopamine metabolite levels in the striatum (Kirsten et al., 2012; Kirsten, Taricano, Florio, Palermo-Neto, & Bernardi, 2010; Soto et al., 2013) as well as to abnormalities in dopamine and serotonin activity in the substantia nigra (SN) (Wang, Yan, Lo, Carvey, & Ling, 2009). These early alterations may affect adult brain functioning leading to abnormalities in dopamine signalling (Eyles, Feldon, & Meyer, 2012). The crucial role of DA in cognitive performance has been well established in prepulse inhibition (Ellenbroek et al., 1996; Mosher et al., 2015), latent inhibition (Diaz, Medellin, Sanchez, Vargas, & Lopez, 2015) as well as working memory (Cools, 2011; Cools & D'Esposito, 2011).

The development of the brain and specific brain structures is sequential (Workman et al., 2013), explaining why timing of the maternal infection is critical for the long-term outcome, as was evident in the present and other studies (Fortier et al., 2007; Meyer et al., 2007). Which brain regions are crucially altered in the early (GD 10/11), but not the later gestational period and how these regions are linked to the cognitive deficits observed in the present study remains to be investigated.

### **The effects of nicotine exposure**

So far the studies modelling maternal infectious agent exposure in rodents have primarily focused on the face validity, for example, does the model lead to abnormalities similar to those seen in schizophrenia. Very few studies have investigated whether pharmacological treatment can reduce these deficits. As mentioned in the Introduction, clozapine, but not chlorpromazine reversed the effects of repeated gestational LPS treatment on prepulse inhibition (Basta-Kaim et al., 2012). Likewise, in the only study examining the effects of nicotine in rodent models of infectious agent exposure, a single acute injection of nicotine was found to reverse the effects of neonatal polyI:C (5 mg/kg, sc, PND 2-6) on object recognition, but not in PPI (Yu et al., 2010). As nicotine has a relatively short half-life and repeated treatments are required to observe its full effect, we studied the effects of repeated nicotine exposure on LPS-induced cognitive deficits. The results revealed that nicotine, as predicted, improved performance, although intriguing differences were found between the three paradigms. With respect to PPI, nicotine induced a global increase in PPI, independent of prenatal treatment. Thus not only was the GD 10/11 deficit ameliorated, but also the normal performance of rats prenatally treated with saline (or LPS on days 15/16 or

18/19) was enhanced. Similar global increases in PPI have been reported in humans, in both healthy volunteers and patients with schizophrenia (Kumari & Postma, 2005; Levin, McClernon, & Rezvani, 2006; Newhouse et al., 2011). However, not all studies have shown an improvement by nicotine on PPI. As aforementioned, Yu et al (2010) found no enhancing effect of nicotine (0.15 or 0.5 mg/kg, sc) in mice prenatally exposed to maternal infectious agents. However, a single injection of nicotine was administered to model an acute effect as opposed to repeated nicotine treatment, which leads to different neurochemical alterations (Brennan et al., 2010) and therefore might affect cognitive performance differently.

Consistent with the findings in PPI, a trend for nicotine-induced increased LI was observed in all sucrose pre-exposure groups, yet this was not statistically significant. Additional experiments, for example, with reduced pre-exposure, are necessary to explain this phenomenon. However, nicotine treatment restored the LI disrupted by LPS on GD 10/11, by selectively increasing the sucrose consumption in the pre-exposed group. Thus, a more specific effect of nicotine was found in this paradigm. As LI has been directly linked to selective attention, these data are consistent with studies in humans (Bates, Mangan, Stough, & Corballis, 1995), where nicotine had a stronger effect in individuals with impairments in selective attention compared to those who displayed normal levels of attention (Hahn et al., 2012; Smucny, Olincy, Eichman, & Tregellas, 2015). A significant reduction in conditioned taste aversion was observed in the LPS GD 18/19 group, a phenomenon, which was also reversed by the administration of nicotine. Thus repeated nicotine reversed both the decreased sucrose intake in PE animals (GD 10/11) and the increased intake in NPE (GD 18/19). Since it did not significantly affect sucrose consumption in any of the other groups, the effect of nicotine on LI appeared to be specific for to LPS-exposed groups.

Finally, there was no change in performance on the DNMTS test, even in the group with a reduced performance (LPS on GD10/11) despite evidence that nicotine can improve working memory performance (D'Souza & Markou, 2012). While, Yu et al. (2010) found a dose-dependent nicotine-induced enhancement in object recognition memory, it should be noted that object recognition is regarded more as a model for episodic memory than working memory (Levin, Christopher, & Briggs, 1997).

A possible explanation for the absence of a nicotine-induced enhancement in the present study may be the presence of a ceiling effect. As with LI, it has been suggested that improvements in memory by nicotine are more pronounced in subjects with a lower baseline (Niemegeers et al., 2014). Whereas some studies report improvements in domains such as learning, memory and attention in healthy volunteers (McClernon, Gilbert, & Radtke, 2003;

Rusted, Trawley, Heath, Kettle, & Walker, 2005), others show a more beneficial and pronounced effect of nicotine in individuals with a reduced baseline performance such as presented by schizophrenic patients (Jacobsen et al., 2004; Jubelt et al., 2008; Myers et al., 2004). Choice accuracy in the present study for GD 10/11 ranged between 79% for the treatment and 90% for the control group. Performance at this level may be difficult to improve. On the other hand, we found a small, but significant increase in performance in the GD 18/19 group (from 87 to 91%). Thus it remains to be investigated whether the lack of effect in the GD 10/11 group could be due to a ceiling effect. One possible way to assess this would be to increase the inter-trial intervals, which would lead to an increased demand on working memory components and reduced accuracy.

### **Mechanisms underlying the effects of nicotine**

The underlying mechanisms how nicotine affects cognitive functioning remain largely elusive. Nicotine rapidly crosses the blood-brain-barrier (10 to 20 seconds) and binds to nicotinic acetylcholine receptors (nAChRs) (Benowitz, 2009), most predominantly to the  $\alpha 7$  and  $\alpha 4\beta 2$  subtypes. The importance of the cholinergic system and nAChRs in mediating cognitive processes has been observed across multiple species (Klinkenberg, Sambeth, & Blokland, 2011; Rezvani & Levin, 2001; Wallace & Porter, 2011) and improvements in humans have been shown in domains relevant to schizophrenia such as sensorimotor gating (Postma et al., 2006; Woznica, Sacco, & George, 2009), attention (Barr et al., 2008; Hahn et al., 2012), memory (Jubelt et al., 2008), and executive control (Petrovsky et al., 2013). Neuronal  $\alpha 7$  receptors, for example, exist at pre- and post-synaptic locations, thus they can rapidly mediate synaptic transmission, plasticity as well as neurotransmitter release relevant to cognitive functioning such as acetylcholine, dopamine, glutamate, serotonin and GABA (Benowitz, 2009; Brennan et al., 2010).

The presence of these receptors on cholinergic as well as dopaminergic neurons in brain areas such as the PFC highlights their significance in modulating a wide range of neurotransmitters crucial to cognition (for review Wallace & Porter, 2011). This is important as prenatal exposure to maternal infectious agents, as aforementioned, has been implicated in long-lasting alterations in most neurotransmitter levels, but in particular in dopamine (DA). Changes in gene expression responsible for the induction and specification of dopaminergic neurons have also been identified (Eyles et al., 2012).

The  $\alpha 7$  subtype has received great attention as a possible drug target to improve cognitive functioning (Keefe et al., 2013). Interestingly, many individuals with schizophrenia show reduced nAChRs levels (such as  $\alpha 7$ ) in particular in brain areas associated with cognitive processing, presumably due to genetic anomalies (for example CHRNA7) (Freedman, 2014). For these reasons it has been suggested that nicotine from cigarette smoke might lead to an optimization of neuronal activity levels in these brain regions by stimulating nAChRs, consequently leading to a beneficial and pro-cognitive effect in schizophrenia patients.

### **Limitations and future research**

The present study demonstrated that compared to saline controls, maternal LPS treatment produced cognitive deficits in offspring, reminiscent of schizophrenia, when the animals were exposed at GD 10/11. However, behaviour was only assessed at a single time point in adulthood (PND 60). Given that the symptoms of schizophrenia typically develop after puberty (although this is may be less evident for the cognitive symptoms), the model could be expanded by additional behavioural analysis earlier in life (i.e. before the period of postnatal days 35-45, which marks the onset of puberty in rats). Additionally, the assessment of other aspects of schizophrenia such as positive or negative symptoms in the maternal infectious agent exposure model would be of interest. In particular, if the timing of the infection during pregnancy affects the development of positive, negative and cognitive symptoms differently.

The main conclusion of this study is that nicotine treatment improves cognitive performance. However, the specific effects depend on the test: whereas nicotine increased PPI in all animals (controls and LPS-exposed), it only reversed the LPS-induced deficits in LI, without altering behaviour in control animals. Although the current study did not include a saline exposed control group when examining the effect of nicotine, as this would have exponentially increased the number of animals utilized to fulfil the minimum group size requirements, we since have replicated our findings that prenatal exposure to maternal LPS treatment (GD 10/11) leads to similar deficits, which did not change after saline treatment (Waterhouse et al., 2016, in preparation). Thus, this data confirms that prenatal maternal LPS leads to long-lasting deficits in these domains and that the enhancing effect observed can be attributed to nicotine exposure.

Additionally, the present study used experimenter-administered nicotine injections to evaluate a cognitive enhancing effect of nicotine on LPS-produced deficits. Since many patients with schizophrenia are heavy smokers, it has been hypothesized that this could be partly due to the self-medicating properties of nicotine (for review Kumari & Postma, 2005; Levin, 2013) and the present data support this hypothesis. However, it is well established that self-administering drugs produces differential neurological effects to those of non-contingently (experimenter) administered drugs (Chen et al., 2008; Hemby, Co, Koves, Smith, & Dworkin, 1997). For these reasons, an extension to the present work would be to investigate the effects of nicotine self-administration on cognitive deficits in the LPS animal model for schizophrenia.

### **ACKNOWLEDGEMENT**

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### **AUTHORS CONTRIBUTIONS**

UW and BE were responsible for the study concept and design. UW and VR contributed to the acquisition of animal data. UW was responsible for the data analysis and UW and BE assisted with the interpretation of findings. UW drafted the manuscript and KAB and BE provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

### **DISCLOSURE**

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

## SUPPLEMENTARY MATERIAL

**Table S1 – Experimental design**

<b>Prenatal</b>	<b>Postnatal</b>		
GD 10/11 GD 15/16 GD 18/19	COMMENCING PND 60	COMMENCING PND 100	COMMENCING PND 110
Maternal sc injections LPS (0.5 mg/kg)	PPI, LI, DNMTS	Daily sc nicotine injections (0.6 mg/kg)	PPI, LI, DNMTS

*Table S1* Time line for experimental design from prenatal treatment (maternal LPS exposure and saline) to adult assessment (before and after repeated nicotine exposure)

**Table S2 – Group sizes**

<b>Gestation day (GD)</b>	<b>Treatment group</b>	<b>n = group size</b>
10/11	LPS / LPS-NIC	11
	SAL / SAL-NIC	12
15/16	LPS / LPS-NIC	7
	SAL / SAL-NIC	11
18/19	LPS / LPS-NIC	11
	SAL / SAL-NIC	12

*Table S2* Group sizes per treatment group

**Table S3 – Basal startle amplitudes PPI**

<b>Treatment Group</b>	<b>GD 10/11</b>	<b>GD 15/16</b>	<b>GD 18/19</b>
LPS before nicotine	69.71	131.50	110.55
SEM	16.34	61.46	28.53
SAL before nicotine	416.61	220.09	149.45
SEM	74.37	85.66	31.44
LPS after nicotine	237.30	258.50	276.91
SEM	40.24	119.90	62.77
SAL after nicotine	368.72	581.45	364.27
SEM	73.69	354.63	181.08

*Table S3* Basal startle amplitudes in prepulse inhibition (PPI) as group averages for 3 gestational periods

## APPENDIX 5-1

### MATERIALS AND METHODS

#### Apparatus/Procedure

##### Pre-pulse Inhibition (PPI)

Average startle response (ASR) and PPI of acoustic startle were assessed using four startle chambers of San Diego® Instruments (San Diego, CA, USA). Each sound-attenuated startle chamber was equipped with a transparent Plexiglas cylinder (diameter: 8.2 cm, length: 25 cm) mounted onto a platform with a piezo-electrical element that measured animal movements. Rats were placed individually into the testing apparatus and the PPI sessions began with a 5-minute acclimatisation period during which the animals were exposed to constant white noise of 70 dB (Ellenbroek et al., 2001). The testing session per animal included a total of 71 trials and lasted about 15 minutes. Each session started with 5 startle trials (P120), where each trial consisted of a single acoustic burst of white noise (120 dB, 20 ms). Following the startle trials were 10 blocks of pre-pulse trials commencing with a “no stimulus” trial where no acoustic stimulus was present, but animal movements were measured to establish differences in baseline activity. Pre-pulse trials included pre-pulses with different intensities (72 dB, 74 dB, 78 dB, or 86 dB) of white noise for a duration of 20 ms followed by a startle trial after a latency of 100 ms. Each session finished with 5 consecutive startle trials. Basic startle amplitude (P120) was determined as the average amplitude of the first 5 startle trials. Percent Pre-pulse Inhibition (% PPI) was calculated as follows:  $(1 - [\text{startle amplitude on pre-pulse trial} / \text{mean startle amplitude on startle trial}]) \times 100$ .



*Figure 5.A1* Pre-pulse inhibition startle chamber



### Latent Inhibition (LI)

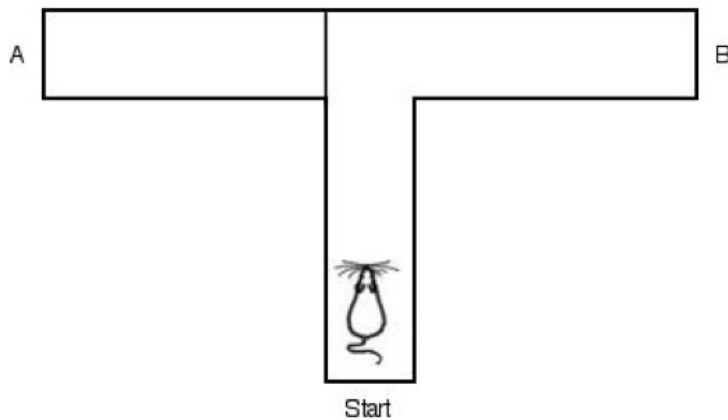
LI was assessed using the conditioned taste aversion paradigm (Ellenbroek et al., 1996). Water bottles were removed from all cages 23.5 hours prior to the experiment to ensure that the animals readily drank the provided fluid during the following 5 days. LI consisted of 3 days of pre-exposure, one conditioning day (day 4), and one testing day (day 5). Within groups, animals were randomly assigned to a pre-exposure (PE) or non-pre-exposure (NPE) group. As animals were housed in groups in their home cage, animals were allowed to habituate daily in the experimental room in individuals, clean cages for 30 minutes prior to the experiment. Animals assigned to the pre-exposure group had once daily (3 days) access for 30 minutes to a drinking bottle with 100 ml of a 5% sucrose solution, the non-pre-exposure group had access to a drinking bottle containing 100 ml of plain water. Bottles were weighed daily before and after access to determine liquid consumption (ml) per animal. During conditioning (day 4), all animals had access to a bottle filled with 100 ml of a 5% sucrose solution for 30 minutes, followed immediately after by an injection of lithium chloride (LiCl) (75 mg/kg, in 10 ml/kg, ip) to induce conditioned taste aversion. The following day (testing day, day 5), all animals had access to both, a bottle with 100 ml of 5% sucrose solution and a bottle with 100 ml plain water. The ratio of sucrose water consumption was calculated as follows:  $\text{Day 5 sucrose consumption} / (\text{day 5 sucrose} + \text{water consumption})$ .

### Delayed non-matching to sample (DNMTS)

Prior to the beginning of the DNMTS experiment, ceramic dishes with sugar pellets were placed into the home cages (2 days) to familiarise the animals with the food reward. DNMTS was assessed using a wooden T-maze with the dimensions: arm length 30 cm, width 9cm, and wall height 10 cm. The T-maze was raised 71 cm off the floor. Animals were individually placed into the maze for 5 minutes before the start of the experiment for maze-habituation during which the rats could freely move around. Days 1 to 3 were maze and food reward habituation days where animals learned to retrieve sugar pellets from small pellet holders located at the end of each vertical arm. Animals were removed from the maze after all sugar pellets had been retrieved or 5 minutes had passed. Basal training commenced on day 4 which consisted of 4 sessions per day. Each session included 2 trials where the first trial required a “forced” arm visit (one of the vertical arms of the maze was blocked off) to retrieve a sugar pellet. Animals were removed from the maze as soon as they had retrieved the food reward and placed into a holding cage for 5 seconds before trial 2 commenced.

During trial 2 both arms were accessible (block was removed) and the animal had to visit the opposite arm compared to trial 1 to retrieve a second food reward. The order of baited arms in trial 1 was randomly assigned. After the criterion of 75% accuracy in 2<sup>nd</sup> arm visits (trial 2) on 3 consecutive days was reached, animals commenced with working memory testing on the following day. During working memory assessment, 4 sessions with 2 trials each for 3 consecutive days were performed. The inter-trial-intervals varied (5, 30, 60, and 120 seconds). The sequence of intervals was randomly assigned over the 4 sessions per day with the restriction that every interval was selected once per day per animal. Variable of interest was percent (%) correct of initial arm visits in trial 2.

Trial 1 (forced arm visit)



Trial 2

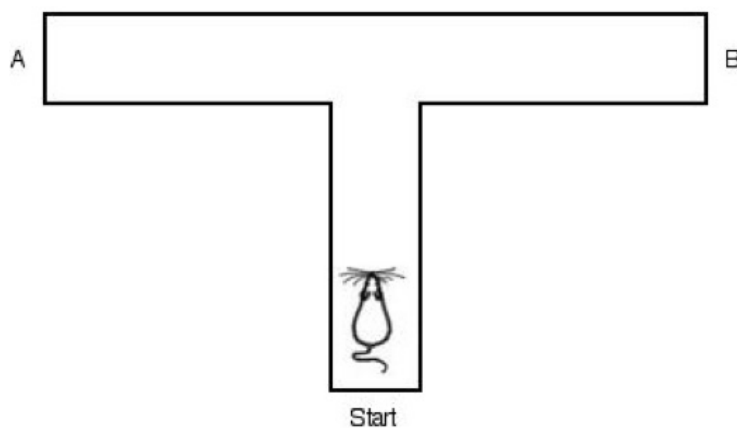


Figure 5.A2 T-maze trial 1 and 2

## RESULTS

### LI

#### *Total fluid intake on testing day (day 5)*

An analysis of total fluid intake (sucrose plus water) on testing day (day 5) revealed a main effect of nicotine exposure (before and after nicotine exposure) at GD 10/11:  $F(1,19) = 4.627$ ,  $p = 0.04$  as animals consumed significantly more fluid after nicotine exposure independent of prenatal treatment and LI pre-exposure. In addition, there was a main effect of LI pre-exposure (PE versus NPE):  $F(1,19) = 7.688$ ,  $p = 0.01$ . Those animals that were not pre-exposed to sucrose (NPE group) showed a higher total fluid intake independent of prenatal treatment or nicotine exposure, but there was no significant interaction.

There was also a main effect of nicotine exposure at GD 15/16:  $F(1,13) = 8.521$ ,  $p = 0.01$  as well as an interaction between nicotine exposure and LI pre-exposure:  $F(1,13) = 19.302$ ,  $p < 0.001$ . Those animals that were not pre-exposed to sucrose (NPE group) consumed significantly more fluid compared to pre-exposed animals after nicotine exposure independent of prenatal treatment. There was no statistical difference between groups at GD 18/19 in total fluid intake.

#### *Sucrose consumption on conditioning day (day 4)*

There was no statistically significant difference at GD 10/11. However, there was a three-way-interaction at GD 15/16 between prenatal treatment, LI pre-exposure and nicotine exposure:  $F(1,13) = 5.672$ ,  $p = 0.03$ . Animals exposed to MIA and LI pre-exposed to sucrose showed reduced intake of sucrose on conditioning day after nicotine exposure, whereas non-pre-exposed animals increased sucrose intake. The opposite occurred in prenatal controls as rats pre-exposed to sucrose displayed increased sucrose intake after nicotine exposure, whereas the non-pre-exposed group showed a decrease in consumption.

There was a main effect of nicotine exposure at GD 18/19:  $F(1,19) = 8.279$ ,  $p < 0.01$ . Animals consumed significantly less sucrose on conditioning day after nicotine exposure independent of prenatal treatment and LI pre-exposure. Furthermore, there was a main effect of LI pre-exposure:  $F(1,19) = 9.760$ ,  $p < 0.01$ . Total sucrose consumption was significantly increased in animals pre-exposed to sucrose during pre-conditioning compared to the non-pre-exposed groups independent of prenatal treatment and nicotine exposure.

*Sucrose consumption during pre-conditioning in PE groups*

There was a main effect of prenatal treatment at GD 10/11:  $F(1,10) = 8.876$ ,  $p = 0.014$  where animals prenatally exposed to MIA consumed more sucrose during pre-conditioning days 1 to 3 compared to controls. There was a statistically significant interaction between prenatal treatment and nicotine exposure at GD 15/16:  $F(1,7) = 6.371$ ,  $p = 0.04$ . Whereas nicotine treatment had no effect in animals prenatally exposed to MIA, total sucrose consumption was significantly reduced after nicotine exposure in controls. There was no statistically significant difference at GD 18/19.

## **CHAPTER 6                      CONTINGENT VERSUS NON-CONTINGENT DRUG ADMINISTRATION**

The traditional method for studying the effects of a drug in animals is via experimenter-administration. This non-contingent administration protocol allows for tight control over the dose and time of drug administration and is technically easy. Conversely, experimental subjects can also be allowed to contingently self-administer the drug as a more representative model of human drug addiction. Experiments have clearly shown that contingency can determine neurobiological and behavioural drug effects. In contrast to non-contingent (passive) administration, contingent (or active) drug administration paradigms incorporate cognitive processes that are associated with drug-taking behaviour (for review Jacobs, Smit, de Vries, & Schoffelmeer, 2003). One of the main focuses of the present study was to investigate why individuals with schizophrenia have high smoking rates. Thus, a contingent paradigm was deemed the most representative model of schizophrenic patients with the option to smoke.

Administration method-dependent differences in neuroadaptation are commonly examined by comparing self-administration paradigms and ‘yoked-designs’, where animals receive passive infusions (non-contingent control group) at the same time as self-administering animals obtain (contingent) drug infusions. Intravenous (iv) self-administration procedures are considered the ‘gold-standard’ in examining reinforcing effects of psychoactive drugs such as nicotine in animals. This model closely represents the human condition of drug taking such as smoking, thus provides high face validity (for review Goodwin, Hiranita, & Paule, 2015). In short, self-administration is assessed in operant chambers (see Appendix A) and usually requires animals to elicit a response such as pressing a lever to obtain a drug infusion. The self-administration model is utilized to examine reinforcing properties of drugs of abuse. Reinforcing properties refer to the “ability of a drug to increase the subsequent probability of a response that precedes its delivery” (O'Connor, Chapman, Butler, & Mead, 2011, p. 913). Self-administration in non-human primates preceded the use of rats in this paradigm. However, the self-administration model in rats has been evaluated in a large number of studies and high concordance within studies as well as in comparison to non-human primate studies has been established. Thus the rat self-administration model has high predictive validity in the assessment of a drug’s abuse potential (abuse liability) (for review O'Connor et al., 2011).

In addition to the pharmacological effect of a substance of abuse, other variables associated with drug taking are evaluated in the self-administration paradigm. For example, individuals who are smokers often report the desire to smoke when exposed to environmental cues associated with smoking such as drinking coffee or simply by being exposed to the smell of coffee (Garcia-Rodriguez, Pericot-Valverde, Gutierrez-Maldonado, Ferrer-Garcia, & Secades-Villa, 2012). Self-administration paradigms in preclinical research commonly utilize a light or a tone cue, which is paired with the drug infusion to model these environmental stimuli in human drug taking (Goodwin et al., 2015). The importance of associative learning in drug addiction has been highlighted by Everitt and Robbins (2005) and was already discussed in Chapter 2. Relevant to self-administration, the underlying process is based on Pavlovian conditioning, where the repeated pairing of an environmental cue such as a light with the primary reinforcer (drug of abuse such as nicotine) leads to the previously neutral stimulus (light) becoming a conditioned stimulus. Subsequently, the conditioned stimulus can elicit conditioned responses similar to those elicited by the primary reinforcer. Additionally, instrumental learning (or operant conditioning) plays a crucial part. The underlying concept of instrumental learning is that behaviours that lead to positive or satisfying outcomes are reinforced and strengthened and therefore increase in frequency. Responses with negative consequences, however, are weakened and will most likely decrease in occurrence (for review Rupprecht et al., 2015).

As aforementioned, many studies have utilized a ‘yoked control-operant paradigm’ to compare effects of contingent and non-contingent drug administration. This paradigm represents parameters of self-administration with an added component of passive drug administration in another subject. The number of received passive drug infusions (response-independent) depends on the number of lever presses performed by an animal actively responding for the substance (response-dependent). Therefore, this paradigm ensures an identical environment with the only distinguishing factor being the control over drug infusions (Jacobs et al., 2003). As yoked control animals have exactly the same pharmacological exposure (in terms of dose and frequency/timing of the administration) to the self-administering animals, the observed differences in neuroadaptations cannot be explained by the pharmacological properties (including the primary reinforcing effects) of the drug. Instead, these differences are most likely related to differences in the above mentioned Pavlovian and instrumental learning processes (Jacobs et al., 2003; Rupprecht et al., 2015).

Differences in neuroadaptive effects of psychoactive drugs such as cocaine, heroin, or amphetamine depending on the administration paradigm have been observed in neurotransmitter release such as dopamine, acetylcholine, serotonin and glutamate. Additionally, differences have been found in neurotransmitter turnover, for example, in dopamine, GABA, and acetylcholine. Differences in neuroadaptation are mainly assessed in brain regions associated with the reward pathway such as the nucleus accumbens or the ventral tegmental area (for review Jacobs et al., 2003). For example, Hemby and colleagues (1997) found differences in extracellular dopamine concentrations in the nucleus accumbens (NAcc). Although baseline dopamine concentration levels and cocaine concentration did not differ between groups, rats that self-administered cocaine (0.33 mg/infusion, 6 hour session, 25 days) showed significantly enhanced dopamine concentrations in the NAcc compared to littermates that received response-independent cocaine infusions (Hemby et al., 1997).

An example of differential neuroadaptations in receptor expression was a study by Stefanski and colleagues (1999). The effect of methamphetamine self-administration (0.1 mg/kg, nose pokes, 5 weeks) and passive drug injections (yoked design) were compared in rats. A significant decrease in somatodendritic dopamine D2 autoreceptor levels were found in the VTA (34%), the medial (31%), as well as the dorsal (21%) part of the substantia nigra pars compacta in animals self-administering methamphetamine compared to yoked controls. Additionally, a significant downregulation was observed in dopamine D1 receptors in the NAcc shell after self-administration (Stefanski, Ladenheim, Lee, Cadet, & Goldberg, 1999). Likewise, Chen and colleagues (2008) examined the effect of active (self-administration, 0.25 mg/kg/infusion, 2 hour session for 14 to 19 days) and passive (yoked design without paired stimulus or experimenter-administered ip injections) cocaine administration in rats. As opposed to animals that received cocaine infusions passively, animals that self-administered cocaine showed enhanced glutamatergic functioning in DA neurons in the VTA. Interestingly, this enhancement was resistant to behavioural extinction and was still evident after prolonged abstinence from cocaine (7, 21 and 90 days of abstinence) after three months (Chen et al., 2008).

Although similar yoked studies utilizing nicotine are not as numerous, variations such as alterations in neurotransmission in the mesocorticolimbic system as well as in glutamatergic plasticity in the hippocampal area have been revealed. For example, Metaxas and colleagues (2010) found administration paradigm and brain region dependent changes in nAChRs density utilizing a yoked control paradigm in mice. Animals self-administering nicotine (0.03 mg/kg/infusion, 1 hour sessions daily for 12 days, FR1) compared to those

animals that self-administered saline or passively received nicotine showed higher levels of nAChR binding (specifically the  $\alpha 4\beta 2$  subtype) in the dorsal-lateral geniculate nucleus and the VTA (Metaxas et al., 2010).

Donny and colleagues (2000) examined the effect of nicotine on the hypothalamic-pituitary-adrenocortical axis using a yoked design. Rats were allowed to self-administer either saline or nicotine (0.03 mg/kg/infusion, 1 hr daily session) or passively received nicotine infusions. Compared to saline controls, both response contingent and non-contingent groups displayed increased corticosterone levels within 15 minutes after exposure. Thus, consistent with previous findings, nicotine activated the hypothalamic-pituitary-adrenocortical axis shortly after exposure. Interestingly, corticosterone levels were back to baseline levels at the end of the session in subjects that actively administered the drug, yet levels remained elevated in animals that passively received nicotine. Likewise, increased plasma epinephrine and norepinephrine levels were observed in the yoked control group (Donny et al., 2000). Together these data suggest that animals that had control over the injections showed a reduced stress response (or a more rapid recovery of this response) compared to animals that had no control.

Similarly, Buczynski et al., (2013) studied the effect of active and passive nicotine in rats on cannabinoid-1 receptors (CB1). These receptors are part of the endocannabinoid system, which is involved in processes of motivation and pleasure such as appetite, pain sensation, mood and memory. Of relevance to nicotine, it has been suggested that CB1 receptors are involved in mediating the effect of nicotine in the brain leading to addiction (Chen et al., 2008). Buczynski and colleagues found that active but not passive nicotine self-administration led to significantly increased levels of anandamide (an endocannabinoid that activates CB1 receptors) in the VTA. Thus, their results demonstrate differences in endocannabinoid signalling based on the nicotine administration paradigm utilized (Buczynski, Polis, & Parsons, 2013).

These studies highlight the importance of administration paradigm-type on resulting neuroadaptations. Thus it is important to investigate nicotinic effects in response-independent and dependent settings before general conclusions regarding nicotinic effects can be made. The focus of previous research was mainly on brain areas associated with addiction, yet contingent nicotine compared to non-contingent exposure could also lead to differential neuroadaptive effects in areas associated with cognitive functioning.



## **CHAPTER 7            NICOTINE SELF-ADMINISTRATION REVERSES COGNITIVE DEFICITS IN A RAT MODEL FOR SCHIZOPHRENIA<sup>2</sup>**

The focus of the second part of this PhD research was firstly, to replicate findings from part 1 (Chapter 5), namely that prenatal exposure to maternal immune activation on GD10/11 leads to cognitive deficits. Secondly, to determine whether nicotine self-administration leads to pro-cognitive effects similar to those observed in the previous study where experimenter-administered nicotine was utilized. And finally, the self-administration protocol can be utilized to show whether animals prenatally exposed to MIA are more susceptible to the reinforcing properties of nicotine. This part of the study is designed to determine whether common underlying pathways may, at least in part, be responsible for high smoking incidence in individuals with schizophrenia as suggested by explanatory theory three (Chapter 3).

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<sup>2</sup> This chapter is composed of a manuscript with the following bibliographic details: Waterhouse, U., Brennan, K. A., & Ellenbroek, B. A. (submitted). Nicotine self-administration reverses cognitive deficits in a rat model of schizophrenia. *Addiction Biology*.

## **Nicotine self-administration reverses cognitive deficits in a rat model for schizophrenia**

### **ABSTRACT**

**Background** High comorbidity between schizophrenia and tobacco addiction has been well established. Explanatory theories include nicotine as a cognitive enhancer ameliorating symptoms of schizophrenia and underlying shared substrates increasing susceptibility to addiction in these individuals.

**Methods** To test these non-mutually exclusive theories, the maternal immune activation (MIA) model was utilized. To this end, pregnant Sprague Dawley rats were subcutaneously (sc) injected with a bacterial endotoxin, lipopolysaccharide (LPS, 0.5 mg/kg), on gestation days 10 and 11. Selective attention and working memory in adult male offspring were subsequently assessed using the latent inhibition (LI) and delayed non-matching to sample (DNMTS) paradigms both before and after nicotine or saline self-administration.

**Results** MIA led to deficits in both LI and DNMTS in male offspring. Further, these animals showed a small but significantly increased responding for nicotine during self-administration acquisition, although there was no difference in dose response effect or in progressive ratio testing. However, nicotine, but not saline self-administration significantly ameliorated the cognitive deficits induced by MIA.

**Conclusion** While the male offspring of mothers prenatally exposed to LPS were only slightly more sensitive to the reinforcing effects of nicotine, after self-administration the MIA induced cognitive deficits significantly improved. These data lend support for the self-medication hypothesis of schizophrenia.

## INTRODUCTION

Smoking tobacco remains the single most preventable cause of death worldwide despite declining smoking rates (Eriksen et al., 2012; World Health Organization, 2015). Similar to other substances of abuse, an intriguing aspect of smoking is that not everyone is equally susceptible to become addicted. Some individuals engage in periodic use, whereas others develop an addiction more rapidly (Stolerman & Jarvis, 1994). Individuals with a mental disorder (50%) and especially schizophrenic patients, have substantially higher smoking rates (70-90%) compared to the general population (25-30%). Moreover, more than half of the patients with schizophrenia who smoke, are heavy smokers (30+ cigarettes/day) (de Leon & Diaz, 2005) and they seem to smoke more “efficiently”, as evidenced by higher (urinary/saliva) levels of the nicotine metabolite cotinine (Olincy et al., 1997; Strand & Nybaeck, 2005).

Several theories have been proposed to explain the high comorbidity between schizophrenia and tobacco addiction. First, there is evidence that nicotine reduces some of the side effects of antipsychotic medication (Barr et al., 2008; Zhang et al., 2013). Second, nicotine, as a cognitive enhancer could ameliorate some of the cognitive deficits (for review Dome et al., 2010; Levin, 2013; Newhouse et al., 2011), and thus smoking could be seen as a form of self-medication. Third, the underlying aetiology and neurobiology of schizophrenia and substance use disorder (SUD) could partly overlap, and thus vulnerability for one would automatically infer increased vulnerability for the other. In agreement with this, common alterations in neurotransmitter activities and dysfunctions in receptors (D'Souza & Markou, 2012; Esterlis et al., 2014) have been reported in both disorders, as has reduced activity of the prefrontal cortex and reduced volume of the insular and anterior cingulate cortex (Goodkind et al., 2015). It is important to note that these theories are not mutually exclusive. However, as it is difficult to separately investigate these theories in humans, the present study examined the cognitive enhancer and common biological substrate theory, using a well-established animal model for schizophrenia.

Prenatal exposure to maternal infection (for example rubella, measles, influenza) has been identified as a risk factor for offspring to develop schizophrenia later in life. As most infectious agents cannot cross the blood-placenta-barrier, a common process such as maternal immune activation (MIA) is thought to interfere with early neuronal development. Specifically, elevated levels of pro-inflammatory cytokines play a crucial role in the immune response (Curfs et al., 1997) as well as in normal brain development (Howard, 2013) and have been suggested as the most parsimonious common process (for review Meyer et al.,

2009) leading to functional and structural changes such as those identified in individuals with schizophrenia (Brown, 2012; Meyer, 2013). Based on findings in human studies, MIA models using prenatal exposure to either polyI:C (polyriboinosinic-polyribocytidilic acid, which mimics a viral infection) and LPS (lipopolysaccharide, which mimics a bacterial infection) have been successfully utilized in animal research (for review Boksa, 2010; Brown & Derkits, 2010).

Recently we showed that maternal exposure to LPS (0.5 mg/kg, sc) at gestation days (GD) 10/11 (but not on GD15/16 or GD18/19) resulted in significant deficits in prepulse inhibition (PPI), latent inhibition (LI), and working memory (delayed non-matching to sample, DNMTS). Further, some of these deficits were ameliorated by repeated experimenter-administered nicotine (0.6 mg/kg/day, sc) (Waterhouse, Roper, Brennan, & Ellenbroek, 2016). This supports the hypothesis that schizophrenic patient might smoke to alleviate cognitive deficits. However, as there are important differences in neuroadaptation between experimenter- and self-administered drugs (Chen et al., 2008; Hemby et al., 1997), in the present study we aimed to investigate whether these cognitive deficits could be alleviated by nicotine self-administration. Moreover, this paradigm allows us to investigate whether MIA exposed rats are more sensitive to the reinforcing properties of nicotine, in agreement with the common underlying substrate theory.

To the best of our knowledge, this is the first study to conduct multiple cognitive tests and nicotine self-administration in a longitudinal animal model of MIA.

## **MATERIALS AND METHODS**

### **Subjects**

Subjects were male Sprague Dawley rats exposed to either MIA (N=32, 6 litters) or saline (N=26, 8 litters). All animals (dams and male offspring) were bred in the vivarium at Victoria University of Wellington. The offspring were weaned at postnatal (PND) 21 and housed in groups of 2-4 in a humidity (77%) and temperature (21°C) controlled room. From PND 50 onwards, animals were housed in a reversed light dark cycle (lights on: 19:00-7:00) and placed on a mild food restriction (85 – 90% of their normal body weight). Rats were fed approximately 20 g of food pellets per day (Diet 86, Sharpes Stockfeeds, Carterton/New Zealand) following testing (Brennan et al., 2015). No statistically significant weight differences were found between any of the treatment groups throughout the experiment. Cognitive testing started around PND 60, followed by catheter-surgery to prepare animals for self-administration (PND 100). Cognitive tests were repeated in the late stages of self-administration from PND 140 onwards (see supplementary Table S1 for a detailed timeline).

### **Prenatal drug treatments**

Pregnant female rats were administered subcutaneous (sc) injections once daily on two consecutive days with either LPS (0.5 mg/kg) or saline on GD 10/11 (Waterhouse et al., 2016). The subjects of this study were male offspring born to these dams. This group will also be referred to as “MIA group” for simplification purposes.

### **Drugs**

(-)-Nicotine hydrogen tartrate salt and LPS (*Escherichia coli* 0111:B4) were obtained from (Sigma-Aldrich®, Dorset, UK) and dissolved in 0.9% sterile saline. The nicotine solution was adjusted to pH 7.2 - 7.4 with NaOH and further diluted with saline to produce four doses (7.5, 15, 30, 60 µg/kg/infusion) with doses refer to the base weight. Lithium Chloride (LiCl) was obtained from SciChem (Bilston, West Midlands, UK) and dissolved in 0.9% sterile saline.

## **Apparatus/Procedure**

### Cognitive tests

Testing occurred between 06:00 and 17:00, Monday-Sunday. Each test session began with a general habituation to the experimental room of 15 minutes. LI and DNMTS were administered in a random and counter-balanced order during re-testing to account for differences in nicotine levels and age differences as these tasks required days/weeks of training/testing. During the re-testing, animals were maintained on an FR5 schedule. Self-administration was performed in the morning, followed (30 minutes) later by cognitive testing.

### LI

LI was assessed using the conditioned taste aversion paradigm. Within groups, animals were randomly assigned to a pre-exposure (PE) or non-pre-exposure (NPE) group and water deprived 24 hours prior to the experiment (Ellenbroek et al., 1996). The pre-exposure group had once daily (3 days, 30 minutes) access to 100 ml of a 5%-sucrose solution; the non-pre-exposure animals had similar access to plain water. During conditioning, all animals were given access to the sucrose solution, followed by an injection of lithium chloride (LiCl) (75 mg/kg; in 10 ml/kg, ip) to induce conditioned taste aversion. The following day (testing day) all animals had access to two bottles, one containing a 5% sucrose solution and one containing plain water. The sucrose preference was calculated as follows:  $\text{Day 5 sucrose consumption} / (\text{Day 5 sucrose} + \text{water consumption})$  (Ellenbroek et al., 1996).

### DNMTS

DNMTS was assessed using a T-maze with the dimensions: arm length 50 cm, width 25 cm, wall height 30 cm. Animals were habituated to the maze and familiarized with retrieving sugar pellets from both arms before training commenced. Once animals reached an accuracy of 75% correct second arm visits on 3 consecutive days during training, working memory (WM) testing commenced, which included 4 sessions with 2 trials each daily for 5 consecutive days. The inter-trial intervals varied (1, 2, 5, 10 minutes) and the sequence was randomly assigned over the 4 sessions per day with the restriction that every interval was selected once per day/animal. The dependent variable was percent (%) correct of initial arm visits in trial 2.

### Self-administration apparatus

For a more detailed description of self-administration apparatus/procedures please refer to supplementary material and (Brennan et al., 2015).

Nicotine self-administration was assessed in operant chambers (ENV 001, Med Associates, St. Albans, VT, USA) placed in sound-attenuating boxes in a temperature controlled (21°C) room. Each chamber was equipped with two levers. Depression of the right lever (active) activated a stimulus light and an intravenous drug infusion (0.25 ml). Depression of the left lever (inactive) had no consequences. All lever responses were recorded. The self-administration parameters were based on those used by (Brennan et al., 2015).

### **Surgical procedures**

Surgical procedures involved the insertion of a catheter into the jugular vein under anesthesia. Catheters were flushed daily with 0.2 ml of a sterile/heparinized saline solution containing penicillin. Catheter functionality was tested weekly, and in the event of failure, rats underwent repair surgery before returning back to self-administration after a 3-day recovery period (Brennan et al., 2015).

### **Self-administration procedures**

Rats from both treatment groups (MIA and SAL) were assigned to self-administer either vehicle control (MIA n=6, SAL n=6) or nicotine (30 µg/kg/infusion; MIA n=26, SAL n=20) during daily 2-hour sessions (Monday-Friday). To start the session, one experimenter-administered prime was initiated to fill the catheter with the drug.

### Fixed ratio (FR) schedules of reinforcement to establish self-administration

FR1 (days 1-10), followed by FR2 (days 11-15) and FR5 (days 16-25) were used to establish self-administration (training dose 30µg/kg/infusion). Nicotine self-administration was considered acquired when, during the final FR5 sessions, an animal responded at least 20 times/session more on the active lever than the inactive lever for a minimum of three consecutive days. Animals that did not reach this criterion, were not subjected to dose-response or progressive ratio testing, but continued on the FR5 schedule while re-tested on the three cognitive tests (see Table S2 for experimental design).

### Dose-response testing (FR5)

Those animals that acquired nicotine self-administration were tested on four doses of nicotine (7.5, 15, 30 and 60  $\mu$ /kg/infusion) for 3 days each. The sequence of nicotine doses was randomly assigned.

### Progressive ratio (PR)

PR testing followed DR testing and established an average breakpoint for each dose/animal and included successive response requirements (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, etc.) for 3 days, where each PR trial-day was followed by an FR5 day. The PR sessions timed out when 30 minutes had elapsed without a drug infusion (Brennan et al., 2015).

### **Statistical analysis**

Mixed analyses of variance (ANOVAs) with the within-subject factor Time (before and after self-administration) and the between-subject factor Group (prenatal MIA versus Saline exposure) and Drug (Nicotine versus Saline) was used to determine whether there was an effect of 1) prenatal MIA on cognition and 2) nicotine on cognitive deficits. Repeated ANOVAs with the within-subject factors of Day x Lever (active versus inactive lever) and the between-subject factor of Group were used to analyse differences during self-administration acquisition (FR1, 2 and 5 were considered separately). Differences in DR and PR breakpoints were analysed with the within-subject factor of Dose and Group as the between-subject factor.

All methods and procedures used in this study were in accordance with the guidelines and have been approved by the Victoria University of Wellington Animal Ethics Committee (reference number AEC2013-R7).



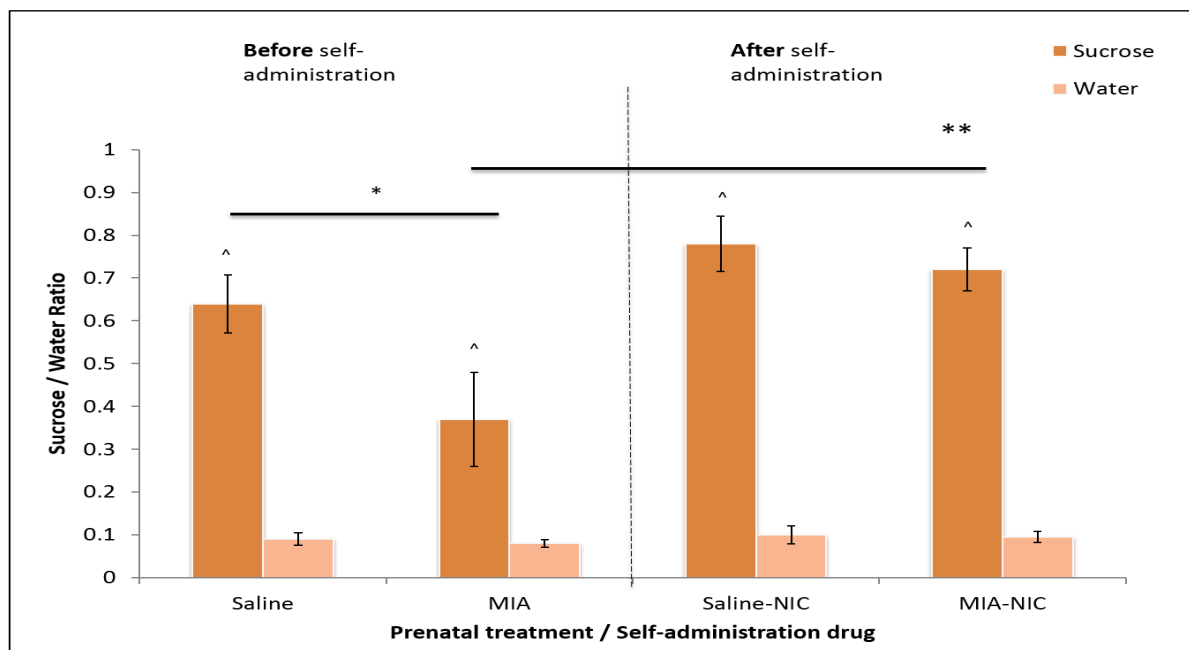
## RESULTS

The results of the cognitive tests before and after nicotine are presented in Figures 1, 2 and 7 followed by the effects of prenatal treatment on nicotine self-administration, which are presented in Figures 3-6.

### MIA induced cognitive deficits in rats

#### LI

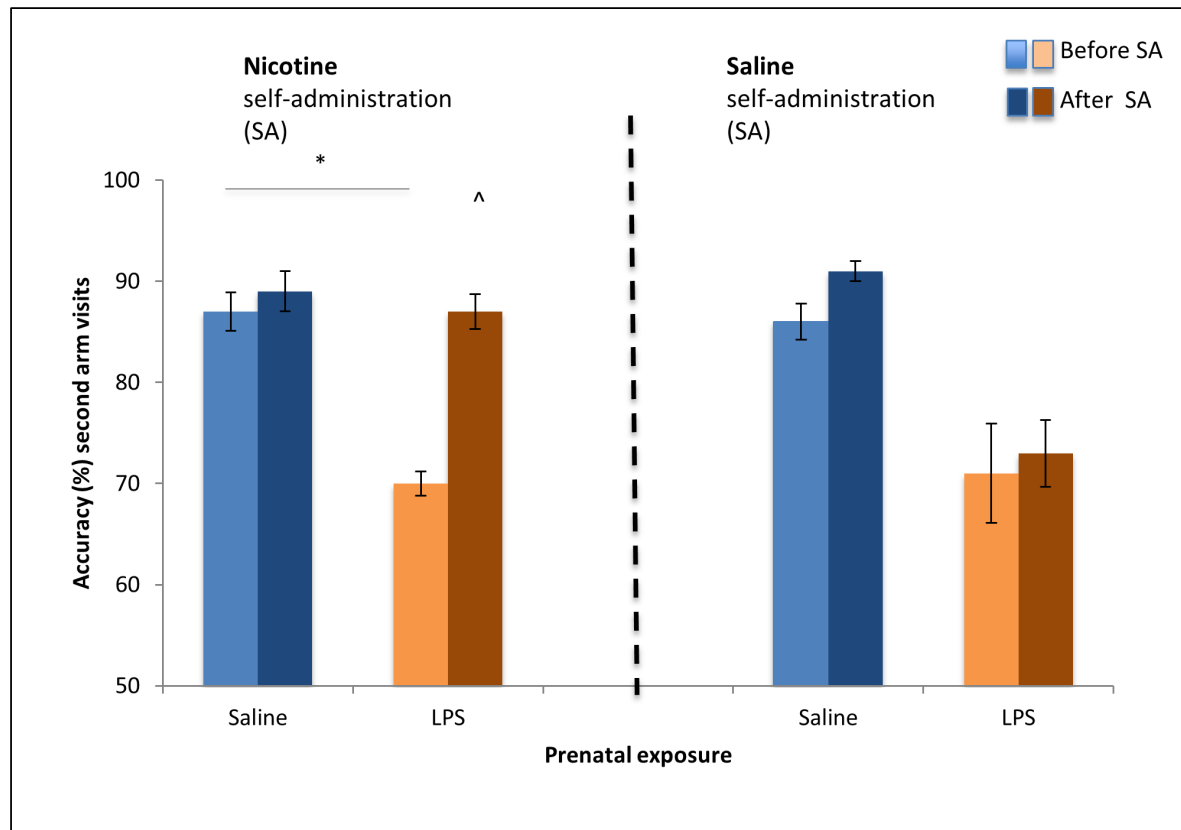
There was a significant main effect of Prenatal treatment:  $F(1,47) = 6.747$ ,  $p = 0.012$  and LI-exposure:  $F(1,47) = 67.370$ ,  $p < 0.01$  as well as an interaction between these factors:  $F(1,47) = 6.807$ ,  $p = 0.012$ . Inspection of Figure 1 revealed that control animals pre-exposed to sucrose drank significantly more of the sucrose at the final test day than non-pre-exposed groups, thus showing the expected latent inhibition effect. However, sucrose/water ratio was significantly lower following sucrose pre-exposure in the MIA group compared to the saline exposed controls, indicating a reduced LI. There was no statistically significant difference in sucrose intake during pre-conditioning between animals prenatally exposed to MIA and controls.



*Figure 1* Latent inhibition at 2 time points (before/after nicotine self-administration). Columns represent the group average for each treatment group (saline/sucrose  $n=10$ , saline/water  $n=10$ , MIA/sucrose  $n=13$ , MIA/water  $n=13$ ) in consumption-ratio sucrose versus water on testing day (mean  $\pm$  s.e.m.). The accent (^) indicates a significant difference for LI Pre-exposure (between subject factor, sucrose exposure versus water, mixed ANOVA,  $P < 0.01$ ). The asterisk (\*) indicates a significant difference for Prenatal treatment (between subject factor, prenatal MIA versus saline,  $P = 0.012$ ). Two asterisks (\*\*) indicate a significant interaction between Time and LI Pre-exposure (within subjects factor, before/after nicotine self-administration,  $P = 0.04$ ).

## DNMTS

A significant effect of Prenatal treatment on accuracy was observed:  $F(1,53) = 48.362$ ,  $p < 0.001$ . Animals prenatally exposed to MIA made significantly more errors in second trial arm visits compared to controls.



*Figure 2* Delayed non-matching to sample measured at 2 time points (before/after nicotine/saline self-administration). Columns represent the group average for each treatment group (prenatal saline/nicotine  $n=20$ , MIA/nicotine  $n=26$ , prenatal saline/saline  $n=6$ , MIA/saline  $n=6$ ) in percent (%) correct second arm-visits over 4 trials/day on 5 consecutive days (mean,  $\pm$  s.e.m.). The first column represents group average for each treatment group before nicotine treatment and the second column represents group average after nicotine/saline self-administration. The asterisk (\*) indicates a significant difference for Prenatal treatment (between subject factor, MIA versus saline, mixed ANOVA,  $P < 0.001$ ). Two asterisks (\*\*) indicate a significant three-way interaction between Prenatal treatment, Time (before/after self-administration) and Drug (nicotine/saline self-administration,  $P = 0.04$ ). The accent (^) indicates a significant difference in Time (within subject factor, before/after self-administration,  $P = 0.002$ ).

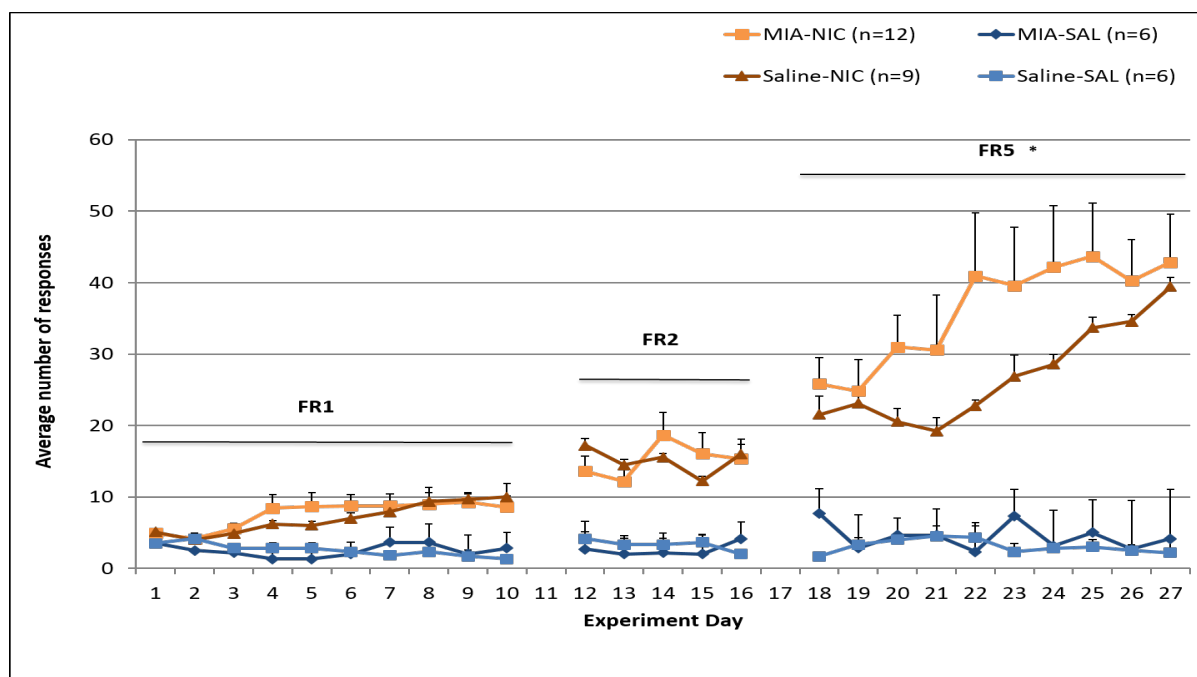
## **MIA had little effect on nicotine self-administration**

### FR schedules

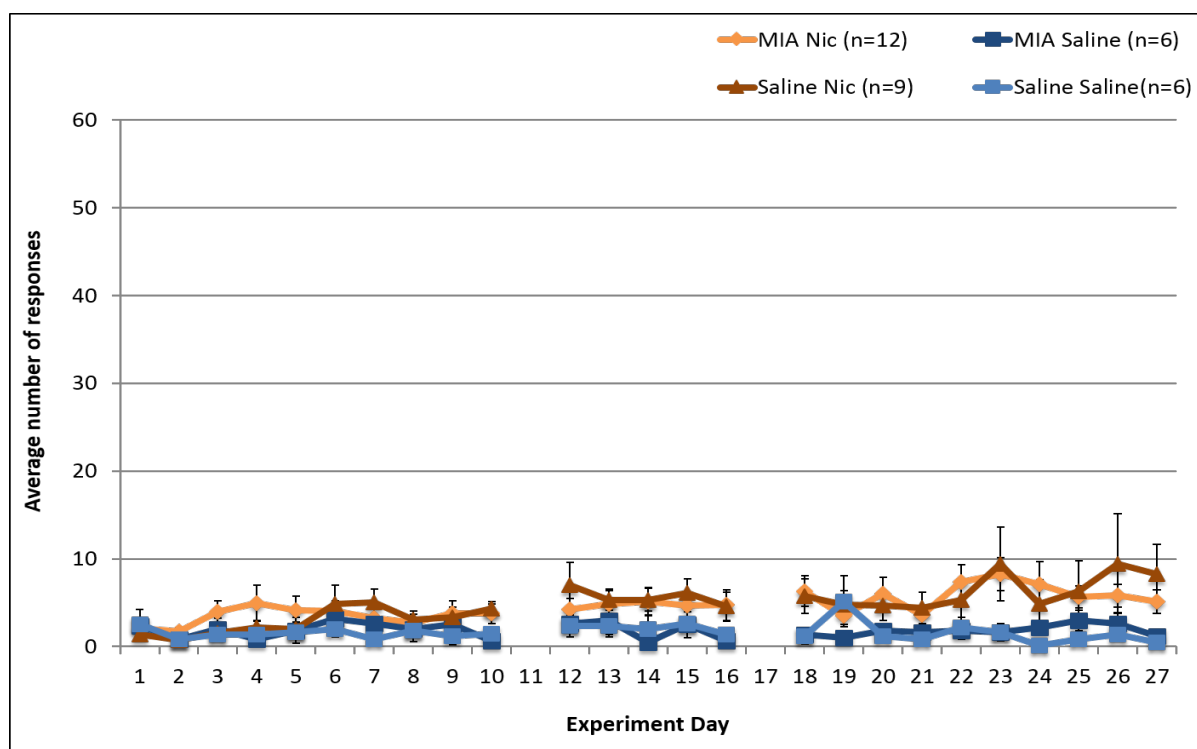
During the first 10 days, responding changed significantly on the FR1 schedule as there were main effects of Day:  $F(9,522) = 2.522$ ,  $p = 0.008$ ; Lever:  $F(1,58) = 15.235$ ,  $p < 0.001$  and Drug:  $F(1,36) = 93.432$ ,  $p < 0.001$ . Further, there was a significant interaction between Day and Drug:  $F(9,522) = 3.853$ ,  $p < 0.001$ , as only those animals that self-administered nicotine increased responding on the active lever (Figure 3) compared to the inactive lever (Figure 4). However, there was no effect of Prenatal treatment.

During FR2, there was a main effect of Lever:  $F(1,58) = 17.143$ ,  $p < 0.001$  and Drug:  $F(1,16) = 264.521$ ,  $p < 0.001$  as well as a significant interaction between these factors:  $F(1,58) = 11.677$ ,  $p = 0.001$ . There was no effect of Day or Prenatal treatment. Animals self-administering nicotine responded significantly more on the active lever independent of Prenatal treatment.

During the FR5 phase, there were main effects of Prenatal treatment:  $F(1,36) = 10.547$ ,  $p = 0.003$ ; Drug:  $F(1,58) = 41.688$ ,  $p < 0.001$ ; Day:  $F(9,522) = 2.834$ ,  $p = 0.003$  as well as Lever:  $F(1,58) = 30.450$ ,  $p < 0.001$ . In addition, there was a significant interaction between Prenatal treatment and Drug:  $F(1,36) = 5.723$ ,  $p = 0.022$  as well as Day and Drug:  $F(9,522) = 248.107$ ,  $p < 0.001$ . Inspection of Figure 3 revealed that, over time, the MIA group responded significantly higher on the active lever compared to all other groups.



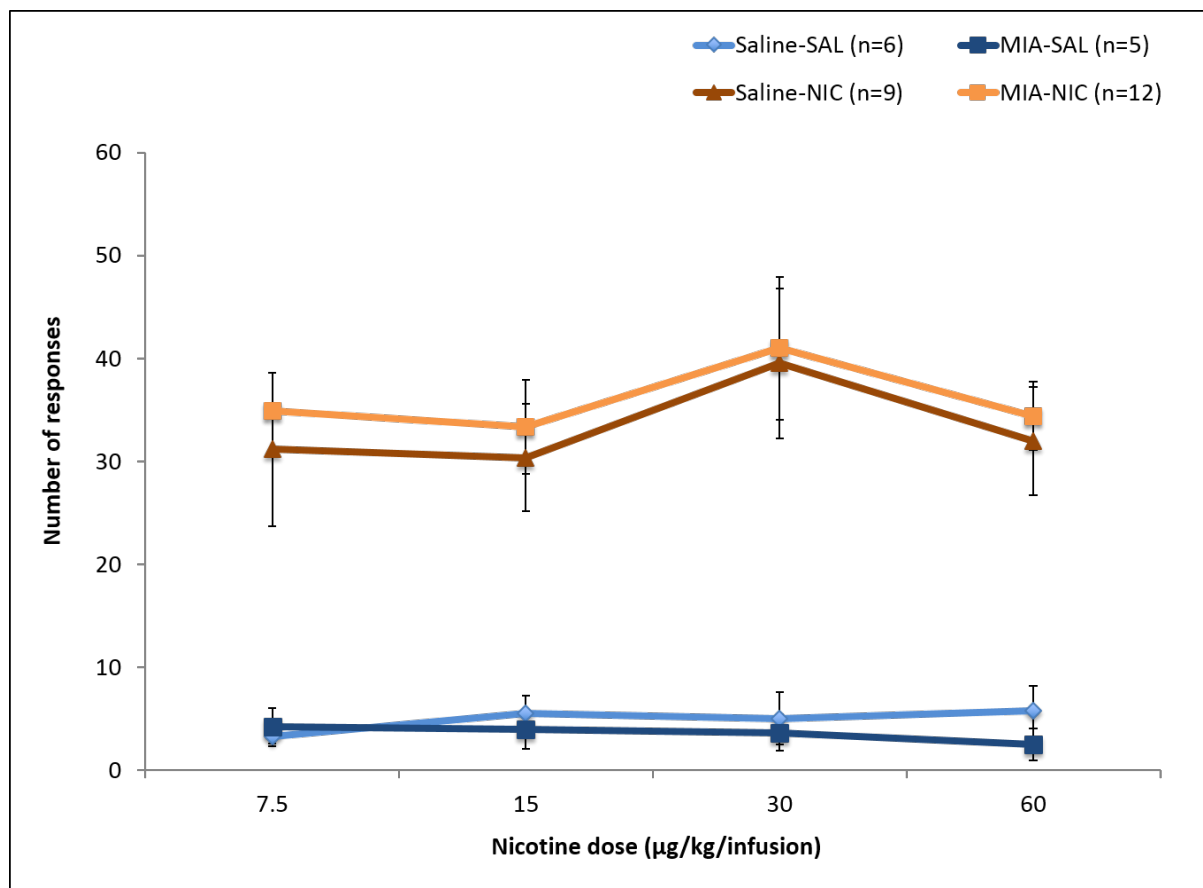
*Figure 3* Self-administration on a fixed ratio (FR) schedule. Data points represent the average number of total responses on the active (right) lever during daily 2-hour sessions for nicotine or saline (mean,  $\pm$  s.e.m.). The asterisk (\*) indicates a significant interaction between Prenatal treatment and Drug (repeated ANOVA,  $P = 0.02$ ). Animals prenatally exposed to MIA responded significantly higher on the active lever compared to all other groups.



*Figure 4* Self-administration on a fixed ratio (FR) schedule. Data points represent the average number of total responses on the inactive (left) lever during daily 2-hour sessions for nicotine or saline (mean,  $\pm$  s.e.m.)

### Dose-response curves

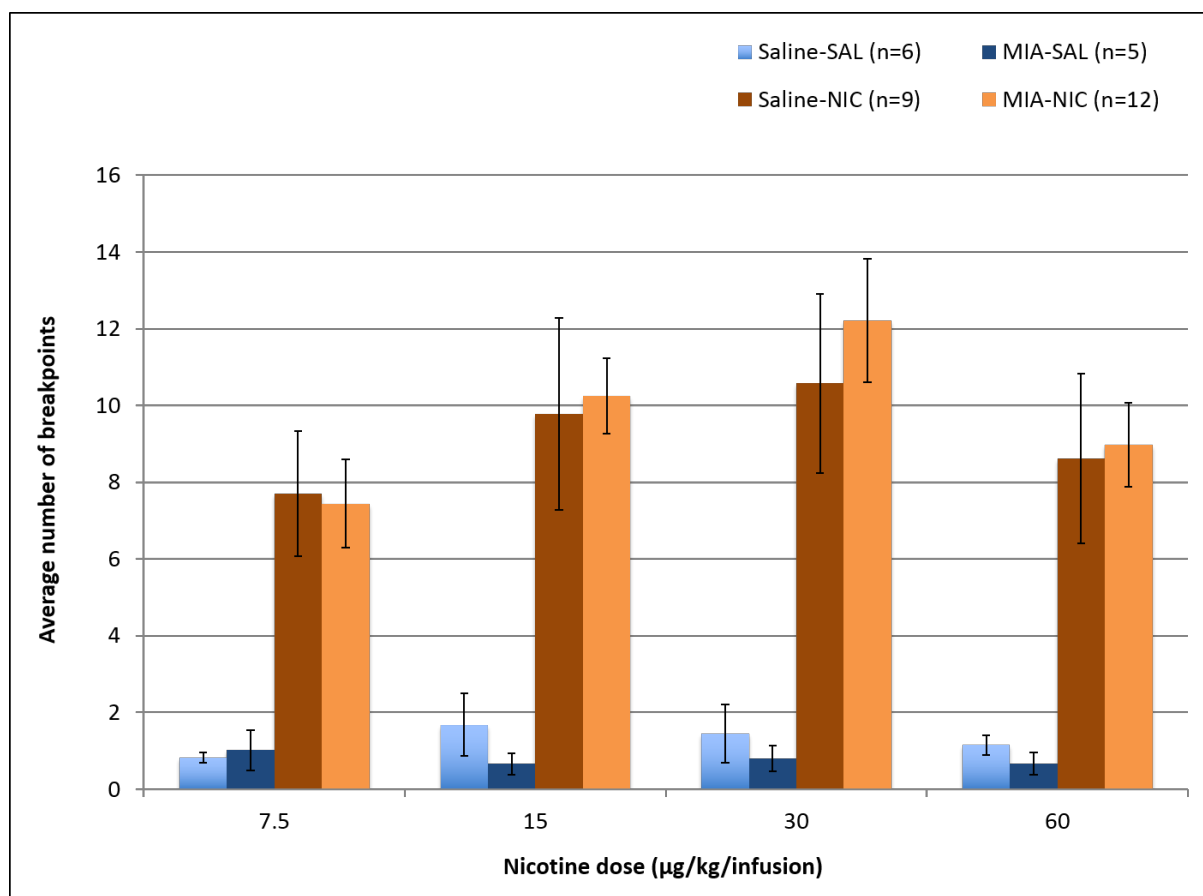
There was no effect of prenatal treatment on self-administration acquisition rate (SAL = 45%, MIA = 46%) or total nicotine intake (data not shown). Animals that successfully acquired self-administration were subsequently subjected to dose response testing. There were main effects of Lever:  $F(7,196) = 22.561$ ,  $p < 0.001$  and Drug:  $F(1,28) = 45.155$ ,  $p < 0.001$  as well as an interaction between these factors:  $F(7,196) = 18.348$ ,  $p < 0.001$ . However, there was no effect of Dose or Prenatal treatment. The nicotine groups responded significantly higher on the active lever compared to the inactive lever independent of prenatal exposure or nicotine dose (Figure 5).



*Figure 5* Dose-response curve on FR5. Data points represent the average number of active lever responses at each dose of nicotine (mean,  $\pm$  s.e.m.).

### Progressive-ratio schedules

The average breakpoint scores were not affected by Prenatal treatment or Dose (Figure 6). During the intermittent FR5 schedule main effects of Drug:  $F(1,56) = 49.044$ ,  $p < 0.001$  and Lever:  $F(1,56) = 46.229$ ,  $p < 0.001$ , but not Prenatal treatment were found. Further, interactions were observed between Drug and Dose:  $F(3,168) = 2.803$ ,  $p = 0.04$  and Drug and Lever:  $F(1,56) = 30.976$ ,  $p < 0.001$ . Animals self-administering nicotine showed a preference for the active lever and responding for the nicotine dose of 30  $\mu\text{g/kg/infusion}$  was significantly greater compared to other nicotine doses, yet there was no effect of Prenatal treatment (data not shown).



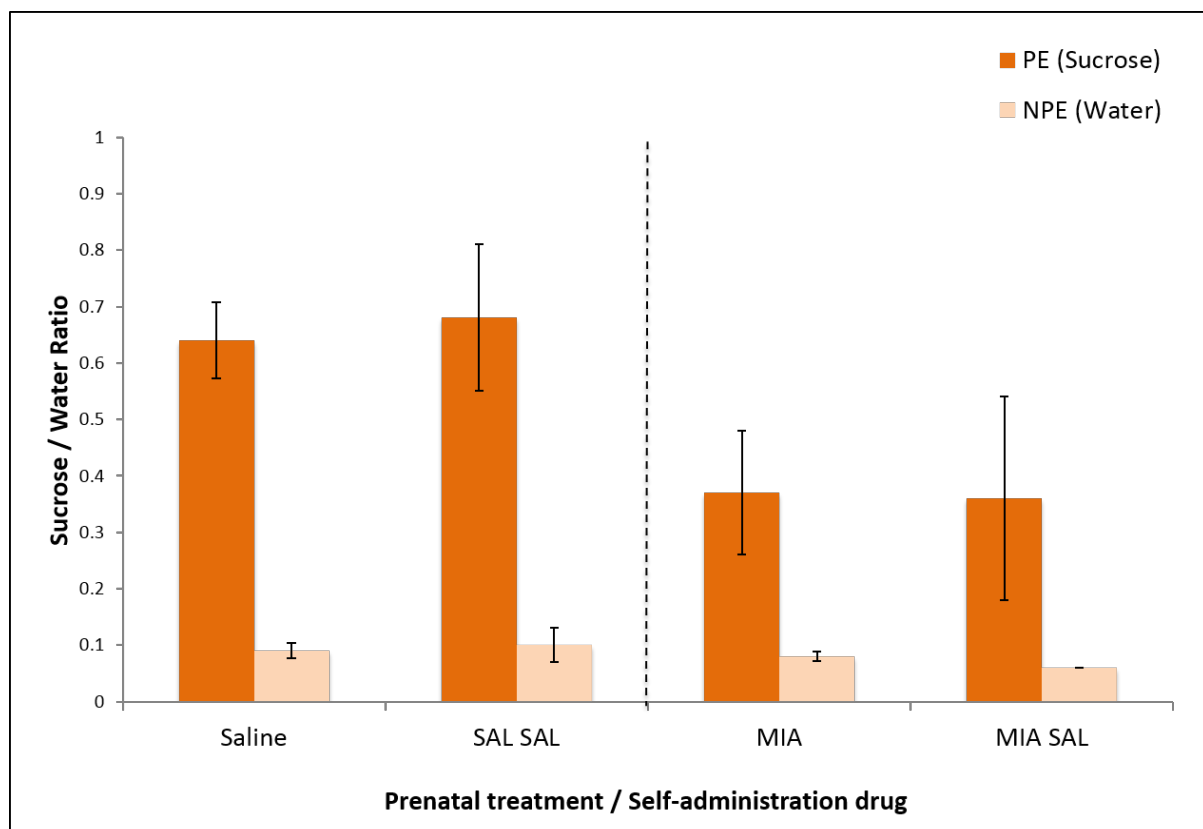
*Figure 6* Nicotine/saline self-administration on a progressive ratio (PR) schedule. Columns represent the average breakpoint at each dose per treatment group (mean,  $\pm$  s.e.m.).

## Self-administered nicotine ameliorated MIA induced cognitive deficits

The results for cognitive testing after nicotine exposure were collapsed into group averages as there were no statistically significant differences observed between both self-administration acquirers and non-acquirers, independent of prenatal treatment (data not shown). Importantly, during cognitive testing, all animals were maintained on a FR5 ratio of nicotine self-administration. Moreover, there were no differences between the prenatal saline and MIA exposed groups in nicotine self-administration during the re-test phase.

### LI

There was a statistically significant interaction between Time (before/after nicotine) and LI-exposure:  $F(1,47) = 4.443$ ,  $p = 0.04$ . As evident in Figure 1, nicotine self-administration ameliorated LI deficits in the MIA group pre-exposed to sucrose, but had no effect on any of other groups. Saline self-administration had no effect (Figure 7).



*Figure 7* Latent inhibition before and after saline self-administration in animals prenatally exposed to MIA or saline. Columns represent the group average for each treatment group (saline/sucrose  $n=3$ , saline/water  $n=3$ , MIA/sucrose  $n=3$ , MIA/water  $n=3$ ) in consumption-ratio sucrose (PE) versus water (NPE) on testing day (mean,  $\pm$  s.e.m.). There was no significant difference before versus after saline self-administration in any treatment group

## DNMTS

Deficits in second arm visits as observed before nicotine exposure were ameliorated by nicotine exposure as there was a main effect of Time:  $F(1,53) = 10.391$ ,  $p = 0.002$ . More importantly, there was a three-way-interaction between Prenatal treatment, Time and Drug:  $F(1,53) = 4.273$ ,  $p = 0.04$ . Inspection of Figure 2 shows that those animals exposed to MIA significantly improved accuracy after nicotine self-administration, whereas saline self-administration had no effect on choice accuracy.



## **DISCUSSION**

The main findings of the present study are: 1. Consistent with previous findings, prenatal exposure to maternal LPS treatment (MIA) on GD 10/11 led to significant deficits in LI and DNMTS in the offspring in adulthood. 2. These animals responded at a significantly higher level during nicotine self-administration acquisition (FR5) compared to all other groups, yet were similar to the controls on DR and PR testing. 3. Cognitive deficits evident before nicotine exposure were ameliorated by nicotine, but not saline, self-administration, without affecting performance in other groups. Thus, in the present study we found strong support for the self-medication hypothesis of nicotine, but only weak support for the common biological substrate hypothesis of schizophrenia.

### **MIA induced cognitive deficits in rats**

The data obtained in this study are consistent with our previous research (Waterhouse et al., 2016) and others (Zuckerman & Weiner, 2003). Animals prenatally exposed to MIA showed diminished LI. Specifically, the MIA pre-exposed group consumed significantly less sucrose solution during testing compared with the saline pre-exposed group. This suggested that repeated pre-exposure to the sucrose taste was less effective in the MIA pre-exposed animals to impede associative learning between the negative consequences of LiCl and the taste conditioned stimulus following conditioning. These findings are consistent with an impairment in selective attention as observed in individuals with schizophrenia and manifest in, for example, a reduced ability to “tune out irrelevant information” (Lubow, 2005).

Similar to the results obtained in LI, the MIA group made significantly more errors in second arm-visits in the DNMTS task compared to controls. As we and others have demonstrated (Graciarena et al., 2010; Hao et al., 2010), maternal exposure to LPS during gestation can lead to schizophrenia-like long-lasting deficits in aspects of working memory such as in goal maintenance and interference control. Interestingly, as seen in Figures 1, 2 and 7 both these cognitive deficits (LI and DNMTS) are very long lasting (up to PND 200) and can be repeatedly assessed in the same animals, thus they can be used to assess the therapeutic efficacy of drugs or other treatments.

### **MIA had little effect in nicotine self-administration**

During the first 10 days on FR1, animals developed a general preference for the active lever with the exception of saline control groups. The difference in preference for nicotine over saline continued into FR2 and FR5, confirming the reinforcing properties of nicotine

(Brennan et al., 2015). Intriguingly, the MIA group responded significantly more for nicotine during the FR5 schedule compared to all other groups.

Consistent with previous nicotine self-administration research, responding following several dose changes resulted in a nicotine-typical flat dose-response curve (Corrigall & Coen, 1989). Although not statistically significant, a tendency for more pronounced responding was observed at the 30 µg/kg/infusion dose independent of prenatal treatment. These dose-response results were consistent with the data from the PR intermittent FR5 schedule, where responding for the 30 µg/kg/infusion dose yielded highest rates of responding (data not shown). Nicotine is considered a weak reinforcer and self-administration in rodents depends on various factors such as infusion rate and dose. Optimal nicotine self-administration rates have been observed at 15 and 30 µg/kg/infusion doses (Corrigall & Coen, 1989).

Thus, the present results showing higher responding on FR5 exhibited by the MIA group during acquisition, but no other effects, are consistent with the idea that nicotine was not substantially more reinforcing to the MIA group.

Speculatively, the enhanced responding on the FR5 schedule might have been a temporally dependent event, suggesting that differences between MIA and control animals can only be observed at a specific period before a critical amount of nicotine exposure required to produce neuroadaptations had been achieved. In line with this, the enhanced responding effect disappeared during the later PR testing phase (even on the FR5 days, in between the PR test days, data not shown). This indicates that the difference between the two groups is only small and only occurs during a specific phase of acquisition.

Although, the present study is not comparable due to numerous methodological differences, Berg and colleagues (2013), using the early ventral hippocampal lesioned (NVHL) model for schizophrenia, also observed increased responding during nicotine self-administration acquisition. Though the authors concluded that nicotine was more addictive in the NVHL rat (Berg et al., 2013), a close inspection of their data shows that, as in our model, increased responding was also seen in the FR5 regime. However, the authors did not perform any PR experiment. Thus, the theory of common underlying substrates to explain increased susceptibility to tobacco addiction in individuals with schizophrenia does not have strong support from either animal model.

In contrast to this, a previous study using prenatal polyI:C treatment found an increased amphetamine-induced behavioural sensitization as well as conditioned place

preference (Borcoi et al., 2015), although self-administration was not investigated. The difference between these two studies may be related to differences in the MIA procedure (LPS vs PolyI:C), the drug used or the paradigms investigated. Importantly, tobacco smoke consists of over 4,000 different chemical compounds, some of which can significantly influence the addictive properties of nicotine. In line with this, research from our own laboratory has shown that tobacco particulate matter (TPM, which consists of the total combustion product actively inhaled by smokers), especially that from roll-your-own tobacco (TPM-RYO) is more reinforcing than nicotine alone (Brennan et al., 2015). Thus subsequent studies using TPM and/or TPM-RYO are required to unequivocally determine whether MIA rats are more sensitive to the reinforcing effects of smoking tobacco.

### **Self-administered nicotine ameliorated MIA-induced cognitive deficits**

In a previous study, we showed that the cognitive deficits induced by MIA were ameliorated after repeated experimenter-administered nicotine (Waterhouse et al., 2016). The findings of the present study extend this to self-administered nicotine. Thus, deficits in LI were ameliorated in the second test-phase after contingent nicotine exposure, yet nicotine had no effect in any other group, suggesting a selective cognitive enhancing effect. Importantly, saline self-administration had no effect. These data therefore confirm that the cognitive deficits are long-lasting in the MIA model and can be repeatedly assessed without any signs of a habituation effect. Our data are also consistent with clinical studies, that showed that with respect to selective attention, nicotine normalized an existing deficit, without affecting normal performance (Hahn et al., 2012; Smucny et al., 2015).

In line with the data on LI, we found that working memory deficits, assessed in the DNMTS test, were significantly improved after self-administered nicotine (see Figure 2): Choice accuracy was significantly enhanced in the MIA group after nicotine, but not saline, self-administration, yet performance in other groups remained unaffected. These results match clinical studies showing that nicotine is more effective in normalizing cognitive deficits than in increasing normal performance (Niemegeers et al., 2014). In the previously mentioned study (Waterhouse et al., 2016), we did not find a significant effect of experimenter-administered nicotine on DNMTS performance. Although this may (in part) be related to the difference between experimenter and self-administered nicotine, the most parsimonious explanation may be the inclusion of longer inter-trial intervals. Thus, while in the previous study the intervals were between 5 and 120 seconds, the present study employed intervals up to 600 seconds. As a result, the overall accuracy, especially in the MIA groups,

was lower in the present study (approximately 70% compared to about 82% in the previous study) and thus a potential ceiling effect could be prevented.

Nicotine binds to nicotinic acetylcholine receptors and alters the release of neurotransmitters such as acetylcholine, dopamine, glutamate, serotonin and GABA, which are crucial for normal cognitive functioning (dos Santos Coura & Granon, 2012; Mansvelder et al., 2002). Of particular importance are the receptor subtypes  $\alpha 7$  and  $\alpha 4\beta 2$ . These receptors are critical in cognitive processing such as selective attention or memory (Klinkenberg et al., 2011), yet many individuals with schizophrenia show reduced levels in brain areas relevant to cognitive functioning, presumably due to genetic polymorphism (for example CHRNA7). For these reasons, nicotine from cigarette smoke might lead to an optimization of neuronal activity levels in these areas and consequently to a pro-cognitive effect (Freedman, 2014).

To our knowledge, this is the first pharmacological study to examine the effect of nicotine self-administration in animals prenatally exposed to MIA. In summary, we found that the male offspring of rats exposed to LPS during pregnancy show significant deficits in LI and DNMTS, which were significantly ameliorated by nicotine, but not saline, self-administration. On the other hand, these same rats were not significantly more sensitive to the reinforcing properties in nicotine. Cognizant of the limitations of animal models, these data tend to provide more support for the self-medication hypothesis of smoking than for the common neurobiological substrate hypothesis.

## **ACKNOWLEDGEMENTS**

The authors gratefully acknowledge support and expert advice provided by the animal lab coordinators Jiun Youn and Joyce Colussi-Mas, as well as the animal technician Michael Roberts, and Sophie Muller for advice in self-administration techniques. The authors are especially grateful for the assistance in surgery provided by Jeremy Webster.

## **DISCLOSURE**

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

SUPPLEMENTARY MATERIAL

Supplementary Table S1


Prenatal	Postnatal				
 GD 10/11	Male offspring PND 60-62 (approximately)	PND 63 commencing	PND 100-110	PND 110 commencing	PND 140 (earliest) commencing
Maternal sc injections either LPS (0.5 mg/kg) or saline	LI testing before self-administration	DNMTS testing before self- administration	Catheter surgery in preparation for self- administration and recovery period	Nicotine/saline self- administration	Cognitive re-testing in late stages of self- administration

Table S1 Timeline of the overall experimental design

Supplementary Table S2

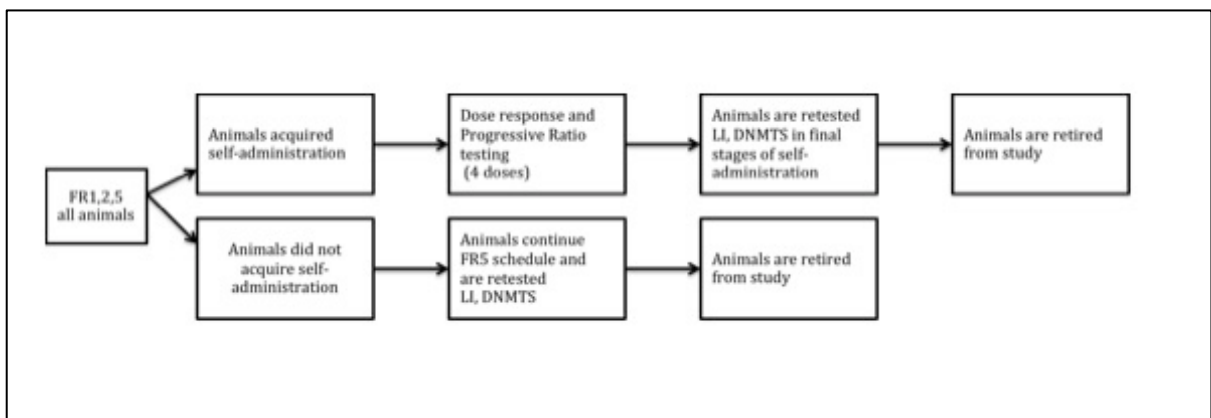


Table S2 Timeline of the experimental design self-administration

## **METHODS AND MATERIAL**

### **Self-administration apparatus**

Nicotine self-administration was assessed in operant chambers (ENV 001, Med Associates, St. Albans, VT, USA) in sound-attenuating boxes in a temperature controlled (21°C) room. Each chamber was equipped with two levers (83 mm apart from closest chamber wall, 72 mm above metal grid floor). Depression of the right lever (active) illuminated a stimulus light located above this lever as well as an intravenous delivery of a drug infusion (0.25 ml). Depression of the left lever (inactive) had no consequences. All lever responses were recorded. The self-administration parameters were based on those used by Brennan et al., 2013 (drug infusion time 30 seconds, 120 second “time out” period). A Med-PC software program controlled drug delivery and data collection. Each chamber was connected to a mechanical pump (Razel, Model A with 1 rpm motor), equipped with a 20.0 ml syringe (Georgia, VT, USA), to deliver the drug infusions.

### **Surgical procedures**

Animals received an intraperitoneally injection (ip) of a mixture of ketamine and xylazine to induce anaesthesia. A small incision in the jugular vein allowed for a silastic catheter to be inserted and the other end of the catheter to be passed through to an exposed section of the skull to be held in place using jeweler’s screws and dental acrylic. Immediately following surgery, rats received 10ml sodium lactate solution (sc injection) for rehydration. Post-surgery-care included a daily (sc) injection of an anti-inflammatory (Carprofen®, 5mg/kg, Norbrook New Zealand LTD, Auckland, New Zealand) for two days following the surgery. Following the catheter implantation, catheters were flushed daily with 0.2ml of a sterile 0.9% heparinized (30 UL/ml) saline solution containing penicillin G potassium (100 000 UL/ml). To ensure catheter patency during self-administration, functionality was tested weekly. In case of catheter patency failure, rats underwent repair surgery and returned to self-administration after a three-day post-surgery recovery period.

### **Self-administration procedures**

The self-administration procedures used in this study model those used in a previous study in our laboratory. Rats from both treatment groups (maternal LPS versus SAL) were assigned to self-administer either vehicle control or nicotine (30 µg/kg/infusion) during daily

2-hour sessions (Monday to Friday). At the beginning of each session, animals were placed into the operant chambers where tubing extending from the drug-containing syringe in the pump was attached to the metal head-piece of the implanted catheter. To start the session, one experimenter-administered prime was initiated to fill the catheter with the drug. Additionally, on the first three days extra experimenter-administered primes (infusions) (day 1 = 3, day 2 = 2, day 3 = 1) were delivered.

## APPENDIX 7.1

### RESULTS

#### LI

An analysis of total fluid intake (sucrose plus water) on testing day (day 5) revealed a main effect of Time (before versus after self-administration):  $F(1,47) = 15.730$ ,  $p < 0.001$  but there was no statistically significant interaction. Animals showed a higher total fluid intake after self-administration independent of prenatal treatment, LI pre-exposure, or self-administration drug.

There was a main effect of Time:  $F(1, 47) = 5.201$ ,  $p < 0.03$  and a statistically significant interaction between Time (before versus after self-administration) and LI pre-exposure for sucrose intake on conditioning day (day 4):  $F(1,47) = 4.530$ ,  $p < 0.04$ . Those animals that were not pre-exposed to sucrose (NPE group, exposed to plain water) showed reduced sucrose intake on conditioning day after self-administration independent of prenatal treatment or self-administration drug.

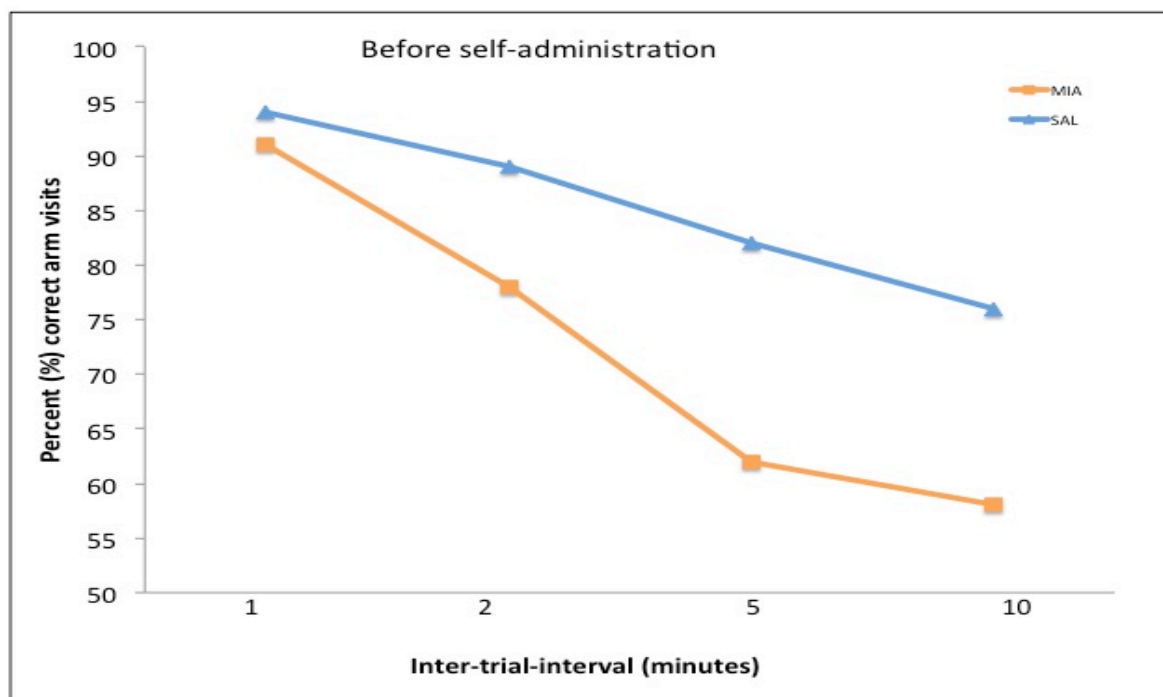
There was no statistically significant difference in sucrose intake during pre-conditioning (days 1 to 3) between animals prenatally exposed to MIA and controls.

#### DNMTS

An analysis of the inter-trial-intervals (ITI) revealed a significant main effect of Delay:  $F(3,165) = 18.845$ ,  $p < 0.001$  and Group:  $F(1,55) = 26.695$ ,  $p < 0.001$  before self-administration, but no statistically significant interaction. Inspection of Figure 8 revealed that accuracy in second arm visits decreased over longer delays. A follow up independent t-test revealed that animals prenatally exposed to MIA made significantly more errors at the 2 minute ITI:  $t(55) = -2.586$ ,  $p = 0.012$ , 5 minute ITI:  $t(55) = -3.464$ ,  $p < 0.001$ , and 10 minute ITI:  $t(55) = -2.576$ ,  $p = 0.013$  compared to controls.

There was a statistically significant main effect of Delay after self-administration:  $F(3,159) = 5.540$ ,  $p = 0.001$  and self-administered Drug:  $F(1,53) = 12.797$ ,  $p = 0.001$ , but no interaction. Animals self-administering nicotine were more accurate in second arm-visits compared to animals self-administering saline independent of prenatal treatment (data not shown).





*Figure 7.A1* Delayed non-matching to sample before self-administration. Data points represent the group average for each treatment group (prenatal saline  $n=26$ , prenatal MIA  $n=32$ ) in percent (%) correct second arm-visits (over 5 consecutive days) over four inter-trial-intervals (mean,  $\pm$  s.e.m.).

## CHAPTER 8                      GENERAL DISCUSSION

The current PhD research project had three aims. Firstly, to establish an animal model that produces general neurocognitive deficits similar to those commonly found in individuals with schizophrenia. To this end, a neurodevelopmental approach, prenatal exposure to maternal infection, was utilized. Pregnant rats were injected with lipopolysaccharide (LPS), which mimics a bacterial infection and reliably initiates a maternal immune activation (MIA). Male offspring were assessed in adulthood in sensorimotor gating (PPI), selective attention (LI), and working memory (DNMTS). Secondly, the self-medication theory suggests high smoking incidence in patients with schizophrenia because nicotine, the primary addictive constituent in tobacco smoke, might ameliorate some of the symptoms of the disorder such as cognitive deficits. Thus, animals were repeatedly exposed to nicotine before being re-tested on the same tasks to determine whether experimenter and self-administered nicotine has a pro-cognitive effect on MIA-induced deficits. Finally, the third aim was to examine the propensity for animals prenatally exposed to MIA to self-administer nicotine compared to control groups. This part of the study investigated the common substrate theory, which suggests shared underlying pathways may increase susceptibility to tobacco addiction in these individuals.

The main findings of the current PhD research project are firstly, prenatal exposure to maternal LPS treatment on GD 10/11 led to long-lasting schizophrenia-like cognitive deficits in male offspring in sensorimotor gating (PPI), selective attention (LI), and working memory (DNMTS). Secondly, non-contingent or contingent nicotine exposure ameliorated these cognitive deficits. Thirdly, a small but significant effect was found during nicotine self-administration acquisition (FR5 schedule) where animals prenatally exposed to MIA were more susceptible to the reinforcing properties of nicotine compared to controls. However no differences were found in dose-response and progressive ratio testing. Thus, overall these findings support the self-medication hypothesis (theory 2, discussed in Chapter 3), yet only offer weak support for the common substrate theory (theory 3, discussed in Chapter 3).

### **8.1 Prenatal exposure to maternal LPS treatment leads to long-lasting schizophrenia-like deficits**

Based on human epidemiological findings, it has been well established that early disruptions in neurodevelopment can predispose an individual to develop schizophrenia later in life. A number of animal models have been used to examine the effect of these early

stressors and insults on the brain and behavioural development. Thus, the current research project utilized a neurodevelopmental model to induce cognitive deficits in animals to examine the effect of nicotine on these impairments. Neurodevelopmental animal models can be categorised into prenatal, perinatal and neonatal models. Prenatal models include stress models such as restraint stress (pregnant females are repeatedly restrained during the last stages of pregnancy), maternal malnutrition, prenatal exposure to methylazoxymethanol acetate (a neurotoxin which reduces DNA synthesis), as well as prenatal exposure to MIA as used in this study. Perinatal and neonatal models include stress models such as 24-hour maternal deprivation as well as so-called pharmacological and lesion models such as administration of phencyclidine (PCP) or ketamine (NMDA antagonists) within the first week after birth, neonatal ventral hippocampal lesions (NVHL) induced by ibotenic acid, exposure to nitric oxide inhibitor (NOS, inhibition), and administration of antimetabolic agents (for review Wilson & Terry, 2010).

The NVHL model is one of the most widely used neurodevelopmental models of schizophrenia. Lesions in the hippocampal area prevent hippocampal innervation and therefore impact on synaptic connectivity and function of other brain areas such as the prefrontal cortex (PFC). The NVHL model leads to behavioural, molecular and physiological alterations similar to those commonly found in schizophrenia. Further, pharmacological studies show that some of the symptoms induced by this model are responsive to treatment by antipsychotic medication (for review Mouri et al., 2013; Tseng, Chambers, & Lipska, 2009). However, one of the major limitations of this model is the lack in construct validity as there is no evidence of a widespread hippocampal lesion in humans.

Prenatal exposure to MIA in animals as utilized in the current project, however, models the human condition of a neurodevelopmental predisposition for schizophrenia more closely, and therefore exerts substantially higher construct validity. As discussed in Chapter 1, like most infectious agents, the bacterial endotoxin LPS or the viral mimic polyI:C, which are commonly used in animal models to induce MIA, do not cross the blood-placenta-barrier. Thus, it is generally accepted that the resulting changes in brain and behaviour of the offspring are the result of the activation of the maternal immune system (Ashdown et al., 2006; Oskvig et al., 2012). Part of the maternal immune response is the activation of chemical messengers such as cytokines, which are involved in the breakdown and neutralisation of infectious agents. In addition, antibodies are produced to protect the organism from future invasions by the same or similar pathogens (Bear et al., 2007; Cicchetti & Walker, 2003). Increased cytokine levels in the peripheral system lead to the stimulation

of neurons and the production of microglia in the brain. Microglia are part of the brain's immune response and, most importantly, increase cytokine release in the brain. Cytokines mediate the communication between cells during inflammation and are responsible for the elimination of infectious agents (Altamura et al., 2013). However, these molecules are also involved in normal brain development such as in neurogenesis (production of neurons) and synaptogenesis (neuronal networks). For example, cytokines regulate the production of Major Histocompatibility Complex cells, which are particularly important in synaptic plasticity, influencing an individual's learning and memory abilities, especially in the developing brain (Boulanger, Huh, & Shatz, 2001). In addition to the involvement in synaptogenesis, cytokines have been implicated in the process of 'pruning'. Pruning is a process where cells that have not been integrated in synaptic connections to a certain extent undergo apoptosis (programmed cell death). Therefore, disturbances in synaptogenesis subsequently might lead to increased or excessive pruning. Further, cytokines control myelination, a process of neuronal insulation to ensure more efficient neurotransmission (Howard, 2013). Thus, the "cytokine hypothesis" states that elevated levels during an infection might lead to disruptions in early neurodevelopment (for review Miller et al., 2013).

This theory is supported by evidence from human studies that show abnormally high base rates of cytokines in individuals with schizophrenia (Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011; Potvin et al., 2008; Schwieler et al., 2015). Such elevated levels are associated with an increased susceptibility to over-stimulation by external stimuli leading to cognitive deficits and psychosis (Meyer & Feldon, 2009; Patterson, 2011). Compelling evidence for the involvement of cytokines in neurodevelopmental disruptions derives from studies that administered cytokines directly to pregnant animals. For example, Smith and colleagues (2007) examined the effect of maternal exposure to cytokine interleukin-6 (IL-6) on GD 12.5 in mice (0.005 mg/kg, ip, single injection). They found impairments in PPI and LI in offspring in adulthood. Further, the authors found no effect of MIA (polyI:C, 20 mg/kg, ip, GD 12.5) in IL-6-knock-out mice (genetically modified mice that lack IL-6) compared to wild-type animals (Smith et al., 2007). These findings provide support for the involvement of specific cytokines (and especially IL-6) in the neuropathological effect of prenatal exposure to MIA.

Neurodevelopmental processes including cell proliferation, differentiation and maturation follow a distinct time course (Workman et al., 2013). Thus, it can be expected that disruptions in neurodevelopment differ depending on the timing of the insult during gestation. Consistent with the findings of the current study, it has been suggested that early

disruptions are more damaging compared to later disturbances (Meyer et al., 2007). Although it was not the primary focus of this PhD research to examine differences in cognitive deficits depending on the timing of the exposure to MIA, those animals that were prenatally exposed to maternal LPS treatment on GD 10/11 showed consistent deficits in sensorimotor gating (PPI), selective attention (LI), as well as working memory (DNMTS), but no effect or a very limited effect was found when MIA was initiated on GD 15/16 or GD 18/19. Prenatal exposure to MIA on GD 18/19, however, increased sucrose intake in those animals that were not pre-exposed to the stimulus before conditioning, which suggests a diminished conditioned taste aversion in this group. More precisely, these animals did not associate the stimulus sucrose water with the LiCl-induced ill-feeling during conditioning. Subsequently, this group consumed almost equal amounts of the sugar liquid during testing compared to those animals that were repeatedly pre-exposed to sucrose prior to testing. However, it is important to note that as is common for latent inhibition experiments, a LiCl-free (saline) control condition was not included. Therefore, in order to proof that prenatal MIA at GD 18/19 reduced CTA, the experiment should be repeated including a saline control condition.

Most importantly, however, the findings of the first part of the study where prenatal exposure to MIA early during gestation (GD 10/11) led to cognitive impairments in all tasks, were replicated in the second part of this research project in an independent sample.

An interesting aspect of the MIA model is the emergence of behavioural abnormalities in offspring mostly in late adolescence/early adulthood. It is known that cognitive deficits are prominent in the prodromal phase of schizophrenia well before the onset of psychosis and the diagnosis of the disorder (see Chapter 1). Disruptions in early brain developmental processes may also limit later higher order maturational processes, which proceed into adolescence and early adulthood (Bear et al., 2007; Weinberger & Lipska, 1995), thus the full extent of disruptions induced by prenatal exposure to MIA in later developmental processes might only be evident in early adulthood. Support for this hypothesis derives from studies that examined grey matter volume in individuals with schizophrenia. For example, Thompson et al. (2001) examined grey matter volume reduction in individuals with childhood onset schizophrenia in a longitudinal design and found early deficits in the form of thinning in the parietal brain regions. Over a time course of five years, deficits progressed into other areas such as the anterior temporal lobes and at the later stages included the dorsolateral prefrontal cortex. Further, Nesvag and colleagues (2008) examined the effect of antipsychotic medication and age on grey matter volume reduction in

schizophrenic patients (schizophrenia n=81, schizoaffective disorder n=15) and healthy controls (n=107) in a study utilizing MRI and computer image analysis. No significant effect of age was found, where mean age at assessment was 42 years of age (range 17 to 57 years). Thus, these findings are consistent with the hypothesis that neurodevelopmental alterations influence maturational processing. Age-related reduction in thickness was similar in patients and healthy controls. Furthermore, no significant effect of antipsychotic medication was observed. More importantly, individuals with a diagnosis of schizophrenia/schizoaffective disorder displayed volume reduction in prefrontal and temporal regions in both hemispheres compared to controls. Thinning was also evident in parietal and occipital brain regions, yet less pronounced compared to prefrontal and temporal areas. The authors suggest that differences in volume reduction between brain areas might be due to the developmental trajectory, where the parietal and occipital regions develop significantly earlier compared to the cerebral cortex and the temporal regions. More precisely, although there is still debate amongst scholars whether neurogenesis occurs in the cerebral cortex throughout adulthood, more pronounced deficits in these areas could be due to disruptions in early neurodevelopmental processes, which subsequently affect neurogenesis and synaptogenesis later. Increased synaptic pruning in the cerebral cortex due to a lack of synaptic formations could explain, at least in part, the observed volume reduction in patients (for review Nesvag et al., 2008) and significantly affect executive functioning.

Overall, the findings of the present study are consistent with previous research supporting a role for maternal infection in neurodevelopmental abnormalities in psychiatric disorders such as schizophrenia. As evident in the present research, this model allows multifaceted (multiple cognitive domains) and longitudinal (prenatal impact leads to long-lasting deficits in adulthood) monitoring of cognitive behavioural deficits and offers a fundamentally strong basis to examine pharmacological impacts such as the nicotinic effect on cognitive impairments.

## **8.2 Contingent and non-contingent nicotine exposure ameliorates cognitive deficits**

The second aim was to assess whether repeated nicotine exposure ameliorates cognitive deficits as suggested by the self-medication hypothesis. To this end, the cognitive assessment paradigms were administered both before and after nicotine treatment. In part 1 (Chapter 5), nicotine exposure included experimenter-administered (0.6 mg/kg) sc injections for 10 days before re-testing commenced. Daily nicotine injections continued during re-testing to ensure adequate nicotine levels during the cognitive assessment. Tasks were re-

administered in a counter-balanced order to account for age differences and levels of nicotine exposure. This was necessary, as some tasks require days/weeks of training and testing. It is important to highlight that the assessment paradigms utilized are not affected by learning effects during testing. Thus changes in cognitive performance observed after repeated nicotine exposure would represent true effects of the drug. The first part of this study did not include an experimenter-administered saline control group when evaluating the effect of nicotine on cognitive performance. This would have exponentially increased the number of animals to fulfil the minimum group size requirements for each gestational period investigated. However, the second part of this study replicated the findings that prenatal exposure to maternal LPS treatment on GD 10/11 leads to deficits in LI and DNMTS and these impairments were not affected by saline self-administration. Thus, this shows firstly, that prenatal exposure to MIA leads to long-lasting deficits in these domains as evident by cognitive evaluation around PND 60 as well as re-testing up to PND 200 and secondly, that the same animals can be repeatedly assessed in these domains/paradigms to examine the therapeutic efficacy of drugs or other treatments. This is of particular importance considering the lack of effective pharmacological treatments for cognitive deficits in schizophrenic patients.

Repeated non-contingent (i.e. experimenter administered) nicotine treatment led to pro-cognitive effects in two of three tests. Nicotine had a global cognitive enhancing effect in sensorimotor gating (PPI) as all treatment groups increased in their ability to inhibit startle response. In LI there was a more selective effect of repeated nicotine exposure. Animals prenatally exposed to MIA on GD 10/11 showed diminished LI before nicotine, which was ameliorated in the second test-phase. Further, nicotine normalized conditioning in animals prenatally exposed to MIA on GD 18/19, but had no effect in any other group. There was no pro-cognitive effect of nicotine in the working memory task (DNMTS). A possible explanation for the absence of a nicotinic effect in the DNMTS task might be a ceiling effect. Choice accuracy before nicotine treatment across all three gestational periods was on average 88% for saline controls and approximately 82% for animals prenatally exposed to MIA. The effect of nicotine depends on baseline performance (see below) and, arguably, performance at this level is difficult to improve. For that reason, in the second set of experiments, the inter-trial interval was prolonged to 10 minutes to enhance the cognitive load and decrease task performance.

High comorbidity between schizophrenia and tobacco addiction has led to a number of studies over the last decades in humans and animals examining the effect of nicotine on

cognition, especially, as smokers have repeatedly reported perceived cognitive benefits after nicotine exposure (Heishman et al., 2010). However, findings are inconsistent and it has been suggested that the effect of nicotine on cognitive performance depends on factors such as baseline performance, task demands and smoking status. Some of the cognitive domains commonly impaired in schizophrenic patients and the effect of nicotine on these functions have been researched more than others and it is beyond the scope of this thesis to evaluate all of these studies. As evident in the present study, nicotine can lead to task-dependent selective improvements where only subjects with suboptimal functioning benefitted from the drug. Thus, emphasis in this chapter will be on findings highlighting the importance of baseline performance in the effect of nicotine on cognitive functioning. Further, preclinical studies that examined the effect of nicotine on cognitive impairments induced by neurodevelopmental models will be discussed, although the number of studies is limited and findings are mixed.

Baseline-dependent effects of nicotine in individuals with known suboptimal cognitive functioning such as patients with schizophrenia compared to healthy controls has been widely researched. Jacobsen and colleagues (2004) evaluated performance in a working memory (WM) task in smokers with schizophrenia and non-psychiatric smokers (withdrawn from tobacco) after nicotine (nicotine patch) or placebo in a within-subject design. An improvement in performance in individuals with schizophrenia in the more difficult task condition (higher working memory load) was revealed, whereas nicotine decreased performance in this condition in healthy controls (Jacobsen et al., 2004). In line with these findings, Hahn and colleagues (2012) utilized a cognitive test battery including Continuous Performance Test, identical pairs, Attentional Network Test and Wisconsin Card Sorting Test in schizophrenic patients (n=104) and matched controls (n=104), both smokers and non-smokers. In healthy volunteers, smoking history led to declined processing efficiency, yet chronic smoking proved to be beneficial in individuals with schizophrenia. Thus, nicotine improved performance in individuals with suboptimal functioning, yet decreased performance in healthy subjects with normal functioning (inverted U-curve). The authors concluded that nicotine from cigarette smoke may reduce distractibility in patients and therefore may lead to improvements in selective attention in these individuals (Hahn et al., 2012). Likewise, Woznica and colleagues (2009) examined prepulse inhibition in individuals with schizophrenia: smokers (n=14) and non-smokers (n=15), and matched controls: smokers (n=11) and non-smokers (n=10). Impaired PPI was observed in individuals with schizophrenia who were non-smokers. Schizophrenic patients who were smokers had similar



PPI compared to smoking controls. Based on these results, the authors suggest that deficits in sensorimotor gating in individuals with schizophrenia were ameliorated by nicotine (Woznica et al., 2009), although this was a cross-sectional (between-subjects) and not a longitudinal (within-subject) design.

Speculatively, nicotine-induced alterations in neurotransmitter levels such as dopamine might play a crucial role. Nicotine increases dopamine (DA) functioning in schizophrenic patients and therefore might normalize attentional processes in these individuals, yet an increase in DA might lead to excessive levels in controls resulting in decreased performance. The importance of dopamine and its contrasting effects on cognitive functioning, in particular in the PFC, has been extensively researched. Evidence elicited from both, human (Mattay et al., 2003) and animal (Granon et al., 2000) studies, has repeatedly shown that the impact of DA, in particular in tasks such as WM and tests that examine selective attention, is best described by an inverted U-shaped curve. Lower baseline performance in these domains is associated with a higher sensitivity to pharmacological manipulations leading to increased DA levels. Yet individuals with higher performance levels might be less susceptible to such manipulations as cognitive functioning is already at an optimal state. Further, a DA increasing drug could potentially lead to imbalances that disrupt prime functioning in these individuals (Robbins, 2005).

However, baseline independent cognitive enhancing effects of nicotine have also been observed. In line with clinical studies that show similar nicotine-induced pro-cognitive effects in patients and in non-psychiatric individuals, nicotine enhanced PPI in all animals in the present study. For example, Baschnagel and Hawk (2008) investigated the eye blink startle reflex after acoustic stimuli in healthy non-smokers using transdermal nicotine patches (7 mg) and found an overall increase in pre-pulse inhibition (Baschnagel & Hawk, 2008). Likewise, Postma and colleagues (2006) showed a nicotine-induced (12 mg/kg) enhancement in pre-pulse inhibition in all participants (schizophrenic patients and healthy controls). These findings were supported by neuroimaging (fMRI) data, which suggest an increase in activity in limbic regions as well as the striatum through nicotine, leading to enhanced sensorimotor gating in all groups (Postma et al., 2006). Interestingly, both studies Postma et al., (2006) as well as Woznica et al., (2009) examined the effect of nicotine in individuals with schizophrenia as well as healthy controls in pre-pulse inhibition, yet findings are inconsistent. Whereas Postma and colleagues found a global pro-cognitive effect in all participants, Woznica and colleagues observed a nicotine-related beneficial effect only in schizophrenic patients (smokers). Methodological variations may explain differences in findings as Postma

et al. utilized tactile stimuli in a within-subject design where participants were tested twice, once after nicotine exposure and once after placebo (14 days apart). Woznica et al. on the other hand, elicited startle responses using acoustic stimuli in a between subject design where individuals from both groups (schizophrenic patients and controls) were either smokers or non-smokers. Thus, despite identical outcome measures (eye-blink reflex), comparability of these studies is limited.

In sum, these studies show that nicotine's enhancing effects can be baseline dependent and independent and are most likely influenced by other variables such as task demands. Speculatively, it is possible that the nicotinic effect depends on the model utilized to induce cognitive impairment, which is further discussed below.

Very few studies have examined the effect of nicotine on cognitive functioning in neurodevelopmental models similar to the one used in the present study. For example, Chambers and colleagues (1996) utilized the neonatal ventral hippocampal lesion model (NVHL) to induce schizophrenia-like cognitive deficits. They assessed spatial learning and memory in the radial-arm maze (RAM) in lesioned rats and controls. Interestingly, nicotine treatment (0, 0.1, 0.2, 0.4 mg/kg, sc) in adulthood (PND 70) showed no significant improvement in choice accuracy (Chambers, Moore, McEvoy, & Levin, 1996). These findings are consistent with a more recent study from the same group that utilized the same neurodevelopmental model for schizophrenia. Experimenter administered nicotine injections (10 injections of 0.5 mg/kg, sc) did not ameliorate lesion-induced cognitive deficits (Berg et al., 2013).

A study by Yu and colleagues (2010), on the other hand, utilized the model of neonatal exposure to infectious agent (poly I:C, 5 mg/kg, PND 2-6, sc) to examine the effect of a single injection of nicotine (0.15 or 0.5 mg/kg, sc) in rats. They revealed a dose-dependent cognitive enhancing effect of nicotine (0.5 mg/kg) in novel object recognition (memory), yet no improvement was found in PPI (Yu et al., 2010). Although not utilizing nicotine, but a partial nAChR  $\alpha 7$ -agonist, Barak and colleagues (2009) examined the effect of SSR180711 (0.3, 1, 3 mg/kg, ip) in a conditioned emotional response paradigm of LI. To induce schizophrenia-like cognitive deficits, animals were exposed to either neonatal nitric oxide synthase inhibitor (L-NoArg, 10 mg/kg, sc, PND 4 and 5, a NOS inhibitor) or MK801 (0.05 mg/kg, ip), a NMDA receptor antagonist. Their results demonstrated that the partial  $\alpha 7$  agonist reversed abnormalities in LI in both neonatal models (Barak et al., 2009).

Overall, these studies show the diversity and complexity of nicotine's effect on cognitive functioning. It is clear from the above that the effect of nicotine depends on a number of different influencing factors such as task-demands and baseline performance. Although existing research is limited, it seems that cognitive enhancing effects of nicotine are less evident in neurodevelopmental models. However, the neurodevelopmental NVHL model, most frequently used in pharmacological studies that examined the effect of nicotine on cognitive functioning, has its limitations. Speculatively, hippocampal lesions might produce broad irreversible damage to processes crucial to the effect of nicotine on cognitive deficits that may be incompatible with what is seen in patients with schizophrenia. The aforementioned study by Yu and colleagues (2010) utilized the neonatal infection model and results are more promising as nicotine had a pro-cognitive effect in one of two tests where nicotine ameliorated deficits in memory, but had no effect in PPI. This limited effect may have resulted from the fact that cognitive performance was studied after a single injection of nicotine. As discussed at length in chapter 5, the effects of nicotine can vary strongly with repeated injections.

The study presented in the present thesis was the first to examine the effect of nicotine on cognitive deficits induced by prenatal exposure to MIA, assessing multiple cognitive domains. Moreover, both, contingent and non-contingent nicotine administration, has been utilized. As highlighted in Chapter 6, different neurobiological effects have been found dependent on the administration paradigm. Thus, nicotinic effects in brain areas associated with cognitive functioning after experimenter-administered nicotine as discussed above could differ significantly from those obtained after nicotine self-administration. For these reasons, the second part of the study (Chapter 7) examined the effect of self-administered (contingent) nicotine on cognitive performance. Further, this is of particular relevance as nicotine self-administration models the human condition of smoking cigarettes more closely.

The current study showed that both non-contingent and contingent nicotine administration ameliorated deficits observed prior to nicotine exposure in attention (LI). Interestingly, a nicotine-induced increase in working memory performance (DNMTS) was observed after nicotine self-administration, which was not evident after non-contingent nicotine treatment. This might be (in part) related to the differences in neuroadaptations between experimenter and self-administered nicotine, yet the most parsimonious explanation seems to be the difference in baseline performance. To avoid a possible ceiling effect in the second part of the study, the inter-trial intervals were increased from 5, 30, 60, and 120 seconds as used in the study in Chapter 5 to 1, 2, 5, and 10 minutes in Chapter 7. Consistent

with a more difficult task demand and subsequently lower baseline levels, choice accuracy in the second part of the study in the MIA group GD 10/11 was significantly lower compared to the first study (approximately 70% compared to about 80%). Thus, choice accuracy was significantly increased in those animals prenatally exposed to MIA after nicotine, but not saline administration. Further, in line with the suggestion that nicotine is more beneficial in subjects with suboptimal baseline performance levels compared to individuals with normal functioning, neither nicotine nor saline self-administration had an effect in prenatally exposed saline controls.

### **8.2.1 Pro-cognitive effects of nicotine – underlying mechanisms**

The underlying mechanisms how nicotine enhances cognitive performance remain elusive. It is known that the cholinergic system is central to normal cognitive functioning and a number of factors such as the stimulation of nAChRs and subsequent alterations in neurotransmitter release in brain areas associated with cognitive functioning are crucial (Levin et al., 2006). In particular, there is substantial evidence from animal studies highlighting the importance of  $\alpha 4\beta 2$  and  $\alpha 7$  nAChRs subtypes (for details see Chapter 2). Although the  $\alpha 4\beta 2$  is the most abundant nAChR in the CNS, its role in cognition is less well studied compared to  $\alpha 7$  (Wallace & Porter, 2011). The  $\alpha 4\beta 2$  receptor subunits are encoded by the *CHRNA4* gene located on chromosome 20 (20q13.2) and *CHRNA2* gene located on chromosome 1 (1q21.3), whereas the *CHRNA7* gene, located on chromosome 15q14, is responsible for the encoding of  $\alpha 7$  subunits in humans (for review Hurst et al., 2013). The primary role of these receptors is to modulate the release of neurotransmitters crucial for cognition such as dopamine (DA), glutamate, and GABA due to their presynaptic locations. As nicotine binds with high affinity to these receptor subtypes, it is not surprising that the stimulation of these receptors may influence cognition by, for example, altering essential neurotransmitter activity levels in brain areas associated with cognitive functioning. However, it is beyond the scope of this thesis to discuss the effects of nicotine on all brain areas and neurotransmitters, thus the remainder of this section will focus on the frontostriatal pathway and the influence on dopamine and GABA neurotransmission and their effects on cognitive processing.

The frontostriatal pathway connects the frontal lobes with the basal ganglia including structures such as the dorsal (for example caudate nucleus, putamen) and ventral striatum (for example NAcc), and which subsequently innervate the globus pallidus, the substantia nigra

and the ventral palladium. Dopamine influences several aspects of the frontostriatal pathway, including the frontal cortex, as well as the dorsal and ventral striatum. Frontostriatal dysfunction has been associated with elevated DA levels in the ventral striatum, which can lead to disruptions in processes in the PFC resulting in impairments in working memory, selective attention, and sensory gating (Robbins, 1990). For example, deficits in PPI in individuals with schizophrenia have been directly linked to frontostriatal pathology (Kumari et al., 2007; Swerdlow, Caine, Braff, & Geyer, 1992). Kumari and colleagues examined prepulse inhibition (eye-blink startle response) in individuals with schizophrenia (n=30, on antipsychotic medication) and healthy controls (n=12). Overall, schizophrenic patients showed reduced PPI compared to controls. More importantly, utilizing fMRI, Kumari et al. found increased activity in brain areas including the striatum, hippocampal, as well as temporal, inferior frontal and parietal regions in healthy controls. Patients on atypical antipsychotics (risperidone, olanzapine) showed milder deficits in PPI and significantly more activity in these regions was observed compared to activity patterns in patients on typical antipsychotics (Kumari et al., 2007).

The role of dopamine in the medial PFC in PPI has mainly been established in animal research. For example, Ellenbroek et al. (1996) utilized local injections (medial PFC) of the selective antagonist of the dopamine D1-like receptors SCH 39166 (250 and 500 ng/0.5µl) as well as the selective antagonist of the dopamine D2-like receptor sulpiride (12.5, 25, 50, 100 ng/0.5µl). Both antagonists dose-dependently reduced PPI. SCH39166 significantly decreased PPI at low-intensity prepulses and the highest dose led to deficits in PPI in all intensities. There was no effect of sulpiride at the lowest dose (12.5ng), whereas the dose of 25ng showed reduced PPI at low-intensity prepulses. The two highest doses, however, resulted in significant deficits in PPI at all intensities.

Although the importance of the dopaminergic system in LI has also been well established, there is still debate amongst scholars, which neural substrates are mainly involved in the different stages of LI. The ability of nicotine to disrupt LI when administered prior to conditioning has been attributed to a nicotine-induced release of dopamine in the NAcc (for review Moser, Hitchcock, Lister, & Moran, 2000). However, Ellenbroek and colleagues (1997) suggest that the substrate underlying LI may depend on the learning paradigm. For example, evidence has accumulated that the substrate underlying conditioned taste aversion as utilized in the present study is mainly the dorsal striatum as opposed to the nucleus accumbens. Amphetamine administration into the dorsal striatum led to diminished

LI, yet had no effect when administered into the NAcc. In sum, Ellenbroek and colleagues showed that “amphetamine affects latent inhibition by stimulating dopamine release in the dorsal striatum” (Ellenbroek et al., 1997, p. 118).

The role of dopamine in WM has been more extensively researched in humans compared to PPI and LI (Kharitonova, Winter, & Sheridan, 2015). For example, Manoach et al. (2000) compared WM performance in individuals with schizophrenia (n=9) and healthy volunteers (n=9). Overall, schizophrenic patients made significantly more errors, evident in a significantly lower response accuracy compared to healthy controls. In addition, an fMRI analysis showed that both control subjects and individuals with schizophrenia activated the dorsolateral PFC during WM testing. However, schizophrenic patients also activated the basal ganglia as well as the thalamus, yet this was not evident in controls. Mattay et al. (2003) examined WM performance in individuals based on *COMT* gene polymorphisms, which has been introduced in Chapter 1. Based on findings in animal studies, the involvement of the *COMT* gene in modulating DA levels in the PFC has been well established. This polymorphism is, in general, associated with diminished DA functioning in the PFC in individuals with the val allele compared to subjects with the met allele type, who usually are associated with optimal DA levels, thus normal cognitive functioning. The effect of amphetamine, which increases DA levels in the PFC (for review Castner, Goldman-Rakic, & Williams, 2004), was examined on the Wisconsin Card Sorting Task (WCST) in individuals with the val/val (n=45) and val/met allele controls (n=52). As hypothesised, individuals with the val/val allele made significant more perseverative errors compared to controls. Amphetamine administration (oral, dextroamphetamine, 0.25 mg/kg of body weight) significantly improved performance in these individuals while performance in individuals with prior normal baseline levels deteriorated. These findings highlight the importance of baseline levels in cognitive enhancing effects of pharmacological manipulations, which is further strengthened by animal studies, which have revealed that the effect of dopamine on WM functioning might be mediated predominately by dopamine D1 receptors in the PFC (for review Goldman-Rakic, Muly, & Williams, 2000). For example, a beneficial effect on cognitive functioning through D1 receptor activation by DA agonists such as SKF 38393 (local administration into the medial PFC) showed baseline dependent effects, best described by an inverted U-shape curve (Granon et al., 2000).

In summary, these data clearly indicate that dopamine is involved in all three cognitive processes described in the present thesis, and as nicotine can influence dopamine release, makes this neurotransmitter a prime candidate for further investigation in this model.

In this respect, there is evidence for a direct causal link between maternal exposure to LPS and reduced DA levels in rats in the PFC (Baharnoori, Bhardwaj, & Srivastava, 2013; Ling et al., 2009). For example, Baharnoori and colleagues (2013) examined the effect of prenatal exposure to MIA induced by LPS (10,000 endotoxin units/kg, ip, GD 10.5-11) on DA development in male rats. Immunohistochemical analysis showed significantly reduced dopamine D2 receptor numbers in the PFC in animals prenatally exposed to maternal LPS treatment compared to controls. These findings suggest significant MIA-induced changes in the dopamine system in offspring (Baharnoori et al., 2013). These findings are of particular relevance to the present study as the gestational time period used to investigate the effect of prenatal MIA on DA is identical to the present research.

de Kloet and colleagues propose that synaptic plasticity in brain areas associated with cognitive functioning such as the PFC is influenced through short- and long-term potentiation of nAChRs, influencing cognitive processes such as attention and working memory (for review de Kloet, Mansvelder, & De Vries, 2015). In line with this, the effect of nicotine on dopamine and GABA activity in these brain areas is modulated mainly through nAChRs activation. For example, in the PFC, nicotine binds to nAChRs located on dopaminergic terminals, predominantly  $\alpha 4\beta 2$  receptor subtypes (Cao, Surowy, & Puttfarcken, 2005). Additionally, stimulation of  $\alpha 7$  receptors on prefrontal glutamate-dopamine synapses can lead to dopamine release in this area (Livingstone et al., 2009).

GABA as the main inhibitory neurotransmitter in the CNS also plays a very important role in the frontostriatal pathway, in particular in the projection from the striatum and the NAcc to the globus pallidus, substantia nigra and ventral palladium. Disruptions in inhibitory circuits have been identified in individuals with schizophrenia in brain areas that are essential for cognitive functioning (for review Lewis, Hashimoto, & Volk, 2005). For example, a decrease of GABAergic function in individuals with schizophrenia in the prefrontal cortex (Radhu et al., 2015) and the hippocampus (Knable et al., 2004) has been observed. Lewis and colleagues (2008) examined the effect of MK-0777 (a benzodiazepine-like agent with selective activity at GABA-A receptors) or placebo in individuals diagnosed with schizophrenia (n=15). Cognitive assessment included working memory and cognitive control measures (N-back, AX Continuous Performance Test, Preparing to Overcome Prepotency). Findings revealed a significant improvement in all three assessment paradigms compared to controls. Thus, the authors suggest that increased GABA neurotransmission can enhance cognitive functioning in the PFC (Lewis et al., 2008).

The importance of GABA neurotransmission in the PFC has also been demonstrated in multiple animal studies. For example, Piantadosi and Floresco examined the effect of intra medial PFC infusions of the GABA-A receptor antagonist bicuculline methobromide (0.5  $\mu$ l/bilateral per side) on latent inhibition in rats. Their findings revealed that receptor blocking before testing, but not before conditioning, diminished LI. Thus, the authors suggest a mediating role of GABA neurotransmission in the retrieval process of LI important information (learned irrelevance of stimuli) (Piantadosi & Floresco, 2014). Utilizing the same GABA-A antagonist bicuculline (12.5 to 50 ng), Auger and Floresco examined the effect of GABA neurotransmission in the mPFC in a reference and working memory task (8 arm maze) in rats. Their results show a significant increase in errors in reference as well as working memory at both doses of bicuculline compared to controls (Auger & Floresco, 2015).

Thus, similar to dopamine, there is ample evidence pointing to a role of GABA in the cognitive processes studied in the present thesis. Moreover, there is evidence that MIA may also affect GABA levels in the brain. This has most often been studied by analysing the expression of the GABA synthesizing enzymes, glutamic acid decarboxylase (GAD) 65 and 67 (Satta et al., 2008). Basta-Kaim and colleagues (2015) examined the effect of prenatal exposure to MIA (LPS, 1 mg/kg, daily sc injections from GD 7 onwards) in rats on GAD 67 in the medial PFC and hippocampus. Immunohistochemical analysis showed GAD67 reduction in female rats in the mPFC, whereas GAD67 decrease in male rats was predominantly found in the hippocampus. Both genders expressed a reduction of GABAergic cells in the mPFC after prenatal exposure to MIA compared to controls. In addition, animals were assessed in sensorimotor gating (PPI) and social interaction (resident-intruder paradigm) in adulthood (PND 90). Compared to saline controls, animals prenatally exposed to maternal LPS showed deficits in PPI in female and male rats. The prenatal LPS group showed a significant increase compared to saline controls in aggressive interactions. Additionally it was observed that prenatal LPS exposed female rats displayed a significant decrease in social grooming (Basta-Kaim et al., 2015).

Interestingly, repeated nicotine administration (4.5 to 22  $\mu$ mol/kg, sc, 4 injections/day, 4 days) results in increased levels of GAD 67 in mice in cortical and hippocampal areas, but not in striatal GABAergic neurons. Thus nicotine-induced stimulation of nAChRs in these brain regions may normalise GABA levels in MIA exposed rats (Satta et al., 2008). Consistent with these findings, Maluku and colleagues (2011)



examined the effect of several partial and full  $\alpha 4\beta 2$  and  $\alpha 7$  agonists and antagonists in mice and found that stimulation of nAChRs containing  $\alpha 4\beta 2$ , but not  $\alpha 7$  subunits, led to an increased expression of GAD67. These findings highlight the importance of  $\alpha 4\beta 2$  in enhancing cognitive functioning in schizophrenic patients (Maloku et al., 2011) and suggests that the cognitive enhancing effects seen in Chapters 5 and 7 of the present thesis may be due to the normalizing effects of nicotine on GABA and/or dopaminergic systems disturbed by MIA.

### **8.3 Nicotine is only slightly more reinforcing in animals prenatally exposed to MIA**

Animals prenatally exposed to maternal LPS treatment on GD10/11 significantly increased nicotine self-administration during acquisition (FR5), but had no effect on dose-response or progressive-ratio testing. Overall, these findings suggest that nicotine was not substantially more reinforcing for animals prenatally exposed to maternal infection.

Nicotine self-administration is commonly assessed in operant chambers and requires experimental animals to perform a behavioural task such as pressing a lever to obtain a drug infusion (see Appendix A). Compared to other psychostimulant drugs, nicotine is only a weak reinforcer as animals readily chose, for example, cocaine over nicotine if given a choice (Manzardo, Stein, & Belluzzi, 2002). Interestingly, nicotine elicits similar reward threshold alterations in the brain as stimulants such as ethanol or caffeine (Bespalov, Lebedev, Panchenko, & Zvartau, 1999). Although nicotine is self-administered by a number of animal species, it requires specific self-administration parameters during acquisition and is influenced by non-pharmacological factors such as environmental cues (Brennan, Laugesen, & Truman, 2014). A number of methodological details such as the dose, infusion-rate, or session length significantly influence nicotine self-administration. For example, Sorge and Clark (2009) established that rats prefer infusion rates of 30 seconds compared to faster rates of 3 seconds or slower rates of 60 or 120 seconds on a fixed ratio (FR) schedule of reinforcement. FR schedules require a fixed or constant number of responses (lever presses) to obtain a nicotine infusion (Rupprecht et al., 2015). The present study utilized three different FR schedules to establish nicotine self-administration (FR 1, 2 and 5), which required one, two or five lever presses to obtain an infusion. These FR schedules were followed by dose response (DR) schedules to examine differences in response behaviour based on four nicotine doses. Finally, a progressive ratio (PR) schedule of reinforcement evaluated the reinforcement or rewarding efficacy of nicotine, as animals were required to perform a sequentially increasing number of lever presses to obtain a drug infusion (1, 2, 4, 6,

9 and so on). The PR schedule establishes the maximum work a subject is willing to perform (breakpoint) for the drug (Brennan et al., 2015).

Utilizing the same equipment, testing procedures and species/age of rats, our laboratory has used these self-administration protocols to compare the reinforcing efficacy of different drugs. When the psychoactive effect of the drug was more reinforcing, higher levels of responding were observed during self-administration acquisition. For example, a study in our laboratory that compared the reinforcing efficacy of different tobacco types, pure nicotine, tobacco particulate matter (TPM, which consists of the total combustion product actively inhaled by smokers), and roll-your-own TPM (TPM-RYO) revealed that TPM-RYO was significantly more reinforcing compared to nicotine and TPM. Although all groups showed significantly increased responding compared to saline controls, those animals that self-administered TPM-RYO showed a different behavioural profile and responded at significantly higher levels during acquisition. Further, PR breakpoints were higher, and there was evidence of dose-related responding. These observations are consistent with an increased 'motivation to work' to obtain infusions (higher breakpoints) and responding based on the pharmacological effects of the drug (dose-dependent-responding) (Brennan et al., 2015). More precisely, animals increased the number of lever presses to compensate for lower doses to obtain the desired level of nicotine. Thus, TPM-RYO was persistently more reinforcing compared to nicotine and TPM as evident in higher levels of responding during early and late stages of self-administration acquisition (FR 2 and 5) and produced significantly higher breakpoints during PR testing.

Although animals self-administering nicotine in the present study showed a trend for higher rates of responding for the 30 µg/kg/infusion dose, this was not significant during the FR acquisition schedules or during DR. These findings are in line with previous studies that revealed that nicotine doses between 15 and 30 µg/kg/infusion showed optimal effects compared to lower doses of 7.5 and higher doses of 60 µg/kg/infusion (Corrigall & Coen, 1989; Sorge & Clarke, 2009). Thus, the results of the present study are consistent with a for nicotine typical relatively flat dose-response curve. Furthermore, and in line with the findings in DR, the present results in PR testing show similar breakpoint levels for nicotine administering animals regardless of prenatal treatment with an average of 10 to 12 breakpoints per session at nicotine doses 15 and 30 µg/kg/infusion. These findings are consistent with the results obtained in the aforementioned study where animals self-

administering pure nicotine had comparable breakpoints with these doses (Brennan et al., 2015).

In sum, these findings only offer weak support for the common-substrate theory as animals prenatally exposed to MIA were not more susceptible to nicotine compared to prenatally exposed saline controls. It is hypothesised that the observed higher responding rate of the MIA group during the FR 5 acquisition schedule was a temporary event. Speculatively, a critical amount of nicotine exposure may be needed to produce neuroadaptations and this may have occurred slightly earlier in the MIA exposed animals, hence the higher FR5 responding during acquisition. However, once these adaptations have been achieved, MIA and control animals perform similarly. In line with this, inspection of Figure 3 (Chapter 7) shows that the difference between both groups in responding decreases towards the final days of the acquisition phase. This interpretation is further supported by the findings during the later PR testing phase where no difference between prenatal treatment groups was observed during the intermittent FR5 schedule. Overall, these data indicate that the difference between the two groups is small and can only be observed during a particular time period during acquisition.

#### **8.4 Future research**

The current study examined the effect of prenatal exposure to MIA in early adulthood (commencing PND 60). Although this time course is in line with the onset of the disorder in individuals with schizophrenia, future research could expand on the current study by investigating the effect of LPS-induced MIA in adolescent rats (PND 35 to 45). Thus far, only very few studies have examined the effect of prenatal exposure to MIA at multiple time points. For example, Basta-Kaim and colleagues (2012) examined the effect of prenatal LPS (1 mg/kg, sc, every second day from GD 7 throughout pregnancy) on PPI on PND 30 and 90 and consistent with findings in the current study found an effect of prenatal treatment in PND 90 (Basta-Kaim et al., 2012). Although no effect was found on PND 30, the adolescent time period in rats ranges usually between PND 35 and 45. Thus, it is possible that an assessment 10 days later (PND 40) might have elicited different results. Further, Zuckerman and Weiner (2003) utilized the polyI:C model (4 mg/kg, GD 15, IV injection) to examine the effect of MIA on LI on PND 35 and 90. Consistent with the schizophrenia-typical onset delay, deficits in selective attention were evident in adulthood, but there was no effect in adolescence (Zuckerman & Weiner, 2003). A more comprehensive evaluation including different prenatal MIA models and additionally multiple outcome measures would

significantly enhance existing knowledge in this field. It is possible that MIA induced disruptions in neurodevelopment may affect cognitive domains differently depending on the time of the insult during gestation.

Further, the current study examined the effect of prenatal exposure to MIA on cognitive performance. It would be interesting to evaluate the effects of prenatal MIA on other aspects of schizophrenia such as negative symptoms. For example, avolition, a lack of motivation (apathy), has been considered as a core element of schizophrenia. Avolition is different from anhedonia, a lack of pleasure, as it is possible that individuals can experience pleasure, yet have a diminished capacity to anticipate whether consequences of actions will be pleasurable (Avolition). This aspect can be assessed in subjects by measuring incentive motivation (Culig & Belzung, 2016). Paradigms that examine impaired incentive motivation include, but are not limited to, PR tasks similar to the PR schedule used in the present study. This paradigm examines a subject's incentive to work (pressing a lever) for rewarding stimuli such as a food reward and establishes the breakpoint as the maximum amount of effort an animal is willing to invest to obtain the reward. Thus, the breakpoint is considered an index of avolition (Ellenbroek & Cools, 2000). Although no effect of prenatal treatment on nicotine self-administration in the PR schedule was obtained in the present study, it would be interesting to assess reward anticipation using natural rewards such as food rewards in this model.

Additionally, the aspect of social interaction could be assessed in utilizing a paradigm such as social choice (social novelty), social approach avoidance, or social selective attention. This paradigm evaluates whether the experimental animal can distinguish between a known and a novel animal by measuring social recognition memory defined by the amount of time the experimental animal spends in close proximity of each. It is expected that normally developed animals spend significantly more time with the unfamiliar animal whereas impaired subjects are expected to spend an equal amount of time with both the familiar and unfamiliar animal (Culig & Belzung, 2016). Although there are a small number of studies that utilized prenatal exposure to MIA as the underlying neurodevelopmental model to induce deficits, the effect of timing of the insult during gestation and social behaviour has not been fully examined.

Furthermore, the current study utilized an environmental factor as a predisposition in the neurodevelopmental model for schizophrenia. However, it is known that schizophrenia develops due to an interaction between various genetic and environmental factors. The present research project could be expanded by utilizing prenatal exposure to MIA in animals

with a genetic predisposition as this would further strengthen construct validity. Although genetic mice models have been around for much longer compared to genetic rat models, more recent advances in understanding the rat genome have facilitated a number of possibilities that could successfully represent rat models for schizophrenia. In general, genetic models can be subdivided into forward and reverse genetic models. Forward models are based on animals displaying a specific phenotype such as deficits in sensorimotor gating. Based on this phenotype selective breeding can be used, which subsequently allows for the identification of the gene(s) involved in this phenotype. However, it is important to note that schizophrenia is a complex disorder characterised by multiple phenotypes. Reverse genetic models, on the other hand, are based on changes in the rat genome and subsequently assessing the impact of this genetic alteration on behaviour. This model allows researchers to target specific genes, however, by now it has been well established that the development of schizophrenia is not due to a single genetic defect (Ellenbroek & Karl, 2016).

For example, the apomorphine susceptible/unsusceptible (APO-SUS/UNSUS) model is an example of a forward genetic rat model. Based on the importance of dopamine in schizophrenia, studies have found that individuals with schizophrenia are more susceptible to apomorphine (selective dopamine agonist). Consistent with these findings in human studies, Wistar rats were selectively bred based on differences in the response to apomorphine. Rats that were more susceptible to apomorphine (APO-SUS) showed a strong, stereotypical gnawing response compared to APO-UNSUS rats that only displayed a weak response (Ellenbroek & Cools, 2002). More importantly, studies have revealed that APO-SUS animals show decreased PPI and LI, thus, the APO-SUS/UNSUS model can be considered as an animal model for schizophrenia. In particular, this model is also associated with schizophrenia relevant neurochemical disturbances (construct validity) in addition to the expression of relevant behavioural anomalies (face validity) (for review Ellenbroek & Karl, 2016).

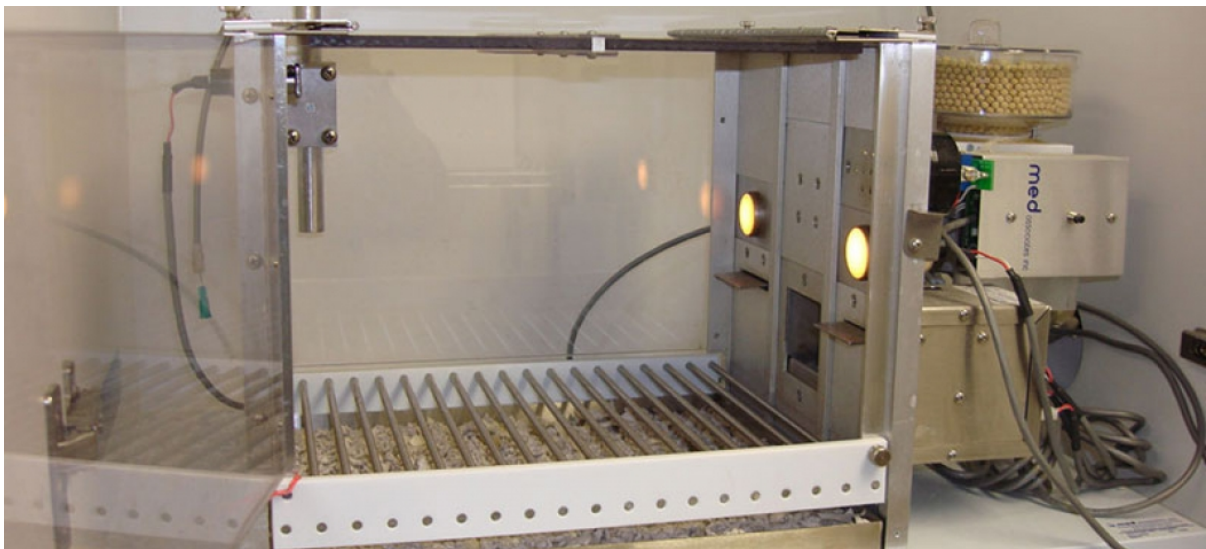
Furthermore, the results in nicotine self-administration in the current study present some interesting questions for future investigations. For example, specific impulsivity testing (i.e. locomotor sensitivity) could be conducted when animals reach the FR5 stage during acquisition to determine whether changes in impulsivity might underlie increased response behavior. Additionally, adjustments to time exposed (i.e. number of days or 6 hour runs compared to 2 hour runs) during the FR1/2/5 periods (i.e. shortening and lengthening) could address the question whether more or less nicotine exposure affects the results and would assist in determining whether there is a “critical time window” to observe these effects.

Further, the present study examined the effect of pure nicotine on cognitive performance and in self-administration. However, tobacco smoke consists of over 4,000 different chemical compounds including nicotine (Fowles & Dybing, 2003) and it has been revealed that compared to pure nicotine and TPM, TPM-RYO was more rewarding and reinforcing and yielded significantly higher levels of responding on all three schedules (Brennan et al., 2015). Thus it is possible that nicotine is the primary driver for cognitive enhancing effects, yet the combination of nicotine and other compounds in cigarette smoke could be responsible for its stronger addictive properties in schizophrenic patients. In addition, morphological studies with immunohistochemistry could inform about existing differences between MIA animals and controls during different stages of the experiment as well as at later stages in, for example, receptor densities (for example nAChRs  $\alpha 7$  and  $\alpha 4\beta 2$ ). Speculatively, prenatal exposure to MIA could affect nAChRs expression and function differently in particular brain areas such as the frontostriatal complex. In addition, nicotine-induced alterations in nAChRs could be affected by prenatal exposure to MIA. For example, nicotine might have a nicotine-typical effect in terminal areas, yet have no or a diminished effect in the VTA and substantia nigra. Additionally, the propensity to self-administer other psychostimulant drugs that are known to be more reinforcing compared to nicotine such as cocaine or heroin could be assessed in animals prenatally exposed to MIA, especially as individuals with schizophrenia usually engage in poly-substance use. Alternatively, utilizing the same neurodevelopmental model, other paradigms that examine reinforcing efficacies of drugs of abuse such as the conditioned place preference (CPP) task could be used. As shown by Borcoi et al. (2015), mice prenatally exposed to polyI:C showed increased amphetamine-induced CPP.

Finally, from a mechanistic point of view it will be important to investigate the underlying neurobiological substrate of MIA and the nicotine induced reversal. As discussed above, both dopamine and GABA have been implicated in the cognitive deficits in schizophrenia and appear to be altered in some MIA models. Moreover, the release of both neurotransmitters is enhanced by nicotine. It will be of interest to see whether the effect of timing on cognitive deficits, described in Chapter 5, is paralleled in alterations in dopamine and GABA. Likewise, it would be of interest to see whether the normalizing effects of nicotine on cognition are associated with comparable improvements in dopamine and/or GABA neurotransmission.

## APPENDIX A.

### Image self-administration chamber



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