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Effects of 2-bromoterguride, a dopamine D_2 receptor partial agonist, in animal models of negative symptoms and cognitive dysfunctions associated with schizophrenia

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Andrea Emilia Tarland
Biologin aus Lund, Schweden

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Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Heidrun Fink
Zweiter Gutachter:	Univ.-Prof. Dr. Christa Thöne-Reineke
Dritter Gutachter:	PD Dr. Bettina Bert

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For Linus

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AD	Alzheimer´s disease
AIL	Amphetamine-induced locomotion
AMPA	a-Amino-3-hydroxy-5-methyl-4-isoazolepropionic acid
APDs	Antipsychotic drugs
ASR	Acoustic startle response
BDNF	Brain-derived neurotrophic factor
BNSS	Brief Negative Symptom Scale
CAINS	Clinical Assessment Interview for Negative Symptoms
CAR	Conditioned avoidance response
CNS	Central nervous system
DA	Dopamine
DISC	Disrupted in schizophrenia
DOI	(±)-2,5-Dimethoxy-4-iodoamphetamine
DRN	Dorsal raphe nucleus
DSM-III	Diagnostic and Statistical Manual of Mental Disorders
DUP	Duration of untreated psychosis
ELISA	Enzyme-linked immunosorbent assay
EPS	Extrapyramidal side effects
FDA	American food and drug administration agency
FRS	First-rank symptoms
GABA	Gamma-aminobutyric acid
GlyT	Glycine transporter
5-HT	5-Hydroxytryptamine; serotonin

ICD	International Statistical Classification of Diseases and Related Health Problems
iGluR	Ionotropic glutamate receptor
ITI	Intertrial interval
KA	Kainate
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
mGluR	Metabotropic glutamate receptor
MRN	Median raphe nucleus
nAChR	Nicotinic acetylcholine receptor
NIMH	National Institute of Mental Health
NMDA	N-Methyl-D-aspartate
NO	Nitric oxide
NOR	Novel object recognition
6-OHDA	6-Hydroxydopamine
PAM	Positive allosteric modulator
PCP	Phencyclidine
PD	Parkinson´s disease
PPI	Prepulse inhibition
PRL	Prolactin
RDC	Research Diagnostic Criteria
RM ANOVA	Repeated measures analysis of variance
SANS	Scale for the Assessment of Negative Symptoms
SEM	Standard error of the mean
SID	Smell identification deficits
SPL	Sound pressure level

VTA	Ventral tegmental area

CHAPTER 1: General introduction

Schizophrenia is a devastating psychiatric disorder associated with a mesolimbic hyperdopaminergic and a mesocortical hypodopaminergic state. The treatment of acute and chronic schizophrenia involves alleviating the positive symptoms, negative symptoms and cognitive deficits. Overall, available antipsychotic drugs (APDs) can successfully silence the positive symptoms such as hallucinations and delusions, as they efficiently elicit antagonistic actions on dopamine D_2 receptors. Unfortunately, the APDs are associated with aversive side effects and there is still severe paucity in the treatment of negative symptoms and cognitive impairments. As a result of these unmet clinical needs, patients with schizophrenia are often permanently excluded from the society.

2-Bromoterguride is a dopamine D_2 receptor partial agonist that shows antipsychotic-like effect in rats (Jantschak et al., 2013) without inducing extrapyramidal side effects (EPS) and metabolic changes (Franke et al., 2016). In addition to its action on D_2 receptors, 2-bromoterguride has affinities for several other receptor types implicated in the pathophysiology of schizophrenia, including the serotonergic and glutamatergic systems, and hence meets the prerequisites for a putative atypical APD.

The aim of this PhD thesis was to investigate the effects of 2-bromoterguride in rat models of negative symptoms and cognitive deficits associated with schizophrenia (**Chapter 4**). First, the effect of 2-bromoterguride on prepulse inhibition (PPI) of the acoustic startle response (ASR), a measure of sensorimotor gating, was assessed. The pre-attentive cognitive process PPI is disturbed in patients with schizophrenia and represents an endophenotype that can be modelled and tested in animals. To induce PPI deficits, the N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) and the mixed D_1/D_2-receptor agonist apomorphine were used. To assess the prospective effects of 2-bromoterguride on cognitive impairments and negative symptoms, male rats were treated with subchronic PCP and tested in the novel object recognition (NOR) test and the social interaction test respectively. With relevance to hyperprolactinemia, the prolactin response following systemic administration of 2-bromoterguride in male rats at three different time-points was measured in vitro by performing a prolactin enzyme-linked immunosorbent assay (ELISA).

In addition, to validate the subchronic PCP model used for the social interaction test, an olfactory habituation/dishabituation test was implemented to control that the PCP-treated animals did not show any signs of anosmia or hyposmia, defects that could have severe impact on social

behaviour in the social interaction test. Notably and despite the popularity of the subchronic PCP rat model for investigating negative schizophrenia symptoms, especially social behaviours, we were the first to conduct a proper study of olfactory function. The results of the olfactory habituation/dishabituation test in this PhD thesis hence entail important conclusions for previous and future studies aiming to investigate social behaviour in the subchronic PCP rat model (**Chapter 5**).

CHAPTER 2: Literature review

2.1 Schizophrenia

The physician Benedict Morel characterized one of the earliest cases of schizophrenia, as he described a young adolescent man suffering from rapid cognitive deterioration in 1852. Morel described the condition as “demence precoce”, referring to the early age onset of the mental deterioration (Adityanjee et al., 1999). In the meantime, Karl Kahlbaum and Ewald Hecker delineated and described the catatonic syndrome respectively hebephrenia (adolescent insanity) (Adityanjee et al., 1999; Tenório, 2016). In the years 1887 to 1927, the German psychiatrist Emil Kraeplin published nine editions of his book of psychiatry, in which he presented a new nosological system (Hoff, 2015). Here, Kraeplin added the category dementia paranoides and incorporated the clinical syndromes previously described by Morel, Kahlbaum and Hecker. Emphasizing on the early onset of symptoms and the degenerating course of the illness, Kraeplin broadened the term demence precoce in 1893 and named it *dementia praecox* (Adityanjee et al., 1999; Hoff, 2015). The swiss psychiatrist Eugen Bleuler modified the Kraeplin concept in 1911 as he believed that the clinical illness described as *dementia praecox* was not one disorder but a cluster of disorders (Carpenter et al., 1999; Jablensky, 2010; Hoff, 2012). Bleuler manifested the term “schizophrenia”, which stems from Greek and translates to “split mind”, or as intended by Bleuler; “splitting of psychic functions*”* (Moskowitz & Heim, 2011; Hoff, 2012). The original intent behind Bleulers term “schizophrenia” was to describe the disintegrated psychological association processes involved in schizophrenia. Nonetheless, the term has led to some public misconception by confounding schizophrenia with the dissociative identity disorder (McNally, 2009; Moskowitz & Heim, 2011). Bleuler distinguished between basic (obligatory) and accessory (delusions and hallucinations) symptoms of the disorder (Jablensky, 2010) and like Kraeplin, Bleuler acknowledged the heterogeneity of the disorder by referring to the “group of schizophrenias”. The multiple aetiological factors and pathophysiological mechanisms in schizophrenia, however, remained neglected by both Bleuler and Kraeplin as they continued to view schizophrenia as a single disease entity (Adityanjee et al., 1999; Bruijnzeel & Tandon, 2011).

The variable phenotypic expression and complex aetiology in schizophrenia is still poorly understood. However, the disorder is believed to be constituted by major genetic influence interacting with several environmental factors (Jablensky, 2010). More than two-thirds of the schizophrenia cases occur sporadically, however, it is well known that schizophrenia is an inheritable disorder. Based on twin study data or data from Scandinavian national population

family and adoption data, the heritability of schizophrenia has been estimated to 60 - 80% (Lichtenstein et al., 2009; Tandon et al., 2010; Cardno & Owen, 2014). The estimated prevalence for schizophrenia is around 1% worldwide but the incidence varies significantly with urbanicity, migration background and gender, with males subjected to a higher risk of developing schizophrenia than females (Tandon et al., 2010).

The neuroanatomy in schizophrenia has been studied since the 1920s and some global brain structure abnormalities and alterations seem to be evident such as reduction in whole brain- and grey matter volume and connectivity alternations, especially in the medial and superior temporal and prefrontal cortices. However, due to the subtle and non-specific nature of the anatomic alterations in schizophrenia, they are not suitable for diagnostic purposes (Keshavan et al., 2008). Several genes and chromosomal regions have been associated with an increased risk of developing schizophrenia. Many of these genes modulate neural connectivity, synaptogenesis and NMDA receptor functions, and their malfunctions result in insufficient information processing at glutamate synapses (Ross et al., 2006). Especially four “susceptibility” genes have strong implications for the development of schizophrenia as they act as key genes in the regulation of neuronal connectivity and synaptogenesis. These genes code for the proteins BDNF (brain-derived neurotrophic factor), dysbindin, DISC-1 (disrupted in schizophrenia-1) and neuregulin, all affecting normal brain development and synapse formation via mechanisms during early neurogenesis, neuronal migration, dendritic organization, myelination and synaptogenesis (Stahl, 2007).

In addition to the genetic influence in the aetiology of schizophrenia, some biological and psychosocial environmental factors, for example cannabis abuse, prenatal infections or malnutrition, and perinatal complications, have been linked to a higher risk of developing schizophrenia (Tandon et al., 2010). It has been proposed that maternal infections during the first and the early second trimester of pregnancy increases the risk of the child developing schizophrenia later in life due to the caused inflammatory responses. Modelled in rodents, prenatal immune challenge results in the activation of immuno-inflammatory pathways coupled to increased microglia activation. This primes early pre- and postnatal alterations in peripheral and central inflammatory response systems and disrupts the normal maturation of neuronal systems including myelination, synaptic pruning, and neuronal remodelling (Meyer, 2013).

Several recent studies have supported the hypothesis of impaired neural synchrony in schizophrenia, i.e. impaired neural circuitry and coordination of distributed processes that involves gamma band synchronization of neurons in multiple brain areas (Spencer et al., 2003; Uhlhaas &

Singer, 2006). Such failure in communication and coordination between cognitive regions may reflect one aspect of schizophrenia and account for some of the impairments, from psychosis to cognitive dysfunction (Ford et al., 2007).

2.1.1 Symptoms

Patients with schizophrenia show substantial heterogeneity regarding course, onset and outcome of the disorder as well as in the symptomology (Bruijnzeel & Tandon, 2011). The symptoms of schizophrenia are subdivided in positive symptoms, negative symptoms and cognitive impairments (Table A.1). In 1959, the German psychiatrist Kurt Schneider claimed in his book “Clinical Psychopathology” that specific symptoms of schizophrenia were pathognomonic (specific for the disorder) and he listed 11 “first-rank" symptoms of schizophrenia as the main criteria for the disorder (Strauss et al., 1974). The Schneiderian first-rank symptoms were later incorporated in the Research Diagnostic Criteria (RDC) (Spitzer et al., 1978), the Diagnostic and Statistical manual of Mental disorders: DSM-III (American Psychiatric Association, 1980) and in the International Statistical Classification of Diseases and Related Health Problems: ICD (World Health Organization, 1992). At present, schizophrenia is diagnosed based on either the ICD-10 Classification of Mental and Behavioural Disorders or the DSM-IV (American Psychiatric Association, 1994) (Table A.2).

2.1.1.1 Positive Symptoms

The onset of schizophrenian psychosis is usually preceded by a prodromal or “prepsychotic” period extending from its stable phase until the onset of direct psychotic symptoms. During this stage, the patient may experience various diffuse subclinical symptoms such as anxiety, moodiness, depression-like feelings as well as cognitive, vegetative and perceptual disturbances (Yung et al., 2003). First episode psychosis interferes substantially with the daily living of the patient due to the lost reality contact which often comes accompanied with blunted or inappropriate emotional expressions and motivational deficits (Larson et al., 2010). The Schneiderian first-rank symptoms (FRS) include auditory thoughts, hearing voices, delusional interpretation of the reality, thought insertion, withdrawal and broadcasting as well as somatic passivity; experiencing that mind, feelings or impulses are controlled by an alien force. It has been argued that patients with schizophrenia suffering from substantial FRS during the acute phase of the disorder are more likely to have poor long-term outcome compared to patients without early FRS (Rosen et al.,

2011). In addition to the FRS, the prognostic value of the DUP (duration of untreated psychosis; the time window from onset of the first psychotic symptom until the patient is prescribed with an adequate therapy), is non-disputable and a longer DUP is implicated with severer cognitive deterioration in first-episode schizophrenia (Amminger et al., 2002). Positive symptoms of schizophrenia can often be effectively treated with available dopamine D_2 antagonistic APDs, however, the risk of relapse is high, and many patients experience one or more psychotic episodes during their lifetime (Wils et al., 2016).

2.1.1.2 Negative symptoms

The negative symptoms of schizophrenia, i.e. asociality, alogia, anhedonia and blunted affect, belong to a distinct and critical symptomatic domain in schizophrenia, much resembling the symptoms of clinical depression (Murphy et al., 2006). The negative symptoms can be further subdivided in the two related, but separate subdomains *diminished expression* and *amotivation* (Foussias et al., 2015). In contrast to positive symptoms, which have received attention during the complete 20th century, it was not until the 1980s that the aetiology and treatment of negative symptoms started to get more attention from psychiatrists and pharmacological companies (Murphy et al., 2006). Nonetheless, negative symptoms significantly affect the patient life-quality and asociality (social withdrawal) is a major contributor to the poor psychosocial functioning and the lack of rehabilitation in patients diagnosed with schizophrenia (Wilson & Koenig, 2014). Around 25 - 30% of the patients with chronic schizophrenia have predominantly negative symptoms and are therefore commonly defined as patients with “deficit” schizophrenia. The subgroup of schizophrenic patients with sustaining primary negative symptoms is smaller than the group of patients with “nondeficit” schizophrenia, i.e. patients who show clinical negative symptoms only when both primary and secondary negative symptoms are included in the ratings (Kirkpatrick et al., 2001). To date, negative symptoms can be rated more extensively using the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982), the Brief Negative Symptom Scale (BNSS) (Kirkpatrick et al., 2011) or the Clinical Assessment Interview for Negative Symptoms (CAINS) (Kring et al., 2013).

Effective treatment of negative symptoms overall is critical in preventing a deteriorating course of schizophrenia, yet the effects of antipsychotic medications has continued giving disappointing results (Murphy et al., 2006).

2.1.1.3 Cognitive impairments

Patients with schizophrenia show impairments in a wide range of cognitive domains including executive function, working memory and episodic memory (Barch & Ceaser, 2012). The cognitive deficits are enduring core features of schizophrenia, strongly correlating with the patient's functional outcome and ability to reintegrate in society (Green, 2006). Consequently, cognition is a much reasonable treatment target in schizophrenia and the National Institute of Mental Health (NIMH) therefore initiated the "measurement and treatment research to improve cognition in schizophrenia" (MATRICS) consensus cognitive battery to standardize clinical trials on cognition for drug development and testing of novel therapeutics (Green & Nuechterlein, 2004). The test battery includes tests for the factors "speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving", as well as social cognition (Nuechterlein et al., 2004).

Symptoms of impaired social functioning were traditionally grouped together with the negative symptoms of schizophrenia. However, a few years ago, social cognition was identified as a contributor to the poor functional outcome in schizophrenia patients and consequently the domain received more attention and was regrouped (Couture et al., 2006). Social cognition can be subdivided in "emotional processing" (perception and use of emotion), "theory of mind/ social intelligence" (ability to understand intentions and beliefs of others), "social perception" (ability to judge social rules and roles as well as social context), "social knowledge/social scheme" (awareness of cues that describe social situations and steer social interactions) and "attributions" (how one explicate the reasons for positive and negative outcomes/events) (Green et al., 2004). Neurocognitive and social cognitive tasks are clearly associated and share cognitive processes such as working memory and perception. Nonetheless, specialized methods and models that separate the two domains tend to give a better fit than a combination of the latter, implicating that social cognition is linked to neurocognition but not redundant with it (Green & Horan, 2010).

2.1.2 Neurochemistry

Schizophrenia is associated with structural and functional abnormalities in different parts of the brain (see Figure 2.1). The symptoms of schizophrenia are believed to derive from altered neurotransmission, i.e. compromised functional integration between cerebral subsystems (Dawson et al., 2014). Due to the known interactions of first generation (typical) APDs with primarily dopaminergic neurotransmission and second generation (atypical) APDs with primarily

serotonergic neurotransmission, the two transmitter systems significantly contribute to the pathophysiology in schizophrenia (Uhlhaas & Singer, 2006). However, the dopaminergic- or serotonergic systems cannot be accountable for all the aspects of schizophrenia, and many patients are unresponsive to dopaminergic compounds (Moghaddam & Javitt, 2012). As such, several other neurotransmitter pathways, for example by glutamate, gamma-Aminobutyric-acid (GABA), and acetylcholine, have been investigated for their involvement in schizophrenia, and for their potential use as drug treatment targets (Karam et al., 2010). The cholinergic system has important functions in cognitive and attentional processing and metabotropic and nicotinic receptors are widespread in the brain. Impairments in this circuit have been reported and smoking, which is frequently seen in schizophrenia patients, has been postulated to aim as a kind of "self-medicating" (Rowe et al., 2015). However, disturbed neurotransmission in the glutamate system has been especially implicated in the development of schizophrenic symptoms (Moghaddam & Javitt, 2012). Many of the known "susceptibility" genes for schizophrenia can be linked to synaptogenesis at glutamate synapses or glutamate neurotransmission, for example at the NMDA receptor (Stahl, 2007).

Neuronal function and connectivity can additionally be disrupted due to neurodevelopmental defects. For example, interruptions during myelination that causes dysfunctions to myelinated fibre pathways, have been reported to trigger the development of psychotic symptoms (Mighdoll et al., 2015). Especially the involvement of oligodendrocytes, (glial cells responsible for the synthesis of myelin in the central nervous system; CNS) have been linked to the pathophysiology of schizophrenia (Chew et al., 2013; Chiappelli et al., 2015). To date, nonetheless, the glutamate and dopamine hypotheses remain the most preeminent theories although, the exhaustive neurobiology of schizophrenia remains to be elucidated.

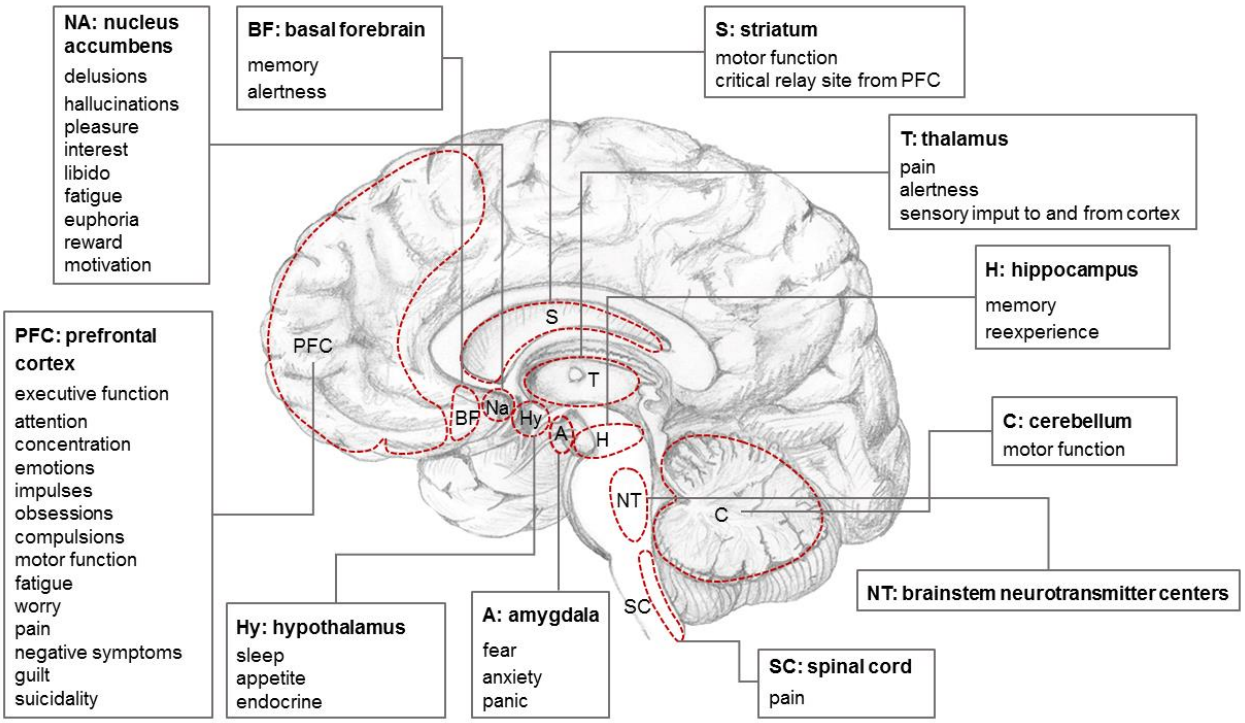

Figure 2.1 Brain region key functions and their implications for schizophrenia (adapted from Stahl, 2008, p.3)

2.1.2.1 The dopamine hypothesis

The dopamine hypothesis of schizophrenia was initially postulated based on the observation that the dopamine agonist amphetamine could induce a schizophrenia-like psychosis in healthy individuals (Reynolds, 2005; Howes et al., 2015). The hypothesis was supported by the fact that all APDs are dopamine D_2 receptor antagonists and that the clinical dose of a drug closely correlates to the antagonistic actions. Since then, the dopamine hypothesis of schizophrenia has been the dominant neurobiological explanation for the numerous symptoms and impairments in schizophrenia, especially the positive symptoms (Reynolds, 2008; Seeman, 2013a).

In humans, five identified dopamine (DA) receptor subtypes D_1, D_2, D_3, D_4 and D_5 are grouped into two major receptor subclasses: D_1-like and D_2-like receptors. The D_1-like receptor subclass (receptor subtypes D_1 and D_5) is predominantly postsynaptic meanwhile the D_2-like receptors (receptor subtypes D_2, D_3, D_4) are found both pre- and postsynaptic throughout the CNS. The dopamine D_1 receptors are found in the prefrontal cortex and striatum. D_2 receptors are situated in the striatum as well as in low concentrations in medial temporal structures, e.g.

hippocampus, entorhinal cortex, amygdala, thalamus and prefrontal cortex. D_3 receptors are found in the striatum and ventral striatum, meanwhile subtype D_4 receptors are located in prefrontal cortex and hippocampus and D_5 receptors in the hippocampus and entorhinal cortex (Kapur & Remington, 1996; Gaur et al., 2008).

The complexity of dopamine receptor function is further augmented by the existence of two isoforms (D_{2Short} and D_{2Long}) in the D_2-like receptor subtype group (Jaber et al., 1996). The two splice variants have separate functions and are differentially distributed. The D_{2Short} receptor serve as a somatodendritic autoreceptor located on central DA neurons meanwhile the D_{2Long} receptor serve as a postsynaptic receptor in vivo (Kahn et al., 1998).

The basal activity state of DA neurons is maintained by a spontaneous, slow depolarizing membrane current, which causes the neurons to fire in a regular pacemaker pattern. This pacemaker fire pattern is in turn changed by different inputs, e.g. GABAergic, into a slow-irregular fire pattern (Grace, 2016). The dopamine system is regulated by strong homeostatic influences. Subcortical dopamine release is controlled via two routes: (A) phasic dopamine release is triggered by DA neuron firing, when these are short-term activated by behaviourally relevant stimulus and (2) sustained tonic release of dopamine regulated by prefrontal cortical afferents. Tonic dopamine regulates the intensity of the phasic dopamine response and sets the level for the background DA receptor stimulation, thereby creating a homeostatic mechanism (Grace, 1991).

DA neurons, originating from the midbrain, distribute projections to different parts of the brain via four main pathways (Figure 2.2). The *nigrostriatal dopamine pathway*, from the substantia nigra to the dorsal striatum is indispensable for muscle movement and motor coordination (Williams et al., 2014). Blockade of striatal dopamine D_2 receptors by APDs hence generates EPS (Reynolds, 2008). It has been hypothesized that positive symptoms arise as a consequence of hyperactive dopamine transmission at D_2 receptors in the midbrain projections to the limbic striatum (*mesolimbic dopamine pathway*), meanwhile negative and cognitive symptoms develop due to deficient dopamine activity and hypostimulation of dopamine D_1 receptors in the *mesocortical pathway* projecting to the frontal cortex (Abi-Dargham, 2004). The *tuberoinfundibular dopamine pathway* sends projections of dopamine from the hypothalamus to the pituitary gland to control hormonal secretion. Dopamine D_2 receptor blockade in this pathway prevents the inhibitory effect on prolactin release, thereby provoking hyperprolactinemia (Freeman et al., 2000).

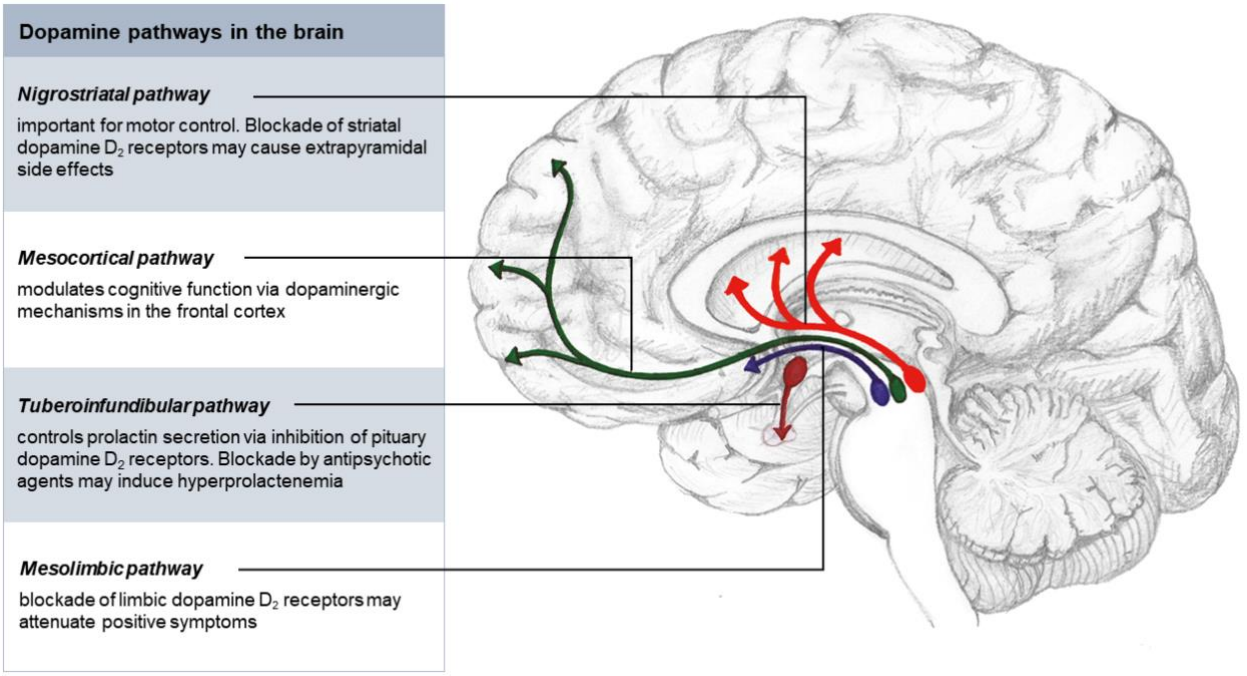

Figure 2.2 Brain dopamine systems and their involvement in the pathophysiology of schizophrenia

The effect of the dopamine projections is mediated by the presynaptic somatodendritic autoreceptors on the neuron cell bodies in the midbrain, which modulate the firing of DA neurons, or by terminal autoreceptors that modulates the release of dopamine from the axonal terminals (Kapur & Remington, 1996). The presynaptic autoreceptors generally respond to changes in extracellular neurotransmitter levels and provide important negative feedback to adjust neuronal firing rate, dopamine synthesis and dopamine release (Wolf and Roth, 1990; Beaulieu & Gainetdinov, 2011). The postsynaptic DA receptors mediate the effect of dopamine on nondopaminergic postsynaptic neurons (Kapur & Remington, 1996). Most dopamine projections occur in the striatum and striatal dysfunctions has been associated with the onset of schizophrenia, severity of the symptoms and with treatment response (Howes et al., 2015).

The association between schizophrenia and a mesolimbic hyperactive dopaminergic state is supported by the fact that human psychosis can be triggered by drugs known to cause striatal dopamine D_2 high states, e.g. amphetamines, phencyclidine, ethanol, steroids, as well as hippocampal, cortical, and entorhinal brain lesions (Seeman et al., 2006). Schizophrenia patients however, do not show substantial pathologic changes in the mesolimbic dopamine system and

hence, the pathologic changes might occur in the upstream dopamine regulating systems (Lodge & Grace, 2011).

Sophisticated circuits, involving other neurotransmitters like glutamate and GABA regulate the transmission of dopamine (Abi-Dargham, 2004). Structural and neurochemical alterations in cortical and hippocampal regions might be the cause behind the abnormal dopamine transmission and the corresponding schizophrenic symptoms (Lodge & Grace, 2011). The deficient levels of dopamine in the cortex appear to upregulate cortical dopamine D_1 receptors in patients with schizophrenia. The upregulation of dopamine D_1 receptors has no apparent functional effect, yet strong predictive value on poor performance in working memory tasks. At the meantime, cortical dopamine has inhibitory effect on subcortical dopamine and abnormal regulation of dopamine that leads to a deficit in cortical dopamine may therefore contribute to the dopamine excess seen in subcortical regions in schizophrenia (Abi-Dargham, 2004).

During the development of psychosis, the synthesis of dopamine progressively increases as well as the dopamine release (Seeman, 2013a). A neuroimaging meta-analysis revealed indeed, that the largest dopaminergic function abnormalities in schizophrenia patients are presynaptic. Consequently, dopamine synthesis capacity, baseline dopamine levels and dopamine release may be entailed in the pathophysiology of schizophrenia (Howes et al., 2015). In addition to presynaptic dopaminergic alterations, postsynaptic hypersensitive dopamine D_2 receptors have been implied in drug dependence and in schizophrenia (Seeman, 2013a). Aberrant levels of neurotransmitter might induce compensatory alterations in the synthesis, neuronal firing and receptor sensitivity for restorage of the original homeostatic state. In schizophrenia patients, the decreased prefrontal cortical activity appears to reduce the tonic dopamine release. The reduced tonic level of dopamine in turn activates homeostatic compensation mechanisms which increase the responsivity to dopamine overall, and hence cause abnormally large responses to subsequent phasic dopamine release (Grace, 1991).

In addition to dysfunctions in the prefrontal cortex, much evidence has linked an overdrive in the hippocampus to increased tonic dopamine neuron firing which consequently causes a dopamine hyperresponsive state (Grace, 2016). Dopamine supersensitivity in turn strongly correlates with high-affinity states of dopamine D_2 receptors. Furthermore, the dopamine supersensitivity commonly seen in schizophrenia is manifested in many drug-induced, lesion-induced and genetically modified animal models of schizophrenia (Seeman et al., 2006; Seeman, 2013b).

2.1.2.2 The role of serotonin

Early studies on the interactions between hallucinogenic drugs and serotonin (5-hydroxytryptamine, 5-HT) yielded the origin of a serotonin hypothesis of schizophrenia (Aghajanian & Marek, 2000). Due to the discredit and ban of hallucinogenic drugs and the discovery of dopaminergic blocking first generation typical APDs, the focus on 5-HT in schizophrenia diminished for some years. However, following the arrival of the second-generation atypical APDs, which elicit their effects by 5-HT blockade, the interest in 5-HT was renewed (Iqbal & van Praag, 1995). Today it is generally accepted that the central effects of psychedelic hallucinogens are mediated at 5-HT_2 receptors and affect complex neurological processes such as mood, cognition and perception (Aghajanian & Marek, 2000).

5-HT is one of the most widely distributed neurochemical in the CNS and derives nearly entirely from the dorsal and median nucleus of the dorsal raphe, situated in the brain stem (Lucki, 1998; Siegel & Crockett, 2013). The number of 5-HT carrying neurons in the brain are relatively few, however, the extensive projections from the raphe innervate regions throughout the complete CNS (Breier, 1995; Figure 2.3). 5-HT neurons are autoactive neurons, which facilitate the central motor tone and fire continuously at a rate of 0.5 to 2.5 spikes/sec. Their partitioned structure indicates their concurrent influential functions in the brain and their activity changes radically in the various states of arousal (Lucki, 1998). The family of 5-HT receptors currently counts to seven (5-HT_{1-7}) and these are comprised by 14 structurally different 5-HT receptor subtypes. The 5-HT receptors are all transmembrane spanning, G-protein coupled metabotropic receptors, except for the 5-HT_3 receptor which is a ligand-gated ion channel (Barnes & Sharp, 1999).

The release of 5-HT can be triggered by a diversity of external stimuli such as environmental stressors or challenges. 5-HT influences a wide range of physiological systems e.g. respiration, cardiovascular regulation and thermoregulation in addition to the effect on several behavioural functions. 5-HT has been attributed functions as modulator of motor behaviour and sensorimotor reactivity, maintainer of circadian rhythm and sleep-wake cycle and for keeping appetite control. In addition, 5-HT acts as the general inhibitor of behavioural responses like aggression and anxiety and influences nociception, sexual behaviour as well as learning, perception and attention (Lucki, 1998; Meltzer, 1999). Intact or enhanced 5-HT function has been linked to social and moral behaviour, moral judgement and harm aversion, meanwhile antisocial behaviour and aggression has been linked to reduced or impaired 5-HT function (Crockett et al., 2010).

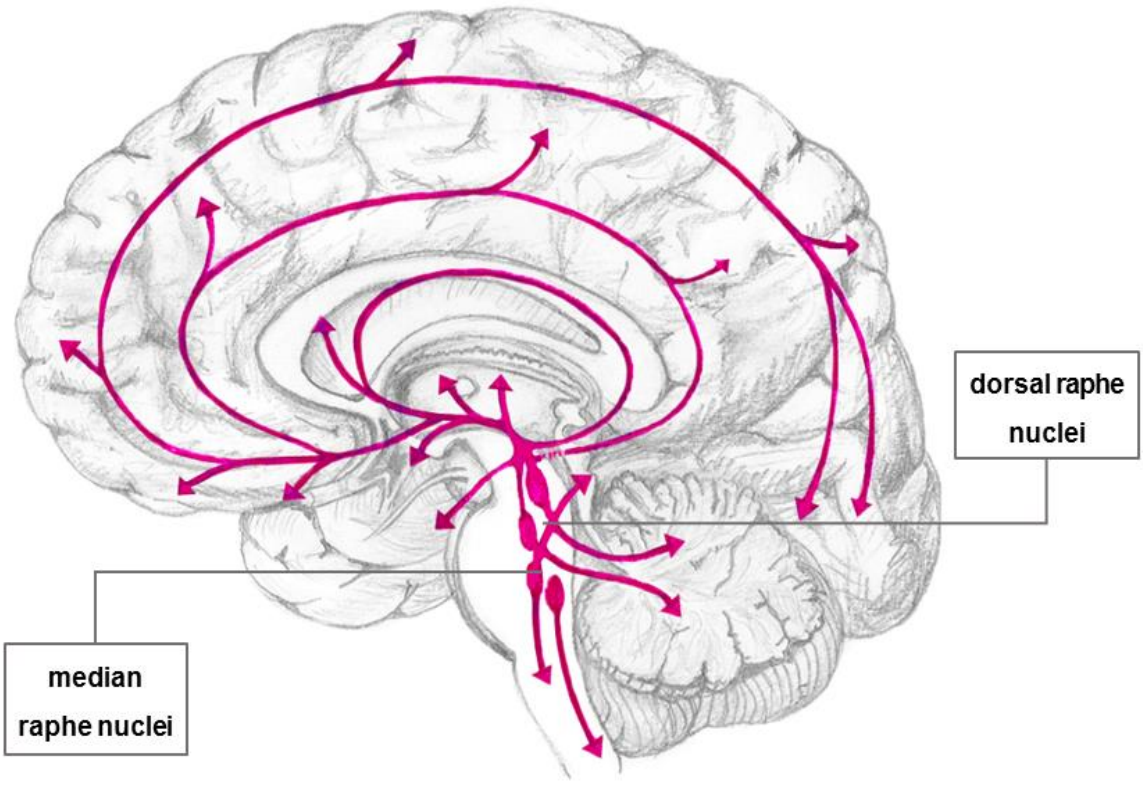

Figure 2.3 5-HT projections throughout the human brain originating from the raphe nuclei in the midbrain

5-HT has a general modulating effect on brain activity and the activation of serotonergic transmission constraints the intensity of information processing signals in other sensory systems in order to regulate the information flow (Spoont, 1992; Frazer & Hensler, 1999). Much evidence indicates a strong correlation between the serotonergic and the dopaminergic system and them being activity modulators of one another. Serotonergic activity predominantly acts as inhibitor of dopaminergic function. The interaction between the serotonergic and dopaminergic systems occurs on the cell body level in the ventral tegmental area (VTA), substantia nigra and median and dorsal raphe as well as on their various terminal areas. The most important receptors involved are the 5-HT_{1A}, 5-HT_{2A} and D_2 receptors (Hensler et al., 2013). Due to the close association between the serotonergic and other neurotransmitter systems, alterations in pre- and postsynaptic function in the serotonergic system are thought to affect many of the behaviours impaired in schizophrenia. Further support for this hypothesis comes from the observations that positive and negative symptoms as well as cognitive- and extrapyramidal function can be reduced or exacerbated by pharmacological agents which interact with the serotonergic system (Meltzer, 1991).

In the mammalian brain, the 5-HT receptors are associated with numerous physiological responses such as modulation of neuronal activity, release of transmitters as well as behavioural alterations (Barnes & Sharp, 1999). The cortical postsynaptic effect of 5-HT varies: 5-HT activation can lead to depolarization, hyperpolarization or no change, gauged by the balance of the co-expressed excitatory 5-HT_2 and inhibitory 5-HT_{1A} receptors (Aghajanian & Marek, 2000). For example, upon dopaminergic transmission, 5-HT_{2A} receptor antagonists alter the influence of 5-HT_{1A} receptor agonists meanwhile the 5-HT_{1A} receptor agonists attenuate the actions of hallucinogens at the 5-HT_{2A} and 5-HT_{2C} receptors (Meltzer, 1999). The most prominent effect of cortical 5-HT is however, to increase the frequency of excitatory postsynaptic potentials. This is in complete opposite to the 5-HT in the piriform cortex, which mainly induces inhibitory postsynaptic potential (Aghajanian & Marek, 2000).

The 5-HT_{2A} receptor is widely dispersed throughout the CNS, with the highest concentration in the cortex. 5-HT_{2A} and 5-HT_{1A} receptors are found situated on cortical and hippocampal pyramidal glutamatergic and additionally on GABAergic interneurons. The 5-HT_{2A} receptor is significant for the regulation and inhibition of neuronal activity due to its stimulating/diminishing effect on GABA release (Meltzer et al., 2003). 5-HT_{1A} receptors act as inhibitory autoreceptors and are found postsynaptic in the forebrain, as well as on serotonergic neurons in the median raphe nucleus (MRN) and the dorsal raphe nucleus (DRN). The serotonergic neurons in the MRN primarily innervate the dorsal hippocampus, septum and hypothalamus meanwhile the DRN located serotonergic neurons innervates the frontal cortex, ventral hippocampus, and the striatum (Barnes & Sharp, 1999; Figure 2.3).

Since 5-HT is involved in practically all behavioural responses, it is not surprising that 5-HT function and regulation is involved in a wide range of psychiatric disorders. In addition to schizophrenia, 5-HT disturbances have been implicated in depression, anorexia nervosa and the spectrum of anxiety- and impulse-related disorders (Lucki, 1998). Decreased density of 5-HT_{2A} receptors has been found in post mortem studies of brain tissue from schizophrenia patients. As the density of 5-HT_{2A} receptors decreases with increased 5-HT_{2A} receptor stimulation, over-active 5-HT_{2A} receptors in patients with schizophrenia might in turn cause a down regulation of the 5-HT_{2A} receptors in the cortex (Meltzer, 1991). In contrast to the decreased density of 5-HT_{2A} receptors, the density of 5-HT_{1A} receptors in many of the brain structures implicated in the aetiology of schizophrenia, e.g. the frontal cortex, hippocampus and thalamus, seems to be increased in schizophrenia patients in comparison to healthy similarly aged subjects. Millan (2000) suggested that schizophrenia patients lack normal age-associated reductions in 5-HT_{1A} receptor density. Pre-

and postsynaptic 5-HT_{1A} receptors are involved in the modulation of motor behaviour, cognition and mood and hence, it is not surprising that these functions are also disturbed in schizophrenia (Barnes & Sharp, 1999). However, as extracellular 5-HT levels and 5-HT_{1A} coding mRNA levels appears unaltered in schizophrenia, the 5-HT_{1A} receptor appears to have linkage to disturbances in post transcriptional processes. In line with the evidence for an involvement of 5-HT_{1A} receptors in the schizophrenia pathogenesis, ligands with actions at 5-HT_{1A} receptors has effect of some of the symptoms (Millan, 2000). In fact, many of the atypical APDs are 5-HT_{1A} partial agonists complimentary to their antagonistic actions at the 5-HT_{2A} receptor (Meltzer, 1999). Furthermore, as some 5-HT_{1A} receptors are found located on pre- and postsynaptic glutamate synapses, there is an implication for a disruption in the glutamatergic activity modulatory effect of 5-HT_{1A} receptors in schizophrenia (Meltzer et al., 2003).

Finally, as the 5-HT receptors interact with other members of the 5-HT family, such as 5-HT_{2A} and 5-HT_{2C} receptors with 5-HT_{1A} receptors (Meltzer, 1999), it seems inevitable that more than one serotonergic receptor type is involved in the schizophrenia pathogenesis.

2.1.2.3 Glutamate hypothesis

The idea of a potential role for glutamatergic mechanisms in the pathophysiology of schizophrenia was first evoked in the 1980s (for reviews see Fonnum, 1984; Watkins & Jane, 2006). The observations that antagonists of the N-methyl-D-aspartate (NMDA) glutamate receptor subtype, such as phencyclidine (PCP), ketamine and MK-801, induce schizophrenia-similar symptoms and cognitive alterations in healthy individuals, led to the formulation of a glutamate hypothesis of schizophrenia (Javitt, 2007; Kristiansen et al., 2007). Recently, disturbances in NMDA receptor-related gene expression and altered glutamate-mediated neurotransmission have been implicated in the pathology of a range of neuropsychiatric disorders including substance abuse, mood disorders, Alzheimer´s disease (AD), and autism-spectrum disorders. For schizophrenia, continuous support for the glutamate hypothesis and the involvement of disrupted glutamate neurotransmission has been provided (Moghaddam & Javitt, 2012).

The amino acid glutamate is the principal excitatory neurotransmitter in the brain (Orrego & Villanueva, 1993; Moghaddam & Javitt, 2012). It has been estimated that over half of all synapses in the brain releases glutamate and that glutamatergic neurons use around 60 - 80% of the total metabolic activity in the brain (Newcomer et al., 2000; Rothman et al., 2003). The inhibition of glutamatergic transmission puts the brain in a state of coma, disabling the complete

nervous system (Verkhratsky & Kirchhoff, 2007). Furthermore, glutamate is a potent endogenous neurotoxin and over-excessive activation of excitatory glutamate receptors on dendritic and somal bodies triggers the destruction of CNS neurons. This pathology is responsible for a variety of neurologic disorders, from stroke to AD and dementia (Newcomer et al., 2000; Verkhratsky & Kirchhoff, 2007).

Glutamate triggered neurotransmission is permitted via metabotropic G protein-coupled receptors and ionotropic ligand-gated ion-channels, each subdivided into three groups (see Figure 2.4). The structure and function of the metabotropic glutamate receptors (mGluR) are diverse and the receptors are found both pre- and postsynaptic, working either excitatory or inhibitory (Blackshaw et al., 2011). The mGluR monitors the glutamate levels by sending either positive- (group I mGlu receptors $_{-1}$ and $_{-5}$) or negative feedback (group II mGlu receptors $_{-2}$ and $_{-3}$, and group III mGlu receptors, $mGlu_4$, $_{-6}$, $_{-7}$ and $_{-8}$) to decrease the release of neurotransmitters or to change the postsynaptic excitability for glutamate (Schoepp, 2001). The ionotropic glutamate receptors (iGluR) received their names from the agonists that selectively activate them: NMDA, a-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) and kainate (KA) (Howes et al., 2015). The postsynaptic activation of ionotropic receptors controls the balance of excitation/inhibition within neuronal circuits by opening K^+, NA^+ and Ca^{2+} permeable ion-channels to create depolarizing excitatory postsynaptic currents when glutamate binds to the receptor (Schoepp, 2001; Lang et al., 2007). The ionotropic NMDA receptor has been extensively studied and frequently implicated in the pathology of various neurological disorders (Newcomer et al., 2000).

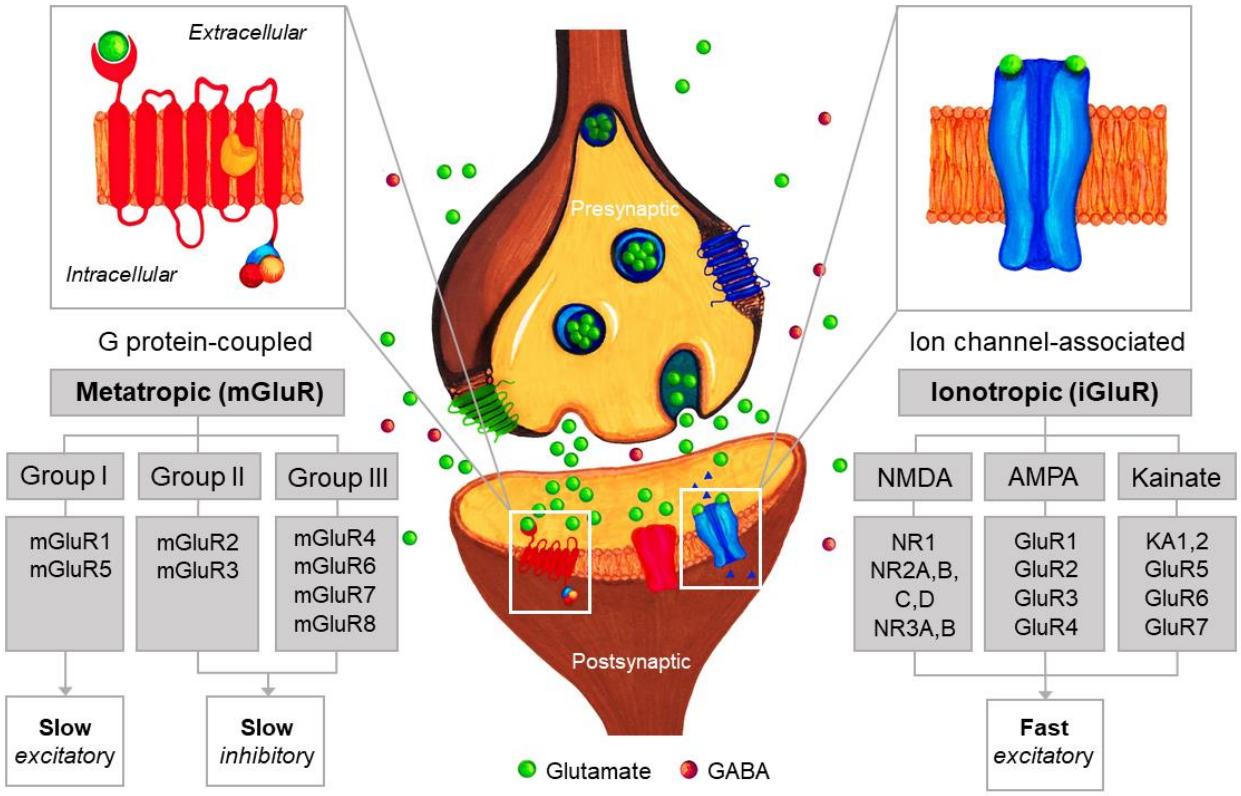

Figure 2.4 Glutamate receptor types. GluR: glutamate receptor; NR: NMDA receptor subtype; KA: kainate receptor subtype

NMDA receptors localized on neurons or glial cells are extensively dispersed throughout the brain and modulated by various endogenous ligands and ions (Lang et al., 2007). The cortical glutamate projections innervate throughout much of the cerebrum (see Figure 2.5). Glutamate pathways are descending from prefrontal to brainstem areas (dorsal- and medial raphe, VTA, substantia nigra); from the prefrontal cortex to striatum and nucleus accumbens; throughout the *thalamocortical pathway* (from thalamus to cortical pyramidal neurons); as well as the backward projections ascending from cortex to thalamus and the intra-cortical glutamate projections. The GABAergic projections are widely distributed throughout the brain (see Figure 2.5), with pathways from the striatum descending to the substantia nigra and the brainstem; innervating the thalamus from the substantia nigra; originating in the hypothalamus descending to the occipital cortex and parietal cortex; projecting from the hippocampus to thalamus and striatum; from nucleus accumbens to thalamus and from the VTA to the prefrontal cortex, nucleus accumbens and other regions (Hnasko et al., 2012; Barth et al., 2015).

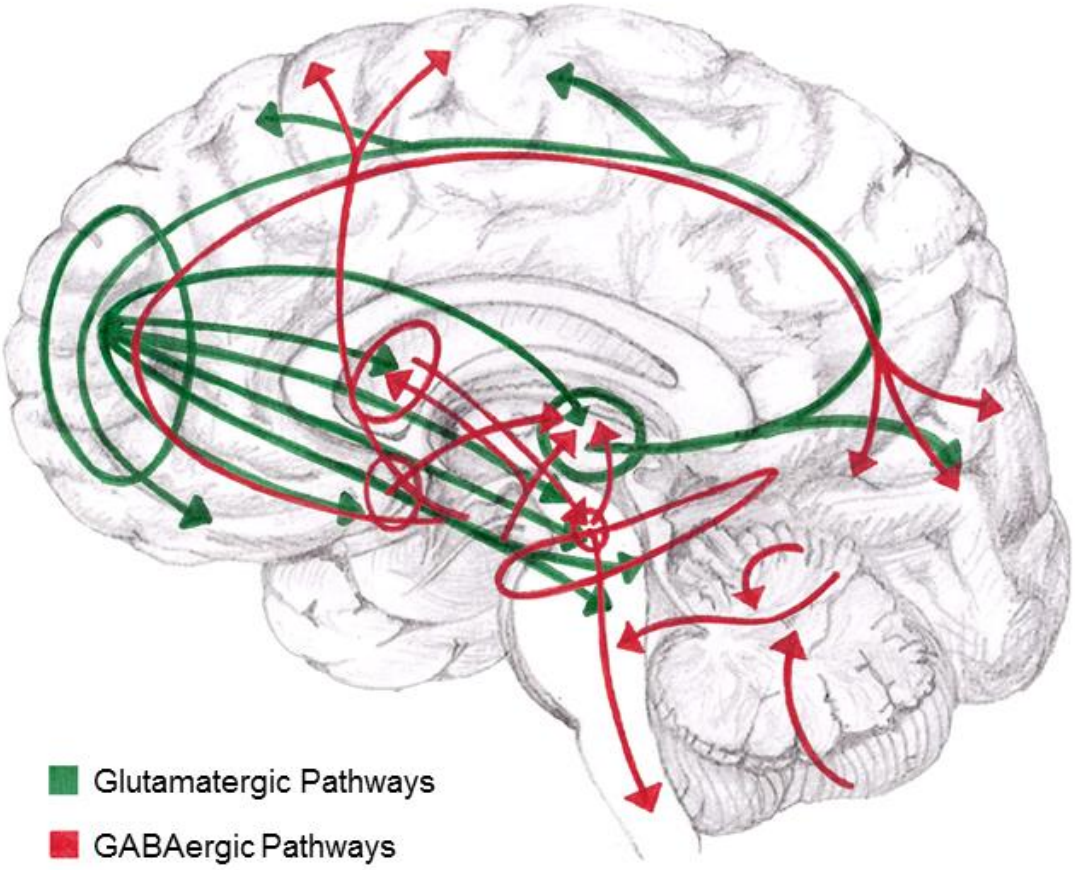

Figure 2.5 Glutamatergic and GABAergic pathways in the brain (adapted from Barth et al., 2015, p. 4)

A considerable body of evidence indicates that the number of synaptic NMDA receptors and their subunit composition change dynamically during development and in response to neuronal activity or sensory experience (Lau & Zukin, 2007). The NMDA receptor have significant function in a variety of neural mechanisms such as cerebrovascular dilation or learning and memory-related signal transduction (Verkhratsky & Kirchhoff, 2007). For example, the activation of postsynaptic NMDA receptors controls the initiations of long-term potentiation, an activity dependent synaptic modification mechanism important for memory formation and for the ability of the brain to store information (Lau & Zukin, 2007). NMDA receptor antagonists, which lead to underexcitation of the NMDA receptor, cause dysfunctional memory and learning performance or, in severe cases of hypofunction, result even in psychosis (Newcomer et al., 2000).

Over the years since first devised, the glutamate hypothesis in schizophrenia has received strong support from morphological, clinical and neuroimaging studies. Glutamatergic

abnormalities, especially those involving NMDA receptor complex hypofunction, are likely to be part of the schizophrenia pathophysiology (Dawson et al., 2014). Especially since the mapping of cognitive deficiencies and alterations in neuronal morphology has indicated the involvement of frontal and cingulate cortices; brain regions that exhibit extensive excitatory glutamatergic neurotransmission (Kristiansen et al., 2007). Furthermore, the NMDA receptor hypofunction hypothesis is compatible with the dopamine hypothesis of schizophrenia, as NMDA antagonists can alter dopaminergic regulation (Jentsch & Roth, 1999) and much evidence has pointed out that the dopaminergic dysfunction in schizophrenia is secondary to glutamatergic dysfunction.

The firing of dopamine neurons in the VTA is controlled by an excitatory system consisting of glutamatergic cells projecting from the cortex, as well as a “brake system” mediated by GABAergic interneurons in the prefrontal cortex, which synchronizes the activity of glutamatergic pyramidal neuron networks. Consequently, any uncoordinated activation or silencing of these systems as a result from abnormalities in glutamatergic pyramidal cells or NMDA receptor hypofunction, could affect the subcortical dopamine transmission. Interrupted excitation-inhibition balance has been extensively described in schizophrenia, in which insufficient stimulation of mesocortical dopaminergic projections causes low cortical dopamine levels, meanwhile the GABAergic brake system fails to reduce the subcortical excessive dopamine resulting in exaggerated sensory flooding in the thalamo-cortical loop, psychotic symptoms and the well-documented alternations in dopamine levels (Abi-Dargham, 2004; Lang et al., 2007; Millan et al., 2016). In addition to pre- and postsynaptic NMDA receptor dysfunctions, disturbance of NMDA receptor-related gene expression and glial NMDA receptor mechanisms has been implicated in schizophrenia (Moghaddam & Javitt, 2012; Howes et al., 2015). Possible interactions between $mGlu_{2/3}$ receptors and dopamine D_2 receptors have been reported studying mice with knocked out $mGlu_2$ or $mGlu_3$ receptors. These mice are supersensitive to dopamine receptor agonists suggesting that group II mGlu receptors are involved in the regulation of dopamine D_2 receptors by reducing high-affinity receptors in cell membranes (Seeman et al., 2009). Furthermore, magnetic resonance spectroscopy has shown associations between altered glutamatergic transmission in several brain regions and schizophrenia (Millan et al., 2016). Therefore, it seems legit to say that a combination of NMDA hypofunction and presynaptic dopamine dysfunction provide the best explanation for the pathology in schizophrenia (Howes et al., 2015).

2.1.3 Treatment

The arrival of drugs with antipsychotic effect revolutionized the treatment of schizophrenia and allowed a change from straitjacket-restrained hospitalization to community treatment with hope of recovery and independence (Bryan, 2011; Dimitrelis & Shankar, 2016). Since the utilization of the first typical and atypical APDs with alleviation of the positive symptoms of the disorder, continuous waves of optimism for efficiency improvements of the APDs have been introduced. Unfortunately, no treatments have been able to completely fulfil the expectations and the APDs remain effective mainly for the treatment of positive symptoms but not for the negative symptoms and cognitive impairments. Furthermore, undesired side effects of varying severity are still major obstacles hampering the safe and effective use of the drugs (Pratt, 2015).

The critical clinical component for antipsychotic action is blockade of dopamine D_2 receptors, either by direct receptor binding (dopamine antagonists) or by the hampering of normal dopamine neurotransmission (partial agonists) (Seeman, 2006). In general, presynaptic D_{2Short} receptors are activated by lower dopamine agonist concentrations than postsynaptic D_{2Long} receptors. Consequently, high doses of APDs might worsen the primary dopaminergic abnormality as presynaptic D_{2Short} receptor blockade triggers a raise in compensatory dopamine synthesis (Howes & Kapur, 2009; Beaulieu & Gainetdinov, 2011).

The clinical efficiacy of currently available first and second generation APDs is reached at 60 - 80% striatal dopamine D_2 receptor occupancy, meanwhile receptor occupancies over 80% triggers the development of EPS (Nordström et al., 1993; Horacek et al., 2006; de Greef et al., 2011). The occupancy-function relationship experienced with dopamine D_2 antagonists does however, not translate directly to partial agonists (Natesan et al., 2006). Compared to the blocking effect of dopamine D_2 receptor antagonists, dopamine D_2 partial agonists may act as either agonists or antagonists depending on the level of endogenous receptor activation and their efficiacy values hence depends on the endogenous dopaminergic tone. For this reason, the optimal properties of partial agonists for antipsychotic efficiacy have proven more difficult to determine in vitro. Nonetheless, it seems that partial agonists such as aripiprazole stabilize the dopamine level rather than blockade the dopaminergic tone (Burris et al., 2002).

Unfortunately, despite the past decades of treatment advances, schizophrenia is still one of the most severe psychiatric disorders associated with chronic relapses and poor functional outcome in many patients. The patient heterogeneity and their different responses to treatments make therapeutic predictability and individualized treatment difficult. However, the ongoing

research and developments in diagnostics and pharmacotherapy will hopefully one day allow for targeted, successful and individualized treatment for schizophrenia (Kane & Correll, 2010).

2.1.3.1 First generation (typical) antipsychotic drugs

The first typical antipsychotic agent, the phenothiazine derivative chlorpromazine, was developed by the French pharmaceutical chemists Paul Charpentier and Simone Courvoisier, researchers for the Rhône-Poulenc Pharmaceutical Group in the 1950s (López-Muñoz et al., 2004). The primary antipsychotic effect of chlorpromazine was produced by dopamine D_2 receptor blockade in the *mesolimbic pathway* and its discovery became the starting point of modern psychopharmacology, improving a significant number of patients with psychosis or mania (López-Muñoz et al., 2004; Bryan, 2011).

In 1958, Paul Janssen and his colleagues at Janssen Laboratories discovered the dopamine D_2 receptor antagonist haloperidol, a substance belonging to the butyrophenone class of APDs. Clinical trials in France demonstrated the efficacy of haloperidol for acute and chronic paranoid psychosis, mania and chronic treatment-resistant schizophrenia. Haloperidol was licensed and marked in Belgium in the end of 1959 under the brand name Haldol® (López-Muñoz & Alamo, 2009). However, with the increased use of the typical antipsychotics chlorpromazine and haloperidol, their drawbacks became obvious. Chlorpromazine has a sedative effect, meanwhile both chlorpromazine and haloperidol induce weight gain and extrapyramidal side effects, such as acute dystonia and Parkinsonism, although the motor symptoms tend to be more frequent for haloperidol (Adams et al., 2005; Leucht et al., 2009). The decreased dopamine activity in brain areas that are not implicated in the pathophysiology of the positive symptoms are thought to be the cause of the debilitating side effects associated with the first generation APDs (Stahl, 2007).

Extrapyramidal side effects emerge at around 80% dopamine D_2 receptor occupancy (Remington & Kapur, 1999), and hyperprolactinemia (elevated levels of secreted prolactin from the anterior pituitary gland) is a consequence of dopamine D_2 receptor blockade (Freeman et al., 2000). The effect of first generation antipsychotics is obtained by the blockade of dopamine D_2 receptors which reduces the dopaminergic activity in the *mesolimbic dopamine pathway*, the route thought to be involved in the development of the positive schizophrenic symptoms. Consequently, the first generation APDs are effective only on symptoms caused by dopamine hyperactivity (Miyamoto et al., 2005).

In the 1960s and 1970s, other similar antipsychotics were introduced but due to the lack of drug monitoring many were over-prescribed as well as overdosed, thereby causing severe side effects in the patients (Bryan, 2011). Nonetheless, chlorpromazine (Adams et al., 2005) and haloperidol has been included in the World Health Organization's list of essential medicines and their introduction lead to significant changes in the treatment of psychiatric disorders. Furthermore, the development of new experimental models to predict the effects of antipsychotics, and the postulation of the first biological hypotheses of schizophrenia pathophysiology are direct and indirect consequences of the introduction of haloperidol in biological psychiatry and neuroscience (López-Muñoz & Alamo, 2009). Although chlorpromazine is a sedating drug, prone to cause various side effects similar to haloperidol, both these APDs are still today low-cost choices for clinicians worldwide and the widely most used treatments for millions of schizophrenia patients, especially in poor countries (Adams et al., 2005; Leucht et al., 2009).

2.1.3.2 Second-generation (atypical) antipsychotic drugs

A new wave of hope for the treatment of schizophrenia and other psychotic disorders came with the preclinical and clinical evidence indicating the involvement of 5-HT receptors in cognitive processes and the arrival of second-generation atypical APDs with additional antagonistic actions at 5-HT_{2A} receptors (Gonzalez et al., 2013; Yamazaki et al., 2014; Aznar & Hervig, 2016; Nikiforuk et al., 2016).

The first atypical APD clozapine was launched in 1989 in the UK and quickly became the established therapeutic gold standard due to its 30 - 61% successful response rate in refractory schizophrenia patients (Meyer & Simpson, 1997). Clozapine has proven therapeutic effect especially on positive symptoms and may in addition elicit beneficial effect on negative symptoms in groups of patients (Kane et al., 1988). Additionally, clozapine is one of the few therapeutic APD exceptions that might improve some of the cognitive impairments in schizophrenia, for example attentional deficits, semantic memory (verbal fluency) and some features of executive function. The reports on working memory and spatial memory improvements by clozapine have nonetheless remained inconclusive (Meltzer & McGurk, 1999). The risks for extrapyramidal side effects and hyperprolactinemia are lesser with clozapine than with typical APDs, however, clozapine treatment increases the risk of agranulocytosis and often causes heavy weight gain (Meltzer et al., 1979; Bryan, 2011).

Clozapine is only a moderate dopamine D_2 receptor antagonist with affinity for several other receptors such as dopamine D_1 and D_4, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors, muscarinic cholinergic receptors, adrenergic α_1 and histaminic H_1 receptors (Abi-Dargham et al., 1997). The superior effect of clozapine has been attributed to the antagonism at 5-HT_2 receptors and this encouraged the development of new antipsychotic agents with similar effects at the 5-HT_2 receptor (Meltzer et al., 2003).

Shortly after clozapine entered the market, risperidone was developed and launched in 1993. Risperidone is an antipsychotic agent with stronger affinity for 5-HT_2 than dopamine D_2 receptors (Abi-Dargham et al., 1997). Risperidone became the first-line treatment choice of many psychiatrists due to its less extent of caused side effects. In therapeutic efficacy, the atypical APD olanzapine, released 1996 in the United States, tends to be slightly superior over risperidone but instead associated with significantly more weight gain (Meyer & Simpson, 1997; Zipursky et al., 2005; Bryan, 2011). Olanzapine is a potent 5-HT_2 antagonist with low dopamine D_2 receptor occupancy and inhibitory effect on histaminic and cholinergic receptors comparable to clozapine. Interestingly, olanzapine is a much more potent α_2-adrenergic receptor antagonist than clozapine and due to its clinical efficacy, which is reached at much lower doses than clozapine, it causes less sedation. On the other hand, olanzapine causes more sever weight gain compared to other APDs (Fulton & Goa, 1997; Nyberg et al., 1997).

Increasing evidence has related the efficacy of atypical APDs to an indirect stimulatory effect mediated by actions at 5-HT receptors involved in the regulation of dopamine cell function (Alex & Pehek, 2007). Most APDs exhibit their clinical efficacy when 60 - 80% dopamine D_2 receptor occupancy is reached, regardless of the 5-HT blocking actions of the drug. Two exceptions however, are clozapine and quetiapine, which both reaches their clinical efficiency already at 10 - 45% D_2 receptor occupation (Remington & Kapur, 1999; Seeman & Kapur, 2000).

In general, both 5-HT_{2A} and 5-HT_{2C} receptors interact with 5-HT_{1A} receptors and because APDs fail to disseminate 5-HT_{2A} from 5-HT_{2C} receptors it has been suggested that blockade of 5-HT_{2C} receptors can compromise the advantages of 5-HT_{2A} receptor antagonism. However, selective blockade of 5-HT_{2C} receptors attenuates EPS and the antipsychotic actions of dopamine D_2 antagonists like haloperidol and might exert favourable actions in psychotic patients due to the anxiolytic properties and augmented dopaminergic transmission in the prefrontal cortex (Millan, 2000; Meltzer et al., 2013). Regardless of which 5-HT receptor that exerts the most important actions, the 5-HT_{1A} receptor might be a key mediator in the pathophysiology and treatment of schizophrenia since dopaminergic transmission can be modified by 5-HT_{2A} receptor antagonists

via 5-HT_{1A} receptors, meanwhile 5-HT_{1A} receptor agonists can attenuate both the 5-HT_{2A} and 5-HT_{2C} receptor-mediated actions (Meltzer, 1999; Meltzer & Sumiyoshi, 2008).

In summary, the atypical APDs share pharmacologic features such as relatively potent antagonistic actions at 5-HT_{2A} receptors combined with weaker dopamine D_2 receptor antagonism, thereby causing less EPS, tardive dyskinesia and hyperprolactinemia than first generation APDs (Meltzer, 1999; Seeman & Kapur, 2000; Meltzer et al., 2003). On the contrary, the properties of second generation APDs at histamine H_1 and 5-HT_{2C} receptors can induce insulin resistance, weight gain, diabetes and other secondary conditions (Kim et al., 2007; Ücok & Gaebel, 2008; Meltzer et al., 2013).

Unfortunately, among the antipsychotic substances permitted for treating schizophrenia in Europe and the US 2016 (Table A.3), the therapeutic effect on cognitive and negative symptoms has remained unsatisfactory. Mainly, clozapine, olanzapine, and risperidone seem to have the ability to improve some of the cognitive deficits in schizophrenia, such as verbal learning and memory, verbal fluency, and executive function. Attentional, working memory, and visual learning and memory deficits however, remains largely unimproved by all available APDs (Meltzer & McGurk, 1999).

2.1.3.3 Third generation antipsychotic drugs

The dopamine D_2 receptor partial agonist aripiprazole was developed in 2002 at the Japanese pharmaceutical company Otsuka and was the first effective partial dopamine agonist APD (Hope et al., 2018). The atypical properties of aripiprazole might derive from its weak dopamine D_2 and 5-HT_{2A} antagonistic actions combined with the agonistic properties at the 5-HT_{1A} receptor (Meltzer et al., 2003). The partial dopamine D_2 agonistic actions of aripiprazole raised the hope and aspirations that aripiprazole would theoretically be able to block dopamine overactivity in the midbrain meanwhile elevating the dopaminergic activity in the prefrontal cortex, leading to a simultaneous amelioration of both the positive and negative symptoms of schizophrenia (Hope et al., 2018).

Aripiprazole can decrease dopamine levels via preferential activity at presynaptic dopamine receptors meanwhile its postsynaptic partial agonistic actions depends on the endogenous dopamine levels and can either decrease or increase dopaminergic transmission (Kikuchi et al., 1995; Natesan et al., 2006). There seems to be a dissociation between receptor occupancy and functional antagonism in the case of aripiprazole compared to other APDs as

aripiprazole becomes clinically effective when receptor occupancies reach over 90% yet, there is no induction of EPS. This might be explained by the intrinsic agonist properties of aripiprazole, which combined with its functional antagonistic properties, lessens the dopamine transmission in total (Kane et al., 2002; Natesan et al., 2006).

Compared to clozapine, aripiprazole seems slightly favorable in the assessments of "life quality", however, there is limited proof of aripiprazole being better than clozapine when measuring clinical response and mental state. General extrapyramidal symptoms are not significantly different between patient groups treated with aripiprazole, clozapine, ziprasidone or olanzapine, however, aripiprazole seems superior to risperidone both in clinical effect and adverse side effect profile although causing more weight gain. Furthermore, more patients on aripiprazole than on olanzapine left the studies early and patients on olanzapine gained significantly more weight (Khanna et al., 2014).

In 2015, the US patent for aripiprazole expired and Otsuka Pharmaceuticals launched their new partial dopamine agonist brexpiprazole for the treatment of schizophrenia. Brexpiprazole is like aripiprazole a dopamine D_2 receptor- and 5-HT_{1A} receptor partial agonist, with antagonistic actions at 5-HT_{2A} and noradrenergic α_{1B} receptors. Brexpiprazole however, show lesser intrinsic activity at the D_2 receptor than aripiprazole. In addition, brexpiprazole binds more potently at 5-HT_{2A}, 5-HT_{1A}, and α_{1B} receptors than aripiprazole, elicits lower activity at histamine H_1 receptors and has no anticholinergic activity (Das et al., 2016; Garnock-Jones, 2016). The two APDs seem comparable in their elicited antipsychotic efficacy but brexpiprazole may be superior in measures of metabolic tolerability and causes less akathisia and less EPS, possibly due to its stronger 5-HT_{2A} receptor antagonism (Das et al., 2016; Hope et al., 2018). Following aripiprazole and brexpiprazole, in September 2015 the D_2 partial agonist atypical antipsychotic cariprazine was approved for the treatment of schizophrenia by the American Food and Drug Administration Agency (FDA).

In summary, dopamine receptor partial agonism seems to be a favorable characteristic of an APD, so far yielding the best results in the treatment of schizophrenia (Dimitrelis & Shankar, 2016).

2.1.3.4 Pipeline

Several novel agents are currently being tested in schizophrenia patients at different clinical trial stages. Some of the most prominent agents are summarized in Table A.4. Due to the latest

advances of the dopamine partial agonists aripiprazole, brexpiprazole and cariprazine, a few agents with similar properties are currently being investigated. Importantly, for a number of schizophrenia patients who exhibit persistent and treatment resistant symptoms (refractory schizophrenia), neither first- or second-generation antipsychotics have effect. The same is true for the debilitating cognitive deficits seen in many schizophrenia patients. This chapter therefore focuses on drugs in the pipeline, which might address these symptoms especially, or agents with novel receptor profiles or pharmacological actions.

Lumateperone (ITI-007), a tetracyclic quinoxaline derivative, showed therapeutic efficacy in clinical trials for schizophrenia, meanwhile having a side effects profile comparable to placebo. ITI-007 has a unique pharmacological profile combining dose-related modulation of monoamines and intracellular signaling protein phosphorylation (Lieberman et al., 2015). ITI-007 is a selective and high-affinity 5-HT_{2A} receptor antagonist with modest dopamine D_2 receptor antagonism when given in higher doses to adult schizophrenia patients (Dimitrelis & Shankar, 2016; Fellner, 2017). Due to its stronger affinity for 5-HT_{2A} receptors than dopamine receptors, ITI-007 can fully saturate the 5-HT_{2A} receptors meanwhile only moderately occupying dopamine receptors. Moreover, the presynaptic partial agonistic and postsynaptic antagonist actions of ITI-007 at the D_2 receptors allows for functional blockade of dopamine and antipsychotic efficacy without increasing dopamine turnover and inducing motor side effects (Lieberman et al., 2015). In addition, the minimal interaction of ITI-007 with histamine, 5-HT_{2C}, and muscarinic acetylcholine receptors, prevents the drug from inducing sedation, weight gain and cognitive dysfunction (Davis et al., 2015).

RP5063, also known as RP5000, is a dopamine/5-HT system stabilizer with partial agonist activity at dopamine D_2, D_3, D_4, 5-HT_{1A}, and 5-HT_{2A} receptors, as well as antagonist activity at 5-HT_{2B}, 5-HT_6 and 5-HT_7 receptors (Rajagopal et al., 2017). In clinical trials, RP5063 improved multiple efficacy outcome measures better than aripiprazole meanwhile having a side-effect profile similar to placebo (Cantillon et al., 2017). Due to its receptor affinity profile, the developer hopes that RP5063 could be effective in the treatment of negative symptoms and cognitive deficits (Dimitrelis & Shankar, 2016).

LuAF35700 is a second-generation antipsychotic that targets the dopamine D_1 receptor in addition to its 5-HT_{2A} and 5-HT_6 receptor antagonistic actions. It has been hypothesized that the effect and efficacy of LuAF35700 in treatment-resistant schizophrenia, would be comparable to clozapine but induce less weight gain (Fellner, 2017; Lundbeck, 2017)

5-HT receptor ligands are implicated in the treatment of several psychiatric conditions and traditionally 5-HT_{2A} antagonism is one of the main properties that distinguishes atypical from

typical APDs (Dimitrelis & Shankar, 2016). 5-HT_6 antagonism has been reported to ameliorate PCP-induced deficits in rat reversal learning meanwhile risperidone and clozapine had no effect (De Bruin et al., 2013). AVN-211 is an antagonist at the 5-HT_6 receptor family, a family which received attention as a potential treatment mediator for cognitive impairments in schizophrenia. Targeting the serotoninergic system for add-on treatment represents a promising approach and AVN-211 added antipsychotic and to some degree, pro-cognitive effect in a clinical trial with patients who were stabilized on antipsychotic medication (Morozova et al., 2014; Dimitrelis & Shankar, 2016).

MIN-101 is the first combined antagonist at the 5-HT_{2A} and sigma-2 receptor, which are involved in the regulation of mood, sleep, cognition and anxiety. 5-HT_{2A} receptor blockade is believed to minimize hallucinations, delusions, agitation, and thought and movement disorders. In addition, sigma-2 receptor antagonism could help to regulate dopamine and increase the calcium levels in neurons thereby eliciting a pro-cognitive effect. MIN-101 has been reported as tolerable and superior over placebo in clinical trials including schizophrenia patients with pronounced negative symptoms (Davidson et al., 2017; Fellner, 2017).

Theoretically, NMDA receptor stimulation is another mechanism that could provide some relief or protection against negative symptoms and cognitive decline (Dimitrelis & Shankar, 2016). The glutamate synapse with its large number of presynaptic and postsynaptic regulatory proteins is a promising therapeutic target (Moghaddam & Javitt, 2012). A significant amount of research has been invested in the development of glutamatergic antipsychotics whereas several agents, such as glycine, d-serine, and glycine transport inhibitors, has reached clinical trials but not yet further (Javitt, 2007, for review see, Tandon et al., 2010). Diminished group II metabotropic glutamate ($mGlu_2$) receptor activity has been implicated in schizophrenia due to the increased neuronal excitability in prefrontal cortical and hippocampal regions. Group II mGlu receptors seem to be involved in the regulation of dopamine by reducing the proportion of high-affinity D_2 receptors in the neuronal membranes (Seeman et al., 2009). Positive allosteric modulators (PAMs) might have the ability to reinforce the glutamate sensitivity and activity of the $mGlu_2$ receptors, thereby normalizing glutamatergic disruptions (Dimitrelis & Shankar, 2016). In fact, the latter has been shown in animal models where $mGlu_2$ agonists attenuated PCP-induced cortical glutamate efflux and impairments in working memory, stereotypies and locomotion (Moghaddam & Adams, 1998). In clinical trials with residual schizophrenia patients, the $mGlu_2$ PAM named JNJ-40411813 (ADX-71149) showed some beneficial effects and seemed well tolerable by the probands, further strengthening the hypothesis of $mGlu_2$ involvement in schizophrenia (Dimitrelis & Shankar, 2016).

Pomaglumetad methionil (LY404039) is another selective agonist for the $mGlu_2$ receptor that showed potential as putative antipsychotic in animal studies by normalizing excess glutamate levels. LY404039 was terminated from further development in 2012 after it failed phase II clinical trials (Stauffer et al., 2013). The prodrug of LY404039 (LY2140023 monohydrate) was however, continued and tested in schizophrenia patients and turned out to be safe and well-tolerated meanwhile significantly improving both positive and negative symptoms (Patil et al., 2007; Kinon & Gomez, 2012).

D-serine and glycine are neurotransmitters inhibiting amino acids, believed to potentiate the function of NMDA receptors. The two major glycine transporter subtypes, $GlyT_1$ and $GlyT_2$, have been implicated in the pathology and treatment of affective and cognitive disturbances in neurological disorders including schizophrenia (Harvey & Yee, 2013). Blockade of the glycine transporter 1 protein corresponds to elevated glycine levels by inhibiting glycine reuptake and consequently increasing glycine activated NMDA transmission at glycinergic synapses (Moghaddam & Javitt, 2012; Dimitrelis & Shankar, 2016). Bitopertin (RG1678) is a glycine reuptake inhibitor representing a novel treatment option which has a potential ameliorating effect on the negative symptoms of schizophrenia in patients stabilized on APDs (Umbricht et al., 2014). However, evidence from clinical trials supporting the treatment with glycine transporter inhibitors has remained scarce and the involvement of glycine in several biochemical pathways implicates that there might be side effects to glycine inhibition (Singer et al., 2015).

Deficient acetylcholinergic function has been observed in schizophrenia and implicated in disturbed cognitive and attentional processing. Metabotropic and nicotinic receptors are widely dispersed in the brain and the nicotinic acetylcholine receptor (nAChR) has hence received attention as a putative target for improvement of the cognitive deficits in schizophrenia (Rowe et al., 2015). Numerous α7-nACh receptor ligands have been developed for use in schizophrenia but unfortunately their efficacy in the treatment of negative symptoms and cognitive dysfunction has not yet been proved. The α7-nACh receptor agonists ABT-126, AQW051, EVP-6124 are currently undergoing clinical trials and their results will show if nicotinic receptor agonism has a future in the treatment of schizophrenia (Hashimoto, 2015; Dimitrelis & Shankar, 2016). Recent phase II clinical trials with EVP-614 (encenicline) showed that the agent was generally well tolerated and elicited some cognitive improvements in schizophrenia patients (Keefe et al., 2015).

Enhancement of NMDA receptor-mediated neurotransmission is a novel approach to target and ameliorate the NMDA receptor hypofunction in schizophrenia. D-serine, the more potent neurotransmitter for the co-agonist site of the NMDA receptor, is similarly to glycine degraded by

the enzyme D-amino acid oxidase in the central nervous system. One method to increase the NMDA function is to block the metabolism of D-amino acids using the D-amino acid oxidase inhibitor sodium benzoate. The novel sodium benzoate agent NaBen showed promising results in clinical trials by significantly improving cognition and several other symptoms in chronic schizophrenia patients stabilized on antipsychotic medications (Lane et al., 2013; Dimitrelis & Shankar, 2016).

Phosphodiesterases (PDEs) are enzymes involved in hydrolysing the intracellular secondary messengers cGMP and cAMP, which have received attention as potential pharmacological targets in schizophrenia (Garcia et al., 2016). PDE-10A is exclusively found in neurons in the striatum and basal ganglia where it is involved in the regulation of cAMP and cGMP signaling cascades (Kehler & Nielsen, 2011). Since dopamine D_2 receptor transmission requires PDE-10A-dependent cGMP and cAMP signaling, it appears that the use of PDE-10A inhibitors is an effective way to inhibit dopaminergic basal ganglia circuitry, a pathway heavily involved in the schizophrenia pathology. Preclinical evidence in animal models has suggested that PDE-10A inhibitors can affect positive, negative and cognitive symptoms of schizophrenia and consequently, PDE-10A inhibitors are being evaluated in clinical trials (Kehler & Nielsen, 2011). In 2014, the pharmaceutical company Omeros conducted a phase II trial of OMS824 to be used as alternative to the available dopamine receptor antagonists (Dimitrelis & Shankar, 2016).

Much recent evidence has pointed out abnormal nitric oxide (NO) and glutamate signalling in schizophrenia. Clinical studies have demonstrated abnormalities in glutamatergic function as well as reduced NO metabolites and cGMP in schizophrenia patients compared to healthy individuals (Hallak et al., 2013). NO has several biological functions whereby one is the activation of guanylyl cyclase, an enzyme that catalyses the conversion from GTP to cGMP. cGMP is an important intracellular secondary messenger that regulates several cellular functions and recent experimental evidence supports the hypothesis of a NO-cGMP pathway dysregulation in schizophrenia (Dimitrelis & Shankar, 2016). The effectiveness and safety of sodium nitroprusside was tested on positive, negative and anxiety symptoms in patients having an acute schizophrenia episode. The results showed a clear therapeutic effect of one sodium nitroprusside infusion which started within 4 hours and lasted up to 4 weeks (Hallak et al., 2013). In another recent study however, sodium nitroprusside failed to reduce positive symptoms or improve spatial working memory performance in patients with schizophrenia and therefore it remains to elucidate if nitroprusside could benefit schizophrenia treatment (Stone et al., 2016).

In conclusion, recent studies continue to show that there is more to schizophrenia than the dopaminergic theory, and consequently the search for novel therapeutic targets has taken on a broader spectrum of neurotransmitter and intracellular messenger systems. In addition, the industry has begun to recognize the unmet need for therapeutic interventions addressing all symptoms of schizophrenia, especially negative and cognitive symptoms. To date the discovery of a tolerable novel drug addressing all of the schizophrenia symptoms seems remote and it is probable that the approach in the immediate future will be focused on combination treatments with drugs addressing specific symptoms of schizophrenia (Dimitrelis & Shankar, 2016). However, the severe lack of reports on common treatment strategies like poly-pharmacological interventions further continues to complicate the challenge that is providing optimal treatment for schizophrenia (Ballon & Stroup, 2013).

2.2 2-Bromoterguride

2-Bromoterguride is a 2-halogenated derivative of terguride [1,1-diethyl-3-(6-methyl-8a-ergolinyl) urea] (Jantschak et al., 2013). Terguride, a C9-10 transdihydrogenated derivative of the ergot alkaloid lisuride, has D_2 receptor partial agonist properties of appreciable intrinsic activity and act as an antagonist at several 5-HT receptors and α_1- and α_2-adrenoceptor subtypes. Terguride was originally used in the clinical management of hyperprolactinemia due to its inhibiting effect on pituitary prolactin (PRL) secretion (Ciccarelli et al., 1988). Due to its neurochemical properties terguride was tested in patients with schizophrenia but failed to significantly ameliorate the positive symptoms of the disorder and was efficacious only in reducing negative symptoms (Olbrich & Schanz, 1991). The relatively high intrinsic activity at D_{2Short} receptors was suggested causative of the insufficient efficacy of terguride in the clinical trials (Natesan et al., 2011). The ability of terguride to reduce negative symptoms could be due to its potent antagonist properties at α_{2C}-adrenoceptors (Jantschak et al., 2013). In 2014, Hashimoto et al. tested terguride as add-on therapy to other antipsychotic medications because of its known effect on circulating prolactin levels. Terguride did indeed lower the prolactin levels but induced a series of unwanted side effects like insomnia, agitation, and/or the aggravation of hallucinations and therefore the study was discontinued.

Compared to terguride, 2-bromoterguride has only half of the intrinsic activity at D_{2Short} receptors and instead mimic aripiprazole as a partial agonist at D_{2Short} receptors and a potent antagonist at D_{2Long} receptors. In comparison to aripiprazole however, 2-bromoterguride show

higher antagonist activity at α_{2C}-adrenoceptors meanwhile lower affinity for H_1 receptors. In addition, both terguride and 2-bromoterguride are potent 5-HT_{2A} receptor antagonists (Jantschak et al., 2013), a receptor that has been implicated in the pathophysiology and treatment of schizophrenia (Meltzer et al., 2012).

In the preclinic, 2-bromoterguride inhibited amphetamine-induced locomotion (AIL) in rats, demonstrating its antidopaminergic activity (Franke et al., 2016). The selective activation of central 5-HT_{2A} receptors induces wet dog shakes in rats and consequently the inhibition of wet dog shakes can be used as a measurement for 5-HT_{2A} antagonism (Schreiber et al., 1995). 2-Bromoterguride effectively reduced wet dog shakes induced by (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) (Franke et al., 2016), indicative of 5-HT_{2A} receptor inverse agonist/antagonist activity and atypical antipsychotic action (Meltzer et al., 2012).

The conditioned avoidance response (CAR) test is a test to predict dopamine D_2 receptor occupancy. Established APDs can effectively suppress CAR without inducing escape failures (Wadenberg et al., 2001). In a previous study, 2-bromoterguride, haloperidol and aripiprazole produced comparable and dose-dependent suppression of CAR. The suppressive effects on CAR elicited by 2-bromoterguride was probably due to the combined effects on dopamine D_2, 5-HT_{2A} and α_{2C}-adrenoceptors, all highlighting the antipsychotic-like effects of 2-bromoterguride in rats (Franke et al., 2016).

It has been proposed that the therapeutic effect of dopamine D_2 partial agonists relies on the combination of the potential to elicit low intrinsic activity and sufficient functional dopamine D_2 receptor antagonism (Natesan et al., 2011). Therefore, a previous study investigated the intrinsic activity of 2-bromoterguride and terguride at human D_2 receptors. The study showed that terguride had a 2- to 3-fold higher intrinsic activity than 2-bromoterguride at D_{2short} receptors and that 2-bromoterguride activated dopamine D_{2Short} receptor-mediated inhibition of cAMP accumulation to the same extent as aripiprazole (Jantschak et al., 2013). Recently, the functional antagonism of 2-bromoterguride was tested via Fos expression. The results showed that 2-bromoterguride enhanced Fos in nucleus accumbens and dorsal dorsolateral striatum mimicking haloperidol, meanwhile also enhancing Fos levels in the medial prefrontal cortex similar to the action of clozapine (Franke et al., 2016). These results imply an eventual beneficial effect of 2-bromoterguride on negative symptoms and cognitive impairments in schizophrenia, as these are known to develop from deficient dopamine activity in the frontal cortex (Abi-Dargham, 2004).

Furthermore, preclinical observations of 2-bromoterguride has predicted its safe and tolerable usage as it did not induce catalepsy, a measurement of EPS, and did not cause any metabolic side effects or weight gain in chronically treated rats (Franke et al., 2016).

2.3 Animal models in schizophrenia research

To increase our understanding of the neurobiological background and pathology in schizophrenia and for the development of improved drugs, it is essential to develop reliable and predictive animal models (Lipska & Weinberger, 2000). Animal models of human disorders should preferable meet the requirements of construct-, face- and translational validity. For schizophrenia, the demonstration of construct validity is problematic due to our incomplete knowledge of the pathophysiology and underlying neurobiology. Face validity composes a real challenge due to the uniquely human features such as thought disorder and hallucinations which cannot be assessed readily in animals. Finally, predictive validity is critical for animal models intended for drug discovery and complicated in schizophrenia research due to the immense lack of positive controls to compare to (Wilson & Terry, 2010).

The current animal models of schizophrenia can be sorted into four categories based on their induction procedure; genetically manipulated, drug-induced, developmental or lesion-induced (Feifel & Shilling, 2010; Jones et al., 2011). Some of the models aim to mirror the full syndrome, however, because of the considerable heterogeneity in the symptoms and clinical course of schizophrenia, which cannot be fully reproduced in animals, these attempts are usually met with scepticism and failure. Instead, the most common animal models mainly share parts of the symptoms or features compared to the clinical picture of schizophrenia (Geyer & Moghaddam, 2002, see Table A.5). Most rodent models of schizophrenia have behavioural phenotypes that reflect altered mesolimbic dopamine function and resembles the positive symptoms of schizophrenia (Jones et al., 2011). However, rodents treated with NMDA receptor antagonists show deficits in social interaction and impaired learning and memory, analogous to negative and cognitive symptoms of schizophrenia (Moghaddam & Jackson, 2003; Bubenikova-Valesova et al., 2008). Naturally, psychotic symptoms such as hallucinations and delusions cannot be precisely modelled or measured in animals. Nonetheless, stress or novelty-induced hyperactivity, as well as psychostimulant hypersensitivity, are potentially useful correlates that can be modelled in animals. Furthermore, perception, attention, learning and memory can be altered and measured

in different rodent behavioural tests (Arguello et al., 2010, see Table A.6 for some of the symptoms in schizophrenia patients and their correlated measures in animals).

A common approach to develop animal models is to use pharmacological treatments, especially drugs known to produce schizophrenia-like symptoms in healthy human individuals. Such drug-induced animal models generally show predictive or construct validity and the utilization of these models has been of great importance for the establishment of the dopamine hypothesis, the serotonin theory, as well as the glutamate hypothesis of schizophrenia (Geyer & Moghaddam, 2002). Similarly, the utilization of pharmacologically induced animal models with schizophrenia analogous deficits enables the study of novel therapeutics and their action on specific symptoms (van der Staay et al., 2009). Due to the heterogeneous manifestation of symptoms there is no ideal animal model of schizophrenia. Instead the current animal models reconstruct only a few features of the disorder and any future models will at best still only be able to represent subpopulations of schizophrenia patients (Powell & Miyakawa, 2006).

For this reason, the next chapters present the most prominent animal models sorted by the main symptomatic and the implicated parameters and measures used to assess these.

2.3.1 Positive symptoms

To model positive symptoms of schizophrenia is undebatable a challenge due to auditory hallucinations and delusions being, to our knowledge, uniquely human. Fortunately, two main categories of rodent behaviour correlate with the positive symptoms in schizophrenia patients; locomotor hyperactivity and disrupted prepulse inhibition (PPI) of the acoustic startle response (ASR). As we do have some insight in the brain mechanisms responsible for causing the positive symptoms, these can be remodelled and induced in animals by drug treatments. Subcortical dopaminergic excess has been implicated in schizophrenia due to the neuropharmacological actions and antipsychotic effect of dopamine receptor antagonistic drugs (Tost et al., 2010; van den Buuse, 2010). Animals and humans are likely to share underlying neuropharmacological mechanism but probably elicit different behavioural responses to them. Consequently, it makes sense to assess these behavioural changes in a construct validity approach rather than by face validity. Hyperdopaminergia induces positive agitation in schizophrenia, and locomotor hyperactivity has been suggested to be analogous in rodents. Because locomotion is relatively easy to measure in rodents, the paradigm has become a widely used model for the positive symptoms of schizophrenia (Nestler & Hyman, 2010).

In theory, enhanced dopaminergic activity in rodents, predominantly in the mesolimbic and nigrostriatal dopamine systems, leads to locomotor hyperactivity which can be measured as horizontal locomotor activity, rearing or when induced by higher doses of dopamine agonists, stereotypic behaviours (van den Buuse, 2010). The role of dopamine in locomotor hyperactivity has been verified in many studies. For example, 6-hydroxydopamine (6-OHDA)-induced lesions in the nucleus accumbens in rats attenuates hyperactivity induced by amphetamines (Kelly & Iversen, 1976). Therefore, AIL in rats may be used to evaluate alterations in mesolimbic dopaminergic neurotransmission with construct validity for the increased dopaminergic activity in schizophrenia (van den Buuse, 2010). Rodent hyperlocomotion as a correlate to human central neurotransmitter system activity can also be induced using NMDA receptor antagonists such as PCP and MK-801 (Jentsch & Roth, 1999). Evidence for the strong construct validity of the rodent hyperactivity model, comes from the demonstration that dissociative anaesthetics like PCP and ketamine, which induce hyperlocomotor activity in rats and mice, also induce schizophrenia-like hallucinations in healthy human individuals (Moghaddam & Jackson, 2003). Nonetheless, a critical point for the locomotor hyperactivity animal model is that the effect of amphetamine may involve other neurotransmitter systems than dopamine. For example, effects on glutamatergic pathways, noradrenaline or 5-HT may all induce changes in locomotor activity (van den Buuse, 2010).

Due to the necessary dopamine D_2 receptor blockage to attenuate psychotic symptoms, antipsychotic activity is traditionally investigated in animals using the conditioned avoidance response (CAR) test; one of the oldest classical tests to predict antipsychotic activity of novel drugs. The CAR test has high predictive validity and can reliably identify novel agents with antipsychotic effect (Wadenberg, 2010). During the CAR test the animals are trained in a shuttle box to avoid an aversive event such as an electric shock. The animal can escape the electric stimulus by moving to the other side of the box. APDs block the CAR when administered in doses that result in around 65 - 70% striatal D_2 receptor occupation, which is similar to the threshold for therapeutic effect in humans (Kapur et al., 2000; Wadenberg et al., 2001).

The acoustic startle response (ASR) is a response to a sudden, relatively intense stimulus causing a partial contraction of skeletal muscles. In small laboratory animals such as rodents, the startle reflex can be assessed as a whole-body startle, meanwhile in humans the startle reflex is assessed with electromyography of the orbicularis oculi measuring the blink reflex (Koch, 1999; Swerdlow & Light, 2016). Assessment and utilization of the simple startle reflex circuit is particularly useful for studies using animal models of psychiatric disorders due to the ability to control the stimulus and therefore the ability to measure both excitatory and inhibitory effects of

drugs or manipulations. In rats, the acoustic startle response circuit reflects a time-confined relationship between the eliciting stimulus and the response only involving a simple neural circuit of three to five central synapses in the auditory nerve/cochlear nucleus/lateral lemniscus/ nucleus reticulars pontis caudalis/reticulo-spinal tract pathway (Geyer & Braff, 1987; Koch, 1999).

Loss of PPI of the ASR is a widely accepted schizophrenia endophenotype and indicative of disrupted sensorimotor gating, a precognitive process that prevents sensory overload and cognitive fragmentation in higher cortical areas (Geyer et al., 2001). PPI is the denoted ability of a non-startling “prepulse” tone elicited 30 - 500ms before a subsequent startling stimulus, to inhibit the startling response to the louder startle eliciting tone (Braff et al., 2001; Powell et al., 2009). PPI is often referred to as a ‘‘cross-species’’ measure of sensorimotor gating due to the suggested overlap of underlying neuronal substrates and pharmacological mechanisms interfering with PPI in human and rodents (van den Buuse, 2010). PPI deficits in sensorimotor gating in schizophrenia patients can be mimicked by surgical neural circuitry manipulations to the limbic cortex, striatum, pallidum, and pontine reticular formation or by treating rats with drugs that activates the dopamine system (Swerdlow et al., 1994; Geyer & Moghaddam, 2002). In general, administration of NMDA receptor antagonists such as PCP, mixed dopamine D_1/D_2 receptor agonists like apomorphine or indirect dopamine agonists like amphetamine, disrupts PPI in rodents (Geyer et al., 2001; Powell et al., 2009; van den Buuse, 2010). In addition to the influence of drug dose and potential drug effect on baseline startle behaviour, the animal species, strain and gender influences PPI and need consideration. Furthermore, PPI protocol measures such as loudness of the pulses, habituation to repeated tones or the interval between the prepulse and the startle pulse, are all factors which has influence on PPI and therefore must be carefully monitored (van den Buuse, 2010; Brosda et al., 2011). Nonetheless, the animal PPI model for impaired sensorimotor gating, has clearly demonstrated its face-, predictive- and construct validity for schizophrenia (Swerdlow & Light, 2016) as well as its relevance in drug development programs as it can predict antipsychotic efficacy and distinguish novel "typical" from "atypical" antipsychotics (Swerdlow & Geyer, 1998; Geyer et al., 2012).

2.3.2 Negative symptoms

Negative symptoms of schizophrenia are major contributors to the poor functional outcome in the patients, yet they cannot be effectively treated with current medications. One key to finding new effective novel agents is the use of improved animal models with predictive validity (Neill et al., 2014). Recently it has been suggested that the negative symptoms in schizophrenia patients may

be divided in the two major subdomains *diminished expression* and *avolition*. The domain *diminished expression* incorporates symptoms like blunted effect and poverty of speech meanwhile the domain *avolition* incorporates lack of motivation, anhedonia as well as asociality (Millan et al., 2014). Such sub-division of negative symptoms implicates that there might be differences in the neuropharmacological basis of the two domains, and that it could be beneficial not to generalize and assess the negative symptoms as one domain. The same argument has been implied for the preclinical research meaning that each negative domain or even symptom should be modelled and tested separately (Neill, et al., 2014). The symptoms asociality, anhedonia and blunted affect can be modelled in specific animal models but as these symptoms also occur in autism and depression, these models are often viewed upon with scepticism (Nestler et al., 2010).

Social dysfunction is a core negative symptom of schizophrenia that cannot be adequately treated using either typical or atypical antipsychotics. Due to the lack of therapeutic effect of dopaminergic substances on negative symptoms and cognitive deficits, the emphasis during the last decade has moved towards the development of animal models which deficits are induced by other pathways than the monoamine system, for example via administration of NMDA receptor antagonists like PCP (Geyer et al., 2012; Neill et al., 2014). Social dysfunction can be induced in rats using several pharmaceutical agents such as amphetamine, cannabinergic and serotonergic receptor ligands or NMDA receptor antagonists (for reviews, see Gururajan et al., 2010 and Wilson & Koenig, 2014). Withdrawal from repeated PCP administration produces social aversion in animals, a symptom that can be readily measured (Seillier & Giuffrida, 2009; Neill et al., 2014). Additionally, subchronic PCP followed by a drug washout phase causes hypofrontality as well as a deficit in parvalbumin-immunoreactive hippocampal- and prefrontal cortical neurons, one of the most robust pathologies in schizophrenia and implication of a GABAergic signalling decrease/deficit (Lodge et al., 2009). These effects cannot be reversed by risperidone respectively haloperidol or clozapine, strengthening the validity of the subchronic PCP model (Jenkins et al., 2008; McKibben et al., 2010).

The accumulating evidence and pharmacological compounds that disrupt social behaviour have implicated the involvement of the dopaminergic, glutamatergic and possibly cholinergic and norepinephrine systems as mediators of social interaction behaviours. However, several compounds such as oxytocin or arginine vasopressin have effect on social behaviours and social recognition (Wilson & Koenig, 2014). Several regions in the hindbrain and forebrain have been

implicated in the facilitation of social behaviour, with special roles for the amygdala, hypothalamus, hippocampus and prefrontal cortex (File & Seth, 2003).

The most commonly used test for assessment of social behaviour in rodents is the social interaction test. This paradigm was first developed 40 years ago (File & Hyde, 1978) to give an aethologically relevant measure for anxiety in rats. The dependent paradigm measure is the time spent in social interaction when a pair of unfamiliar rats is placed together in a box or arena. In a novel setting with bright light, social interaction behaviour is sensitive to both anxiolytic and anxiogenic effects with a decrease in social contacts denoting the higher level of anxiety. The highest degree of social interaction on the other hand, is achieved when the rats are tested in a familiar arena under low light (File & Seth, 2003, Rex et al., 2004). Over the years, the "low-anxiety-test-condition" of the social interaction test has become one of the standard tests for sociability in preclinical research and subchronic PCP treatment reliable induces social interaction deficits in rats when they are tested in the low-anxiety-test version (Sams-Dodd, 1995; Neill et al., 2010; Wilson & Koenig 2014).

Some publications have described that atypical antipsychotics like clozapine may ameliorate the PCP-induced social deficit in rats but not typical antipsychotics (Neill et al., 2010). For this reason, the subchronic PCP rat model of social dysfunction represents a model, which allows distinguishing between typical and atypical novel drugs. Other groups have described the need for balanced 5-HT_{1A} and dopamine D_2 receptor activity of APDs to ameliorate the PCP-induced social deficit and the failure of clozapine to do so (Bruins Slot et al., 2005; Snigdha & Neill, 2008a), showing the problematic drug response variability, which is evident in both preclinical studies and clinical trials. As such, the predictive validity of the model can be debated and must be considered because atypical APDs does normally not have effect on social behaviour deficits in schizophrenia patients (Neill et al., 2014). In addition, aripiprazole and fluoxetine have been shown to ameliorate the social interaction deficit in female rats after withdrawal from subchronic PCP treatment. As this effect was in turn prevented when the rats were injected with the 5-HT_{1A} receptor antagonist WAY100635, this was interpreted indicative of serotonergic system involvement, particularly 5-HT_{1A} receptors in the alleviation of the PCP-induced social interaction deficit (Snigdha & Neill, 2008b). However, WAY100635, an agent which has been widely used as a pharmacological probe for the investigation of 5-HT_{1A} receptor distribution and function, is additionally a potent agonist at the dopamine D_4 receptor and hence not a "selective" 5-HT_{1A} receptor antagonist as previously believed (Chemel et al., 2006; Marona-Lewicka & Nichols,

2009). This implicates however, that the subchronic PCP induced social interaction deficit in rats might be ameliorated by agents that modulate 5-HT_{1A} and/or dopamine D_2/D_4 receptors.

2.3.3 Cognitive deficits

As with the negative symptoms of schizophrenia, the aetiology of the cognitive deficits in schizophrenia remain largely unknown and an unmet clinical need. Translation between animal models of cognitive deficits and schizophrenia has sadly turned out to be troublesome. For example, dissimilarities in the methods to assess the cognitive domains of interest in rodents and humans makes it problematic to study the underlying neural substrates and their relevant behaviours in a way that can be directly translated to schizophrenia patients (Young & Geyer, 2015).

As schizophrenia is a disorder defined by abnormal behaviours, one approach to gain understanding for the schizophrenia pathophysiology is to develop models that show similar behaviours (for review see, Young & Geyer, 2015). During the MATRICS initiative experts in the field identified seven domains of cognition that are deficient in schizophrenia, and several behavioural tests has been developed with the aim to quantify the corresponding behaviour in animals (Young et al., 2009, see table A.7). Declarative memory is one impaired cognitive function in schizophrenia and its analogue in animals is visual learning and object recognition (Meltzer et al., 2013). The novel object recognition (NOR) test is a cognitive task consisting of two short trials separated by an inter-trial interval of variable length and it assesses visual learning and memory in rodents (Ennaceur & Delacour, 1988). Due to its simplicity and the lack of need to pretrain animals or add external motivators, the NOR test has become the most common test to assess recognition memory in rodents and proved its usefulness for testing novel drugs (for reviews see, Lyon et al., 2012; Grayson et al., 2015).

Accumulating evidence support the involvement of glutamatergic dysfunctions in the development of the cognitive impairments in schizophrenia and the NMDA receptor antagonist animal model has proven effective and reliable in producing cognitive deficits that mimic some features and symptoms of schizophrenia (for review, see Gururajan et al., 2010; Neill et al., 2010; Meltzer et al., 2013). In addition to the effects on social behaviour, the non-competitive NMDA antagonists' PCP, ketamine and MK-801 (dizocilpine) cause disruptions in several cognitive domains in rodents. For example, visual memory and learning in the NOR test, reversal learning,

set shifting, attention and speed of processing has frequently been studied and disrupted by administering subchronic PCP to rats (Neill et al., 2010; Redrobe et al., 2010).

Meanwhile subchronic PCP-treated rats show robust deficits in object recognition when tested in the NOR task, it has continuously been shown that only 5-HT_{2A}/5-HT_{1A} and dopamine D_1/D_2 receptor antagonistic atypical APDs can attenuate the PCP-induced object memory deficit in rodents (Grayson et al., 2007; Snigda et al., 2011; Oyamada et al., 2015). Consequently, the NOR test can be used in the preclinic to identify novel agents with atypical characteristics.

2.3.4 Treatment side effects

During APD treatment, it is vital to reach optimal dopamine D_2 receptor occupancy meanwhile keeping a balance between clinical effect and induction of adverse side effects (Horacek et al., 2006). Dopamine D_2 receptor blockade by typical APDs can induce hyperprolactinemia, acute or later developed ("tardive") EPS such as dystonia, Parkinsonism, akathisia, akinesia, and dyskinesia, as well as mentally manifested EPS symptoms like anhedonia, apathy and social withdrawal. Second generation, atypical APDs are causing less EPS due to their clinical effect being reached at lower non-EPS-inducing dosages (Tandon & Jipson, 2002). However, atypical drugs come with their own unique side effect profile and often induce metabolic side effects such as weight gain (Kalinichev et al., 2005). As all current antipsychotics block dopamine D_2 receptors to some degree, it is important to predict at which D_2 receptor occupancy the clinical response is reached, as well as the occupancy that induces extrapyramidal side effects and hyperprolactinemia (Kapur et al., 2000).

To predict the probability of compounds to induce side effects and for understanding the underlying mechanisms of these, animal models can be used. The liability of substances to produce EPS is often investigated via the catalepsy test in laboratory animals. Catalepsy is defined as the failure to correct an unusual posture imposed by an experimenter such as being placed on an inclined grid or with the forepaws on a bar. The scored measurement for catalepsy is the duration of immobility/latency until the animal changes its body posture. Studies in schizophrenic patients have indicated that striatal dopamine D_2 receptor occupancy over 80% induces motor side effects and the catalepsy test has high predictive accuracy due to the identical underlying mechanism for the onset of cataleptic behaviour; dopamine D_2 receptor occupancy, which corresponds well with the induction of EPS in humans (Wadenberg et al., 2001).

Tardive dyskinesia is a serious antipsychotic treatment side effect consisting of continuous, rhythmic, involuntary movements, frequently in the face, mouth and tongue (orofacial type). It is believed that excitotoxic mechanisms and oxidative stress cause striatal neurodegeneration leading to the development of tardive dyskinesia which symptoms often remain present for months after treatment discontinuation (Andreassen & Jörgensen, 2000). Several of the clinical features of tardive dyskinesia can be modelled in rats, measured as orofacial dyskinesia (vacuous chewing movements) (Waddington et al., 1983). Rats exposed to APDs over long periods might develop persisting chewing movements which continue even after prolonged drug withdrawal, giving the model a high validity. Furthermore, the risk for development of tardive dyskinesia is higher in older rats and exacerbated by stress, similarly to observations in patients treated with typical APDs (for review see, Waddington, 1990).

Finally, hyperprolactinemia is one of the more common side effects associated with typical APD treatment and a state that might induce a cluster of sexual and reproductive complications. Decreased bone mineral density, osteoporosis, menstrual disruptions, sexual impairment and infertility, galactorrhea, breast cancer and cardiovascular disorders are some of the secondary effects resulting from hyperprolactinemia (Halbreich & Kahn, 2003). A variety of stimulus such as exercise, breast suckling, stress, sleep, eating, sex and ovarian steroid levels participates in keeping the equilibrium of inhibitory and stimulatory prolactin release and the secretion of prolactin from the anterior pituitary gland is controlled by peptide and steroid hormones as well as neurotransmitters (Peuskens et al., 2014). For example, dopamine D_2 receptors inhibit the prolactin secretion and D_2 receptor blockade elevates blood serum levels of prolactin by counteracting this inhibitory control (Freeman et al., 2000). The normal prolactin blood serum concentration varies with gender, with strong interindividual differences as well as circadian rhythm fluctuations, with the maximum concentration reached during the REM sleep. APD-induced prolactin elevation is usually dose dependent, although some APDs seems to have a stronger potential for causing hyperprolactinemia even at relatively low doses. Hyperprolactinemia is often induced at the beginning of APD treatment but can also be triggered later during ongoing treatment (for review see, Peuskens et al., 2014). In preclinical research, drug-induced prolactin elevation is usually studied in rats which receive different doses of the APD and are sacrificed to collect blood for subsequent in vitro analyses such as enzyme-linked immunosorbent assay (ELISA).

CHAPTER 3: Aims and objectives of the thesis

The overall purpose of this research was to investigate the effect of 2-bromoterguride as a potential novel atypical APD on cognitive deficits and negative symptoms in pharmacological animal models of schizophrenia as well as its side effects. To assess the effects of 2-bromoterguride the specific aims of this research was to study:

I. The effect of 2-bromoterguride on apomorphine- and PCP-induced sensorimotor gating deficits
II. The effect of 2-bromoterguride on subchronic PCP-induced declarative memory/object recognition deficits
III. The effect of 2-bromoterguride on subchronic PCP-induced social aversion
IV. The effect of 2-bromoterguride on prolactin secretion.

The results of these four studies are presented in **Chapter 4**. Additionally, we dedicated a final study to:

V. Validate olfactory function in the subchronic PCP model for social impairments, using the olfactory habituation/dishabituation test.

A literature search revealed a serious lack of studies on olfactory function in subchronic PCP treated rats. To fill the knowledge gap and to investigate sensory function thoroughly, subchronic PCP treated rats from study III were tested in the final experiment presented in **Chapter 5**.

CHAPTER 4: Effects of 2-bromoterguride, a dopamine D_2 receptor partial agonist, on cognitive dysfunction and social aversion in rats

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Effects of 2-bromoterguride, a dopamine D_2 receptor partial agonist, on cognitive dysfunction and social aversion in rats

Emilia Tarland[1] · Robert T. Franke[1] · Heidrun Fink[1] · Heinz H. Pertz[2] · Jan Brosda[1,3]

[1] Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Freie Universität Berlin, Koserstrasse 20, D-14195 Berlin, Germany

[2] Institute of Pharmacy, Freie Universität Berlin, D-14195 Berlin, Germany

[3] Present address: Bundesinstitut für Risikobewertung (BfR), Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

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4.1 Abstract

Rationale 2-Bromoterguride, a dopamine D_2 receptor partial agonist with antagonist properties at serotonin 5-HT_{2A} receptors and α_{2C}-adrenoceptors, meets the prerequisites of a putative atypical antipsychotic drug (APD). We recently showed that 2-bromoterguride is effective in tests of positive symptoms of schizophrenia in rats without inducing extrapyramidal side effects or metabolic changes.

Objective In continuation of our recent work, we now investigated the effect of 2-bromoterguride on apomorphine and phencyclidine (PCP)-induced disruptions of prepulse inhibition (PPI) of the acoustic startle response (ASR), a measure of sensory gating. In addition, we used subchronic PCP treatment to produce cognitive deficits and social aversion and assessed the effect of 2-bromoterguride on the performance in the novel object recognition (NOR) task (model for studying cognitive deficit symptoms of schizophrenia) and the social interaction test (model for studying negative symptoms of schizophrenia). Finally, we extended the side effect profile of 2-bromoterguride by measuring the prolactin response to systemic administration of the drug in rats.

Results Treatment with 2-bromoterguride (0.1 and 0.3 mg/kg) reversed PPI deficits induced by apomorphine and PCP, respectively. Subchronic PCP induced impairments in object memory and social interaction behavior which were ameliorated by 2-bromoterguride but not by clozapine and aripiprazole, respectively. Prolactin concentration in blood serum was not elevated at 1, 2, or 4 h post-2-bromoterguride treatment, which further supports the safe and effective use of this drug.

Conclusions Our data support 2-bromoterguride as a promising APD candidate due to its beneficial effect on cognitive impairments and negative symptoms of schizophrenia.

Keywords: Prepulse inhibition · Cognitive deficit symptoms of schizophrenia · Novel object recognition · Social interaction · Rat · Dopamine D_2 receptor partial agonist · Antipsychotic

4.2 Introduction

The treatment of schizophrenia ideally involves reduction of positive symptoms, negative symptoms, and cognitive deficits. Positive symptoms such as hallucinations and delusions can be treated more or less satisfactorily with currently available antipsychotic drugs (APDs). However, negative symptoms (affective flattening, alogia, avolition, and anhedonia) and cognitive impairment often fail to respond to typical (first generation) APDs (Dunlop and Brandon 2015; Vreeker et al. 2015).

Emerging evidence from preclinical and clinical studies using atypical (second generation) APDs with additional affinities for multiple serotonin (5-HT) receptors, predominantly the 5-HT_{2A} subtype, provided renewed optimism for the pharmacological treatment of schizophrenia and other psychotic disorders (Aznar and Hervig 2016). Atypical APDs produce less extrapyramidal side effects (EPS), tardive dyskinesia, and hyperprolactinemia than typical APDs but their antagonistic effects at histamine H_1 and 5-HT_{2C} receptors might induce insulin resistance, weight gain, diabetes, and other secondary conditions (Meltzer 2013; Kim et al. 2007; Ücok and Gaebel 2008). Additionally, and most importantly, atypical APDs are apparently no more effective than typical APDs regarding negative and cognitive symptoms of schizophrenia (Leucht et al. 2013).

Dopamine D_2 receptor partial agonists such as aripiprazole represent the latest advancement in the treatment of schizophrenia. The intrinsic dopamine-stabilizing effect of partial agonists can adjust the levels of dopamine through decreased postsynaptic transmission in the mesolimbic system when dopamine is elevated, and through increased transmission in the mesocortical system when the dopamine level is low (de Bartolomeis et al. 2015). 2-Bromoterguride (Figure 4.1) is a dopamine D_2 receptor partial agonist that mechanistically resembles aripiprazole. 2-Bromoterguride is the dihydro derivative of the dopamine antagonist bromerguride (2-bromolisuride), a compound that behaved as an atypical APD (Löschmann et al. 1992). Interestingly, 2-bromoterguride possesses a higher affinity for 5-HT_{2A} receptors and α_{2C}-adrenoceptors, and a lower affinity for histamine H_1 receptors than aripiprazole (Jantschak et al. 2013). We recently demonstrated that 2-bromoterguride inhibits amphetamine-induced locomotion (AIL) and conditioned avoidance response (CAR) in rats, suggesting antipsychotic action. Furthermore, neither acute nor chronic treatment with 2-bromoterguride induced catalepsy or altered body fat composition and body weight in rats (Franke et al. 2016; Jantschak et al. 2013).

To investigate the prospective effects of 2-bromoterguride as a clozapine-like atypical APD, we induced disruptions of prepulse inhibition (PPI) of the acoustic startle reflex using the

mixed dopamine D_1/D_2 receptor agonist apomorphine and the noncompetitive NMDA receptor antagonist phencyclidine (PCP). PPI is a pre-attentive process regulated by multiple neurotransmitter systems including dopaminergic, serotonergic, cholinergic, GABAergic, and glutamatergic systems (Geyer 1998). Apomorphine induces loss of PPI in a robust manner when administered to rats, and its effect can be blocked by typical APDs like haloperidol (Mansbach et al. 1988). In contrast, loss of PPI induced by PCP is insensitive to either dopaminergic or serotonergic antagonists but can be attenuated by selective α_{2C}-adrenoceptor antagonists such as JP-1302 or atypical APDs such as clozapine and quetiapine (Sallinen et al. 2007; Swerdlow et al. 1996; Bakshi et al. 1994).

In contrast to acute PCP administration, subchronic PCP treatment triggers prefrontal cortical dopaminergic hypoactivity and a hyper-responsive state in the mesolimbic dopamine system, resembling the pathophysiology of schizophrenia (Jentsch et al. 1998). Behaviorally, subchronic PCP administration induces memory and learning deficits (for reviews, see Jentsch and Roth 1999; Meltzer et al. 2013; Neill et al. 2010). Atypical APDs in contrast to typical ones successfully attenuate the object memory-disrupting effects of PCP in rodents (Grayson et al. 2007; Horiguchi et al. 2012; Oyamada et al. 2015, Snigda et al. 2011). To study the effect of 2-bromoterguride on these cognitive disruptions, we used the one-trial object recognition test, commonly known as the novel object recognition task (NOR). NOR is a well-established model for assessment of visual learning and recognition memory in rodents (Ennaceur and Delacour 1988; Ennaceur 2010) and to evaluate the general efficacy of novel APDs to alleviate cognitive deficits (Grayson et al. 2015). In addition to its disrupting effect in rodent object memory, subchronic PCP induces social interaction impairments, which can be attenuated by compounds with combined D_2/5-HT_{2A} antagonist and D_1/5-HT_{1A} agonist properties but not by dopamine D_2 selective agents (Bruins Slot et al. 2005; Neill et al. 2014; Snigdha and Neill 2008a,b). To assess the effect of 2-bromoterguride on PCP-induced social aversion, we used the social interaction test, an established paradigm to demonstrate the effect of putative therapies on the negative symptoms of schizophrenia (for review, see Wilson and Koenig 2014).

Finally, we wanted to know whether 2-bromoterguride is a prolactin-elevating APD. Elevated levels of serum prolactin may induce sexual dysfunction worsening negative schizophrenic symptoms (Leucht et al. 2013).

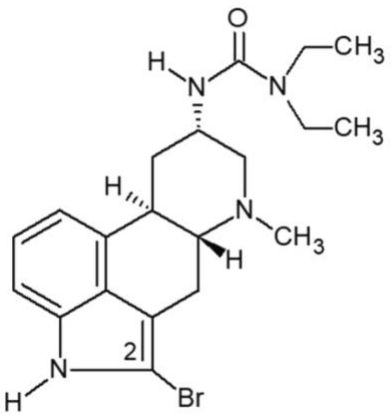

Figure 4.1 Chemical structure of 2-bromoterguride

4.3 Materials and methods

4.3.1 Animals

Naïve male Sprague-Dawley rats (Élevage Janvier, Le Genest Isle, France) aged 10 weeks and with a mean weight of 450 g by the beginning of the experiments were used for the PPI (*N* = 98), NOR (*N* = 70) and social interaction (*N* = 98) experiments. Rats from the PPI study were additionally used for prolactin determination (*N* = 74). At this point of the study, the aim was to investigate the overall effectiveness of 2-bromoterguride on cognition and sociality in vivo. Therefore, and for reasons of comparability to data of our previous study (Franke et al. 2016), only one gender (males) was included. The animals were housed in groups of 3 - 4 per cage (type open-top IV polycarbonate cages; Ehret, Emmendingen, Germany) under standard laboratory conditions (room temperature, 22 ± 2 °C; relative humidity 55 ± 10%) on a 12 h light-dark schedule (lights on at 6:00 am). All experiments were conducted during the light phase between 9:00 am and 2:00 pm. Water and laboratory chow (Ssniff, Soest, Germany) was freely available and the cages were enriched with metal tubes and paper tissues. The animals were allowed 1 week of acclimatization upon arrival and gently handled 5 min/day (during the second week) by the person performing the experiments. All experimental procedures were approved by the Berlin State Authority (Landesamt für Gesundheit und Soziales) and performed in compliance with the German Animal Protection Law and the EU Directive 2010/63/EU for animal experiments.

4.3.2 Drugs

2-Bromoterguride (Alfarma sro, Cernosice, Czech Republic) was suspended in 15% Cremophor® EL (Sigma-Aldrich, Steinheim, Germany), phencyclidine hydrochloride (Sigma-Aldrich, Steinheim, Germany) and haloperidol (Janssen Pharmaceuticals, Beerse, Belgium) were dissolved in 0.9% saline. Apomorphine hydrochloride hemihydrate (Sigma-Aldrich, Steinheim, Germany) was dissolved in 0.2% ascorbic acid solution, aripiprazole (Toronto Research Chemicals, Toronto, Canada) in 30% N,N-dimethylformamide and blended in 0.5% acetic acid. Clozapine (Abcam Biochemicals, Cambridge, UK) was dissolved in 0.1 N HCl and adjusted to pH neutrality. Compound doses and the elicited effect on behavior were thoroughly evaluated in pilot studies prior to the PPI, NOR, and social interaction experiments. All drugs were freshly prepared on the day of injection and administered in a volume of 1 ml/kg body weight.

4.3.3 Prepulse inhibition of the acoustic startle response (ASR)

We measured PPI of the acoustic startle response (ASR) using a two-unit SR-LAB startle response system (San Diego Instruments, San Diego, CA) placed in an experimental chamber with 43 dB ambient noise level. The startle response cabinets were sound isolated and contained an acrylic cylinder (non-restrictive, 9 cm in diameter) attached to a platform. The delivery of acoustic stimuli was operated by the SRLAB interface system and emitted via loudspeakers above the cylinder. A piezoelectric accelerometer sensor installed beneath the platform transduced cylinder vibrations into analogue signals, which were digitized and stored by the SR-LAB software. A short test session was performed 2 - 4 days prior to the PPI experiment to distribute the rats into balanced treatment groups based on their mean baseline startle magnitudes. For the PPI experiment, the rats were pre-treated with 2-bromoterguride (0.1 or 0.3 mg/kg, i.p.), clozapine (5.0 mg/kg, i.p.), haloperidol (0.1 mg/kg, i.p.), or vehicle (saline 0.9%, i.p.) 30 min prior to the test, followed by either PCP (1.5 mg/kg, s.c.), apomorphine (0.5 mg/kg, s.c.), or vehicle (saline 0.9%, s.c.) 20 min later. The animals were placed into the startle chambers and the session initiated with a 5-min acclimatization period. Background noise of 70 dB was emitted during the complete session. A total of 100 trials were presented in a pseudorandom order with variable inter-trial intervals (7–23 s). The protocol consisted of 20 startle trials (pulse-alone, 118 dB sound pressure level (SPL), duration 40 ms), 10 prepulse trials (86 dB SPL, duration 20 ms), 10 no-stim trials, and 40 prepulse-pulse trials. Prepulse-pulse trials consisted of a single 118 dB pulse preceded by a 74-, 78-, 82-, or 86-dB prepulse (20 ms duration) emitted 120 ms before the pulse onset. In addition, 10 pulse-alone trials were carried out at the beginning (startle block 1) and the end of the

session (startle block 2) to measure habituation to the startle stimulus. These trials were excluded from startle magnitude calculations. Data was measured during a 100-ms time window after stimulus onset and averaged for each animal and trial type. PPI of the ASR was calculated for prepulse-pulse trials as a percentage of pulse-alone startle magnitude [(mean startle magnitude for pulse-alone trials − mean startle magnitudes for prepulse-pulse trials)/mean startle magnitude for pulse-alone trials] × 100. Pilot experiments were conducted to define doses of apomorphine and PCP which induced a robust loss of PPI, and to examine the effect of 2-bromoterguride on startle activity and PPI alone.

4.3.4 Novel object recognition

The rats were administered with PCP (5.0 mg/kg) or vehicle (0.9% saline) i.p. twice a day (at 8:00 am and 5:00 pm) for 7 days. After a 14-day drug wash-out period, the animals were habituated to a 50 × 50 × 32 cm-sized dark colored acrylic arena placed in a sound-isolated chamber with dimmed lightning (5 lux) over 3 days. The first habituation was performed in littermate groups for 15 min. On days two and three, the rats were placed alone in the arena for 10 min. On day four, the rats were injected with either 2-bromoterguride (0.1 or 0.3 mg/kg, i.p.), clozapine (5.0 mg/kg, i.p.), or vehicle (0.9%saline, 1 ml/kg, i.p.) 30 min prior to the experiment.

In the acquisition trial (3 min), each rat was exposed to two identical objects, placed in opposite diagonal corners of the arena (positioned 12 cm from the walls). Subsequently, the rat was placed in a holding cage for a 1-min inter-trial interval; meanwhile, both objects were replaced (novel object + triplicate of familiar object). Finally, during the retention trial, the rat was re-introduced to the arena and allowed to explore the objects for 3 min. We used bright-colored glass bottles with metal caps, 12 × 12 × 6 cm and dark colored glass bottles with a blue plastic wrap, 12 × 12 × 6 cm, respectively. Objects were counterbalanced as novel or familiar objects in the treatment groups. Object attribute sensitivity and animal preferences was thoroughly evaluated in pilot studies to ensure that the objects elicited the same level of spontaneous investigation. The position of the novel object was counterbalanced (left/right) to eliminate spatial bias. The arena (after the acquisition and retention trial) and objects (after each animal) were cleaned with a 1:3 mix of isopropyl alcohol (70%) and meliseptol® (B. Braun Melsungen AG, Germany) to remove olfactory traces. The experiments were video recorded and scored by a blinded experimenter. Object exploration was defined as sniffing, licking, biting, or touching the object from < 1 cm distance, but not climbing on the object. Animals which failed to explore one or both of the objects for less than 4 s during the acquisition or retention trial were excluded from the data analysis.

Discrimination index was calculated from: [(time spent exploring the novel object − time spent exploring the familiar object)/total exploration time]. Track length was measured with the software Videomot2 (TSE-Systems, Berlin, Germany).

4.3.5 Social interaction

Half of the rats were treated with PCP (5.0 mg/kg, i.p.) or vehicle (0.9% saline, i.p.) twice a day (at 8:00 am and 5:00 pm) for 7 days. The other half remained untreated, housed in an adjacent room, and were brought into the lab 30 min prior to experimental procedures. After a 14-day drug wash-out period, all animals were habituated to a 50 × 50 × 32 cm-sized arena as described for the NOR test. On the test day, PCP- or vehicle-treated rats received a dose of either 2-bromoterguride (0.1 or 0.3 mg/kg, i.p.), aripiprazole (3.0 mg/kg, i.p.), or vehicle (0.9% saline, i.p.) 30 min prior to the social interaction test. During the test, each PCP- or vehicle-treated animal was paired with a weight matched (to max 30 g difference) untreated animal, placed together in the arena for 10 min, and video recorded for subsequent behavior analysis (Videomot2; TSE-Systems, Berlin, Germany). An inanimate object (a 33-cl aluminum soda can) was placed in the arena to measure the preference for interacting with an unfamiliar animal opposed to an unfamiliar object. The following social and non-social behaviors were scored by a blinded experimenter: following (the subject rat moves behind the unfamiliar conspecific), sniffing (investigative sniffing the snout, body, or anogenital region of the unfamiliar animal), climbing (climbing over the back of the conspecific or pushing the head and/or forepart beneath the conspecific), avoiding (actively turning away when approached by the conspecific) and object exploration (sniffing the object from < 1 cm distance). An overall social behavior parameter was calculated as the sum of times engaged in the abovementioned social behaviors. To assess locomotion, line crossings were manually scored using a 9 × 9 squared grid. Treated rats were marked with dark stripes on the back to distinguish them from untreated rats during the video analysis.

4.3.6 Prolactin

The rats received an injection of 2-bromoterguride (0.1 or 0.3 mg/kg, i.p.), haloperidol (0.5 mg/kg, i.p.), or vehicle (saline 0.9%, i.p.), and were sacrificed 1, 2, or 4 h later by decapitation. To avoid stress-related prolactin release, the rats were only handled by a familiar experimenter. Trunk blood was collected into standard 2 ml Eppendorf® tubes and left to clot for 40 min at room temperature. The samples were centrifuged 10 min at 21 °C with 4000 rpm, blood serum collected into aliquots

and stored at −80 °C until prolactin determination. Enzyme-linked immunosorbent assay (ELISA) was performed to assess prolactin levels using a commercial available rat prolactin ELISA kit following the instructions of the kit manufacturer (DRG Instruments, Marburg, Germany). Samples were analyzed in duplicates in the same assay (MTPL-Reader BE-LizaMat^ 3000, DRG Instruments, Marburg, Germany) and two rat prolactin control samples (DRG Instruments, Marburg, Germany) containing a mean of 15.8 ng/µl respective 29.6 ng/µl prolactin were used for internal quality verification. The assay sensitivity was 0.6 ng/ml and the intra- and inter-variability coefficients were 3.7 and 10.4%, respectively.

4.3.7 Data presentation and analysis

Statistical analysis was performed with SigmaPlot 11 (Systat Software, Erkrath, Germany). Two-way repeated measures (RM) analysis of variance (ANOVA) with treatment as between-subjects factor and prepulse intensity as within-subjects factor was conducted to determine whether pretreatment with 2-bromoterguride, haloperidol, or clozapine reversed the effects of apomorphine or PCP on PPI. Startle habituation data were analyzed with two-way repeated RM ANOVA with treatment as between-subjects factor and startle block as within-subjects factor. Mean startle magnitude data for pulse-alone trials, for prepulse-elicited reactivity, and reactivity on no-stim trials were analyzed with one-way ANOVA. NOR and social interaction data were analyzed by one-way or two-way ANOVAs according to the parameter and experimental design. Prolactin quantity data were analyzed by two-way ANOVA with treatment and time as between-subjects factors. Post hoc pairwise comparisons (Holm-Sidak method) were performed when appropriate. *P* values < 0.05 were considered significant and all data presented as mean ± standard error of the mean (SEM).

4.4 Results

4.4.1 Prepulse inhibition of the acoustic startle response

Acute apomorphine administration induced a robust loss of PPI compared to controls, which was attenuated by 2- bromoterguride or haloperidol treatment. We observed significant main effects for the factors treatment ($F(4,132) = 12.7$, $p < 0.001$) and prepulse intensity ($F(3,132) = 113.9$, $p < 0.001$). 2-Bromoterguride (0.3 mg/kg) prevented the apomorphine-induced PPI deficits at 78, 82, and 86 dB ($p < 0.003$ each), an effect that was elicited by haloperidol (0.1 mg/kg) at 74 ($p = 0.044$), 78, 82, and 86 dB ($p < 0.002$ each) (Figure 4.2a). Further, 2-bromoterguride and clozapine

attenuated PPI deficits induced by PCP. ANOVA revealed significant effects for the factors treatment ($F(4,132) = 6.4$, $p < 0.001$), prepulse intensity ($F(3,132) = 207.8$, $p < 0.001$), and the interaction of these factors ($F(12,132) = 2.2$, $p = 0.016$). Post hoc comparisons revealed that both doses of 2-bromoterguride and clozapine ameliorated the PPI impairment at 82 dB (0.1 mg/kg: $p < 0.007$; 0.3 mg/kg: $p < 0.009$; clozapine: $p = 0.017$). Additionally, 0.3 mg/kg 2-bromoterguride was also effective with a prepulse of 86 dB ($p < 0.034$) (Figure 4.2b). The ASR magnitude was affected by the factor treatment ($F(4,132) = 4.5$, $p = 0.004$). Apomorphine alone ($p = 0.013$), or in combination with 0.1 mg/kg 2-bromoterguride ($p < 0.027$), increased the startle reaction (Table 1). All treatment groups habituated to the startle stimuli as illustrated by the mean startle reactivity in the first 10 pulse-alone trials (startle block 1) compared to the last 10 pulse-alone trials (startle block 2) ($p < 0.001$). We observed a main effect of startle block ($F(1,117) = 233.7$, $p < 0.001$) and an interaction between treatment and startle block ($F(10,117) = 3.6$, $p < 0.001$) (Table 1). Pilot experiments showed that (i) the used doses of apomorphine/PCP induced a PPI-disruptive effect and (ii) the used doses of 2-bromoterguride alone did not affect startle activity or PPI.

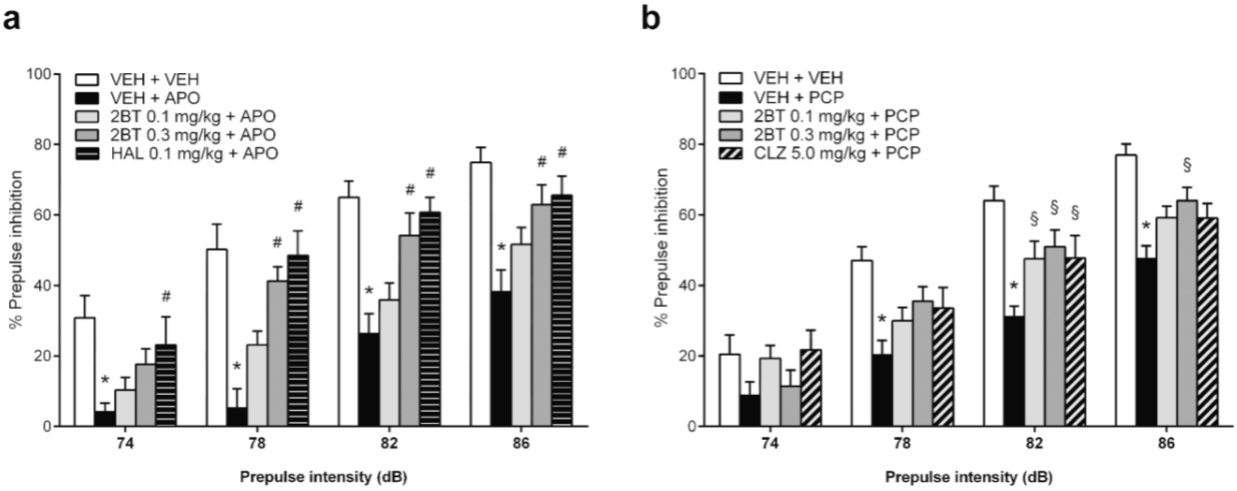

Figure 4.2 Effects of **(a)** 2-bromoterguride (0.1 and 0.3 mg/kg) and haloperidol (0.1 mg/kg) on acute apomorphine (0.5 mg/kg)-induced prepulse inhibition (PPI) deficits, and **(b)** 2-bromoterguride (0.1 and 0.3 mg/kg) and clozapine (5.0 mg/kg) on acute phencyclidine (1.5 mg/kg)-induced PPI deficits in male rats. Data are expressed as mean + SEM of $n = 9 – 10$ rats per group. *$p < 0.05$ versus controls (VEH + VEH); $^{\#}p < 0.05$ versus apomorphine (VEH + APO); $^{\S}p < 0.05$ versus phencyclidine. 2BT: 2-bromoterguride; APO: apomorphine; CLZ: clozapine; HAL: haloperidol; PCP: phencyclidine; VEH: vehicle

Table 4.1 Effects of apomorphine (0.5 mg/kg) and phencyclidine (1.5 mg/kg) in the absence or presence of 2-bromoterguride, haloperidol and clozapine on startle habituation and startle reactivity

Treatment	Mean startle reactivity		
	Startle block 1 (First ten pulse-alone trials)	Startle block 2 (Last ten pulse-alone trials)	Startle reaction (Pulse-alone trials)
VEH + VEH	4393 ± 568	#2655 ± 817	2973 ± 486
VEH + APO	7640 ± 540	#4050 ± 682	*6150 ± 621
2BT (0.1 mg/kg) + APO	8519 ± 736	#3251 ± 600	*5860 ± 657
2BT (0.3 mg/kg) + APO	5625 ± 925	#3533 ± 576	4019 ± 633
HAL (0.1 mg/kg) + APO	5196 ± 1094	#3130 ± 622	4019 ± 883
VEH + VEH	5160 ± 737	#3430 ± 532	3684 ± 573
VEH + PCP	6237 ± 732	#3284 ± 526	4425 ± 681
2BT (0.1 mg/kg) + PCP	5400 ± 795	#3387 ± 570	4211 ± 636
2BT (0.3 mg/kg) + PCP	5199 ± 443	#3635 ± 471	3715 ± 573
CLZ (5.0 mg/kg) + PCP	5392 ± 806	#2972 ± 735	4278 ± 834

Data (in mV) are expressed as mean ± SEM. #$p < 0.001$ versus startle block 1; *$p < 0.05$ versus controls (VEH + VEH). 2BT: 2-bromoterguride; APO: apomorphine; CLZ: clozapine; HAL: haloperidol; PCP: phencyclidine; VEH: vehicle, (n = 9 – 10 rats per group)

4.4.2 Novel object recognition task

2-Bromoterguride ameliorated subchronic PCP-induced cognitive impairment in the NOR task. A main effect of treatment on discrimination index was observed ($F(6,55) = 6.6$, $p < 0.001$). Subchronic PCP led to a robust decrease in object recognition memory compared to controls ($p = 0.027$). 2-Bromoterguride resulted in levels similar to the control group (0.1 mg/kg: $p = 0.44$; 0.3 mg/kg: $p < 0.001$), an effect not elicited by clozapine. 2-Bromoterguride alone had no effect on NOR performance in control animals (Figure 4.3a). Distance traveled during the retention trial was affected by treatment ($F(6,55) = 5.8$, $p < 0.001$). Subchronic PCP in combination with 0.3 mg/kg

2-bromoterguride (p = 0.001) and 2-bromoterguride alone (0.1 mg/kg: p = 0.013; 0.3 mg/kg: p < 0.001) induced a reduction in track length compared to controls (Figure 4.3b). However, 2-bromoterguride did not cause a decrease in object exploration time in the acquisition (Figure 4.3c) or retention (Figure 4.3d) trial. In the latter, ANOVA revealed significant main effects for the factor object (F(1,55) = 122.2, p < 0.001) and the interaction of the factors object and treatment (F(6,55) = 3.7, p = 0.004). Animals of all treatment groups favored the novel object over the familiar one (p < 0.001), with the exception of subchronic PCP in combination with vehicle and clozapine (Figure 4.3d).

4.4.3 Social interaction test

Treatment affected our measures of social behavior (F(6,42) = 4.4, p = 0.001). Subchronic PCP administration induced deficits in social interaction compared to controls (p = 0.003), which were ameliorated by 2-bromoterguride (0.3 mg/kg: p = 0.009) but not aripiprazole (Figure 4.4a). Further, 2-bromoterguride alone did not affect social interaction, implying that the D_2 receptor partial agonist does not negatively influence sociality in naive rats (Figure 4.4a). Drug treatment also affected the mean number of line crossings (distance traveled; F(6,42) = 7.8, p < 0.001). PCP in combination with aripiprazole and 2-bromoterguride (0.3 mg/kg), or 0.3 mg/kg 2-bromoterguride alone, reduced the number of line crossings compared to controls (p < 0.05; Figure 4.4b). The average duration investigating a novel object placed in the arena during the social interaction test was not affected by treatments (Figure 4.4c). Finally, treatment affected sniffing behavior (F(6,42) = 4.6, p = 0.001). PCP treatment significantly decreased time sniffing the unfamiliar rat compared to the control group (p = 0.011). This effect was ameliorated by 2-bromoterguride (0.3 mg/kg: p = 0.014) (Figure 4.4d). Climbing and avoiding behavior were unaffected by treatments (data not shown).

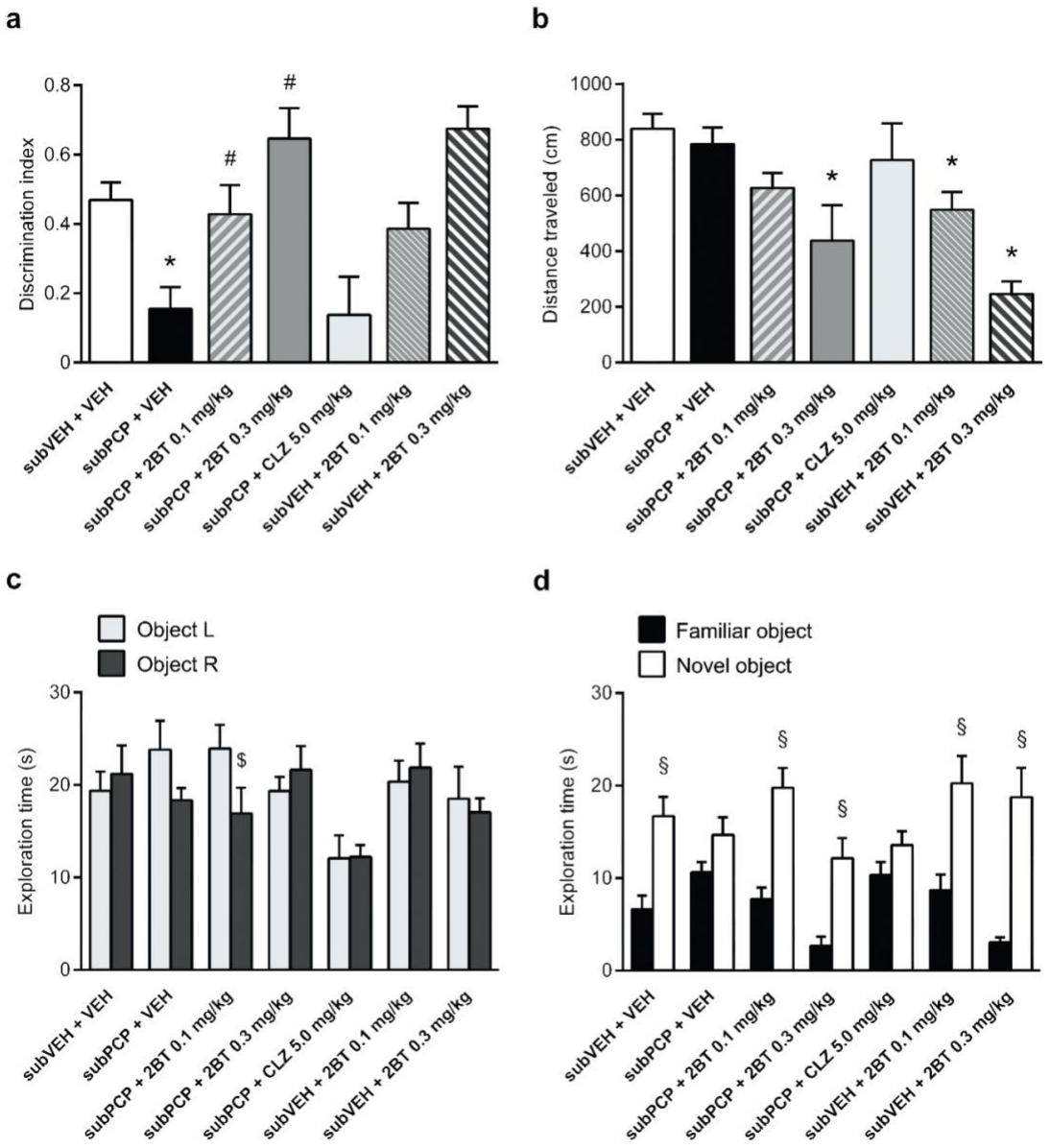

Figure 4.3 Effects of 2-bromoterguride (0.1 and 0.3 mg/kg) and clozapine (5.0 mg/kg) after subchronic phencyclidine (5.0 mg/kg) treatment, and 2-bromoterguride (0.1 and 0.3 mg/kg) alone on the **(a)** discrimination index, **(b)** distance traveled during the 3 min long retention trial, **(c)** exploration time of two identical objects (L and R) during the 3 min long acquisition trial and **(d)** exploration time of the familiar versus the novel object during the 3 min long retention trial in the novel object recognition task (NOR) in male rats. Data are expressed as mean + SEM of n = 7 – 10 rats per group. *p < 0.05 versus controls (subVEH + VEH); $^{\#}p$ < 0.05 versus phencyclidine (subPCP + VEH); $^{\$}p$ < 0.05 versus the second identical object; $^{\S}p$ < 0.05 versus familiar object. 2BT: 2-bromoterguride; CLZ: clozapine; PCP: phencyclidine; VEH: vehicle

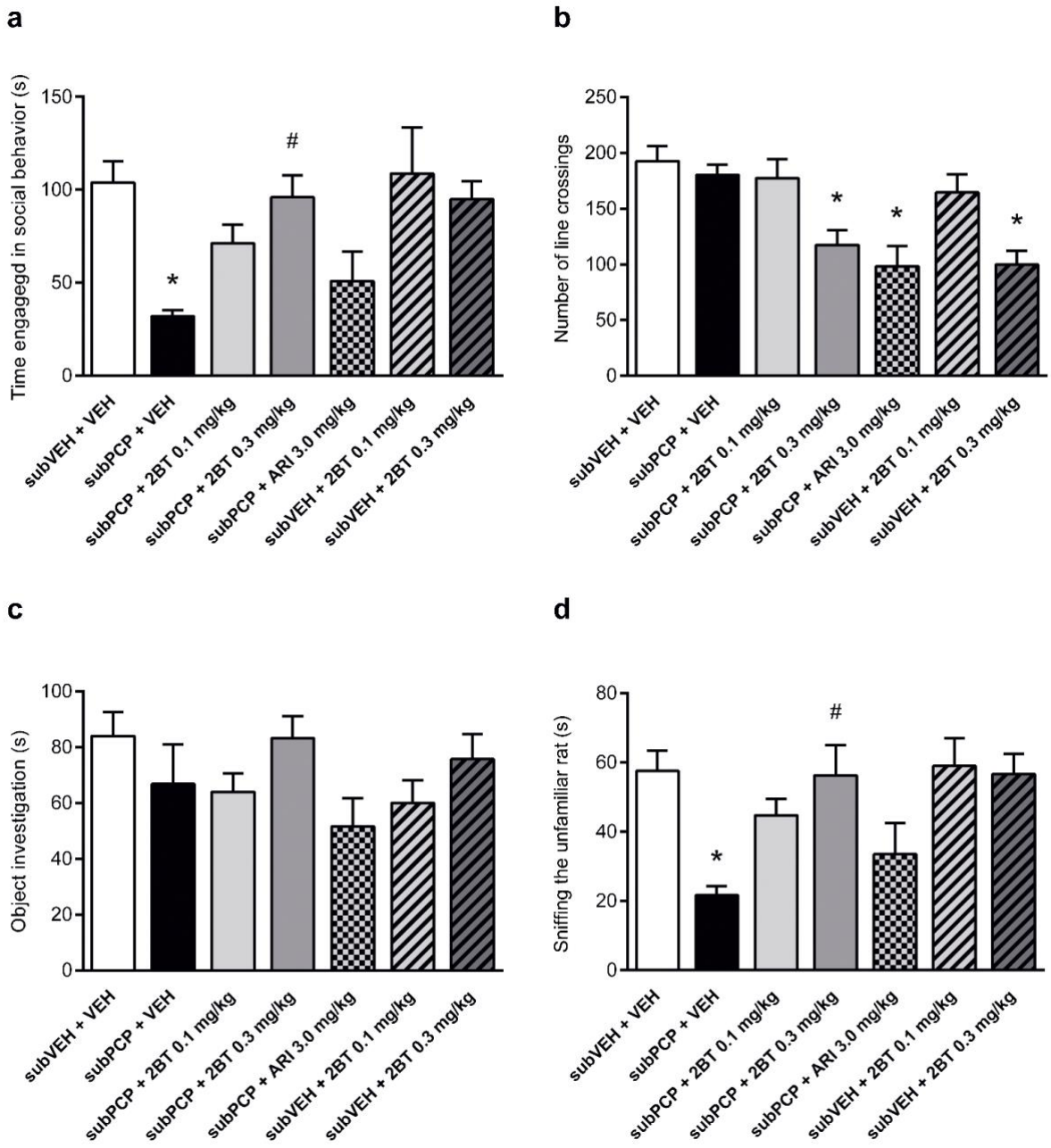

Figure 4.4 Effects of 2-bromoterguride (0.1 and 0.3 mg/kg) and aripiprazole (3.0 mg/kg) after subchronic phencyclidine (5.0 mg/kg) treatment, and 2-bromoterguride (0.1 and 0.3 mg/kg) alone on the **(a)** social interaction behavior, **(b)** total number of line crossings, **(c)** exploration time of a novel object and **(d)** investigative sniffing time towards the unfamiliar rat in the social interaction test in male rats. Data are expressed as mean + SEM of $n = 7$ pairs of unfamiliar rats per group. *$p < 0.05$ versus controls (subVEH + VEH); #$p < 0.05$ versus phencyclidine (subPCP + VEH). 2BT: 2-bromoterguride; ARI: aripiprazole; PCP: phencyclidine; VEH: vehicle

4.4.4 Prolactin

Treatment affected prolactin concentration in rat blood serum ($F(3,37) = 78.0$, $p < 0.001$). We observed an interaction between the factors treatment and time ($F(6,37) = 2.9$, $p = 0.021$). Administration of haloperidol but not 2-bromoterguride (0.1 and 0.3 mg/kg) or vehicle resulted in elevated prolactin concentrations at all three time points ($p < 0.001$) (Figure 4.5).

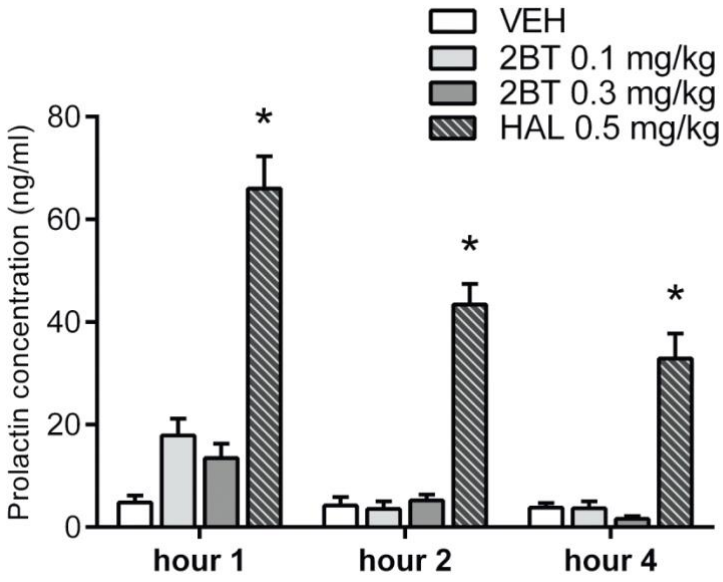

Figure 4.5 Effects of 2-bromoterguride (0.1 and 0.3 mg/kg) and haloperidol (0.5 mg/kg) on prolactin concentration, 1, 2 or 4 hours after administration, in blood serum of male rats. Data are expressed as mean + SEM of $n = 6 – 8$ rats per group. *$p < 0.05$ versus controls (VEH)

4.5 Discussion

Achieving cognitive improvement in patients with schizophrenia represents a critical challenge as cognitive impairment diminish the patient's functional outcome and ability to reintegrate into society (Green et al. 2004; Young and Geyer 2015; Vreeker et al. 2015). Deficits in attention, information processing, and ability to filter out redundant environmental stimuli have been identified in patients with schizophrenia, and PPI has been extensively investigated in patients as well as in preclinical animal models (for review, see Swerdlow et al. 2008). Based on the observation that the startle response magnitude is reduced when a startle eliciting acoustic stimulus is preceded by a weaker acoustic prepulse, the PPI paradigm in rodents represents a

preclinical test with face, predictive, and construct validity (Swerdlow et al. 1994; Geyer et al. 2001; Leumann et al. 2002). In this study, the ergoline derivate 2-bromoterguride prevented the apomorphine-induced loss of PPI to the same extent as the dopamine D_2 antagonist haloperidol, verifying its dopamine D_2 antagonistic profile and antipsychotic-like effect in rats. PPI disruptions induced by NMDA antagonists seems to be more sensitive to clozapine-like atypical APDs than to typical APDs and hence, the NMDA model of disrupted PPI can aid the identification of novel and atypical antipsychotics (Geyer et al. 2001). Interestingly, 2-bromoterguride also antagonized the PCP-induced loss of PPI to the same extent as clozapine, highlighting the atypical character of 2-bromoterguride. It should be mentioned that the PPI loss induced by NMDA receptor antagonists is mediated by systems other than the central dopamine systems (Keith et al. 1991). The highly potent α_{2C}-adrenoceptor antagonist properties of 2-bromoterguride (pA2 = 10.5; Jantschak et al. 2013) may contribute to the reversal of PCP-induced impairment of PPI. This is in line with observations using selective α_{2C}-adrenoceptor antagonists such as JP-1302 and ORM-10921 as inhibitors of PCP-induced PPI deficits (Sallinen et al. 2007, 2013).

Although the complete aetiology of the negative and cognitive symptoms of schizophrenia are not yet understood, the involvement of a dysfunctional glutamatergic system is supported by the observation that NMDA receptor antagonists effectively and reliably produce behavioral and cognitive deficits that mimic features and symptoms of schizophrenia (for reviews, see Neill et al. 2010; Gururajan et al. 2010). The NOR task offers a relative simple method to assess recognition memory as it does not require external motivation or pretraining of animals and relies on the innate explorative behavior of rodents and their preference for novel over familiar objects (Ennaceur and Delacour 1988; for review, see Grayson et al. 2015). Several studies have repeatedly found that NMDA receptor antagonists such as PCP induce memory and learning deficits in animals, and the NOR test is frequently accommodated to assess the effects of novel drugs on PCP-induced deficits (for reviews, see Jentsch and Roth 1999; Meltzer et al. 2013; Neill et al. 2010). It has been suggested that an elevated acetylcholine and dopamine tone in the prefrontal cortex due to 5-HT_{1A} and D_1 receptor activation may explain why some atypical APDs rescue NOR performance after PCP treatment (Snigdha et al. 2011; Guo et al. 2009; Nagai et al. 2009; for review, see Lyon et al. 2012). Interestingly, 2-bromoterguride shows no affinity for 5-HT_{1A} receptors in vitro (unpublished data). However, the results in this study, showing that 2-bromoterguride attenuates subchronic PCP-induced NOR deficits, indicate that this drug may have effects via D_1 receptors in addition to its high affinity for 5-HT_{2A} receptors.

Asociality, anhedonia, blunted affect, alogia, and avolition are negative core symptoms of schizophrenia, yet available treatments have only inadequate therapeutic effect (Neill et al. 2014). Negative symptoms as well as cognitive symptoms of schizophrenia impact the patient tremendously. As a consequence, efforts to find reliable research models have promoted the development of various tests for social behaviors in animals (Millan and Bales 2013). In this study, we performed a social interaction test following subchronic PCP treatment in rats to investigate the effect of 2-bromoterguride on social aversion. Our results show that 2-bromoterguride efficiently antagonized the effect of subchronic PCP and restored social behaviors. Surprisingly, in our study, aripiprazole did not ameliorate the social interaction deficits. Line crossings were affected by aripiprazole as well as by 2-bromoterguride; however, sniffing time towards the unfamiliar rat was ameliorated by 2-bromoterguride but not by aripiprazole. A plausible explanation for these differences may be that the 2-bromoterguride-treated rats remained stationary during sniffing bouts towards the unfamiliar rat. As a dopamine D_2 receptor partial agonist, 2-bromoterguride resembles aripiprazole; however, 2-bromoterguride possesses higher affinity for 5-HT_{2A} receptors than aripiprazole. In addition, 2-bromoterguride is a potent α_{2C}-adrenoceptor antagonist (see above). α_{2C}-adrenoceptor blockade has been shown to contribute to improvement of cognitive and social function in rats (Marcus et al. 2005; Wadenberg et al. 2007; Sallinen et al. 2013; Uys et al. 2016). These mechanistic properties of 2-bromoterguride may explain why this drug was effective in the social interaction test.

Finally, to extend the side effect profile of 2-bromoterguride, we examined the effect of 2-bromoterguride on prolactin secretion, as dopamine D_2 receptor blockade may result in elevated levels of secreted prolactin from the anterior pituitary gland (Freeman et al. 2000). It has been hypothesized that elevated prolactin induced by APDs is associated with a cluster of sexual and reproductive complications (for review, see Peuskens et al. 2014). Interestingly, aripiprazole, a D_2 partial agonist just like 2-bromoterguride, has the potential to improve sexual dysfunction (Hanssens et al. 2008), which is commonly associated with the negative symptoms of schizophrenia (Leucht et al. 2013). Our results presented in this study indicate that 2-bromoterguride does not cause hyperprolactinemia in the acute state of treatment and thus might be associated with less sexual dysfunction than APDs which induce hyperprolactinemia.

In conclusion, our data demonstrate that the dopamine D_2 receptor partial agonist 2-bromoterguride does not only inhibit amphetamine-induced locomotion (AIL) and conditioned avoidance response (CAR) in rats without inducing catalepsy or causing weight gain (Franke et al. 2016); it also elicits a positive impact on cognitive impairments and social aversion in rats.

2-Bromoterguride prevented the PPI disrupting effects of apomorphine and PCP similar to haloperidol and clozapine, respectively. However, 2-bromoterguride also attenuated object recognition memory deficits, in contrast to clozapine. Moreover, 2-bromoterguride ameliorated subchronic PCP-induced social interaction impairments, an effect that was not shown by aripiprazole. The mechanistic properties and antipsychotic-like effects of 2-bromoterguride previously shown (Jantschak et al. 2013; Franke et al. 2016) and the results presented herein confirm our opinion that 2-bromoterguride represents a very promising third-generation antipsychotic candidate.

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Author contributions

H.F., H.H.P., and J.B. designed the research studies; E.T. and J.B. validated and established the experimental procedures; E.T., R.T.F., and J.B. conducted experiments and acquired data; J.B. provided resources and materials; E.T., H.H.P., and J.B. analyzed data and wrote the manuscript.

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CHAPTER 5: Male rats treated with subchronic PCP show intact olfaction and enhanced interest for a social odour in the olfactory habituation/ dishabituation test

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Male rats treated with subchronic PCP show intact olfaction and enhanced interest for a social odour, in the olfactory habituation/dishabituation test

Emilia Tarland[a*] and Jan Brosda[a]

[a]*Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Freie Universität Berlin, 14195 Berlin, Germany*

**Corresponding author: Emilia Tarland, E-mail: emilia.tarland@fu-berlin.de; +491778632537*

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5.1 Abstract

The olfactory system participates in many sensory processes, and olfactory endophenotypes appear in a variety of neurological disorders such as Alzheimer´s and Parkinson´s disease, depression and schizophrenia. Social withdrawal is a core negative symptom of schizophrenia and animal models have proven to be invaluable for studying the neurobiological mechanisms and cognitive processes behind the formation of social relationships. The subchronic phencyclidine (PCP) rat model is a validated model for negative symptoms of schizophrenia, such as impaired sociability. However, the complete range of social behaviour and deficits in the model are still not fully understood. Intact rodent olfaction is essential for a wide range of social behaviour and disrupted olfactory function could have severe effects on social communication and recognition. In order to examine the olfactory ability of male rats treated with subchronic PCP, we conducted an olfactory habituation/dishabituation test including both non-social and social odours. The subchronic PCP-treated rats successfully recognized and discriminated among the odours, indicative of intact olfaction. Interestingly, the subchronic PCP-treated rats showed greater interest for a novel social odour compared to the saline-treated rats and the rationale remains to be elucidated. Our data indicate that subchronic PCP treatment does not disrupt olfactory function in male rats. By ruling out impaired olfaction as cause for the poor social interaction performance in subchronic PCP-treated rats, our data supports the use of NMDA receptor antagonists to model the negative symptoms of schizophrenia.

Highlights

- Subchronic PCP treatment did not interfere with olfactory function in male rats
- Olfactory exploratory motivation was unaltered by subchronic PCP
- Male rats treated with subchronic PCP spent more time investigating a novel social odour

Keywords

Olfactory habituation/dishabituation test; subchronic PCP; schizophrenia; animal model; sociality

5.2 Introduction

The neuronal circuitry involved in social behaviour is immensely complex and normal social interactions require a high degree of flexibility, making social behaviour especially vulnerable to disruption [1]. Animal models have proven to be invaluable tools for studying the neurobiological mechanisms and cognitive processes behind the formation of social relationships [2]. Social interaction deficits and social withdrawal are crucial early indicators for autism [3], core behavioural symptoms of schizophrenia and important readouts in animal models of negative symptoms of schizophrenia [4,5].

Rodents utilize olfactory cues for a wide range of social behaviours, including recognition of individuals and for the expression of appropriate sexual behaviours [6,7,2]. Blockade of olfactory social cues by anosmia or hyposmia would hence have major impact on rodent social behaviour by making it impossible for the animal to differentiate and recognize individual conspecifics. Therefore, to prevent data misinterpretation it is vital to verify normal olfactory function in animals used for tests with an odour component, such as social interaction, recognition and novelty preference tests [3,7,8].

The olfactory habituation/dishabituation test represents a simple yet sophisticated method for assessment of olfactory function in rodents [9]. The olfactory habituation test relies on the animals' tendency to investigate novel odours. It evaluates the animals' ability to detect, recognize, and differentiate between odours, including both non-social and social odours. It also assesses olfactory responsiveness and the capacity to habituate to the odours, as defined as a progressive decrease in olfactory investigative behaviour over sequential trials. Dishabituation on the other hand, implies that the animal can recognize a novel olfactory stimulus and show reinstated interest and investigation of the new odour [10,7].

The olfactory system participates in sensory functions, emotionality and in memory formation [11]. Hence, not surprisingly, olfactory endophenotypes appears in several neurological disorders like Alzheimer´s disease (AD), Parkinson´s disease (PD), depression and schizophrenia [12,13,14]. In many neurodegenerative disorders, particularly AD and PD, dysfunction of the olfactory system represents one of the earliest symptoms of the disorder [15,16]. In schizophrenia, patients often demonstrate reduced odour detection threshold and odour identification ability [17]. Accumulating evidence furthermore suggests that social dysfunction and the well-described smell identification deficits (SID) share a common pathophysiology. Especially patients with deficit syndrome schizophrenia (characterized by severe and enduring negative symptoms, including

disturbed social functioning; particularly reduced social drive) has been associated with more SID [18,19,20,21].

The subchronic phencyclidine (PCP) rat model represents a validated pharmacological model with relevance for the negative symptomatic in schizophrenia, especially social withdrawal [22,23,24,25,26]. Subchronic PCP treatment reliable induces social interaction deficits in rats, when they are tested in the low-anxiety-test version of the social interaction test following a drug-withdrawal phase [27,28, for review on the social interaction test, see 29].

Surprisingly, the effect of subchronic PCP on sociality is nonetheless explained in regards of which social behaviour deficit that causes the reduced interaction [30]. It has been suggested that social withdrawal [28] or lack of social approach resulting from a lack of social motivation [30] in the subchronic PCP rat, causes the reduced interaction in the dyadic social interaction test. However, these parameters are principally indistinguishable from each other in the social interaction test setup and other processes such as impaired social cognition cannot be assessed [26]. Some studies have therefore aimed to disseminate the social deficits in the subchronic PCP rat model using the sociability and preference for social novelty paradigm. However, this test can measure either social motivation or lack of social cognition, hence producing ambiguous results [31,26].

To further extend our understanding of the complex neurobiology of the negative symptoms and social impairments seen in schizophrenia, we need to first understand the animal models we use [32,33]. Explicitly ruling out the presence of basic sensory abnormalities, such as anosmia, in the subchronic PCP model is therefore of utter importance. There is however a striking lack of publications on olfactory function in PCP-treated animals. The effect of acute PCP on odour detection was investigated in 1981 by Kesner [34], who found profound disruptions after injections of 12, 16 and 24 mg/kg PCP in male rats. For subchronic PCP-treated rats however, the literature is scarce of tests explicit for olfactory function. Nonetheless, Seillier and Giuffrida [26] who used a modified version of the sociability and preference for social novelty test [3,35], did include a two-phase social odour discrimination task to control for olfactory sensory function and the ability of PCP-treated rats to discriminate between neutral and social odours. During the task each rat could investigate petri dishes containing either clean bedding, dirty bedding from the familiar home cage, dirty bedding from a cage with unfamiliar rats or no bedding at all. The result showed that both PCP and vehicle treated rats preferred the quadrants where the social odours were contained, and the authors interpreted this as a sign of intact olfactory function. However, both the PCP and vehicle rats failed to show preference for the novel smell of dirty bedding from the unfamiliar cage

and the lack of novelty preference was interpreted as a sign of deficient recognition memory, similarly to the deficit seen in PCP-treated rats during the novel object recognition task [26]. Nonetheless, the novelty preference deficit was equally evident in both the PCP- and vehicle treated groups, and this could indicate a methodological problem, such as the bedding not being soiled enough to enable the rats to detect the novel odour or due to a high degree of overlapping elements present in the stimuli, such as food or the bedding material itself.

Furthermore, other studies implicitly investigated olfactory function after subchronic PCP treatment as they used odours in the attentional set shifting task and found no impairments in intradimensional shifts between odours, indicating intact olfactory function [36,37,38,and 39]. Sahin et al. [32] used two newly developed preclinical tests for the often-neglected domain "blunted effect", to assess anticipatory motivation and affective state. In the optimistic- and affective-bias tests, female rats treated with subchronic PCP, were trained to associate cues and odours with high-valued and less-valued rewards. The results showed that subchronic PCP-treated rats performed fewer optimistic choices during the optimistic-bias test and that PCP-treatment diminished the ability of the rats to form a preference for any of the reward-containing bowls during the affective-bias test, implicating a lack of a positive affective state [32].

In both the above-mentioned tasks, an association between an odour and a food reward was formed; meanwhile Audet et al. [40] used a cat odour to investigate the emotional response and anxiety when rats treated with subchronic PCP were exposed to the smell of a predatory threat. The result showed that the PCP-treated rats sniffed the cat collar significantly less than the vehicle treated rats, what the authors concluded an effect of heightened anxiety in the PCP-treated rats as these also spent more time inside the dark compartment during the emergence test for anxiety [40].

To our knowledge, there is no previous study investigating the effect of subchronic PCP on the performance of rats in the olfactory habituation/dishabituation test. We recently showed that subchronic PCP treatment significantly reduced the time spent in social interaction with an unfamiliar conspecific rat. The previously performed social interaction test was part of a recent drug-treatment study in our lab [41] in which the rats tested for olfactory function here, served as the positive (subchronic PCP-treated) and negative (subchronic saline-treated) controls.

With the aim to further disseminate the subchronic PCP rat model and validate the absence of anosmia or hyposmia, we performed the olfactory habituation/dishabituation experiment presented here, three days after completion of the social interaction test.

The results from the olfactory habituation/dishabituation test have important implications for several previously performed, and future studies on the social behaviour in the subchronic PCP rat model. Finally, these data are important to verify that the social interaction deficit in subchronic PCP-treated rats is not merely a consequence from deficient olfactory function, but truly mimic the complex negative symptoms of schizophrenia.

5.3 Experimental procedures

5.3.1 Animals

The olfactory habituation/dishabituation experiment was carried out with 16 male Sprague-Dawley rats (Élevage Janvier, Le Genest Isle, France) with mean weight 450 g at 10 weeks of age. Groups of three to four rats were housed together in standard type open-top IV polycarbonate cages (Ehret, Emmendingen, Germany) under standard laboratory conditions (room temperature 22 ± 2°C; relative humidity 55 ± 10%). The cages were enriched with metal tubes and paper tissues. Water and food (standard laboratory chow; Ssniff, Soest, Germany) were available ad libitum. The lightning followed a 12 h light-dark schedule (light on at 6 a.m.) and all experiments were conducted during the first half of the light phase (between 9 a.m. and 2 p.m.). The animals were permitted one week of habituation to the premises before being gently handled by the person performing the experiments during the second week (5 min/day). The Berlin State Authority ("Landesamt für Gesundheit und Soziales") approved all experimental procedures and they were performed in compliance with the German Animal Protection Law and the EU Directive 2010/63/EU for animal experiments.

5.3.2 Drugs and treatment regime

We recently established the subchronic PCP model in our laboratory in order to study the effect of a novel antipsychotic drug on PCP-induced social interaction and object recognition deficits [previously published data see, 41]. Therefore, we conducted pilot experiments and studied the effect of different doses subchronic PCP (2.0 or 5.0 mg/kg) with different washout period lengths (1, 2 or 6 weeks) on behavioural test performance. In our laboratory setting and for both above-mentioned test paradigms, the most robust deficits were detected after the rats were treated with 5.0 mg/kg PCP twice a day over 7 days with a subsequent 2-week drug washout period before behavioural testing begun. Based on the results from the pilot experiments, prior to

the social interaction study the rats were randomly assigned to either the vehicle (0.9% saline) or PCP (5.0 mg/kg) group. Phencyclidine hydrochloride (Sigma-Aldrich, Steinheim, Germany) was dissolved in 0.9% saline and each rat received two injections of the assigned treatment per day (PCP or saline; i.p. injection given at 8 a.m. and 5 p.m.) for a period of 7 days, followed by a 14-day washout period.

5.3.3 Social interaction test

Three days prior to the olfactory habituation/dishabituation test, the rats which represented the two control groups (subchronic PCP + vehicle and subchronic saline + vehicle), were tested in a social interaction test included in a larger drug-treatment study in our laboratory [41]. Due to the partially presented and previously published social interaction procedure, the method is only mentioned here briefly but can be fully accessed elsewhere [41]. However, in short, each rat was placed in a familiarized arena together with an unfamiliar male conspecific and allowed to interact freely during a 10 min session. Because the original social interaction test was part of a pharmacological treatment investigation, each subject rat was injected with saline (i.p.) 30 min prior to the social interaction test. The social behaviours “sniffing, climbing, following, and avoiding” were scored by an experimenter blinded for treatments and “total social interaction” was calculated from the cumulative duration engaged in social behaviours. In addition, the duration exploring an unfamiliar object placed in the arena (a 33cl aluminium soda can) and the number of line crossings was scored.

5.3.4 Olfactory habituation/dishabituation test

The olfactory habituation/dishabituation test was performed following Yang and Crawley [10], with some minor methodological changes to the protocol. Orange and almond baking aroma (OSNA Nährmittel GmbH, Osnabrück, Germany), were prepared freshly and diluted 1:100 with distilled water on the test day. For each trial, a fresh q-tip was dipped and kept 2 s in the diluted aroma sample. The social odour was prepared immediately before each trial by swiping a q-tip in the dirty cage bedding from unfamiliar male conspecifics. In order to avoid contamination of the cage lid between trials and to minimize the spreading of the aroma in the cage, the tip of a 15 ml transparent centrifuge tube (Carl Roth GmbH, Karlsruhe, Germany) was cut open, making a hole through which the q-tip was inserted. By removing the tube plug and placing the tube with the opening downwards on the cage lid, a simple “nose poke” hole was formed in which the rats could

sense the volatile odour, but not gnaw on, lick or retract the applicator and contamination of the top cage lid was minimized. Each rat was placed in a standard type II polycarbonate cage (Ehret, Emmendingen, Germany) in which the cage lid food bin had been covered by a layer aluminium foil leaving only a small hole where the tube end was fitted (Figure 5.1). The test began with a 5 min habituation period in which a tube with a sterile q-tip was placed in the cage lid. The test session began with three consecutive trials of presenting an odour neutral stimulus (distilled water) for 2 min per trial, followed by each three 2 min long consecutive trials of the non-social odours orange and almond presented in a counterbalanced order. Each trial was separated by a 1-minute intertrial interval in which the tube and q-tip was removed from the cage lid and the next trial/odour was prepared. The test ended with three consecutive trials of the social odour. Each test session was video recorded from a horizontal angle, which allowed a blinded experimenter to measure the duration in which the rat sniffed on the odour containing tube from less than < 2 cm distance.

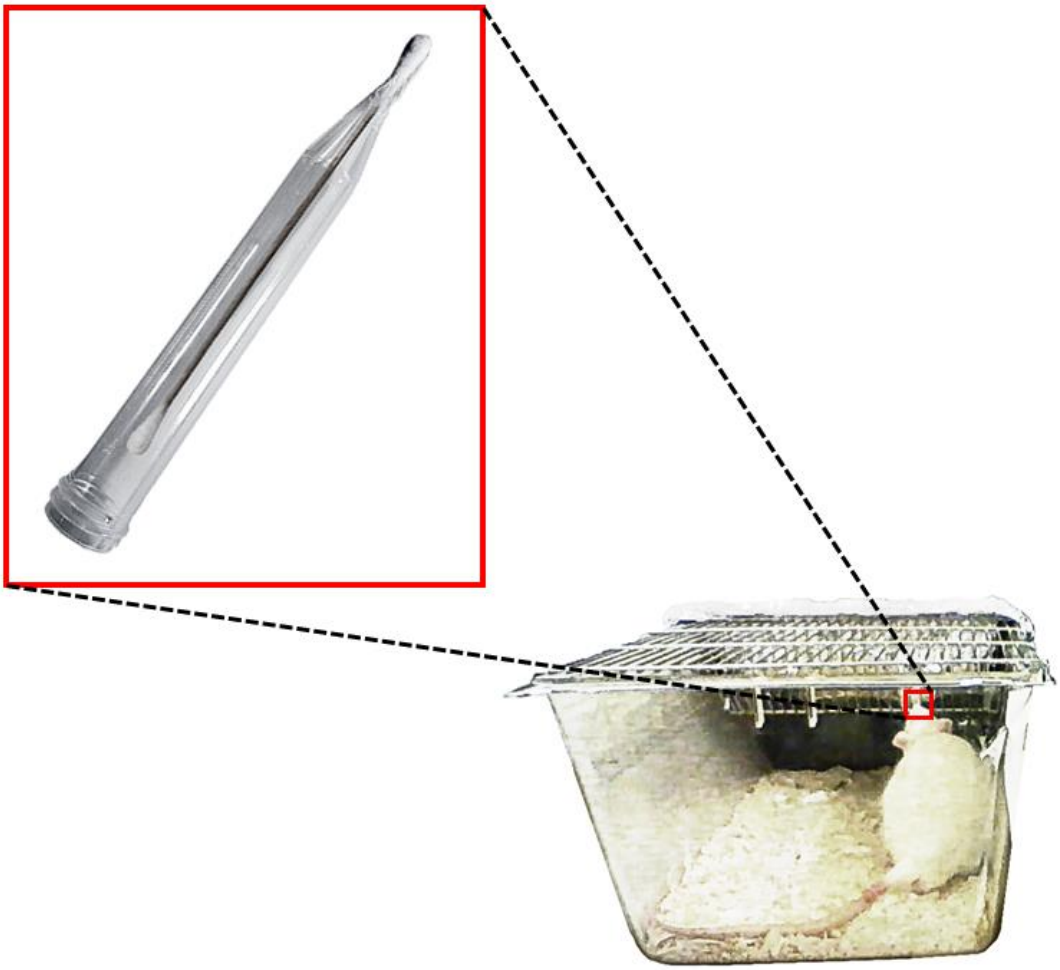

Figure 5.1 Experimental setup for the olfactory habituation/dishabituation test

5.3.5 Statistical analysis

The data were analyzed with SigmaPlot 11 (Systat Software, Erkrath, Germany). The social interaction data was reanalysed for the two control groups only, using unpaired sample t-tests with homogeneity of variance to compare the mean duration for each behaviour respectively [for the original analysis see, 41]. One-way or two-way repeated measures ANOVA were utilized to determine odour habituation and dishabituation during the olfactory habituation/dishabituation test. Post-hoc Holm-Sidak multiple pairwise comparison analysis was performed when appropriate. Student's t-test with homogeneity of variance was used to analyse the total exploration time for each odour. *P* values < 0.05 were considered significant and data are presented as mean ± standard error of the mean (SEM).

5.4 Results

5.4.1 Social interaction test

The subchronic PCP-treated rats engaged in significantly less social interaction behaviour compared to the subchronic vehicle-treated rats (Figure 5.2). The PCP treatment induced robust deficits in all the scored social behaviours in comparison to the vehicle group [total social interaction duration $t(12) = 6.05$, $p < 0.0001$; sniffing duration $t(12) = 5.6$, $p = 0.0001$; climbing duration $t(12) = 3.06$, $p < 0.01$; following duration $t(12) = 2.76$, $p = 0.02$; avoiding duration $t(12) = 2.52$, $p = 0.03$]. There was no detected effect of PCP treatment on the duration investigating an unfamiliar object placed in the arena during the social interaction test or on general locomotion assessed by the number of line crossings.

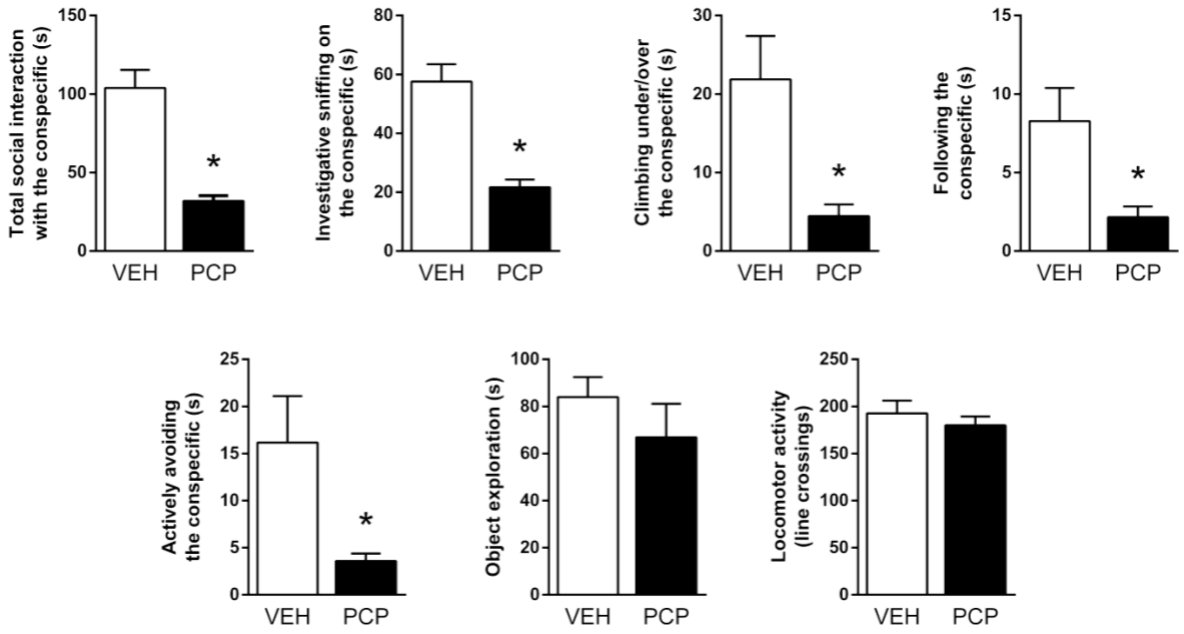

Figure 5.2 Effects of subchronic phencyclidine (5.0 mg/kg) or saline on social and non-social behaviours during the dyadic social interaction test. Data are expressed as mean ± SEM of $n = 7$ pairs of rats per group. *$p < 0.05$ versus the subchronic saline group. PCP: phencyclidine; VEH: vehicle

5.4.2 Olfactory habituation/dishabituation test

General olfactory ability was assessed by studying the odour recognition- and discrimination ability of rats treated with subchronic PCP in comparison to rats treated with subchronic saline (vehicle group). Both PCP and saline treated animals habituated to each of the tested odours as indicated by the decrease in exploration time over the consecutive presentations of the same odour (Figure 5.3; upper section). One-way repeated measures ANOVA revealed a progressive reduction in exploration time over the three consecutive trials, for each of the presented odours in both treatment groups. The PCP-treated rats showed significant olfactory habituation [almond $F(2,14) = 16.9$, $p < 0.001$, post-hoc trial 1 to trial 3: $p < 0.001$; orange $F(2,14) = 41.6$, $p < 0.001$, post-hoc trial 1 to trial 3: $p < 0.001$; social $F(2,14) = 6.8$, $p = 0.009$, post-hoc trial 1 to trial 3: $p = 0.003$], similar to the habituation of saline treated rats [almond $F(2,14) = 4$, $p = 0.042$, post-hoc trial 1 to trial 3: $p = 0.018$; orange $F(2,14) = 29.4$, $p < 0.001$ post-hoc trial 1 to trial 3: $p < 0.001$; social $F(2,14) = 6.2$, $p = 0.012$ post-hoc trial 1 to trial 3: $p = 0.004$]. To assess the effect of treatment on the habituation pattern for each odour respectively, olfactory habituation data was analyzed with two-way repeated measures ANOVA. There was a significant effect of the PCP treatment only in

the case of the social odour [treatment: $F(1,28) = 7.2$, $p = 0.018$; trial: $F_(2,28) = 12.9$, $p < 0.001$, post-hoc trial 1 to trial 3: $p < 0.001$] with no significant interaction between the factors treatment and trial. For the other odours there was no effect of PCP treatment on habituation and no interaction of the factors treatment x trial. To further elucidate the effect of PCP on olfactory exploratory motivation, the total exploration time per odour were calculated for each animal respectively and analyzed with Student's t-tests (Figure 5.3; bottom section). Interestingly, the only significant difference between the treatment groups was detected for the social odour. PCP-treated rats spent significantly more time investigating the social odour than rats treated with saline [$p = 0.018$].

Olfactory dishabituation (detection of olfactory novelty) was evaluated by comparing the exploration of the third consecutive presentation of one odour to the time in the first trial presenting a new odour. Due to the randomization of the order of the first presented non-social odour, dishabituation data is presented separately (Figure 5.4). One-way repeated measures ANOVA revealed that PCP-treated rats effectively detected olfactory novelty, as implicated by the significant increase in exploration time towards the new odour [water to odour1 $F(1,7) = 52.5$, $p < 0.001$; odour1 to odour2 $F(1,7) = 37.4$, $p < 0.001$; odour2 to social $F(1,7) = 30.2$, $p < 0.001$]. The saline treated rats discriminated the novel odours in a similar manner [water to odour 1 $F(1,7) = 26.2$, $p < 0.001$; odour1 to odour2 $F(1,7) = 17.2$, $p = 0.004$; except at the shift from odour2 to social where the homogeneity of variance test failed and a Friedman repeated measures ANOVA on ranks could not detect a significant difference, $p = 0.07$]. Last, to evaluate the effect of PCP treatment on each odour shift we analyzed the dishabituation data by two-way repeated measure ANOVA. There was as significant effect of trial [$F(5,70) = 27.4$, $p < 0.001$] but no effect of treatment and no significant interaction of the factors treatment and odour shift. Post-hoc Holm-Sidak multiple comparisons showed that both groups significantly dishabituated to each of the odour shifts [PCP: water to odour1: $p < 0.001$; odour1 to odour2: $p < 0.001$; odour2 to social: $p < 0.001$ and saline: water to odour1: $p = 0.003$; odour1 to odour2: $p < 0.001$; odour2 to social: $p = 0.003$]. Taken together, the results in the olfactory habituation/dishabituation test verifies that subchronic PCP treatment did not cause anosmia nor deficits in olfactory recognition and discrimination.

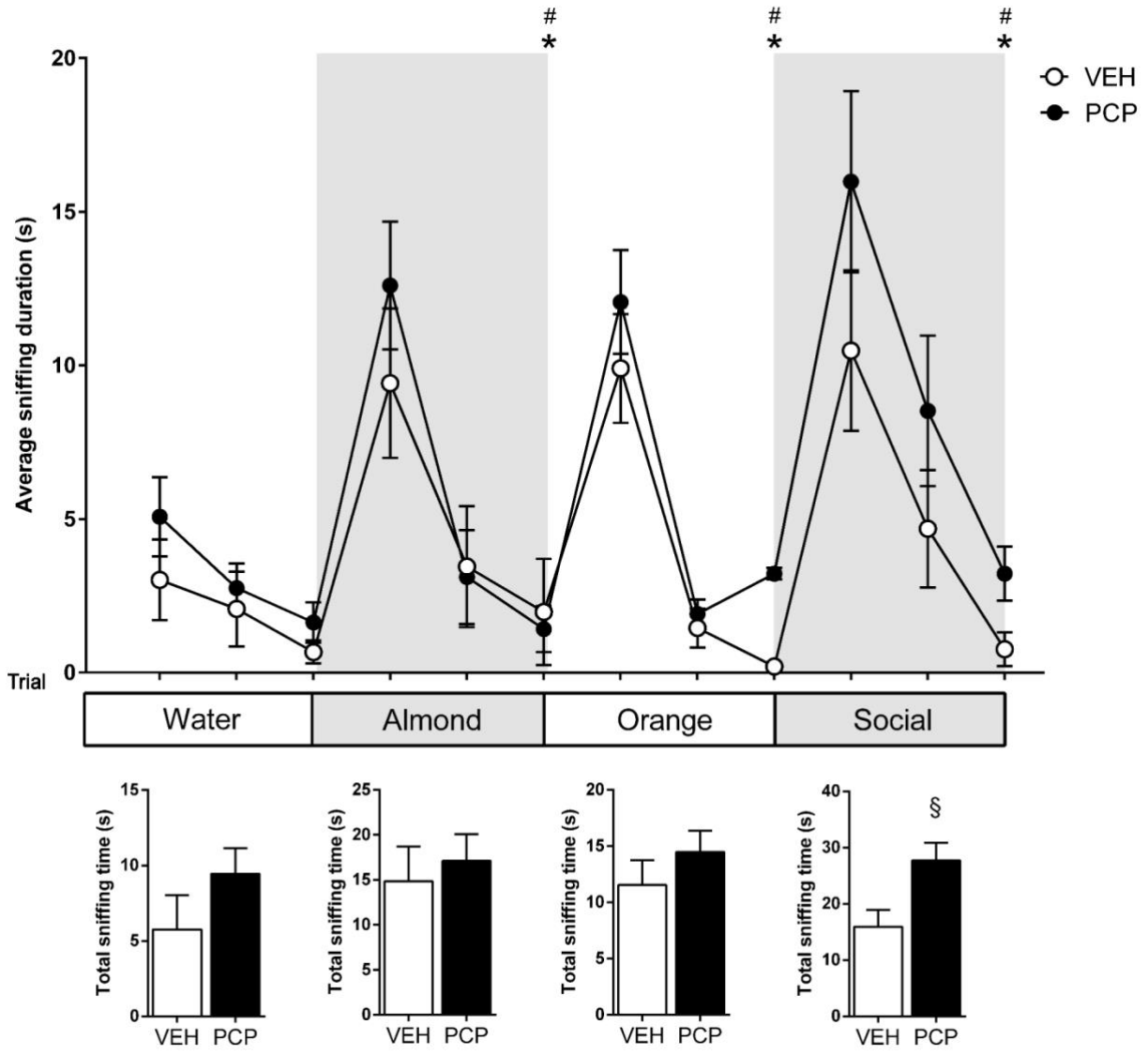

Figure 5.3 Effects of subchronic phencyclidine (5.0 mg/kg) or vehicle on olfactory function and odour discrimination tested in the olfactory habituation/dishabituation test. ***The upper section*** shows the habituation over three consecutive presentations per odour. Color change indicates an odour change. *p < 0.05 trial 1 versus trial 3 for each odour in the PCP group, $^{\#}p$ < 0.05 trial 1 versus trial 3 for each odour in the vehicle group. ***The lower section*** shows the total exploration time per olfactory cue. Data are expressed as mean ± SEM of n = 8 rats per group. $^{\S}p$ < 0.05 versus vehicle; PCP: phencyclidine; VEH: vehicle

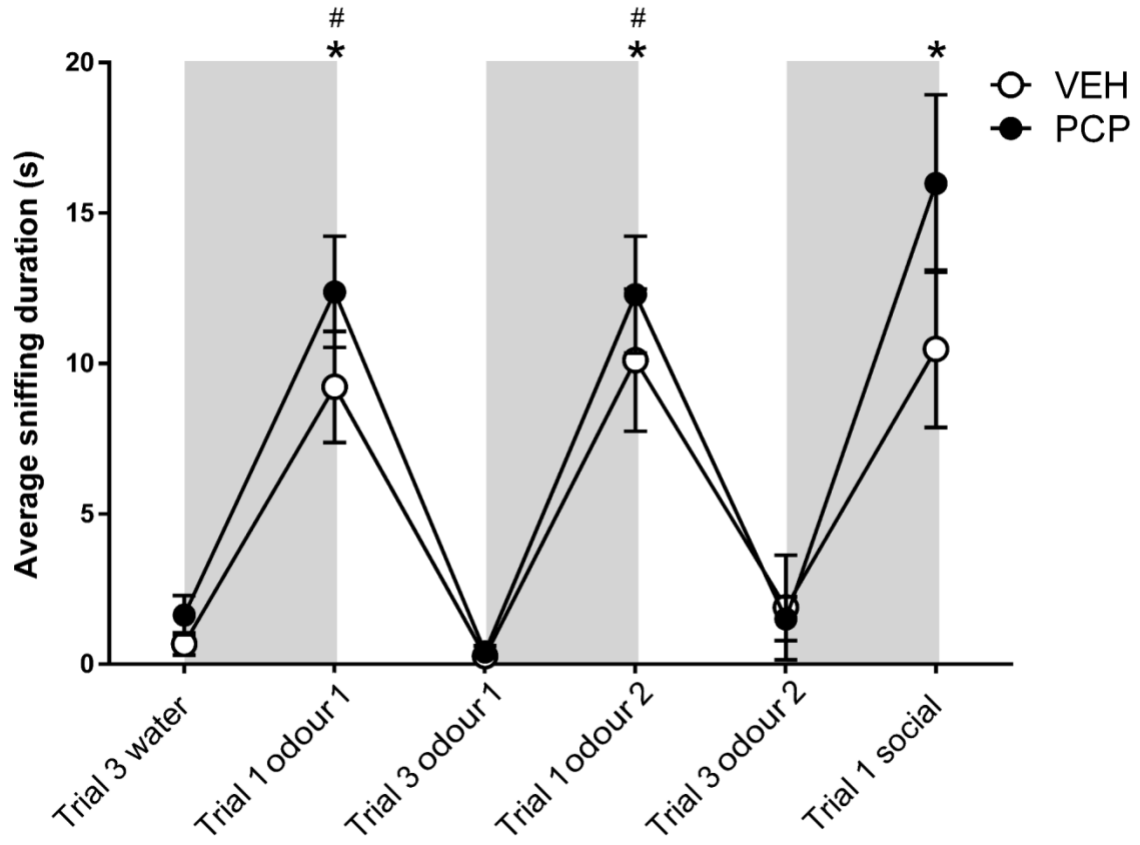

Figure 5.4 Effects of subchronic phencyclidine (5.0 mg/kg) or vehicle treatment on the ability to detect olfactory novelty (dishabituation). Color change indicates an odour change. Data are expressed as mean ± SEM of n = 8 rats per group. *$p < 0.05$ versus the last trial of the previously presented odour for PCP, $^{\#}p < 0.05$ versus the last trial of the previously presented odour for vehicle. PCP: phencyclidine; VEH: vehicle

5.5 Discussion

In the present study we demonstrated that groups of male rats treated with subchronic PCP did not differ in their habituation and dishabituation to non-social odours compared to controls. The subchronic PCP-treated rats did however, spend more time investigating a social odour.

Furthermore, another intriguing finding in this study was the enhanced investigatory behaviour of the PCP-treated rats towards the social odour, considering that the same rats showed significantly reduced social interaction behaviour when tested in the social interaction test three days earlier [Figure 5.2].

A possible rationale for the contrasting results in social interaction behaviour and investigative behaviour of social odours presented herein, might be the distinct conceptual differences of the two experimental setups. It has been suggested that in social environments, the uncertainty of the situation influences decision-making between multiple of rewards such as choices to interact with another animal, food or objects [42,43]. Consequently, the experimental setup has impact on the motivational state of the animal and the behavioural outcome in tasks investigating sociability. For example, the dyadic social interaction test where two unfamiliar animals are placed together and allowed to interact freely, represents an uncertain social setup due to the risk of physical threat. Meanwhile, the three-partite-chamber test for sociability and social novelty preference represents a "socially safe" environment where it is more likely that an animal rely on its own motivational state for social interaction [43]. During the olfactory habituation/dishabituation test, the animal had no visual or tactile contact with the unfamiliar animal, and the social odour is hence presented in a "socially safe" environment. This might explain why the PCP-treated animals showed enhanced interest for a novel social odour using this setup, meanwhile they failed to elicit normal social interest for an unfamiliar rat during the social interaction test [41]. In agreement with the hypothesis that the social motivation of PCP-treated rats reflects the uncertainty in the experimental condition, Seillier and Giuffrida [26] used a modified version of Crawley´s three-partite-chamber test for sociability and preference for social novelty to study the social approach tendency by PCP-treated rats. Since there is no physical contact between the subject rat and the stimuli rats during the test, the authors aimed to investigate sociability, preference for social novelty and social motivation in a "socially safe" environment [26]. In contrast to the numerous studies that reported disrupted social behaviour in PCP-treated rats in the "socially uncertain" dyadic social interaction test [23,44,45,46] Seillier and Giuffrida [26] found no deficits in sociability in the three-partite chamber test. The PCP-treated rats preferred the social box (in which an unfamiliar rat was enclosed in a wire mesh cage) to the box holding an empty wire mesh cage. However, when allowed to choose between a familiar and a novel rat in the three-partite chamber arena, the PCP-treated rats showed a selective social novelty preference deficit, a deficit that was independent of olfactory function since the ability to discriminate social over neutral odours was intact in the animals. In the odour discrimination task, the PCP-treated animals showed a preference for a social odour over a neutral non-social odour, but neither the PCP-treated group nor the vehicle group favoured a novel social odour (bedding from a cage with unfamiliar animals) over an "familiar" social odour (bedding from the home cage). Seillier and Giuffrida [26] suggests that the social motivation in subchronic PCP-treated rats might be intact in a "socially safe" condition and it remains to be elucidated whether the lack of novelty

preference in the three-partite chamber test represents a true shortage of social novelty preference or a consequence of impaired social recognition.

A reduction of the time spent engaged in social interaction behaviour with an unfamiliar animal during the low-anxiety dyadic social interaction test, could also be an implication of higher general anxiety in rats treated with subchronic-PCP. In the experiments performed by Audet et al. [40], the lesser investigation of the PCP-treated rats towards the smell of a predatory treat (a cat collar) was interpreted as an indicator of increased anxiety. The same rats additionally spent more time in a dark compartment over a brightly lit one, indicative of higher anxiety [40]. Other studies did however, controversially not find any differences between subchronic PCP-treated and vehicle-treated rats in the dark/light-emergence test or in classical behavioural paradigms for anxiety such as the elevated plus maze and the open field test [47,48]. Data from our laboratory (not shown), further agree with these results as our subchronic PCP-treated rats did not differ from the saline-treated rats when the number of visits- or the duration spent in the centre part of the arena on the first day of habituation was compared, indicating no differences in environmental novelty-induced anxiety between the two groups. Taking these results together, it seems unlikely that the diminished social interaction following subchronic PCP withdrawal is explained by increased anxiety-like behaviour.

The subchronic PCP-treated rats in our study showed intact habituation to the social odour over the three trials. However, habituation and dishabituation are basic non-associative learning processes [7]. The preserved non-associative memory seen in the subchronic PCP-treated rats during the olfactory habituation/dishabituation test hence, does not exclude that they could have impairments in associative/declarative memory. As such, the increased sniffing towards the social stimulus could also be indicative of disrupted social cognition in the PCP-treated animals.

The visual recognition memory disrupting effect of subchronic PCP treatment is well validated and has important implications for the use of NMDA receptor antagonist rodent models to identify novel drugs especially targeting the negative- and cognitive symptoms of schizophrenia [33]. The subchronic treatment regime described in this report, which induced sever social impairments but no olfactory dysfunctions in male rats, did also induce significant disruptions to object recognition memory in our laboratory [41].

Notably, in our study we did not include a second novel social odour in the protocol as we mainly focused on the capacity to detect and discriminate among odours overall. It would hence be very interesting to conduct the olfactory habituation/dishabituation test with two or more social odours to investigate if subchronic PCP-treated rats can differentiate between novel social odours.

However, a lack of dishabituation to a novel social odour in this test could arise for different reasons and the olfactory habituation/dishabituation test does not allow discriminating between a social cognition deficit and a preference for social novelty deficit. For this reason, it is strongly advised that future studies with the aim to further investigate the social impairments in the subchronic PCP-treated rodent model, uses more than one social test to differentiate lack of social novelty response from social cognition dysfunction.

The development of ethologically relevant social test paradigms that allow such detailed dissemination between social cognition and novelty preference as well as social behaviours in situations with conflicting motivations, might help to answer some of the questions regarding the overall social impairment in the subchronic PCP model.

In addition, the variety of used PCP treatment regimen and test protocols used to assess behaviour in the PCP model has created another need for more validation. For example, the length of the drug-withdrawal period prior to behavioural testing seems especially important for the emergence of social- and cognitive deficits and for the induction of NMDA receptor hypofunction in animals resembling the hypofunction and negative symptoms in schizophrenia [49]. Our established PCP treatment protocol with 14 days of withdrawal prior to behavioural testing induced robust deficits in both recognition memory and social interaction behaviour [41]. In agreement with these results, the observation that subchronic PCP induces neurological and behavioural alterations especially after longer withdrawal periods, have been supported by multiple lines of evidence. For example, evident and widespread reductions in NMDA receptor binding, especially in the hippocampus, was found following a 14-day PCP withdrawal period but not after shorter delays of 1 or 24 hours [50]. Snigdha & Neill [45, 46] used a 6-week washout phase following subchronic PCP-treatment to induce social interaction deficits [for a recent review on the NMDA receptor antagonist model, see 33] and additionally, Jenkins et al. [51] found social deficits 24 hours, 1 week, 3 weeks and 6 weeks after PCP treatment. Especially noteworthy, in this study they found reduced expression of parvalbumin immune-reactive neurons in the hippocampus after the 6 weeks withdrawal phase, providing further support for the theory of long-lasting neurological and behavioural changes produced by subchronic PCP.

In line with several recent studies disseminating the social behaviour in rats treated with subchronic PCP [31,30,26,32], our results demonstrate the social behaviour complexity in the subchronic PCP rat, and the need for subsequent elucidation. The development of new tests or fine-tuning already established methods, to give more straightforward measures of social

behaviour, would be of great value for our understanding of the outcome of subchronic PCP treatment on sociability and its translation for the negative symptomatic of schizophrenia.

In conclusion, the present results from the olfactory habituation/dishabituation test rule out basic impairments in olfaction following subchronic PCP treatment in male rats. It is hence unlikely that a sensory deficit explains the poor performance on social interaction tasks in the current, and previously performed PCP research. These data therefore provide further support for the usefulness of the subchronic PCP animal model to study mechanisms of social and cognitive impairments with relevance to negative symptoms of schizophrenia.

Acknowledgments

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Conflicts of Interest

E.T. and J.B. declare no biomedical financial interests or conflicts of interests.

Author contributions

E.T and J.B. designed the research study; E.T. and J.B. validated and established the experimental procedures; E.T. conducted experiments and acquired data; J.B. provided resources and materials; E.T. analyzed data and E.T. and J.B. wrote the manuscript.

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CHAPTER 6: General discussion and conclusions

The negative symptoms of schizophrenia are concerned with the loss or reduction of certain normal functions or behaviours and align in the subdomains *diminished expression* (including poverty of speech and affective flattening) and *amotivation* (including apathy, anhedonia and asociality) (Foussias et al., 2015). The symptoms are directly associated with poorer functioning and clinical outcomes for patients with first episode psychosis (Austin et al., 2013). Being the target of rapidly growing interest over the last decades, the negative symptoms of schizophrenia are now considered central features and core symptoms of the disorder (Remington et al., 2016; Krause et al., 2018). Since we now acknowledge the substantial impact of negative symptoms on functional recovery, which cannot be directly translated to the resolution of mainly positive symptoms, the view on patient recovery has evolved from a strict clinical view (mainly the recovery from positive symptoms) to a functional view (Austin et al., 2013; Foussias et al., 2015; Remington et al., 2016). Interestingly, Austin et al. (2013) found that in addition to the baseline negative symptoms, also the resisting negative symptoms at one year follow up could predict recovery in patients with schizophrenia. Therefore, from a treatment perspective, focusing on interventions for negative symptoms could assist in the recovery process and the maintenance of long-term functioning. Sadly, despite the advances in our understanding of the impact of the negative symptoms for recovery, there is still a tremendous lack of effective treatments for the negative symptoms and cognitive impairments in schizophrenia (see, review in **Chapter 2**).

Disrupted social cognition is another character of schizophrenia which correlates closely with poor functional outcome. The disruptions in social cognition, i.e. the inability to monitor and interpret social signals, emotions, intentions and states of minds of others and to respond with the appropriate social behaviour, are related to the cognitive impairments and negative and positive symptoms of schizophrenia, yet there is a lack of preclinical experimental procedures as well as effective clinical interventions (Millan & Bales, 2013). Albeit there have been significant improvements to diagnosing and measuring negative symptoms in schizophrenia patients since the 1980s, further advances and development of objective paradigms for both humans and laboratory animals are required. For many negative symptoms, which can be assessed readily in humans, straightforward test paradigms in animals are still lacking. For example, facial emotion expressions can be analysed with *video-based automated analysis of affective expressiveness* (FACS/FACES) or *electromyography-based assessment of affective expressiveness* in schizophrenia patients but there are no objective animal paradigms. The same is true for expressive gestures and vocal inflections which can be automatically analysed from video

recordings or assessed with acoustic analyses but naturally, due to the lack of language in animals, cannot be equivalently studied in laboratory animals (Foussias et al., 2014). However, behaviour assays for social communication such as olfactory or tactile communication as well as ultrasonic vocalization, have been developed and used in translational studies of psychiatric disorders such as schizophrenia and autism (for review, see Silverman et al., 2011).

In the meantime, methods investigating animal motivation, for example by assessing the preference for a sucrose solution over normal drinking water, have yielded important insights in the neurobiology of hedonic capacity (Foussias et al., 2014), although a reduction of the consumption of a pleasurable drink or food stimulus may also reflect reward-unrelated factors such as low overall motor function or ingestion (Neill et al., 2014). Other animal paradigms are technically complicated and time consuming (for example reward- and prediction learning and rodent decision making- or gambling tasks) or known to produce ambiguous results (Gilmour et al., 2013). Social interaction tasks on the other hand, represent another extremity as social motivation and withdrawal can be measured in laboratory animals but no objective analogue human paradigms exist. It is hence possible that the lack of success in clinical drug development programs and trials for negative symptoms of schizophrenia reflect the constraints in the assessment of the latter, and not mainly lack of therapeutic effect (Foussias et al., 2014).

As a natural consequence of the positive symptoms and thought disorder being to our knowledge uniquely human, the animal models in schizophrenia often have translational validity but less face validity. However, in some cases the models share both similar pathophysiology and behaviour, as in the case of NMDA receptor antagonist-induced animal models, which show deficits in social interaction and learning and memory, similar to symptoms in schizophrenia patients (Moghaddam & Jackson, 2003; Bubenikova-Valesova et al., 2008; Cadinu et al., 2017). Fact is, animal models in schizophrenia have been and still are of immense value for our deeper understanding of the pathophysiology of schizophrenia and we need thoroughly validated and robust models to continue the search for novel therapeutic agents (Neill et al., 2010; 2014; Cadinu et al., 2017). The last part of this thesis (**Chapter 5**) was therefore dedicated to the validation of the subchronic PCP animal model for negative symptoms and cognitive deficits, especially to shed light over the social impairments induced by the PCP treatment. These results will hence be discussed in detail at the end of the main discussion.

For this thesis main part in **Chapter 4** however, the mechanisms and character of the potential novel antipsychotic drug 2-bromoterguride was investigated in animal models of negative symptoms, cognitive defects as well as on prolactin secretion. 2-Bromoterguride is the

2-halogenated derivative of terguride (Jantschak et al., 2013), a substance which was effective in treatment of negative- but not positive schizophrenic symptoms in clinical trials and therefore cancelled from further development for the use in schizophrenia (Olbrich & Schanz, 1991). Terguride has potent antagonist properties at the α_{2C}-adrenoceptor and 5-HT_{2A} receptor, which could possibly explain its effectiveness in treating the negative symptoms of schizophrenia (Jantschak et al., 2013). Similarly, 2-bromoterguride is a potent antagonist at α_{2C}-adrenoceptors and 5-HT_{2A} receptors, yet in addition, 2-bromoterguride has less than half of the intrinsic activity at D_{2Short} receptors and potent antagonist activity at D_{2Long} receptors, more like the properties of aripiprazole (Jantschak et al., 2013).

Due to previous studies which showed the in vivo effects of 2-bromoterguride on central 5-HT_{2A} receptors (via wet dog shake behaviour), antidopaminergic efficiacy (via amphetamine-induced locomotion), antipsychotic activity (Fos protein expression) and antipsychotic-like effect (CAR test) in rats without inducing metabolic changes (Jantschak et al., 2013; Franke et al., 2016), this thesis was dedicated to examining the effect of 2-bromoterguride on negative symptoms and cognitive deficits in schizophrenia. Furthermore, terguride was originally developed to treat hyperprolactinemia (Ciccarelli et al., 1988; Hashimoto et al., 2014) and hence, 2-bromoterguride could belong to the group of dopamine D_2 partial agonists that do not cause a D_2 receptor blockade-induced increase in prolactin secretion and therefore do not induce the unwanted side effects, which coincides with hyperprolactinemia.

The shared pharmacologic features of effective atypical APDs seems to be potent 5-HT_{2A} receptor antagonism in combination with weaker dopamine D_2 receptor antagonism (Seeman & Kapur, 2000; Meltzer et al., 2003). In the meantime, histamine H_1 and 5-HT_{2C} receptor antagonism might cause insulin resistance, weight gain and diabetes (Kim et al., 2007; Ücok & Gaebel, 2008; Meltzer et al., 2013). 2-Bromoterguride however, has lower affinity for the histamine H_1 receptor than aripiprazole (Jantschak et al., 2013) and did not cause weight-gain or metabolic side effects when administered chronically to rats (Franke et al., 2016). Most importantly, like aripiprazole, the dopamine D_2 partial agonist 2-bromoterguride can behave as either D_2 receptor agonist or as antagonist dependent on the dopamine concentration and receptor population (Burris et al., 2002; Tamminga, 2002).

To investigate the prospective effects of 2-bromoterguride on a set of schizophrenia-like behaviours, in this thesis male rats were pharmacologically treated to induce deficits in sensorimotor gating, declarative memory and social withdrawal. Due to the many existing protocol variations, ambiguous previously published results and problem with reproducibility, pilot studies

were conducted prior to each experiment. In the pilot experiments each rat model and intended behavioural test procedure was carefully validated to ensure the induction of robust deficits and effective detection of statistical differences between the two main treatment groups i.e. the schizophrenia related symptom deficient *drug-vehicle* group and the normal behaving *vehicle-vehicle* group. The pilot-studies performed in the frame of this thesis, turned out immensely important for the development of effective testing procedures. Although the methods used herein, have been utilized and published frequently, direct methodological translations to our laboratory setting, used rat strain and test equipment rarely worked immediate and the procedures had to be carefully fine-tuned. Additionally, and based on the existing literature, a control treatment APD was chosen for each study. However, due to the lack of comparable positive control substances (Wilson & Terry, 2010), the main goal with each conducted pilot-study in **Chapter 4** was to assure proper function of the experimental methods and the possibility to effectively and objectively assess the behaviours.

Throughout the study 2-bromoterguride was administered in two dosages (0.1 resp. 0.3 mg/kg). The doses were selected based on the results from previous studies, which showed that 1.0 mg/kg 2-bromoterguride caused significant sedation meanwhile 0.1 or 0.3 mg/kg doses were adequate to inhibit AIL, decrease CAR and reduce DOI-induced wet dog shakes in rats (Jantschak et al., 2013; Franke et al., 2016).

The results for 2-bromoterguride and the respectively used “control” APD for each main experiment will be discussed on the next pages in order to allow detailed dissemination of the results and the implicated involved neurotransmitter systems.

Effect of 2-bromoterguride in rat models of sensorimotor gating deficits

Prepulse inhibition of acoustic startle response (PPI of ASR, described in detail in **Chapter 2.3.1**) is an operational measure of the pre-attentive mechanism sensorimotor gating. Sensory gating, i.e. the inhibition of sensory and cognitive information, is often disrupted in schizophrenia and the direct translation of PPI among species has made the paradigm very popular (for reviews see, Braff et al., 2001; Swerdlow et al., 2008). It has been hypothesized that PPI is mediated via the pons but can be regulated by dopaminergic receptors located in several different forebrain limbic regions such as nucleus accumbens, amygdala, dorsal hippocampus and in the medial prefrontal cortex (Bakshi & Geyer, 1998; Geyer et al., 2001; Swerlow et al., 2008). The mesolimbic

hyperdopaminergic state seen in schizophrenia patients has been indicated as the PPI disruptive force and this deficit can be modelled in rats using a single injection of dopamine receptor agonists (Mansbach et al., 1988; Swerdlow et al., 1996). The ability of a substance to block the PPI disrupting effect of dopamine agonists represents a well-validated preclinical model with face and construct validity additional to the prediction of antipsychotic efficacy (Geyer et al., 2001; Geyer et al., 2012). Apomorphine is a mixed dopamine D_1/D_2 receptor agonist known to induce a robust loss of PPI when administered to rats. The PPI disrupting effect of apomorphine can be blocked by typical APDs with strong antagonistic activity at dopamine receptors (Mansbach et al., 1988; Davis et al., 1990; Swerdlow et al., 1990).

Due to the known activity of 2-bromoterguride at dopamine D_2 receptors (Jantschak et al., 2013; Franke et al., 2016), in **Chapter 4.2** we hypothesized that 2-bromoterguride (0.1 or 0.3 mg/kg, i.p.) would attenuate the PPI disruptive effect of apomorphine (0.5 mg/kg, s.c.), similar to the effect of haloperidol (0.1 mg/kg, i.p.). Meanwhile the effect of 2-bromoterguride on apomorphine-induced PPI disruption aimed to further validate the antipsychotic-like activity of 2-bromoterguride as a putative typical APD, the ability of 2-bromoterguride to block PPI disruptions induced by the noncompetitive NMDA receptor antagonist phencyclidine (PCP, 1.5 mg/kg, s.c.) aimed to investigate if 2-bromoterguride resembles the atypical APD clozapine (5.0 mg/kg, i.p.).

The PPI deficit and hyperglutamatergic state induced by acute PCP administration results from a series of indirect effects on monoaminergic and cholinergic pathways by PCP and it is believed that the PPI-disruptive effect of noncompetitive NMDA antagonists are modulated in limbic forebrain regions such as the amygdala and the dorsal hippocampus (Bakshi & Geyer, 1998; Swerlow et al., 2008). Importantly, the PCP-induced PPI disruption allows closer investigation of the receptor profiles of novel agents, as the PCP-induced PPI deficit is insensitive to compounds with strict dopamine or 5-HT antagonistic actions but can be attenuated by α_1-adrenergic receptor antagonists or agents with clozapine-like features. Consequently, modelling PPI deficits with PCP allows the distinguishing of compounds with atypical character from compounds with mainly typical character, such as haloperidol (Bakshi & Geyer, 1997; Geyer et al., 2001).

As illustrated in **Chapter 4.4.1** (Figure 4.2a), apomorphine significantly reduced PPI compared to controls. In agreement with the observations of Davis et al. (1990), the largest reductions in PPI by apomorphine were detected for the weaker prepulse tones (4 and 8 dB above background). It has been hypothesized that direct dopamine agonists like apomorphine alter sensory gating by affecting the detectability of the prepulse, a process known as central masking,

and therefore cause the largest PPI disruption when the prepulse is elicited at less than 10 dB above the background (Davis et al., 1990). However, in our PPI study (**Chapter 4.4.1**), apomorphine reduced PPI for all our used prepulse levels including the loudest prepulse at 16 dB over background, with the largest difference to the saline treated rats for the prepulse 8 dB over background. The PPI disruptive effect of apomorphine was effectively blocked by the dopamine D_2 receptor antagonist APD haloperidol, strongly agreeing with previous studies where apomorphine effectively disrupted PPI in rats and haloperidol in turn, blocked the sensorimotor gating disruptive effect of apomorphine (Svensson, 1990; Swerdlow et al., 1996; Nakai et al., 2008). Importantly, like haloperidol, 2-bromoterguride succeeded in blocking the effect of apomorphine on PPI. This result hence supports the hypothesis that dopamine D_2 receptor partial agonists such as 2-bromoterguride can act as a D_2 receptor antagonist during a mesolimbic hyperdopaminergic state.

Acute treatment with PCP also caused a significant reduction of PPI (Figure 4.2b). The atypical APD clozapine attenuated the effect of PCP, a result strongly agreeing with results from other studies (Bakshi et al., 1994; Swerdlow et al., 1996; Bakshi & Geyer, 1997). Interestingly, 2-bromoterguride (especially for the higher dose of 0.3 mg/kg) ameliorated the PPI-disrupting effect of PCP in trials where the startle stimulus was preceded by an 82 or 86 dB prepulse. These results hence indicate that 2-bromoterguride resembles clozapine and has antagonistic effect on several neurotransmitter systems indirectly activated by PCP. However, due to the several possible interaction sites which may account for the effect of 2-bromoterguride on the PCP-induced PPI deficit, to draw further conclusions on the receptor profile of 2-bromoterguride would be speculative. Nonetheless, the result is a strong indication that 2-bromoterguride exhibits an atypical profile.

Furthermore, two animal groups were treated with 2-bromoterguride alone (0.1 resp. 0.3 mg/kg dose) to assess the effect of 2-bromoterguride on basal PPI and startle magnitude. These parameters, especially the startle magnitude, directly influence the calculated PPI and could cause misinterpretation of therapeutic effect if left unassessed and unaccounted for. In fact, only sensorimotor gating changes arising in the absence of startle magnitude alterations, can be viewed as truly informative and unbiased (for review, see Swerdlow et al., 2000). Our data showed however, that 2-bromoterguride did not itself affect startle magnitude or baseline PPI levels (see, Table 4.1). Apomorphine on the contrary, caused a significant increase in baseline startle magnitude. This result agrees with findings of previous studies in which apomorphine to some

degree increased startle reaction magnitude in the subject animals (Yee et al., 2004; Nakai et al., 2008).

Another issue that comes with serious implications for the correct interpretation of the PPI data is a potential impact of the prepulse itself. The prepulse can as such only be considered a true “prepulse” if it does not evoke a startle response on its own, i.e. prepulse-induced startle activity. For this reason, a set of ”prepulse-only”-trials were additionally and randomly included in the test protocol. During these trials, the loudest of the prepulses (16 dB above background; 86 dB) was elicited in the absence of a startle stimulus and therefore we could conclude that our implemented prepulses did not alone induce startle responses, adding further validity to our data (Yee et al., 2004; Swerdlow et al., 2008).

Effect of 2-bromoterguride on a subchronic PCP-induced declarative memory/object recognition deficit

Declarative memory is frequently impaired in patients with schizophrenia and the disruptions can only partially be ameliorated by some atypical APDs (Meltzer & McGurk, 1999). The animal analogue to human declarative memory, object memory, can be effectively impaired by subchronic treatment with PCP (bidaily over a 7-day period, followed by a wash-out phase) in rodents and assessed in the novel object recognition test (NOR, for reviews on NOR performance after PCP treatment, see Jentsch & Roth, 1999, Neill et al., 2010; Meltzer et al., 2013). The hypothesis that subchronic treatment with the NMDA receptor antagonist PCP triggers prefrontal cortical dopaminergic hypoactivity and a hyper-responsive state in the mesolimbic dopamine system (Jentsch et al., 1998) adds construct validity to the subchronic PCP model of schizophrenia (for reviews, see Gururajan et al., 2010; Neill et al., 2010).

During the framework of this thesis, subchronic PCP treatment (5.0 mg/kg, twice daily for 7 days followed by a 2-week withdrawal phase) effectively disrupted object memory in the treated animals. Our results hence, agree with several other studies showing that subchronic PCP significantly disrupts NOR performance i.e. the ability to discriminate novel and familiar objects, when a 1-minute intertrial interval (ITI) was used (Grayson et al., 2007; Snigdha et al., 2011; Horiguchi et al., 2012; Horiguchi & Meltzer, 2012; Oyamada et al., 2015). 2-Bromoterguride administrated 30 min prior to the NOR test efficiently ameliorated the cognitive disruptive effect of subchronic PCP. Although the larger dose of 2-bromoterguride (0.3 mg/kg) did cause a reduction in horizontal locomotor behaviour as illustrated by the distance travelled during the retention trial,

there was no effect on explorative behaviour and motivation to explore the objects. Clozapine (5.0 mg/kg) however, failed to attenuate the NOR deficit when a 1-minute ITI was used. Other studies instead utilized longer delays between the acquisition and retention phase, such as one hour, and detected a significant NOR deficit in the subchronic PCP-treated rats (Vigano et al., 2009; McKibben et al., 2010; Redrobe et al., 2010; Pyndt Jørgensen et al., 2015). Due to some critical aspects of only using a 1 minute ITI, such as the increased risk of artefacts arising from disruptions in the concentration ability or perceptional changes (Lyon et al., 2012), we additionally investigated the effects of subchronic PCP and the respective APD treatments with a one hour ITI protocol (unpublished data, see Figure A.8) In our laboratory, subchronic PCP treatment caused a significant interruption to the novel object recognition performance of male rats also when a one hour inter trial interval was implemented. In this additional test version, 2-bromoterguride again successfully ameliorated the PCP-induced deficits, but not clozapine, further strengthening the hypothesis that 2-bromoterguride is a novel agent that might be valuable in the treatment of cognitive impairments in schizophrenia.

Although the results from many studies have shown that atypical APDs like clozapine improve the subchronic PCP-induced NOR deficit in rats, a few studies agree with our results. In the study by Le Cozannet et al. (2010) the effects of PCP and clozapine in an object-place-context recognition test was investigated to further disseminate the reason why clozapine has been shown to ameliorate the PCP induced NOR deficit in rats but has less beneficial effect on cognitive symptoms in humans. For this study the same subchronic PCP treatment protocol was used as in our study and additionally, the dose of clozapine was the same (5.0 mg/kg). Interestingly, the authors concluded that subchronic PCP induced disruptions in memory performance and that clozapine failed to attenuate the memory disruption. Furthermore, Grayson et al. (2007) did not detect significant attenuation of the discrimination index by clozapine, however, found that clozapine attenuated the time difference exploring the novel respective familiar object.

Due to the differences in test settings, treatment regime, drug dose or rat strains and gender, it is however not possible to know the exact reason why clozapine failed to ameliorate the subchronic PCP induced NOR deficit in our lab. Nonetheless, it seems that the preclinical effect of clozapine on PCP-induced memory deficits in rats is less direct than often assumed in the literature.

It has been hypothesized that subchronic PCP treatment mainly increases cortical 5-HT_{1A} receptor binding meanwhile decreasing striatal dopamine D_1 receptor density and that subchronic PCP does not cause changes in 5-HT_{1A} levels in other brain regions nor changes in 5-HT_{2A} or D_2

receptors in any brain region (Choi et al., 2009). Furthermore, it has been suggested that the improving effect of some atypical APDs on cognition, comes from either their direct or indirect agonistic actions at the 5-HT_{1A} receptor which increases dopamine release in the medial prefrontal cortex and hippocampus, or from the secondary effect on 5-HT efflux by combined blockade of 5-HT_{2A} and dopamine D_2 receptors (Meltzer et al., 2011; Horiguchi & Meltzer, 2012). Interestingly however, 2-bromoterguride effectively ameliorated the cognitive disruptions following subchronic PCP but has no affinity for 5-HT_{1A} receptors in vitro (unpublished data). As such, these results indicate the lesser importance of direct interactions with the 5-HT_{1A} receptor and that 2-bromoterguride exerts its procognitive effect by interacting with either dopamine D_1 receptors, or dopamine D_2 receptors in addition to 5-HT_{2A} receptors.

In agreement with this theory, several studies of the effects of NMDA receptor antagonists on NOR performance has indicated that 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptor antagonism might ameliorate the cognitive impairments and contribute to the superior effects of atypical APDs over typical APDs (Meltzer et al., 2011; Meltzer et al., 2013). Furthermore, there are implications for involvement of dopamine D_1 receptors in the reversal of subchronic PCP induced object recognition deficits as the dopamine D_1 agonist SKF-38393 successfully ameliorated subchronic PCP-induced NOR impairments (McLean et al., 2009; for review, see Lyon et al., 2012).

Nonetheless, the translational aspect of the subchronic PCP model for cognitive deficits has been partially questioned due to the efficacy of atypical APDs to reverse the NOR deficit meanwhile they continuously show less robust effect on cognitive deficits in schizophrenia patients (for reviews see, Meltzer et al., 2011, 2013; Gilmour et al., 2012 and Rajagopal et al., 2014). For this reason, the failure of clozapine to ameliorate the NOR deficit in our lab adds some translational and predictive value to the model. This in turn has important implications for the results of 2-bromoterguride, which continuously attenuated the NOR deficits for both the used test ITIs, indicating the potential of this novel putative atypical drug.

Effect of 2-bromoterguride on subchronic PCP-induced social aversion

To investigate the putative effect of 2-bromoterguride on negative schizophrenic symptoms, the subchronic PCP treatment regimen that induced cognitive deficits in male rats in our laboratory, was used in another batch of animals subsequently tested in the social interaction paradigm. As reviewed in **Chapter 2.3.2**, the dyadic social interaction test is a paradigm that allows the

assessment of novel agents in an established model for negative symptoms of schizophrenia (see also, review by Wilson and Koenig, 2014).

In agreement with several previously performed studies (Sams-Dodd, 1995; Lee et al., 2005; Snigdha & Neill, 2008a, 2008b; Seillier et al., 2013, Seillier & Giuffrida, 2016), subchronic PCP treatment induced robust social deficits in the treated rats (see Figure 4.4). The PCP treated rats interacted significantly less in social behaviours like sniffing, following and climbing, however, there were no differences in exploration of a novel object or line crossings during the test. These parameters are important and implicate that the decreased social interaction in the subchronic PCP-treated animals did not arise mainly as a secondary effect of decreased locomotor activity or overall explorative motivation.

It has been suggested that social withdrawal (Sams-Dodd, 1995) or lack of social approach resulting from a lack of social motivation (Peters et al., 2017) in the subchronic PCP rat, causes the reduced interaction in the dyadic social interaction test. However, these parameters are principally non-distinguishable from each other in the social interaction test setup and other processes such as impaired social cognition cannot be assessed (Seillier & Giuffrida, 2016). To further assist in the validation of the subchronic PCP-induced social interaction deficient rat model for negative symptoms of schizophrenia, the social behaviour of the model was thoroughly investigated in **Chapter 5**.

As discussed in **Chapter 5**, social interaction behaviour might decrease in the event of anxiety, however, several studies have shown that PCP treatment does not increase anxiety-related behaviours compared to vehicle treated animals in the open field and light/dark emergence test (Lee et al., 2005; McLean et al., 2009). The results in this thesis strongly agree with the previously mentioned studies, as the rats in our laboratory showed no decrease in either locomotor activity or time spent in the centre of the arena during the first habituation trial (i.e. open field test). These measures can be used to assess general anxiety of animals placed in a novel environment (Christmas & Maxwell, 1970; Bailey & Crawley, 2009; Seibenhener & Wooten, 2015).

Several animal studies have confirmed the importance of 5-HT_{2A}/5-HT_{1A} and dopamine D_1/D_2 receptor antagonism to attenuate PCP-induced social interaction deficits and the failure of dopamine D_2 selective agents (Bruins Slot et al., 2005; Neill et al., 2014). Our results showed that the dopamine D_2 receptor partial agonist 2-bromoterguride effectively restored the social interaction behaviours disrupted by subchronic PCP (Figure 4.4). Furthermore, administration of 2-bromoterguride to subchronic saline-treated animals did not interfere with their repertoire of

social behaviours, a result which can be interpreted as another implication of acute 2-bromoterguride treatment tolerability.

Interestingly, as a dopamine D_2 receptor partial agonist, 2-bromoterguride resembles aripiprazole. However, in our study, aripiprazole (3.0 mg/kg) failed to attenuate the PCP-induced social deficit. An extra dosage pilot study for aripiprazole was therefore implemented additionally to ensure that the given dose was not to low (data not shown). However, neither 3.0 mg/kg nor 5.0 mg/kg had any effect on the social aversion seen in the subchronic PCP treated rats. Due to the lack of difference in the effect for the two tested dosages, and due to the results from our previous study in which 3.0 mg/kg aripiprazole had effect on CAR performance (Franke et al., 2016), we continued to use the 3.0 mg/kg dose for the social interaction study.

Other studies, in contrary to our results, found that aripiprazole ameliorated the social deficit induced by subchronic PCP treatment. For example, Bruins Slot et al. (2005) found that aripiprazole (0.04 and 0.16 mg/kg), administered daily for 3 days together with PCP, reversed the PCP-induced disruption of social interaction behaviour. The 5-HT_{1A} receptor antagonist WAY100635 blocked the effect of aripiprazole indicating the importance of a balanced activity at 5-HT_{1A} and dopamine D_2 receptors by APDs for them to have effect on PCP-induced social deficits (Bruins Slot et al., 2005). Additionally, Snigdha & Neill (2008b) showed that an acute dose of aripiprazole (5.0 mg/kg) reduced subchronic PCP-induced social deficits in female rats when tested after a 7-day washout phase. In the same study, the results from Bruins Slot et al. (2005) were reproduced, showing that pre-treatment with WAY100635, prevented aripiprazole from reversing the social deficit, again suggesting an effect mediated via interaction with 5-HT_{1A} receptors (Snigdha & Neill, 2008b). However, as mentioned in **Chapter 2.3.2**., WAY100635 is a combined 5-HT_{1A} receptor antagonist and dopamine D_4 receptor agonist, and therefore the ability of WAY100635 to prevent aripiprazole from ameliorating a subchronic PCP-induced social deficit might not solely be allocated to its actions at the 5-HT_{1A} receptor (Chemel et al., 2006; Marona-Lewicka & Nichols, 2009). It is possible that the inconsistency of the results for aripiprazole in the present study and the studies of Bruins Slot et al. (2005) and Snigdha & Neill, (2008b), can be explained by the differences in drug dosing and/or dosing regimen, the rat gender or in the length of the utilized washout phase. Possibly, our experimental procedure caused a severer social impairment in our rats, and aripiprazole therefore failed to reverse the deficit in our laboratory. Female rats have been shown to differ from males in the biotransformation of PCP i.e. females have slower PCP metabolism making them more susceptive to PCP treatment (Nabeshima et al., 1984). Therefore, a stronger antipsychotic effect might be needed to ameliorate a social deficit as

severe as the PCP-induced impairment, seen after a two weeks drug withdrawal period in the male rats in our laboratory.

The difference of 2-bromoterguride and aripiprazole in their ability to ameliorate the subchronic PCP-induced social withdrawal, might also be a possible result from the higher affinity for 5-HT_{2A} receptors by 2-bromoterguride than aripiprazole. Additionally, another difference is the higher α_{2C}-adrenoceptor antagonist potency of 2-bromoterguride (Jantschak et al., 2013). In agreement with this theory, some previous studies have shown a contribution of α_{2C}-adrenoceptor blockade in enhancing cortical glutamatergic transmission and the attenuation of cognitive and social functional deficits in rats (Marcus et al., 2005; Sallinen et al., 2013). Furthermore, it has been postulated that the atypical properties of aripiprazole might derive from its combined actions as weak dopamine D_2/5-HT_{2A} antagonist and 5-HT_{1A} receptor agonist (Meltzer et al., 2003). Yet, as 2-bromoterguride effectively ameliorated the subchronic PCP-induced social deficit but has no affinity for 5-HT_{1A} receptors in vitro (unpublished data), the 5-HT_{1A} receptor seems to be less implicated in the reversal of the subchronic PCP-induced social aversion.

Most importantly, these results again showed a superior performance by 2-bromoterguride compared to renowned atypical APDs in tests with translational value for the negative symptoms of schizophrenia. Together with the continuously seen well tolerability of 2-bromoterguride in the performing animals, this further implies that 2-bromoterguride could be a new successful putative atypical APD worth further investigation.

Effect of 2-bromoterguride on prolactin secretion

For the last study in **Chapter 4**, the effect of 2-bromoterguride on prolactin secretion was studied.

It is well known that dopamine D_2 receptor antagonists induce hyperprolactinemia and that the magnitude of prolactin elevation depends on the extent of D_2 receptor blockade (Freeman et al., 2000; Cosi et al., 2006). Elevation of secreted prolactin has direct effects on the brain and other organs and hyperprolactinemia can induce several sexual dysfunctions and abnormalities making the APD treatment unendurable for the patients (Meaney & O´Keane, 2002; Leucht et al., 2013; Peuskens et al., 2014). All typical antipsychotics and the atypical antipsychotics amisulpride and risperidone have been reported to elevate serum prolactin levels (Lu et al., 2008). The typical APD haloperidol is known to induce massive hyperprolactinemia in both experimental animals and patients (Feigenbaum et al., 1982; Haddad & Wieck, 2004) and was therefore included as positive control in this study.

Interestingly, terguride was originally developed to be used for the treatment of hyperprolactinemia, and add-on treatment with terguride has been suggested to relief some of the side effects in APD treated patients experiencing high prolactin levels. Sadly, terguride itself showed low tolerability and its use in schizophrenia treatment was therefore diminished (Hashimoto et al., 2014). Aripiprazole is an atypical APD, which like terguride has been reported to reduce prolactin levels in patients suffering from hyperprolactinemia (Lee et al., 2006). Aripiprazole, unlike haloperidol or risperidone, does not show a dose/occupancy-dependent elevation of prolactin but instead shows a divergent relationship between occupancy and functional antagonism, something which has been attributed to its partial agonistic actions (Natesan et al., 2006). It has been proposed that the prolactin releasing ability of a drug is dependent on its dissociation rate from dopamine D_2 receptors, with compounds showing fast dissociation triggering more prolactin release regardless of receptor affinities and occupancies (Cosi et al., 2006; Carboni et al., 2012).

Interestingly, our results showed that the terguride derivate 2-bromoterguride acts as a non-prolactin-elevating drug in experimental animals as the prolactin concentration did not significantly differ from vehicle treated rats at any of the tested doses or timepoints. The ELISA study showed that blood serum prolactin concentrations were affected mainly by haloperidol, which caused elevated prolactin concentrations for all three time points (Figure 4.5; Table A.8). 2-Bromoterguride did raise the prolactin level weakly at one-hour post injection, however, no statistical difference to the vehicle group could be detected. In our study the prolactin concentration in blood serum remained < 4.8 ng/ml for vehicle-treated animals at all time points, a level corresponding well with the results of other studies or being lower (< 4.3 ng/ml in the study of Cosi et al., 2006; 3.5 ng/ml in the study of Kapur et al., 2002; 10.1 ng/ml in the study of Inoue et al., 1996). Our results for haloperidol matches the study of Feigenbaum et al. (1982) who measured a prolactin concentration around 65 ng/ml following a single 0.5 mg/kg dose of haloperidol one-hour post injection. The same dose and time in our study resulted in a haloperidol-induced prolactin elevation up to an average of 66 ng/ml, thereby showing our results as highly reliable.

In the study of Cosi et al. (2006), aripiprazole was found to partially elevate prolactin up to 32 ng/ml in male rats. Consequently, as 2-bromoterguride only elevated prolactin up to 17.9 (0.1 mg/kg dose) respective 13.5 ng/ml (0.3 mg/kg), it demonstrates a prolactin-sparing profile.

Validation of olfactory function in the subchronic PCP model for social impairments, using the olfactory habituation/dishabituation test

Chapter 5 was dedicated to further validate the subchronic PCP rat model used for the NOR- and social interaction test in **Chapter 4**. A thorough literature review showed the alarming lack of behavioural control tests for the model, i.e. tests aimed specifically to investigate behaviours and factors that could influence the performance of the rats in other test paradigms. **Chapter 5** is hence not directly dedicated to the study of 2-bromoterguride, yet indirectly **Chapter 5** is a prerequisite to correctly assess and interpret the results of 2-bromoterguride in **Chapter 4.4.3** on social interaction behaviour.

To adequately perform behavioural assays in small rodents, an array of environmental factors and possible artefacts must be considered and controlled, such as general health, motor function, anxiety and sensory ability (Crawley, 2008).

As described in more detail in **Chapter 5.2**, olfaction is necessary for rodents to perform their repertoire of social behaviour and olfactory function must be tested to avoid the misinterpretation of a social deficit when in fact a physical odour detection inability makes it impossible for the animal to recognize other conspecifics (Leypold et al., 2002; Yang & Crawley, 2009). Rodents depend on olfactory cues to recognize individuals and for the expression of appropriate social and sexual behaviours (Leypold et al., 2002; Lim & Young, 2006; Arbuckle et al., 2015). Most chemosensory cues are detected by the olfactory epithelium, which also allows the discrimination among thousands of various odours that enters the nasal cavity. The dendrites of bipolar olfactory receptor neurons projects from here to the main olfactory bulb (Kelliher & Wersinger, 2009). The process of social recognition in rodents operates via the vomeronasal organ, which detects pheromones by projecting olfactory information to the accessory olfactory bulb. From the olfactory bulb, the projections flow downstream to the amygdala, lateral septum, and cortex (Lim & Young, 2006). Particularly the amygdala has been linked to the processing of social recognition and social emotions (Ferguson et al., 2001). The interruption of olfactory social cues by anosmia (the absence of sense of smell) or hyposmia (reduced ability to smell and to detect odours) would therefore have major impact on rodent social behaviour and olfactory function must be validated in the subject animals (Moy et al., 2004; Crawley, 2008).

In **Chapter 5**, normal olfactory function in male rats treated with subchronic PCP was validated as the rats successfully habituated and dishabituated to non-social odours similarly to the vehicle treated animals (see, Figure 5.3 and 5.4). The subchronic PCP treated rats in the study did habituate to the social odour, however, spent more total time investigating the social stimulus. This result could have important implications for the background of the social deficit seen in the subchronic PCP rat. As shown in **Chapter 4.4.2**, the same PCP treatment protocol was used to study cognition in the NOR test and caused a severe working memory deficit. The increased interest for the social odour could hence be an implication that social cognition is interrupted by subchronic PCP. However, if this theory is correct the intriguing question why the animals did not sniff the strange rat more during the dyadic social-interaction test remains. As hypothesized in more detail in **Chapter 5.5**, the social interaction test situation itself with its forced interaction with another animal could have caused the social aversion and therefore not interfered with the motivation to explore a social odour in the safe environment during the olfactory habituation/dishabituation test. These questions remain to be answered in future experiments and could help to shed light on the reason for the social aversion of the subchronic PCP rat.

Nonetheless, the most important finding in **Chapter 5**, is the result that the rats suffered no olfactory disruptions from the subchronic PCP treatment. These results support the continued use of the NMDA receptor antagonist model for negative symptoms of schizophrenia. It also adds validity to our study in **Chapter 4**, as well as a wide range of other published studies from other laboratories in which subchronic PCP was used to model social deficits without having (reported) knowledge of intact olfactory sensory function in the studied animals.

Conclusion

The results from this PhD thesis strongly support the in vivo dopamine D_2 receptor partial agonistic actions of 2-bromoterguride. Acting as either an antagonist or agonist, 2-bromoterguride successfully attenuated deficits in animal models with dopaminergic hyperactivity (PPI study) and dopaminergic hypoactivity (NOR and social interaction test). 2-Bromoterguride showed a prolactin-sparing profile and throughout the study, it proved its tolerability in male rats. Taken together with our previously published studies which showed an absence of induced metabolic changes in rats after chronic 2-bromoterguride treatment (Franke et al., 2016), 2-bromoterguride represents a novel agent, that might be effective in treating the positive and negative symptoms as well as cognitive deficits in schizophrenia without inducing hyperprolactinemia, EPS or weight gain.

CHAPTER 7: Summary/Zusammenfassung

Summary of the PhD Thesis:

Effects of 2-bromoterguride, a dopamine D_2 receptor partial agonist, in animal models for negative symptoms and cognitive dysfunctions associated with schizophrenia.

As described shortly in the general introduction (**Chapter 1**) of this PhD thesis, schizophrenia is a severe psychiatric disorder, which affects about one percent of the human world population. To treat schizophrenia, positive symptoms, negative symptoms and cognitive deficits must be effectively ameliorated. However, todays available antipsychotic drugs (APDs) are successful mainly in the silencing of the positive symptoms meanwhile there is still a serious lack of effective treatments for the negative symptoms and cognitive impairments in schizophrenia.

The first part of this PhD thesis (**Chapter 2**) was dedicated to study the literature to give an overview about the history of schizophrenia, its diagnosis and to summarize the literature on the neurochemistry theories for the development of schizophrenic symptoms, mainly the dopamine, serotonin and glutamate hypotheses. **Chapter 2** was furthermore committed to give a literature overview on the therapeutic developments in schizophrenia, summarized as the first, second and third generation APDs, including a section on the present drug discovery pipeline. Being the reason and purpose of this PhD thesis, the theory and present scientific knowledge of the dopamine D_2 receptor partial agonist *2-bromoterguride*, is described in the later part of **Chapter 2**, together with a summary on the theory and use of different animal models in preclinical schizophrenia research.

Chapter 3 shortly explains the aims and hypotheses of this PhD thesis, which main work consists of the two published manuscripts that constitutes **Chapter 4** and **5**. The main goal of this PhD study was to investigate the effect of the novel putative APD 2-bromoterguride on negative symptoms and cognitive deficits in drug-induced animal models. Therefore, a series of behavioural experiments were conducted.

In the first experiment described in **Chapter 4**, the effect of 2-bromoterguride on sensory motor gating was measured via the prepulse inhibition of the acoustic startle response (PPI). Deficits in PPI in male rats were induced using acute treatment with either the mixed dopamine D_1/D_2 agonist apomorphine or the N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP). The pre-attentive cognitive process PPI which is disturbed in schizophrenia

patients, represents an endophenotype that can be readily modelled and tested in animals. The results of the study showed that 2-bromoterguride was successful in blocking the effect of both apomorphine and PCP similarly to the first generation APD haloperidol respectively the atypical second generation APD clozapine. The results are the first indication that 2-bromoterguride has an atypical character in vivo, as first-generation dopamine D_2 antagonists are known to fail to block the effects of PCP on prepulse inhibition. Furthermore, the study on PPI showed that treatment with 2-bromoterguride alone caused no changes in startle magnitude, habituation to repeated startle stimuli or PPI in the absence of other drugs.

In the second experiment described in **Chapter 4**, a subchronic PCP treatment protocol was established which caused serious cognitive deficits in male rats when these were tested for object memory in the Novel Object Recognition (NOR) test after a subsequent drug-washout phase. Subchronic PCP treatment has been reported to induce a prefrontal hypodopaminergic state in rats mimicking the pathophysiology of the negative symptoms and cognitive deficits in schizophrenia. Our results showed that 2-bromoterguride was effective in ameliorating the object recognition deficit in the NOR test, induced by subchronic PCP treatment and a 2-week drug-withdrawal phase, an effect which the atypical APD clozapine failed to elicit. Furthermore, in our NOR experiment 2-bromoterguride itself caused no cognitive interruptions and even, for the higher dose (0.3 mg/kg), showed a slight pro-cognitive effect.

In order to evaluate the effect of 2-bromoterguride on negative symptoms of schizophrenia, in the social interaction behaviour study in **Chapter 4**, the same subchronic PCP treatment protocol which induced cognitive impairments in the NOR test, was used to induce social aversion in male rats. The study showed an effective disruption of all measured social behaviours in the rats following subchronic PCP and that 2-bromoterguride, but not the dopamine D_2 partial agonist aripiprazole, was successful in attenuating the social deficit.

In the final study described in **Chapter 4**, the impact of acute 2-bromoterguride treatment on secreted prolactin in male rats was evaluated at one, two or four hours post treatment by performing a prolactin enzyme-linked immunosorbent assay (ELISA). The result of the experiment suggests that 2-bromoterguride belongs to the non-hyperprolactinemia-inducing APDs and implicates that 2-bromoterguride has a profitable side-effect profile.

Taking all the results from **Chapter 4** into consideration, this PhD study strengthens the hypothesis of the beneficial effects elicited by dopamine D_2 partial agonists like 2-bromoterguride for the treatment of the complete range of schizophrenia symptoms. Whether the promising effects of 2-bromoterguride seen in the rat models used in this PhD study, can be translated for the

treatment of schizophrenia patients, remains to be elucidated. However, not only the efficacy of 2-bromoterguride as a putative third generation APD but also its safe use and tolerability was continuously proven throughout each experiment in **Chapter 4**.

Finally, **Chapter 5** was dedicated to the validation of the subchronic PCP model for social withdrawal in schizophrenia. For this purpose, an olfactory habituation/dishabituation test with both non-social and social odours was conducted to control the absence of PCP-induced anosmia or hyposmia in the animals used to study social interaction in **Chapter 4**. Olfactory defects would have severe impact on the social behaviour of the rats in the social interaction test and this needed to be validated in more detail due to a serious lack of such control measures in the literature of previous PCP-induced social interaction research. The results, which indicated normal olfactory function in the rats treated with subchronic PCP, have important implications for the correct interpretation of the social interaction deficit of the model. Interestingly, the study did leave some intriguing queries for future research on the social impairments in the PCP rat model. For example, in contrary to the shown lack of social interest in the dyadic social interaction test, the study revealed an enhanced interest for the social odour by the PCP-treated rats during the olfactory habituation/dishabituation test. Nonetheless, whether this effect was expressed because of a social cognitive deficit, or if it can be explained by differences in motivational or anxiety-driven parameters during the two behavioural tests remains unelucidated and calls for continuation and further dissemination of the subchronic PCP rat model for negative symptoms of schizophrenia.

Zusammenfassung der Dissertation:

Effekte von 2-Bromtergurid, einem Dopamin-D_2-Rezeptor-Partialagonisten, in Tiermodellen der Negativsymptomatik und kognitiver Dysfunktionen der Schizophrenie

Wie in der allgemeinen Einleitung (**Kapitel 1**) dargestellt, ist die Schizophrenie eine schwere psychiatrische Störung, von der etwa ein Prozent der Weltbevölkerung betroffen ist. Eine effektive Behandlung der Schizophrenie beinhaltet eine Abmilderung bzw. Aufhebung der Positivsymptomatik, der Negativsymptomatik und kognitiver Defizite. Allerdings sind die heute verfügbaren Antipsychotika (APDs) hauptsächlich bei der Behandlung der Positivsymptomatik erfolgreich. Wirksame Behandlungsoptionen für die Negativsymptomatik und kognitive Beeinträchtigungen liegen nicht vor.

Der Literaturteil der Dissertation (**Kapitel 2**) gibt einen Überblick über die Geschichte und die der Schizophrenie zu Grunde liegenden neurochemischen Störungen (Dopamin-, Serotonin- und Glutamat-Hypothese). Zusätzlich werden die Entwicklung von typischen und atypischen APDs und die Erforschung von Testsubstanzen aufgezeigt. Das Kapitel schließt mit einer Vorstellung des Dopamin-D_2-Rezeptor-Partialagonisten 2-Bromtergurid und einer Übersicht verschiedener Tiermodelle der präklinischen Schizophrenieforschung.

Kapitel 3 erläutert die Ziele und Hypothesen der vorliegenden Doktorarbeit. Zentraler Teil sind die beiden wissenschaftlichen Veröffentlichungen, die in **Kapitel 4** und **5** vorgestellt werden. Das Hauptziel der Doktorarbeit war es, die Wirkung des Dopamin-D_2-Rezeptor-Partialagonisten 2-Bromtergurid auf Substanz-induzierte Verhaltenseffekte mit Relevanz zur Symptomatik der Schizophrenie (Negativsymptomatik, kognitive Defizite) in tierexperimentellen Studien an der Ratte zu untersuchen.

In der ersten Veröffentlichung (**Kapitel 4**) wird die Wirkung von 2-Bromtergurid auf die Präpulsinhibition (PPI) der akustisch ausgelösten Schreckreaktion (ASR) beschrieben. PPI-Defizite wurden durch eine akute Behandlung mit dem Dopamin-D_1/D_2-Rezeptor-Agonisten Apomorphin oder dem nicht-kompetitiven N-methyl-D-aspartat (NMDA)-Rezeptor-Antagonisten Phencyclidin (PCP), induziert. Die der PPI zu Grunde liegende sensomotorische Integrationsleistung ist bei Schizophreniepatienten gestört und repräsentiert einen Endophänotyp, der im Tiermodell leicht modelliert und getestet werden kann. Die Ergebnisse der Studie zeigen, dass 2-Bromtergurid die Apomorphin- und PCP-induzierten PPI-Defizite, ähnlich wie das typische

APD Haloperidol und das atypische APD Clozapin, antagonisieren konnte. Die Ergebnisse sind ein erster Hinweis auf den atypischen Charakter von 2-Bromtergurid, da typische APD keinen Effekt auf PCP-induzierte PPI-Defizite aufweisen. Darüber hinaus zeigt die PPI-Studie, dass eine akute 2-Bromtergurid-Behandlung keine Veränderung der Schreckreaktion, der Habituation an den Schreckreiz oder der PPI induzierte.

Das zweite Experiment der Arbeit beschreibt die Etablierung und Anwendung einer subchronischen PCP-Behandlung im Novel Object Recognition (NOR)-Test. Die wiederholte Gabe von PCP führte, im Anschluss an eine Auswaschphase, zu kognitiven Defiziten. Vorangegangene Studien zeigen, dass eine subchronische PCP-Behandlung einen präfrontalen hypodopaminergen Zustand bei Ratten induzierte, der die Pathophysiologie der negativen Symptome und kognitiven Defizite der Schizophrenie nachahmt. Die Ergebnisse der vorliegenden Arbeit zeigen, dass 2-Bromtergurid die durch eine subchronische PCP-Behandlung induzierten kognitiven Defizite im NOR-Test signifikant reduzierte, eine Wirkung, die das atypische APD Clozapin nicht auslösen konnte. Darüber hinaus zeigte 2-Bromtergurid in einer höheren Dosis (0,3 mg/kg) eine leichte pro-kognitive Wirkung.

Für den dritten Versuch der Arbeit wurde die oben beschriebene subchronische PCP-Behandlung verwendet, um bei männlichen Ratten eine soziale Aversion zu induzieren. Die Studie zeigt, dass die PCP-Behandlung das Sozialverhalten der Ratten reduzierte und 2-Bromtergurid erfolgreich das soziale Defizit abschwächte, eine Wirkung, die nicht durch den Dopamin-D_2-Partialagonisten Aripiprazol ausgelöst werden konnte.

Im abschließenden Experiment der Arbeit wurde die Wirkungen von 2-Bromtergurid auf die Prolaktinsekretion mittels eines Enzyme-linked Immunosorbent Assays (ELISA) untersucht. Die Ergebnisse des Experiments legen nahe, dass 2-Bromtergurid zu den nicht-Hyperprolaktinämie-induzierenden APDs gehört und ein vorteilhaftes Nebenwirkungsprofil besitzen könnte.

Die Ergebnisse der in **Kapitel 4** dargestellten Arbeit stärken die Hypothese das Dopamin-D_2-Partialagonisten wie 2-Bromtergurid für die Behandlung der gesamten Bandbreite der Schizophreniesymptomatik eingesetzt werden könnten. Ob die vielversprechenden Effekte von 2-Bromtergurid auf die Behandlung schizophrener Patienten übertragen werden kann, ist an dieser Stelle nicht zu beantworten. Jedoch wurde nicht nur die Wirksamkeit von 2-Bromotergurid als potentielles atypisches Antipsychotikum, sondern auch dessen sichere Verwendung und Verträglichkeit, in den Experimenten der Studie nachgewiesen.

Die in **Kapitel 5** dargestellte zweite Veröffentlichung widmete sich der Validierung des in der ersten Arbeit (**Kapitel 4**) eingesetzten subchronischen PCP-Modells, mit dem u. a. ein Defizit des Sozialverhaltens induziert wurde. Zu diesem Zweck wurde ein olfaktorischer Habituationstest mit sowohl nicht-sozialen als auch sozialen Gerüchen durchgeführt, um zu kontrollieren, ob PCP eine Beeinträchtigung der Geruchsleistung bei den eingesetzten Tieren auslöst. Obwohl jegliche olfaktorischen Störungen eine deutliche Auswirkung auf das Sozialverhalten der Ratten während des sozialen Interaktionstests hätte und die Aussagekraft der gewonnen Ergebnisse hinterfragt werden müsste, wurden solche Kontrollexperimente in vergleichbaren Studien anderer Arbeitsgruppen nicht durchgeführt.

Die Ergebnisse zeigen, dass die wiederholte Gabe von PCP keine olfaktorischen Störungen induzierte und somit von einer korrekten Interpretation der Erkenntnisse ausgegangen werden kann. Interessanterweise ergab die Studie, dass PCP-behandelte Tiere im Gegensatz zu dem gestörten Sozialverhalten im sozialen Interaktionstest ein verstärktes Interesse für den sozialen Geruch im olfaktorischen Habituationstest zeigten. Ob dieser Effekt aufgrund eines sozial-kognitiven Defizits oder durch Unterschiede in der Motivation oder in dem Angstverhalten während der beiden Verhaltenstests erklärt werden kann, müsste durch Nachfolgeuntersuchungen geklärt werden, um eine weitere Validierung des subchronischen PCP-Rattenmodells für die Negativsymptomatik der Schizophrenie zu ermöglichen.

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APPENDIX

Table A.1 Primary symptoms and features of schizophrenia

Positive symptoms	Negative symptoms	Cognitive impairments
Hallucinations	Reduced speech (alogia)	Attentional deficits
Delusions	Lack of pleasure (anhedonia)	Deficits in executive functions
Disorganized behaviour, thoughts or speech	Blunted of flattened affect	Verbal learning and memory deficits
Catatonic behaviour	Anxiety	Visual learning and memory deficits
	Diminished ability to start and sustain activities (avolition)	Working memory impairments
	Social withdrawal	Reasoning and problem solving
		Deficits in social cognition

Table A.2 The main diagnostic criteria following the ICD-10 and DSM-IV

ICD-10	DSM-IV
> one clear symptom listed in category a -d; if less clear symptomatics; a minimum of two category a - d-symptoms > two of the symptoms in category e - i present for at least one month of the psychotic illness	> two of the symptoms listed in category *a* has been present for a minimun of one month category *b* has been the case for > six months criteria in category c - e must be fulfilled
a) thought echo, thought insertions or withdrawal, and thought broadcasting	a) delusions, hallucinations, bizarre behaviour and negative symptoms
b) delusion of control or passivity	b) occupational or social dysfunction
c) hallucinatory voices	c) schizoaffective or mood disorder exclusion
d) persistent delusions	d) disturbance shall not be due to medications or drug abuse
e) significant and consistant change of personal behaviour	e) if the patient has a prevasive developement disorder; prominent hallucinations or delusions must have been present for > one month
f) negative symptoms which are not due to depression or antipsychotic drug treatment	
g) persistent hallucinations accompanied by deletions or persistent over-valued ideas constantly reaccuring for weeks or months	
h) incoherent or irrelevant speech	
i) catatonic behaviour	

Table A.3 Available antipsychotic medications in the USA and Europe year 2016

First generation drugs / Typical antipsychotic agents	Second generation drugs / Atypical antipsychotic agents	Third generation drugs
Thorazine® (Chlorpromazine HCl)	Zyprexa® (Olanzapine)	Abilify® (Aripiprazole)
Serentil® (Mesoridazine Besylate)	Geodon® (Ziprasidone)	
Mellaril® (Thioridazine HCl)	Seroquel® (Quetiapine)	
Haldol® (Haloperidol)	Risperidal® (Risperidone)	
Loxitane® (Loxapine Succinate)	Clozaril® (Clozapine)	
Trilafon® (Perphenazine)	Invega® (Paliperidone)	
Navane® (Thiothixene)	Asendin® (Amoxapine)	
Sordinol® (Clopenthixol)	Latuda® (Lurasidone)	

Table A.4 Novel agents in ongoing research and clinical trials for schizophrenia treatment

Main treatment action	Agent name	Company/ Institution	Mechanism	Main treatment target	Reference
Dopamine partial agonist	ITI-007 (lumateperone)	IntraCellular Therapies	5-HT_{2A} antagonist, presynaptic partial D_2 agonist, postsynaptic D_2 antagonist, SERT antagonism, increased GluN2B phosphorylation	Negative symptoms Less side-effects	Lieberman et al., 2015
	RP5063	Reviva Pharmaceuticals	Partial D_2, D_3, D_4 agonist, partial 5-HT_{1A} and 5-HT_{2A} agonist, 5-HT_6 and 5-HT_7 antagonist	Positive symptoms Negative symptoms (cognitive impairments)	Cantillon et al., 2017
Dopamine D_1 antagonist	Lu AF35700	Lundbeck	D_1, 5-HT_{2A} and 5-HT_6 receptor antagonist	Refractory schizophrenia	Lundbeck, 2017
5-HT antagonist	AVN-211	Avineuro Pharmaceuticals	5-HT_6 receptor antagonist	Cognitive impairments	Morozova et al., 2014
	MIN-101	Minerva Neurosciences	5-HT_{2A} and α_2 receptor antagonist	Negative symptoms	Davidson et al., 2017
mGlu $_2$ selective agonist	JNJ-40411813 (ADX-71149)	Janssen	metabotropic gluR$_2$ positive allosteric modulator, 5-HT_{2A} antagonist	Negative symptoms	Patil et al., 2007
glycine transporter inhibitor	Bitopertin (RG1678)	Hoffman-La Roche	$GlyT_1$ inhibitor	Negative symptoms Cognitive impairments	Umbricht et al., 2014
α_7 nicotinic acetylcholine agonist	ABT-126AQW051	AbbVie (Abbott) Novartis	α_7-nACh receptor agonist	Cognitive impairments	Hashimoto, 2015
	EVP-6124 (encenicline)	EnVivo Pharmaceuticals	α_7-nACh receptor agonist	Cognitive impairments	Keefe et al., 2015
D-amino acid oxidase inhibitor	Sodium benzoate (NaBen)	SyneuRx	D-amino acid oxidase inhibitor	Negative symptoms Refractory schizophrenia (combined with clozapine)	Lane et al., 2013
Phosphodiesterase (PDE)-inhibitor	OMS643762; OMS824	Omeros	PDE-10A inhibitor	Cognitive impairments	Kehler & Nielsen, 2011
Nitric oxide (NO)	Sodium nitroprusside	New York University, School of Medicine	NO-releasing drug	Positive symptoms Negative symptoms Cognitive impairments	Hallak et al., 2013

Table A.5 A subset of animal models used to model schizophrenia

Model	Manipulation	Features and Symptoms
developmental	Mouse prenatal immune challenge: human influenza virus (Fatemi et al., 1999)	Decreased open field exploration; PPI deficits; social interaction deficits; reduced reelin expression in cortex layer 1; increased pyramidal cell density; cortical and callosal atrophy; reduced 5-HT levels in cerebellum
	Rat 24 hour maternal deprivation on postnatal day 9 (Ellenbroek et al., 1998)	PPI deficits develops after puberty; in males: neuronal degeneration and increased GFAP+ cells in cerebellum; increased astrocytes in hippocampus; both sexes: altered cannabinoid receptor expression in hippocampus; increased plasma glucocorticoid levels
	Rat antimitotic agent MAM or AraC (Gourevitch et al., 2004	Post-pubertal increased response to amphetamine and MK-801 and PPI deficits; impaired Morris water maze learning, object recognition, and attentional set-shifting; social interaction deficit; decreased brain size and hippocampus weight; increased neuron density in prefrontal cortex; enhanced NAc DA release response to amphetamine; increased firing of dopaminergic neurons
	Rat isolation rearing (Geyer et al., 1993)	Increased amphetamine-induced locomotion and DA release; PPI deficits; novel object recognition and attentional set-shifting deficits; increased social interaction and aggression in males; reduced PFC volume (neuron numbers unchanged); reduced GAT-1 expression; altered accumbal protein expression; reduced accumbal dendritic length; reduced spine density
	Rodent prenatal immune challenge with Polyl:C (Shi et al., 2003)	Reduced open field center exploration; increased response to amphetamine and MK-801; PPI deficits; changes in latent inhibition; reduced escape latency in Morris water maze; novel object recognition deficits; social interaction deficits; enhanced amphetamine-induced DA release; increased hippocampal pyknotic cells and DA turnover; reduced D_2 receptors binding in striatum; reduced reelin- and parvalbumin- expressing neurons in PFC; reduced D_1 receptors in PFC; increased TH expression in striatum; reduced density of cerebellar Purkinje cells; delayed myelination of hippocampus
drug-induced	Treatment with NMDA-receptor antagonists: MK801, PCP, Ketamine (Jentsch and Roth, 1999)	Enhanced locomotor activity and stereotopy; ataxia; short-term PPI deficits; working memory deficits, impaired fear conditioning learning, and long-term spatial memory; impaired passive avoidance learning; social interaction deficits; impaired long-term-potentiation; decreased AMPA receptor density
	Treatment with dopamine agonists: amphetamine or cocaine (Steeds et al., 2015)	Induces stereotyped behaviours, PPI deficits, enhanced dopamine release in response to amphetamine; some limited but long-term cognitive deficits such as attentional and set-shifting impairments
	Treatment with cannabinoid agonists: WIN 55,212-2 (Schneider & Koch, 2003)	Working memory deficits; PPI deficits; persisting behavioural changes such as anhedonia with underlying neurobiological changes

Table A.5 Continuation

Model	Manipulation	Features and Symptoms
Genetically modified	AMPAR $GluR_1$ subunit knockout (Bannerman et al., 2004)	Hyperactive; PPI deficits; spatial working memory deficit; disorganized social behaviours; slowed clearance of striatal extracellular DA
	BACE1 knockout (Savonenko et al., 2008)	Enhanced novelty-induced hyperactivity; MK-801 induced hypersensitivity locomotor effect; PPI deficits; working memory deficits; impaired inhibitory avoidance task learning; altered social recognition; impaired processing of Neuregulin 1; decreased spine density and mature spines in hippocampus
	DISC1 knockout (Hikida et al., 2007)	Increased locomotor activity; PPI deficits; decreased latent inhibition; social interaction deficit; decreased parvalbumin-containing cells in cortex; accelerated neurogenesis and aberrant connectivity
	Dopamine transporter knockout (Spielewoy et al., 2000)	Increased DA; decreased D_1R, D_2R; hyperactive; PPI deficits; impaired adaption to environmental changes in Morris water maze; social behaviour deficits; decreased long-term-depression in hippocampus
	Dysbindin-1, Sandy mouse (Murotani et al., 2007)	Less active in open field and delayed hyperactivity response to novel environment; object recognition deficit; impaired long-term memory retention; working memory deficits; social interaction deficit DTNBP1 mutation with lack of dysbindin protein; increased DA metabolism in different brain regions; reduced snapin in hippocampus; decreased cortical, hippocampal and hypothalamus DA levels; altered transmitter release kinetics
	Heterozygous Reeler mouse (Ballmaier et al., 2002	Increased mesolimbic DA; cross-modal PPI deficits; decreased working memory; social interaction deficit; reduced GAD 67, increased DNA methylation; increased truncated TrkB receptor, decreased BDNF/TrkB signaling in frontal cortex
	STOP knockout (Fradley et al., 2005)	Hyperactive; altered DA neurotransmission in the mesolimbic pathway; PPI deficits; short term memory and spatial learning deficits; long-term memory and object recognition deficits; social learning and recognition deficits; enlarged ventricles; reduced cortex and diencephalon volume; hypoglutamatergic activity
lesion-induced	Neonatal amygdalar lesion (Daenen et al., 2003)	Increased amphetamine or apomorphine induced locomotion; increased acoustic startle response but impaired PPI after lesion on PND 7 but not PND 21; abnormally persistent latent inhibition; impaired spatial alternation and food hoarding; social behaviour deficits after lesion on PND 7 but not 21; increased lateral ventricular volume; reduced density of D_1- and D_2-like receptors and increased DA turnover in mesolimbic regions
	Neonatal ventral hippocampal lesion (Lipska & Weinberger, 2000)	Increased locomotor respons to amphetamine (post-pubertal onset); increased methamphetamine self-administration; PPI deficits; various learning and memory impairments; social behaviour deficits; reduced presynaptic protein and growth factor expression, reduced NMDA receptor expression, impaired DA receptor expression in frontal cortex; impaired maturation of PFC; brain region- and age-specific changes in GABA receptor expression; reduced PFC spine density; increased nicotine sensitivity

Table A.6 A subset of clinical symptoms of schizophrenia and the correlated behaviours in animal models

Symptom in schizophrenia patients	Behavioural changes in animal models
Psychotic symptoms	Increased locomotor activity
Stereotypic behaviours	Stereotyped behaviours i.e. circkling, sniffing, face washing
Stress vulnerability	Changes in locomotor behaviour induced by stress or novelty
Impaired information processing	Deficient prepulse inhibition of the acoustic startle reflex
Attentional deficits	Deficient latent inhibition
Cognitive impairments	Impaired performance in tests for spatial memory or object recognition
Social withdrawal	Reduced social interaction with novel conspecifics

Table A.7 Tests for cognition in humans and animals

Cognitive domain	Construct	Test in humans	Test in animals	Reference
Attention/ Vigilance + Speed of Processing	Selective attention	Latent Inhibition	Latent Inhibition	Lubow, 2005
	Sustained and divided attention/ speed of processing	Continuous Performance test of Attention	Five-choice Serial Reaction Time test	Robbins, 2002
	Sensorimotor gating	Prepulse Inhibition	Prepulse Inhibition	Swerdlow et al., 1994
Reasoning & Problemsolving	Cognitive flexibility, Executive function	Wisconsin Card Sorting test	Reversal learning and Attentional Set-Shifting	Birrel & Brown, 2000
	Decision-making	Iowa Gambling Task	Iowa Gambling Task	De Visser et al., 2011
Working memory	Acquisition and working memory	Rey Auditory-Verbal Learning Test	T-maze delayed alteration task	Hauber & Schmidt, 1989
	Interference control/ span capacity	n-back task	Delayed matching/ non-matching-to-sample task	Dudchenko et al., 2013
	Spatial working memory	Wechsler Memory Scale–III Faces test	8-arm Radial maze test	Olton, 1987
Declarative/ Long-term memory	Episodic/relational memory	Associative Inference Paradigm	Paired Associated Learning	Talpos et al., 2014
Visual learning and memory	Episodic/recognition memory	Brief-Visual Memory test Revised	Novel Object Recognition test	Young et al., 2009
	Visuospatial learning and memory, recognition memory		Morris water maze test	
Social cognition	Social stimuli processing	Bell Lysaker Emotion Recognition Task	Social Discrimination test	Ferguson et al., 2002

Figure A.8 Results from the NOR test with a one-hour ITI between the acquisition and retention trial

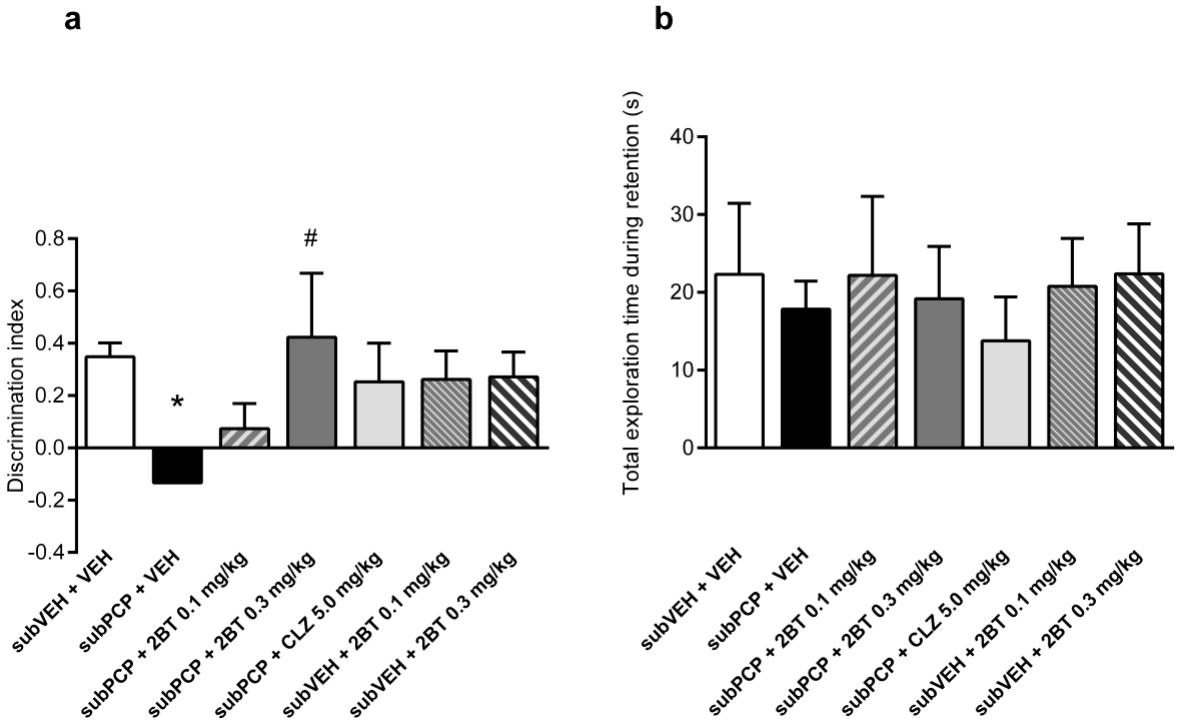

Figure A.9 Effects of 2-bromoterguride (0.1 and 0.3 mg/kg) and clozapine (5.0 mg/kg) after subchronic phencyclidine (5.0 mg/kg) treatment, and 2-bromoterguride (0.1 and 0.3 mg/kg) alone on the **(a)** discrimination index, **(b)** total exploration time during the 3 min long retention trial in the novel object recognition task (NOR) in male rats. 2-Bromoterguride subchronic PCP-induced cognitive impairment in the NOR task. One-way ANOVA detected a significant main effect of treatment on discrimination index ($F(6,50) = 2.97$, $p = 0.0148$). Subchronic PCP treatment caused a sever object recognition memory deficit compared to the subchronic vehicle treated animals ($p < 0.001$). 2-Bromoterguride (0.3 mg/kg) ameliorated the PCP-induced deficit ($p = 0.002$), but not clozapine. Data are expressed as mean + SEM of $n = 6 – 10$ rats per group. $^{*}p < 0.05$ versus controls (subVEH + VEH); $^{\#}p < 0.05$ versus phencyclidine (subPCP + VEH); 2BT: 2-bromoterguride; CLZ: clozapine; PCP: phencyclidine; VEH: vehicle

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Emilia, May 2018

DECLARATION OF ACADEMIC HONESTY

I hereby confirm that the written text herein is solely my own work. I assure that I only used the cited sources and that the thesis has not been submitted in any form for any degree at any other university or institute.

Berlin, 28.11.2018

Emilia Tarland