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KATJA SAVOLAINEN

**NON-CLINICAL MODELING OF
COGNITIVE AND NEGATIVE
SYMPTOMS OF SCHIZOPHRENIA
AS A PART OF THE ASSESSMENT
OF NOVEL DRUGS**

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Katja Savolainen

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ABSTRACT

Schizophrenia is a severely debilitating, multiform neuropsychiatric syndrome. The symptoms of schizophrenia can be divided into three main categories, namely positive, negative and cognitive, although also affective symptoms are common. The positive symptoms, better known as psychotic symptoms, include for example hallucinations and delusions. Social withdrawal, affective flattening and anhedonia are characteristic negative symptoms. The cognitive symptoms consist of a wide range of impairments in cognitive abilities, such as attention, learning, memory, and executive functions. Currently available pharmacotherapies mainly affect the positive symptoms, while there is no effective treatment for the negative and cognitive symptoms causing a loss of social and occupational functioning and reducing the quality of life. In addition to disturbed dopaminergic neurotransmission, hypofunction of glutamatergic *N*-methyl-D-aspartate (NMDA) receptors has been associated with the pathogenesis of schizophrenia, and NMDA antagonists have been shown to induce schizophrenia-like symptoms in humans, as well as in experimental animals. One major challenge in non-clinical drug discovery has been the inadequacy of current animal models to mimic the negative and cognitive symptoms of schizophrenia, i.e. these models have a poor ability to predict the clinical efficacy of novel drugs.

The general objective of this study was to develop and validate animal models mimicking the cognitive and negative symptoms of schizophrenia as a part of the assessment of the efficacy of novel drugs. Schizophrenia-like cognitive and negative symptoms were induced pharmacologically by using an NMDA antagonist phencyclidine (PCP). First, the PCP administration protocols were adjusted in order to induce disturbances in social behavior and deficits in cognitive domains relevant to schizophrenia, i.e. problems in visual learning and memory, and deficits in cognitive flexibility, without inducing non-specific behavioral effects. Second, two rat strains were compared for their pros and cons in the modeling of PCP-induced visual learning and memory deficits in a visually demanding cognitive test. In

addition, the efficacy of established atypical antipsychotics and novel drugs to alleviate PCP-induced schizophrenia-like cognitive and negative symptoms was assessed.

Pigmented Lister Hooded (LH) rats displayed superior visuo-spatial learning and memory in a Morris swim navigation task, and lower repeated PCP doses were needed to impair their performance in comparison to albino Wistar rats which have poorer vision. In a visual discrimination and reversal task, repeated PCP impaired cognitive flexibility in LH rats, and this effect was seen upon repetition of reversals. This protocol could be utilized with a cross-over study design, and thus improve the throughput of the test. In a social interaction test, acute single-dose PCP impaired social interaction in Wistar rats, providing a straightforward model for testing novel drugs. According to earlier observations, atypical antipsychotic drugs ameliorated PCP-induced visuo-spatial learning and memory deficits but were ineffective against PCP-induced social interaction deficits. A novel selective adrenergic α_{2c} receptor antagonist ORM-13070 ameliorated the PCP-induced impairments in both cognitive flexibility and social interaction.

In conclusion, the findings of the present study showed that PCP administration protocol can be adjusted to model impairments in cognitive domains and negative symptoms relevant to schizophrenia. Furthermore, a novel drug ORM-13070 ameliorated the PCP-induced impairments in cognitive flexibility and social interaction indicating that these models could be used in the testing of drugs to treat schizophrenia.

Keywords: Behavioral animal models; Cognition: NMDA antagonist; Non-clinical drug testing; Phencyclidine; Rat; Schizophrenia; Social interaction; Visuo-spatial learning and memory; Visual reversal learning

Savolainen, Katja

Skitsofrenian kognitiivisten ja negatiivisten oireiden nonkliininen mallintaminen uusien lääkeaineiden testaamisessa

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TIIVISTELMÄ

Skitsofrenia on vakava monimuotoinen neuropsykiatrinen oireyhtymä, jonka oireet voidaan jakaa kolmeen pääryhmään: positiivisiin, negatiivisiin ja kognitiivisiin. Myös ahdistus- ja mielialaoireet ovat yleisiä. Skitsofrenian positiivisiin, eli psykoottisiin oireisiin kuuluvat mm. aistiharhat ja harhaluulot. Sosiaalinen vetäytyminen, tunneilmaisun latistuminen sekä kyvyttömyys tuntea mielihyvää ovat tyypillisiä negatiivisia oireita. Kognitiivisiin oireisiin kuuluu häiriöitä lukuisissa kognition osa-alueissa, kuten tarkkaavaisuudessa, muistissa, oppimisessa ja toiminnanohjauksessa. Skitsofrenian positiivisia oireita voidaan hoitaa lääkkeillä, mutta potilaiden toimintakykyä ja elämänlaatua merkittävästi heikentäviin negatiivisiin ja kognitiivisiin oireisiin ei ole tehokasta lääkehoitoa. Dopaminergisen hermovälityksen häiriöiden lisäksi glutamatergisten N-metyyli-D-aspartaatti (NMDA) -reseptoreiden toiminta on häiriintynyt skitsofreniaa sairastavilla. NMDA-reseptorin salpaajilla voidaan aiheuttaa skitsofreniaa muistuttava oirekuva niin ihmisille kuin koe-eläimille. Yksi keskeinen haaste uusien lääkehoitojen kehittämisessä on lääkekehityksen varhaisessa vaiheessa käytettävien eläinmallien puutteellinen kyky jäljitellä skitsofrenian negatiivisia ja kognitiivisia oireita sekä ennustaa lääkkeen tehoa potilaalla.

Tämän tutkimuksen tavoitteena oli kehittää ja validoida skitsofrenian kognitiivisten ja negatiivisten oireiden tutkimuksessa käytettäviä eläinmalleja, jotta ne soveltuvat aiempaa paremmin uusien lääkehoitojen testaamiseen. Oireiden aiheuttamiseen käytettiin tässä tutkimuksessa NMDA-antagonisti fensyklidiiniä (PCP). Aluksi fensyklidiinin annostelua muokattiin siten, että saadaan aiheutettua skitsofrenialle tyypillisiä häiriöitä sosiaalisessa vuorovaikutuksessa ja kognition visuaalisen oppimisen ja muistin sekä kognitiivisen joustavuuden osa-alueilla. Lisäksi arvioitiin kahden rottakannan eroa fensyklidiinillä aiheutetun visuospatiaalisen oppimisen ja muistin häiriöiden mallintamisessa hyvää näkökykyä vaativassa tehtävässä. Lisäksi tutkittiin, voidaanko fensyklidiinillä aiheutettuja

kognitiivisia ja negatiivisia oireita kumota atyyppisillä antipsykooteilla ja uusilla lääkeainekandidaateilla.

Pigmentoidut Lister Hooded (LH) -rotat suoriutuivat paremmin kuin heikompinäköiset albiinot Wistar-rotat hyvää näkökykyä vaativassa visuospatiaalista oppimista ja muistia mittaavassa Morrisin uimasuunnistustehtävässä, minkä lisäksi pienemmät fensyklidiiniannokset riittivät heikentämään LH-rottien kognitiota. Näköerottelukykyä ja kognitiivista joustavuutta mittaavassa testissä fensyklidiinillä saatiin toistuvasti heikennettyä LH-rottien kognitiivista joustavuutta. Tämä mahdollistaa testin käytön vaihtovuoroisessa (engl. cross-over) koeasetelmassa, mikä lisää testin käyttökelpoisuutta. Sosiaalista vuorovaikutusta mittaavassa testissä Wistar-rottien sosiaalista vuorovaikutusta saatiin heikennettyä kerta-annoksella fensyklidiiniä. Tämän suoraviivaisen protokollan avulla on mahdollista tehostaa uusien lääkeainekandidaattien testausta. Aiempien havaintojen mukaisesti atyyppiset antipsykootit vähensivät fensyklidiinillä aiheutettuja visuaalisen tilaoppimisen häiriöitä, mutta niillä ei ollut vaikutusta sosiaalisen vuorovaikutuksen häiriöihin. Uusi lääkeainekandidaatti, selektiivinen adrenergisen α_2 c-reseptorin salpaaja ORM-13070 kumosi sekä fensyklidiinillä aiheutettuja kognitiivisen joustavuuden että sosiaalisen vuorovaikutuksen häiriöitä.

Tulokset osoittivat, että fensyklidiinin annostelua voidaan muokata siten, että saadaan mallinnettua skitsofrenian kannalta keskeisten kognitiivisten osa-alueiden toimintahäiriöitä sekä negatiivisia oireita. Uudella lääkeainekandidaatilla ORM-13070:lla pystyttiin kumoamaan sekä kognitiivisen joustavuuden että sosiaalisen vuorovaikutuksen häiriöitä, mikä viittaa mallien käyttökelpoisuuteen uusien skitsofrenialääkkeiden testaamisessa.

Avainsanat: Fensyklidiini; Kognitiivinen joustavuus; Kognitio; Käyttäytymistestit; NMDA-antagonisti; Nonkliininen lääketutkimus; Rotta; Skitsofrenia; Sosiaalinen vuorovaikutus; Visuospatiaalinen oppiminen ja muisti

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Alapitkä, 16th September 2020

Katja Savolainen

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- II Savolainen K, Ihalainen J, Hämäläinen E, Tanila H and Forsberg MM. Phencyclidine-induced cognitive impairments in repeated touchscreen visual reversal learning tests in rats. *Submitted*.
- III Savolainen K, Ihalainen J, Jalkanen AJ and Forsberg MM. Selective adrenergic alpha2C receptor antagonist ameliorates acute phencyclidine-induced schizophrenia-like social interaction deficits in rats. *Psychopharmacology* 236: 1245–1253, 2019.

**Both authors contributed equally to this work*

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ABBREVIATIONS

5-HT	5-hydroxytryptamine or serotonin	MATRICES	Measurement and Treatment Research to Improve Cognition in Schizophrenia
AMPA	α -amino-3-hydroxy-5-methyl-4-isoazolepropionic acid	mGluR	Metabotropic glutamate receptor
ANOVA	One-way analysis of variance	mRNA	Messenger ribonucleic acid
AR	Adrenergic receptor	MWM	Morris swim navigation task (Morris water maze)
CNS	Central nervous system	nAChR	Nicotinic acetylcholine receptor
COMT	Catechol- <i>O</i> -methyltransferase	NMDA	<i>N</i> -methyl- <i>D</i> -aspartate
DAAO	D-amino acid oxidase	PCP	Phencyclidine
GABA	γ -aminobutyric acid	PFC	Prefrontal cortex
GAD ₆₇	67-kDa isoform of glutamic acid decarboxylase	PPI	Prepulse inhibition
GlyT	Glycine transporter	rmANOVA	Analysis of variance for repeated measures
ITI	Inter-trial interval	SEM	Standard error of mean
LH	Lister Hooded	VTA	Ventral tegmental area
mAChR	Muscarinic acetylcholine receptor		

1 INTRODUCTION

Schizophrenia is a debilitating neuropsychiatric syndrome (Salomon et al. 2015) affecting about 1% of the world's population (Perälä et al. 2007; McGrath et al. 2008). In Finland, there are over 50 000 people suffering from the disorder. Although schizophrenia treatments have improved over the years, today less than every second patient achieves remission where there is only mild symptomatology for at least 6 months; even then, there is an 80% risk of relapse within 5 years, especially when pharmacotherapy is discontinued, leading to reduced social, cognitive, and occupational functioning (Carbon and Correll 2014). A considerable proportion of the patients require chronic treatment, and in Finland, about 6% are hospitalized every year (THL 2019), which combined with the loss of productivity, represents a substantial cost burden to society (Hastrup et al. 2019). Furthermore, it is estimated that the life expectancy of patients with schizophrenia is reduced by as much as 15–20 years when compared with the general population (Tiihonen et al. 2009; Laursen et al. 2014; Hjorthøj et al. 2017).

The symptoms of schizophrenia can be roughly divided into three main categories, namely positive, negative and cognitive (van Os and Kapur 2009; Kahn et al. 2015). The positive symptoms, better known as psychotic symptoms, include hallucinations, delusions, and disorganized speech and behavior. Social withdrawal, affective flattening, anhedonia, and decreased activity of daily living are characteristic negative symptoms. The cognitive symptoms consist of a wide range of impairments in cognitive abilities, such as speed of processing, attention, deficits in learning and memory, reasoning and problem solving, and social cognition. Furthermore, affective symptoms, especially depression and anxiety, are common, but also aggression, hostility and suicidal tendencies can be present.

When considered from a pathophysiological viewpoint, schizophrenia can be described as a disorder, where there is abnormal processing of information in the brain. This abnormal processing may result from alterations in neuron to neuron communication and functioning of different neurotransmitter systems, including dopaminergic, glutamatergic, γ -aminobutyric acid (GABA) -ergic and serotonergic nerves. Furthermore, some gross pathological changes have been observed, such as reductions in total gray and white matter volume in prefrontal and temporal regions and increased ventricular volume (Keshavan et al. 2020).

The etiology of schizophrenia still remains elusive. However, a diathesis-stress model is generally accepted (Walker and Diforio 1997; Walker et al. 2008). According to this hypothesis, genetic and environmental risk factors, such as complications, viral infections or malnutrition during fetal life and at birth, affecting early brain development combined with biological adaptation to internal or external stress factors, such as crisis of life, conflicts, insomnia, alcohol or drugs, trigger the disorder; this occurs typically during late adolescence or early adulthood. However, the prodromal phase consisting of subtle changes in cognitive and social functioning and

emerging negative symptoms may begin over a decade before the manifestation of the first signs of psychosis; these have detrimental effects on children and adolescents (Kahn and Keefe 2013; Millan et al. 2014). Furthermore, these symptoms tend to persist also after the acute psychotic phase.

Although current pharmacotherapies and psychosocial interventions help to control the psychotic symptoms, there is a significant unmet medical need in the treatment of negative and cognitive problems since both typical and atypical antipsychotic drugs have shown only limited efficacy in the alleviation of these symptoms (Meltzer and McGurk 1999; Kirkpatrick et al. 2006; Davidson et al. 2009; Hanson et al. 2010; Citrome 2014; Sarkar et al. 2015; Veerman et al. 2017). The inadequate response to the available treatments causes a decline in the social and cognitive capacity of the patients leading to a loss of occupational functioning and a reduced quality of life (Fett et al. 2011; Fervaha et al. 2014; Robertson et al. 2014). Schizophrenia research has thus recently refocused on treating the cognitive dysfunction and negative symptoms.

The non-clinical development of novel pharmacotherapies for schizophrenia utilizes valid animal models. Currently, there is no method for inducing the whole spectrum of the schizophrenia syndrome in experimental animals such as rodents. However, it is possible to approach the syndrome by mimicking some of the symptoms and the associated pathophysiological changes using glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) or ketamine. These compounds have been shown to induce all domains of schizophrenia-like symptoms in healthy individuals as well as exacerbating the symptoms in patients with schizophrenia (Luby et al. 1959; Cohen et al. 1962; Itil et al. 1967; Krystal et al. 1994). Because of their ability to induce schizophrenia-like symptoms also in rodents, NMDA antagonists have become an important tool for modeling schizophrenia in non-clinical studies.

The major challenge in the drug discovery for cognitive dysfunction and negative symptoms of schizophrenia is the inadequate understanding of the pathological processes underlying these symptoms, which also hinders the improvement of the disease models. Although many of the available models mostly predict the efficacy of new drugs against the positive symptoms, their ability to predict the efficacy against cognitive dysfunction and negative symptoms is poorly established. In the following review of the literature, the divergent methods for modeling cognitive impairment and negative symptoms of schizophrenia will be discussed from the perspective of non-clinical drug discovery and development, focusing especially on NMDA antagonist-induced models. Finally, the effects of novel potential drugs to treat the negative and cognitive symptoms of schizophrenia and the clinical relevance of NMDA antagonist rodent model in their assessment will be discussed. The experimental part concentrates on refining the PCP rodent model for schizophrenia.

2 REVIEW OF THE LITERATURE

2.1 SYMPTOMS OF SCHIZOPHRENIA – FOCUS ON COGNITIVE AND NEGATIVE DOMAINS

Patients with schizophrenia have heterogenous symptom dimensions (van Os and Kapur 2009; Kahn et al. 2015). The symptom profiles vary along the trajectory of the disease in individual patients. Furthermore, the heterogenous nature of schizophrenia can be traced to the onset, course as well as the outcome. However, none of the symptom domains, positive, negative or cognitive, are unique to schizophrenia; they have been observed also in other disorders such as in major depression (van Os and Kapur 2009; Millan et al. 2012; Foussias et al. 2014). A schizophrenia diagnosis is based on the criteria proposed by International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10; World Health Organization 1992) or Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5; American Psychiatric Association 2013). The diagnosis criteria are based on the long-term occurrence of positive and/or negative symptoms, which are not preceded by mood disorders, medications, or substance abuse (Table 1).

Positive symptoms of schizophrenia are described as excess of thoughts and emotions than those normally present. Auditory, especially threatening or accusatory, hallucinations are typical, although they can occur also in all other sensory modalities (Tandon et al. 2009). Delusions of reference and persecutory delusions are the most common, but also delusions of control, thought insertion, withdrawal and broadcasting are widespread. Incoherence in speech is evident as disorganization in content, frequent shifts of topics and the usage of irrelevant or meaningless words. Disorganized behavior can be excessive motor activity, unusual and stereotypic movements, catatonia and inappropriate affect or attire. The positive symptoms can be usually managed with the currently available pharmaco- and psychotherapies.

Cognitive impairment and negative symptoms are prevalent in patients with schizophrenia in both the prodromal phase and throughout the course of the illness (reviewed for example in Kahn and Keefe 2013; Millan et al. 2014). They have been shown to gradually develop in the decade before there is any manifestation of the first psychotic episode. Cognitive impairment and negative symptoms respond poorly to treatments effective for psychotic symptoms, and they tend to persist after the acute psychotic phase i.e. during the maintenance treatment period. Unfortunately, the incidence of these symptoms has been linked to poorer prognosis and functional outcome of the patients (Fett et al. 2011; Fervaha et al. 2014; Robertson et al. 2014).

Table 1. The main diagnostic criteria according to ICD-10 and DSM-5.

ICD-10	DSM-5
<p>Criterion A. At least one clear symptom, or if less clear symptomatic, two of the following:</p> <ul style="list-style-type: none"> - Echoing, insertion, withdrawal or broadcasting of thought - Delusion of control or passivity - Hallucinatory voices - Persistent delusions <p>or</p> <p>Criterion B. At least two of the following:</p> <ul style="list-style-type: none"> - Significant and consistent change of personal behavior - Persistent hallucinations in any modality - Incoherent or irrelevant speech - Catatonic behavior - Negative symptoms which are not due to depression or antipsychotic drug treatment <p>Criterion C. Persistent for most of the time for at least 1 month</p> <p>Criterion D. Disorder is not caused by substance use or organic brain disease</p>	<p>Criterion A. Two (or more) of the following (at least one of these should include 1–3):</p> <ol style="list-style-type: none"> 1. Delusions 2. Hallucinations 3. Disorganized speech 4. Grossly disorganized or catatonic behavior 5. Negative symptoms (i.e. diminished emotional expression or avolition) <p>Criterion B. One or more major areas of functioning are markedly below the level achieved prior to the onset.</p> <p>Criterion C. Continuous signs of the disturbance persist for at least 6 months; must include criterion A symptoms for at least 1 month.</p> <p>Criterion D. Schizoaffective disorder and depressive or bipolar disorder with psychotic features ruled out.</p> <p>Criterion E. The disturbance is not caused by substance use or general medical condition</p> <p>Criterion F. If there is a history of autism spectrum disorder or other communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least 1 month (or less if successfully treated)</p>

Abbreviations: ICD-10 – International Statistical Classification of Diseases and Related Health Problems, 10th Revision; DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

Several international consortia have been formed to tackle the challenges in the treatment of cognitive and negative symptoms, e.g., the United States National Institute of Mental Health has funded “Measurement and Treatment Research to Improve Cognition in Schizophrenia” (MATRICS), “Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia” (CNTRICS) and “Treatment Units for Research on Neurocognition in Schizophrenia” (TURNS) programs and the EU’s Innovative Medicine Initiative (IMI) sponsored a “Novel Methods leading to New Medications in Depression and Schizophrenia” (NEWMEDS). The MATRICS initiative reached a consensus about the cognitive domains that form the core of cognitive impairments typically found in patients with schizophrenia. These eight impaired cognitive domains are specified in Table 2 (Nuechterlein et al. 2004). Another result of the MATRICS initiative was a standardized method for the assessment of cognition-enhancing drugs in clinical trials. The MATRICS Consensus Cognitive Battery (MCCB) consists of ten tests assessing all but one of the cognitive domains present in patients; verbal comprehension was omitted from the test battery owing to its extreme resistance to

change (Nuechterlein et al. 2008). In addition, non-clinical counterparts, i.e. rodent behavioral tests, for the assessment of six of these seven remaining domains are shown in Table 2.

Table 2. Characteristics of the key cognitive domains of schizophrenia (Nuechterlein et al. 2004) and tests for the assessment of them in both humans and rodents (Nuechterlein et al. 2008; Young et al. 2009; Nikiforuk 2018).

Domain	Core characteristics	MCCB tests	Non-clinical tests
Speed of processing	Reduced information processing speed. Affects also the performance in other cognitive domains.	Trail making test, part A Brief assessment of cognition in schizophrenia, symbol coding subtest Category fluency test, animal naming	5-Choice serial reaction-time task (5-CSRTT) Olfactory discrimination
Attention/vigilance	Reduced ability to focus attention on relevant, and to ignore less relevant environmental stimuli.	Continuous performance test (CPT), identical pairs version	5-CSRTT 5-Choice continuous performance test (5C-CPT) Rodent continuous performance test (rCPT) Sarter's sustained attention task
Working memory	Declined maintenance of information for a short period of time to guide thought processes (easily distracted and interfered). Affects also other cognitive processes.	Wechsler memory scale, 3 rd ed., spatial span subtest (nonverbal) Letter-number span test (verbal)	Odor-span task Spatial span task Continuous alternation task Discrete paired-trial alternation Radial arm maze Delayed non-match to position (DNM(T)P) Trial unique non-match to location (TUNL)
Verbal learning and memory	Impairments in processes of acquiring, retaining and recalling of verbal material.	Hopkins verbal learning test–revised, immediate recall (three learning trials only) (verbal learning)	–
Visual learning and memory	Impairments in processes of acquiring, retaining and recalling of visual information.	Brief visuo-spatial memory test–revised	Novel object recognition (NOR) Paired-associate learning (PAL) Barnes maze Morris swim navigation

Table 2. (Continued)

Reasoning and problem solving (cognitive flexibility, executive control)	Impairments in rule generation and selection in response to stimuli and overcoming a habit. Hinders the adaptation of behavior to everyday environmental changes.	Neuropsychological assessment battery, mazes subtest	Attentional set-shifting task (ASST) Cross-maze set-shifting task Reversal learning tasks
Verbal comprehension	Impaired ability to listen to orally given information, remember it, understand it and use the information across a range of tasks.	–	–
Social cognition	Impaired understanding of behaviors, gestures, facial expressions and mental states of others. Contributes also to the impaired understanding of verbal and non-verbal communication. Strongly affects social behavior (asociality) of patients with schizophrenia.	Mayer-Salovey-Caruso emotional intelligence test, managing emotions branch	Social preference (3-chambered sociability task) Social recognition Social discrimination USV emission

Abbreviations: 5-CSRTT – 5-Choice serial reaction-time task, MCCB – MATRICS Consensus Cognitive Battery, USV – ultrasonic vocalization

Whereas positive symptoms are described as an excess of thoughts and emotions normally present, negative symptoms could be described as a reduction or a lack of such behaviors, thoughts and emotions (Millan et al. 2014). According to the current understanding, negative symptoms of schizophrenia include blunted affect, alogia, amotivation (avolition), asociality and anhedonia (Kirkpatrick et al. 2006; Millan et al. 2014). However, according to factor analysis studies, negative symptoms can be further divided into two major sub-domains: diminished emotional expression and avolition (described in more depth in Table 3) (Blanchard and Cohen 2006). It has been hypothesized that these two sub-domains may have differential neural underpinnings and treatment responses, and thus, should be examined separately (Marder and Kirkpatrick 2014; Millan et al. 2014). The negative symptom domains and non-clinical behavioral tests for the assessment of different sub-domains of avolition are shown in Table 3.

Table 3. Characteristics of the sub-domains of negative symptoms (modified from Millan et al. 2014) and tests for the assessment of them in animals (Barnes et al. 2014; Wilson and Koenig 2014).

Sub-cluster	Sub-domain (alternative terms)	Core characteristics	Non-clinical tests
Diminished emotional expression	Blunted affect (affective flattening, blunted expression)	Reduced intensity and range of emotional expression as manifested via vocal and non-verbal modes of communication including intonation (prosody), facial expression, hand-gestures and body movements.	?
	Alogia (poverty of speech)	Decreased quantity of speech, reduced spontaneous speech and loss of conversational fluency.	–
Avolition	Amotivation (loss of volition)	Deficits in the initiation and maintenance of goal-directed behaviors like work, study, sport, personal hygiene and daily tasks, especially when requiring an effort (cognitive or physical) and significant organization. Also, deficits in desire to undertake such activities. Related to apathy and lack of energy.	Fixed ratio Progressive ratio Operant or T-maze effort-related choice
	Anhedonia (reduced ability to experience or anticipate pleasure)	The looking forward to a reward, recreational or other pleasurable experience (“wanting”) is more markedly and consistently impaired (anticipatory anhedonia) than the appreciation (“liking”) of the experience itself (consummatory anhedonia) as opposed to depression.	Sucrose consumption
	Asociality (social withdrawal)	Diminished interest in, motivation for, and appreciation of social interactions with others, like family and friends. Also, loss of interest in intimate (sexual) relationships independent of any somatic problems. For children, may correspond to loss of interest in playing together.	Social interaction test

Negative symptoms diverge in terms of origin and course (Millan et al. 2014). Primary negative symptoms, also known as deficit symptoms, are inherent in the disease process itself, whereas secondary, or non-deficit symptoms are a reaction to the psychotic process and/or a result from the treatment, environmental factors or comorbidities. Negative symptoms can be variable or stable and also transient or enduring: 20–40% of patients have persistent negative symptoms (Mäkinen et al. 2008). Although primary negative symptoms often are persistent and stable, it seems that secondary symptoms are more transient owing to changes in the underlying conditions; they can be difficult to distinguish, and they may also coexist (Millan et al. 2014). Clinical trials often do not discriminate between primary and secondary negative symptoms. This may result in overestimating the effectiveness of antipsychotics or novel drugs on primary negative symptoms, since secondary negative symptoms can be alleviated by effectively treating psychotic symptoms and

comorbidities, or simply by adjusting the ongoing treatment. Thus, clinical trials should be carefully designed to highlight the effects of treatment specifically on primary negative symptoms (Kirkpatrick et al. 2006).

There are several different tools for the assessment of the negative symptoms in patients with schizophrenia. Some of the most widely used tools date back to the 1980s and the 1990s i.e. the Positive and Negative Syndrome Scale (PANSS), the Scale for the Assessment of Negative Symptoms (SANS), and the 16-item Negative Symptoms Assessment (NSA-16) scale (Marder and Kirkpatrick 2014). However, our knowledge of the negative symptoms has evolved considerably over the last 30 years (Malaspina et al. 2014; Dollfus and Lyne 2017) and thus, new scales such as Clinical Assessment Interview for Negative Symptoms (CAINS), and Brief Negative Symptom Scale (BNSS) have been developed to answer better the current understanding of negative symptoms of schizophrenia according to the MATRICS consensus statement (Kirkpatrick et al. 2006; Marder and Kirkpatrick 2014).

There are behavioral measures for cognitive and negative-like symptoms also in rodents (Tables 2 and 3) (Young et al. 2009; Barnes et al. 2014; Wilson and Koenig 2014; Nikiforuk 2018). All cognitive domains included in the MATRICS Consensus Cognitive Battery, except verbal learning and memory, can also be assessed in rodents with a substantial resemblance in underlying neural mechanisms as compared to humans (Young et al. 2009; Nikiforuk 2018). It is evident that negative-like symptoms are more difficult to measure. Motivational and social impairments can be modeled in rodents, whereas blunted affect is difficult and alogia may be practically impossible to model. Furthermore, measures of anhedonia may be difficult to separate from depressive-like behaviors, for example the sucrose consumption test is sensitive also for antidepressant drugs. The neural correlates in human and rodent tasks also differ to some extent (Barnes et al. 2014; Wilson and Koenig 2014).

2.2 ALTERATIONS IN NEUROTRANSMITTER SYSTEMS UNDERLYING COGNITIVE AND NEGATIVE SYMPTOMS

Many structural and functional changes in the brain are observed in schizophrenia (Keshavan et al. 2020). Alterations in different neurotransmitter systems contribute to dysfunctional brain networks, and hence, disturbances in processing of information. Here, the most relevant neurotransmitter systems are reviewed.

2.2.1 Dopamine

The observations of the ability of dopaminergic compounds such as amphetamine to induce schizophrenia-like psychotic symptoms were clues leading to the implication that there was dopamine dysregulation in schizophrenia (for a review, see Howes et al. 2015). This dopamine hypothesis was further supported by the finding that the effects of antipsychotic drugs were based on the blockade of dopaminergic receptors.

The five human dopamine receptors can be divided into D₁-like, including D₁ and D₅, and D₂-like receptors, including D₂, D₃ and D₄ (Beaulieu and Gainetdinov 2011). D₁-like receptors are mainly postsynaptic, whereas D₂-like receptors are located both pre- and postsynaptically. The D₂ receptor has two common splice variants, of which D₂-short (D_{2S}) is predominantly expressed in presynaptic locations, whereas D₂-long (D_{2L}) is postsynaptically expressed. Dopaminergic overactivity of D₂ receptors in mesolimbic projections from the midbrain ventral tegmental area (VTA) to the nucleus accumbens has been associated with the positive symptoms, whereas dopaminergic hypofunction of D₁ receptors in target areas of mesocortical projections from VTA to the frontal cortex has been linked with cognitive and negative symptoms of schizophrenia (Schwartz et al. 2012; Howes et al. 2015). There are also alterations in the nigrostriatal dopaminergic pathway with its projections from substantia nigra to dorsal striatum in schizophrenia, as striatal dopamine synthesis and release capacity have been found to be elevated in patients with schizophrenia as compared to healthy controls (McCutcheon et al. 2020). The striatal dopaminergic dysfunction has been speculated to contribute to positive, but also negative and cognitive symptoms (Howes et al. 2015). However, the dopaminergic tuberoinfundibular pathway, which has projections from hypothalamus to the pituitary gland and is involved in controlling the secretion of hormones, e.g. prolactin, is considered to remain relatively unaltered (Schwartz et al. 2012; Howes et al. 2015).

The dopamine hypothesis was later found to be a too narrow approach to pathophysiology of schizophrenia. This is partly supported by treatment resistance, which occurs in about every third patient with schizophrenia despite adequate D₂ receptor occupancy with antipsychotic drugs (Howes et al. 2015). The inadequacy of the hyperdopaminergic models to generate deficits in cognitive and negative symptom domains further emphasized the limitations of the dopamine hypothesis.

2.2.2 Glutamate

NMDA receptor antagonists, such as PCP and ketamine, have been shown to induce all domains of schizophrenia-like symptoms in healthy individuals and to exacerbate these symptoms in patients with schizophrenia (Luby et al. 1959; Cohen et al. 1962; Itil et al. 1967; Krystal et al. 1994). Thus, a hypofunction of the NMDA receptor-mediated neurotransmission was hypothesized to be associated with the pathogenesis of schizophrenia (Olney and Farber 1995; Javitt 2010; Schwartz et al. 2012) and the severity of cognitive impairment (Bustillo et al. 2011). Furthermore, a considerable number of genetic vulnerabilities associated with increased risk of schizophrenia are related to aberrations in glutamate neurotransmission and receptor function (Schwartz et al. 2012).

Glutamatergic pathways include descending projections from cortical pyramidal neurons to the brainstem modulatory neurotransmitter centers, such as raphe nuclei, VTA and substantia nigra, and to the downstream subcortical structures (striatum, nucleus accumbens, thalamus) of equivalent cerebral hemisphere (Schwartz et al.

2012). An ascending thalamocortical pathway originates from thalamus and extends to cortical pyramidal neurons. In addition, there are known to be intra-cortical glutamate projections. Glutamate signaling has been associated with synaptic plasticity and learning and memory processes, but when overactivated, it may induce excitotoxicity in central nervous system (CNS) neurons, perhaps being implicated in the reduced gray matter volume found in schizophrenia (Olney and Farber 1995).

Glutamate exerts its effects through a variety of ionotropic and metabotropic receptors. Ionotropic receptors are also further divided into three subtypes: NMDA, AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate receptors (Kew and Kemp 2005). These are responsible for fast excitatory postsynaptic currents via opening Ca^{2+} , Na^{+} and, to some extent, K^{+} permeable ion channels. The opening of the NMDA receptor channel requires the binding of two co-agonists, glycine and glutamate, to their own binding sites, which makes it unique among ligand-gated ion channels. Currently, also the metabotropic receptor family is known to consist of eight G-protein-coupled receptors (mGluR₁₋₈), which are further divided into three groups modulating excitability via pre- and postsynaptic, together with glial mechanisms (Schoepp 2001; Kew and Kemp 2005). Group I receptors, mGluR₁ and mGluR₅, positively modulate glutamate and GABA transmission. Group II receptors are inhibitory consisting of mGluR₂ and mGluR₃. Group III receptors are also inhibitory, modulating negatively glutamate and GABA transmission.

An NMDA receptor hypofunction in inhibitory parvalbumin-positive GABAergic interneurons may be one factor, and also abnormalities in glutamatergic signaling in cortico-cortical circuits have been observed, which lead to a disinhibition of pyramidal cell firing disrupting the homeostatic excitatory/inhibitory balance (Pratt et al. 2008; Schwartz et al. 2012; McCutcheon et al. 2020) (Figure 1). Cortical neuronal activity can be detected as oscillations resulting from synchronized firing (Gonzalez-Burgos et al. 2015; McCutcheon et al. 2020). Gamma oscillations (~30–80 Hz) seem to be the most important for many cognitive processes, such as working memory and executive control, as they tend to intensify during cognitive tasks in healthy individuals. The dyssynchrony of cortical neuronal activity is evident as attenuated gamma oscillations when performing cognitive tasks in patients with schizophrenia as compared to healthy controls (Figure 1).

Glutamate signaling affects also dopaminergic neurotransmission, as the descending glutamatergic projections innervate areas of dopaminergic activity, including striatum, VTA and substantia nigra. The excitatory/inhibitory imbalance in descending glutamatergic projections leads to impaired regulatory control at these subcortical areas. However, focusing simply on dysfunctional NMDA receptors is also likely to be an excessively narrow approach as there are multiple ways to affect glutamate, and the subsequent dopamine functioning (Schwartz et al. 2012).

A Healthy control

B Individual with schizophrenia

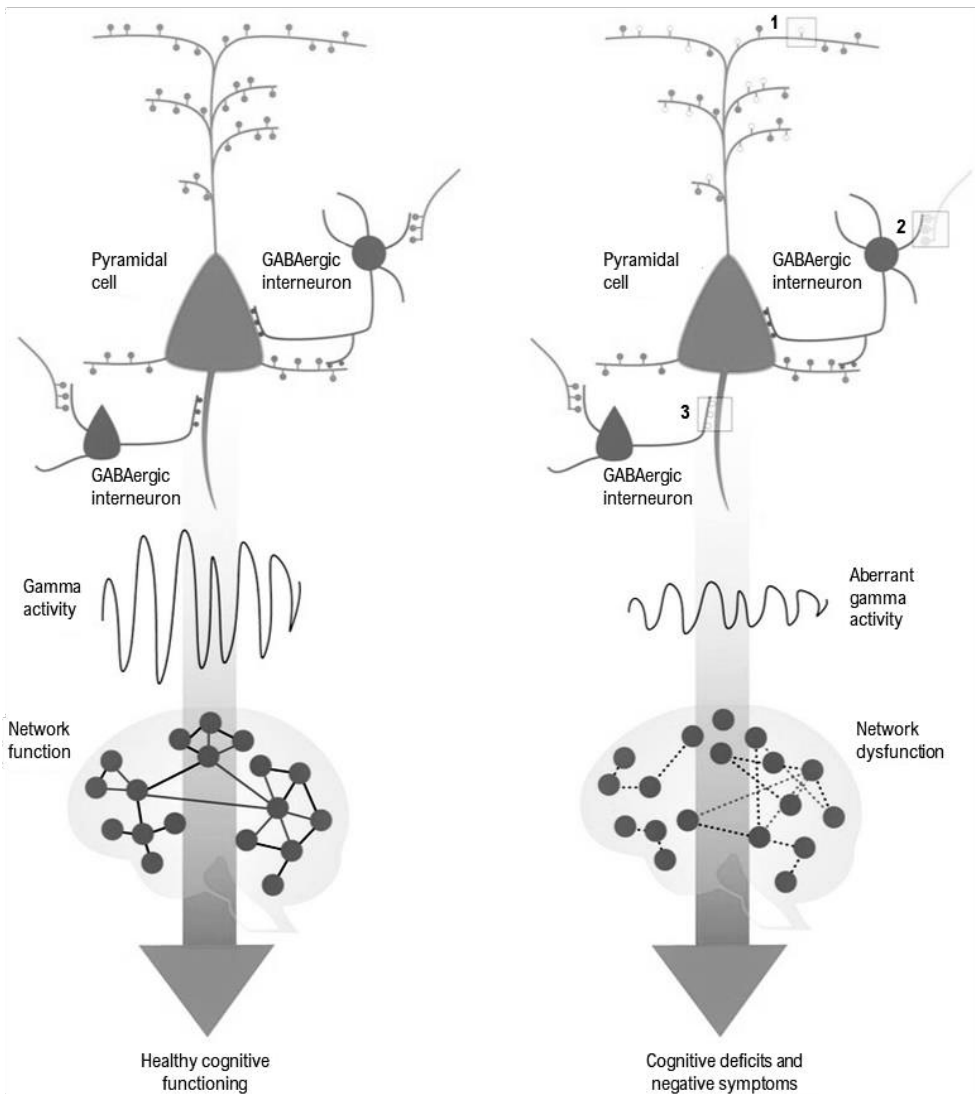


Figure 1. (A) The interplay between cortical excitatory pyramidal neurons and inhibitory GABA interneurons generates gamma oscillations underlying functional brain networks. (B) Several mechanisms within these cortical circuits are altered in individuals with schizophrenia. (1) The loss of pyramidal cell dendritic spines reduces excitatory activity. (2) The excitatory input to GABAergic interneurons is reduced, leading to disinhibition of pyramidal cells. (3) Also GABAergic interneuron inhibition of pyramidal cells is reduced. These changes are believed to lead to aberrant gamma oscillations and further, dysfunctional brain networks contributing to the cognitive and negative symptoms of schizophrenia (McCutcheon et al. 2020). Reproduced with permission from JAMA Psychiatry. 2020. 77: 201–10. Copyright©2020 American Medical Association. All rights reserved.

2.2.3 Other neurotransmitter systems

Serotonin

The role of serotonergic (or 5-hydroxytryptamine, 5-HT) neurotransmission in schizophrenia gained interest as the behavioral effects of hallucinogenic drugs, such as D-lysergic acid diethylamide (LSD), hinted at the involvement of dysfunctional serotonergic neurotransmission in schizophrenia (Aghajanian and Marek 2000). This was further supported by the fact that most atypical antipsychotic drugs bind to serotonergic receptors, especially 5-HT_{2A} and 5-HT_{1A} (Meltzer et al. 2003).

Serotonergic neurons in the CNS are involved in the control of mood, aggression and cognition as well as in the regulation of many physiological functions, such as sleep-wake cycle, appetite, nociception and hypothalamic hormone excretion. The serotonin receptor family is divided into seven major subfamilies (5-HT₁₋₇), and many of them are further divided into subtypes, such as 5-HT_{2A-C}. Serotonergic projections from the medial and dorsal raphe nuclei are spread throughout the brain.

Serotonergic system has been proposed to contribute to schizophrenia via a modification of dopamine transmission (Meltzer et al. 2003; Stahl 2018). An overactivation of 5-HT_{2A} receptors in the cortical regions has been closely associated with mesolimbic dopaminergic overactivity, and thus, psychotic symptoms. However, also the glutamatergic system is involved as the 5-HT_{2A} overactivation is mediated via downstream signaling of glutamatergic pyramidal cells to the VTA (Aghajanian and Marek 2000; Meltzer et al. 2003; Stahl 2018). These cortical pyramidal cells express also inhibitory 5-HT_{1A} receptors. Furthermore, at least 5-HT_{1A}, -2A-C, -3, -6 and -7 receptors are differently modulated by atypical antipsychotic drugs, which may partly explain the differences in the efficacy and adverse effect profiles of these agents (Meltzer et al. 2003).

GABA

The synthesis and reuptake of GABA are decreased in a subpopulation of GABAergic neurons containing the calcium-binding protein parvalbumin in the dorsolateral prefrontal cortex (PFC) of patients with schizophrenia (Lewis and Moghaddam 2006; Pratt et al. 2008). Reduced expressions of messenger RNAs (mRNAs) for the GABA synthesizing enzyme, the 67-kDa isoform of glutamic acid decarboxylase, GAD₆₇, and the GABA membrane transporter GAT1, along with decreased levels of these proteins have been observed. Furthermore, the levels of parvalbumin are reduced. The activity of GABAergic receptors at pyramidal neurons is altered in response to diminished GABAergic tonus (Figure 1). The activity of parvalbumin-positive GABAergic basket cells, which is one type of the GABAergic interneurons, is most strongly coupled with the gamma oscillation cycle, albeit disturbances also in other types of GABAergic interneurons occur in schizophrenia (Gonzalez-Burgos et al. 2015). The dyssynchronization of pyramidal cell activity, which can be detected as attenuated gamma oscillations is suggested to be involved

in the cognitive deficits encountered in schizophrenia (Gonzalez-Burgos et al. 2015; McCutcheon et al. 2020). However, the precise role of GABA dysfunction in schizophrenia remains to be clarified.

Acetylcholine

The cholinergic system is associated with attention, learning and memory, but also with sleep, arousal, body weight and metabolism (Barak 2009). The cholinergic neurones also regulate dopamine, serotonin and noradrenaline neurotransmission. Acetylcholine exerts its effects through metabotropic muscarinic receptors (mAChRs) and ionotropic nicotinic receptors (nAChRs). The metabotropic receptor family is further divided into 5 receptors (M_{1-5}), of which M_1 is especially associated with cognition. Pentameric nAChRs are formed from six different α (α_{2-7}) and three β (β_{2-4}) subunits. The most important subtypes for cognition are heteromeric $\alpha_2\beta_4$ and homomeric α_7 receptors. Thus, cholinergic neurotransmission is closely related to cognition.

Receptor binding studies, experiments examining α_7 receptor protein expression and mRNA levels of gene encoding the α_7 nAChR have shown decreased receptor activity in frontal cortex, thalamic nuclei and hippocampus of patients with schizophrenia (Wallace and Bertrand 2015). Furthermore, the heavy smoking reported among patients with schizophrenia has been considered as an attempt at self-medication. Finally, mAChR antagonists have been shown to induce a psychotic state with a range of cognitive symptoms (Barak and Weiner 2011; Steeds et al. 2015).

Endocannabinoids

Use of cannabis, and especially its psychotomimetic constituent, the cannabinoid CB₁ receptor agonist δ^9 -tetrahydrocannabinol (THC), can induce all of the domains of schizophrenia-like symptoms in healthy individuals and exacerbate the symptoms in patients with schizophrenia (Steeds et al. 2015). Moreover, cannabis use has been associated with an increased risk to develop schizophrenia (Moore et al. 2007). The endocannabinoid system has a regulatory role over many neurotransmitter systems, and it is associated with attention, learning and memory. Furthermore, endocannabinoid system alterations have been observed in schizophrenia. Thus, a dysfunctional endocannabinoid system has been suggested to contribute to schizophrenia pathogenesis.

Noradrenaline

Both an overactivity and an underactivity of noradrenaline system has been suggested to play a central role in positive and negative symptoms of schizophrenia, respectively (Yamamoto and Hornykiewicz 2004). Noradrenaline has been shown to affect a variety of CNS functions such as sleep-wake cycle, arousal, mood, stress,

attention, learning and memory. Noradrenaline projections from locus coeruleus in pons spread widely throughout the CNS. Adrenergic α_2 receptors (ARs) are located both pre- and postsynaptically modulating the release of noradrenaline, but also other neurotransmitters via presynaptic heteroreceptors in the CNS (Langer 2015). Furthermore, the α_2 ARs are important modulators of PFC functioning, and thus, are involved in mediating cognitive processes (Berridge and Spencer 2016). The α_2 AR subtypes are differently distributed in CNS; the postsynaptically located α_{2B} subtype is found primarily in thalamus, about 10% of the α_2 ARs are of the α_{2C} subtype located mainly in the striatum, hippocampus, olfactory tubercle and cortex, while the remaining 90% are α_{2A} ARs which are widespread throughout the CNS (Scheinin et al. 1994; Rosin et al. 1996; Bücheler et al. 2002). The α_{2A} ARs are responsible for modulation of neurotransmission during high levels of endogenous noradrenergic activity, while α_{2C} ARs exert a more subtle modulation of noradrenergic balance during times of low endogenous noradrenergic tone (for a review, see Uys et al. 2017). Furthermore, they appear to exert partly opposing effects in the CNS; whereas α_{2A} AR agonists have been shown to improve spatial and working memory, similar effects have been connected to α_{2C} AR antagonism in rodent models.

2.3 PHARMACOLOGICAL ANIMAL MODELS OF COGNITIVE AND NEGATIVE SYMPTOMS OF SCHIZOPHRENIA

Several animal models mimic some of the features of the complex and heterogeneous syndrome of schizophrenia. Validity is the most important feature to be considered when selecting the most relevant model. Construct validity means that the model reproduces or mimics core pathological mechanisms. Face validity means that the model reproduces the symptoms of the human disease. Predictive validity means that the effects of pharmacotherapy in the experimental animals correlates with clinical findings in patients, or that the model can discover potential novel therapeutics (Figure 2) (Jones et al. 2011). The current inadequate understanding of neurobiological mechanisms of the schizophrenia syndrome complicates the assessment of construct validity. Full face validity cannot be reached since some symptoms, such as hallucinations, delusions, and verbal learning, are unique to humans; in other words, they cannot be determined in animals. The most important, and yet, the hardest feature to accomplish, is predictive validity. There is no efficacious treatment for negative and cognitive symptoms available in the clinical practice, and thus the models being used to mimic cognitive and negative symptoms of schizophrenia lack a real positive control (Wilson and Terry 2010). Nevertheless, a reasonable level of construct and face validity can be accomplished, albeit the predictive validity of these models remain to be resolved (Figure 2) (Jones et al. 2011).

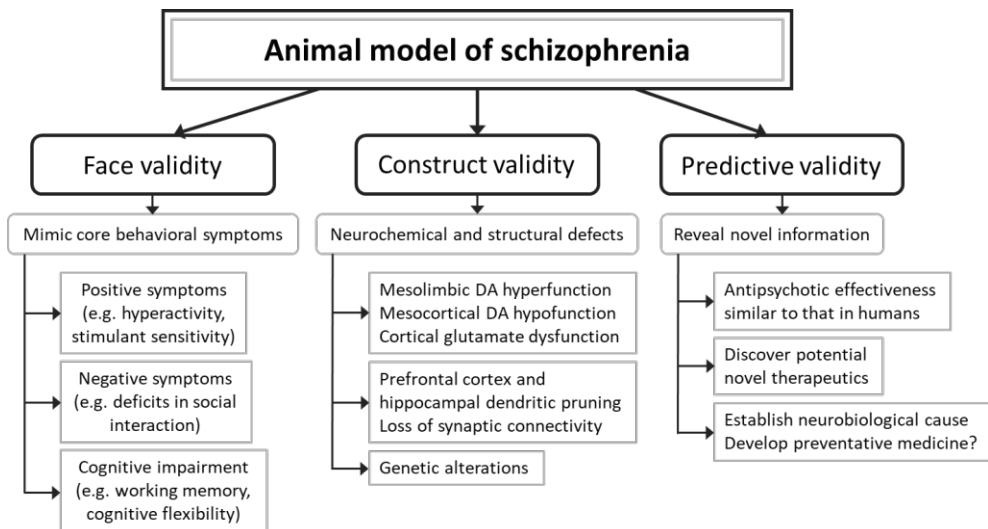


Figure 2. Key changes relevant to schizophrenia in an animal model with face, construct and predictive validity (modified from Jones et al. 2011).

2.3.1 General overview of animal models of schizophrenia symptoms

The methods for modeling cognitive impairment and negative symptoms associated with schizophrenia in animals can be divided into three main categories: genetic, developmental and pharmacological models. Additionally, it is possible to use so called dual- or triple-hit models, which combine two or more different approaches, for example genetic and developmental or developmental and pharmacological, to better replicate the complex etiology of schizophrenia. These methods have been used especially in rodents, but also in non-human primates and lately even in zebrafish. Nevertheless, all animal models consist of two components; the first is a manipulation which reproduces some features of a disease, and the second is a test which measures symptoms induced with the manipulation. For example, by refining the manipulations and carefully selecting suitable behavioral tests, it is possible to produce a condition where positive-like symptoms interfere as little as possible with the symptoms under investigation.

Genetic models

The genetic background of schizophrenia is highly heterogeneous; next-generation sequencing (NGS) and genome-wide association studies (GWAS) have revealed numerous genetic vulnerabilities contributing to schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Nonetheless, most of these genetic aberrations account for only a very minor increase in the schizophrenia risk (Sullivan et al. 2012; Brainstorm Consortium et al. 2018).

The genetic vulnerabilities found in patients with schizophrenia are linked to neuronal development, synaptic function, immune responses, and dopaminergic, glutamatergic and calcium signaling (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). However, the precise consequences of these genetic vulnerabilities to the biological processes relevant to schizophrenia remain to be resolved. Furthermore, clarifications are needed into whether the effects are analogous between humans and experimental animals.

In schizophrenia research, genetically manipulated mice are most often used, but also a zebrafish model has been introduced (Pratt et al. 2012; Gawel et al. 2019). Recently, a comprehensive phenotypic landscape of 132 schizophrenia-associated genes was established in the zebrafish model (Thyme et al. 2019). However, genetically manipulated mouse models offer a more extensive selection of behavioral tests to assess both cognitive- and negative-like symptomatology. The schizophrenia-related genes can be deleted, modified or introduced and the modification can be present either from conception or it can be induced later in the animal's lifespan (O'Tuathaigh et al. 2014).

There are numerous genetically manipulated mouse models, but many of these have still not been adequately validated. The strongest and most replicable mouse models in schizophrenia studies have been shown in models of disrupted in schizophrenia 1 (*DISC1*), neuregulin 1 (*NRG1*), NR1 subtype of NMDA receptor (*GRIN1*), neuregulin receptor (*ERBB4*) and dystrobrevin binding protein 1 (*DTNBP1*; dysbindin), which all are functionally linked to glutamatergic synapses (Pratt et al. 2012). The phenotypic characterization has typically focused on locomotor activity and prepulse inhibition (PPI) of the startle reflex, a measure of sensorimotor gating deficits, while changes in social behavior and cognitive performance have attracted less attention. Nevertheless, there is no single genetic model that robustly replicates all the cognitive and negative symptom domains associated with schizophrenia. Furthermore, as is the case with the phenotypic characterization, also the potential treatment effects have been assessed mainly against PPI deficits and hyperlocomotion. Thus, the effect of available and novel potential pharmacological agents on cognitive impairments and negative symptoms in genetic models remains to be resolved.

Developmental models

Developmental models are based on the evidence suggesting that certain environmental factors affecting pre-, peri- and postnatal developmental stages can promote the development of schizophrenia later in life (Young et al. 2009; Jones et al. 2011; Pratt et al. 2012; Moser 2014). These environmental factors include for example prenatal exposure to infection (immune activation) and malnutrition, obstetric complications like hypoxia, and abnormal fetal growth reflected as low birthweight and small head circumference (Brown 2011). Non-clinical models can be roughly

divided into two main categories, prenatal and neonatal insults (Pratt et al. 2012; Moser 2014).

There are several methods to disrupt neurodevelopment in rodents. The best established method consists of disruption of neurogenesis during a critical gestational period with the mitotoxic agent methylazoxymethanol (MAM), typically between gestational days (GD) 9.5 and 17. In addition, neonatal bilateral ventral hippocampal lesions with locally infused ibotenic acid typically a week after birth, post-weaning social isolation starting 3 weeks after birth, and perinatal or maternal immune activation with a viral-like immune response-eliciting agent polyriboinosinic-polyribocytidilic acid (PolyI:C) have been used (Jones et al. 2011; Pratt et al. 2012; Moser 2014). The timing of the prenatal insult is considered to significantly affect the phenotypic outcome of the models, owing to the typical phases of ontogenesis of rodents. These models have shown a variety of negative or cognitive symptoms resembling schizophrenia. Both atypical antipsychotic drugs and novel potential pharmacological agents have exhibited some alleviating effects against these symptoms (Jones et al. 2011; Moser 2014).

Pharmacological models

Pharmacological models aim to reproduce the abnormal neurotransmission observed in patients with schizophrenia. The pharmacological manipulations used to induce these changes can vary in their duration. Since schizophrenia is a neurodevelopmental syndrome, it has been claimed that acute administration will likely fail to mimic the slowly occurring neurobiological changes encountered in schizophrenia (Steeds et al. 2015). Nevertheless, subchronic treatment either during adolescence or in the peri- or postnatal developmental period can help to overcome this shortcoming.

Several pharmacological models have been used to induce a schizophrenia-like state. Probably the most interesting models regarding cognitive and negative symptom domains are glutamatergic and cholinergic models. However, pharmacological agents acting also on other neurotransmitter systems such as serotonin and cannabinoids, have been used.

2.3.2 Dopaminergic models

Dopaminergic models are based on the original dopamine hypothesis of schizophrenia, especially on the hyperactive mesolimbic dopamine neurotransmission (Jones et al. 2011; Steeds et al. 2015). Psychostimulants have been shown to induce psychotic symptoms in healthy individuals and exacerbate them in patients with schizophrenia. These drugs act as indirect dopamine agonists, increasing the dopamine levels in synapses. However, they have also similar effects on noradrenaline and to a lesser extent also on serotonin.

Dopaminergic agents, such as apomorphine, methylphenidate, cocaine and particularly amphetamine, have been used to model schizophrenia also in rodents

(Jones et al. 2011; Steeds et al. 2015). They induce stereotyped behaviors, which are thought to relate to the positive symptoms of schizophrenia (Steeds et al. 2015). In addition, impaired PPI and enhanced dopamine release (increased sensitivity), which both are seen also in patients with schizophrenia, have been observed after acute stimulant administration (Jones et al. 2011; Steeds et al. 2015). Amphetamine has been shown to induce also cognitive deficits in PFC-dependent tasks, such as attention, set-shifting and reversal learning. However, hippocampus-dependent tasks, such as visuo-spatial learning and memory, seem to remain unaffected. Amphetamine has also been reported to induce negative-like symptoms in social interaction tasks (Wilson and Koenig 2014), although also opposite results have been reported (Jones et al. 2011; Wilson and Koenig 2014).

Chronic amphetamine rodent models seem to robustly replicate findings related to positive symptoms (Jones et al. 2011; Steeds et al. 2015). Some of these symptoms have been either alleviated or prevented with haloperidol and clozapine, but also with mGluR_{2/3} agonists (Jones et al. 2011). However, the cognitive and negative symptoms are more difficult to reproduce also in the chronic amphetamine model. Furthermore, an acute low dose of amphetamine has been shown to enhance certain cognitive processes, such as attention; in fact amphetamine and its derivatives have been utilized in the treatment of attention-deficit hyperactivity disorder (ADHD) (Turner and Burne 2016). Whether the dopaminergic stimulant animal model can truly predict effective treatments with no direct dopaminergic effect, is yet to be resolved even for the positive symptoms.

2.3.3 Glutamatergic models

A hypofunction of the NMDA receptors is thought to play a role in the pathogenesis of schizophrenia (Olney and Farber 1995; Javitt 2010; Schwartz et al. 2012). Two NMDA antagonists, PCP and ketamine, induce all the domains of schizophrenia-like symptoms in healthy individuals and exacerbate these symptoms in patients with schizophrenia (Luby et al. 1959; Cohen et al. 1962; Itil et al. 1967; Krystal et al. 1994). NMDA antagonists interfere with cognitive performance and social behaviors also in rodents (e.g. Neill et al. 2010; Gilmour et al. 2012; Cadinu et al. 2018). PCP, ketamine and a more selective MK-801 (dizocilpine) are the most commonly used noncompetitive NMDA antagonists to induce schizophrenia-like symptoms in rodents. They bind to the PCP site in the NMDA receptor channel pore after AMPA receptor-mediated depolarization releases Mg²⁺ ion from blocking the pore (Kristiansen et al. 2007). However, PCP and ketamine have affinity also for other receptors. These drugs have been suggested to act as agonists at the high-affinity state of D₂ receptors, and PCP also at σ_2 receptors. PCP acts as an inhibitor at serotonin transporter (SERT), but this effect takes place in the micromolar rather than the nanomolar range. The three NMDA antagonists have displayed differing effects on behavioral outcomes, which may partly be explained by the differences in receptor affinities (Kapur and Seeman 2002; Gilmour et al. 2009; Dix et al. 2010a; Roth et al. 2013). It has also been suggested that NMDA antagonists bind differently for

the various subtypes of NMDA receptor NR2 (or GluN2) subunits, and could therefore affect different cell types in CNS (Bygrave et al. 2019).

Both acute and subchronic administration protocols have been used to induce schizophrenia-like deficits in rodents. Acute treatments are typically given either as a single dose or as a repeated (acute) administration protocol, i.e. a subchronic administration, where an acute pharmacological effect is present during behavioral testing. The acute administration of NMDA antagonists may induce non-specific sensorimotor side-effects, especially at higher doses, and these effects may interfere with the assessment of their effects on cognition or social behaviors (Sams-Dodd 1996; Gilmour et al. 2009; Dix et al. 2010b; Smith et al. 2011a). However, a 2- to 4-day pretreatment with an NMDA antagonist has been able to attenuate the undesired sensorimotor side-effects encountered after acute NMDA antagonist treatment (Pouzet et al. 2002; Podhorna and Didriksen 2005; Bruins Slot et al. 2005). It has also been postulated that this pretreatment period is not necessary if one administers low NMDA antagonist doses (Boulay et al. 2004). Usually, subchronic administration is given during adolescence/adulthood and is followed by a drug-free washout period ranging from days to weeks before behavioral testing. However, it can be given also during the vulnerable neurodevelopmental period (developmental model).

Acute NMDA antagonist administration has been shown to increase extracellular glutamate, dopamine and serotonin levels in rat PFC (Moghaddam et al. 1997; Moghaddam and Adams 1998; López-Gil et al. 2007), whereas subchronic treatment has been claimed to decrease the levels of glutamate and dopamine (Jentsch et al. 1997; Zuo et al. 2006). Both acute and subchronic administration have been found to inhibit GABA neurotransmission, reduce GAD₆₇ levels, parvalbumin levels, and parvalbumin mRNA expression, and furthermore subchronic administration can also decrease GAD₆₇ mRNA expression and the number of parvalbumin-positive GABAergic neurons in PFC and hippocampus (Qin et al. 1994; Yonezawa et al. 1998; Cochran et al. 2003; Keilhoff et al. 2004; Abdul-Monim et al. 2007; Romón et al. 2011; Amitai et al. 2012). NMDA antagonists have also been shown to affect gamma oscillations in cortical and hippocampal areas similarly as in patients with schizophrenia (Cadinu et al. 2018; Bygrave et al. 2019). The alterations seen in the subchronic models seem to be more persistent, and thus, more relevant regarding the chronic nature of the human syndrome (Jentsch and Roth 1999). However, the effects of acute administration have been suggested to mimic the first-episode schizophrenia (Adell et al. 2012).

Whereas the subchronic NMDA antagonist models have more comprehensive construct validity, acute and repeated models match the divergent dysfunctions encountered in schizophrenia more reliably, thus displaying better face validity. However, the clinical efficacy has not always been comparable with these models, and thus the question of predictive validity remains to be resolved.

2.3.4 Other models

Serotonergic

Serotonergic agents acting either directly or indirectly on 5-HT receptors have been used to induce schizophrenia-like symptoms also in experimental animals. Furthermore, 5-HT_{2A} receptor antagonism has been suggested to contribute to the alleviation of negative and cognitive symptoms of schizophrenia (Steeds et al. 2015). Nevertheless, acute administration of hallucinogenic 5-HT_{2A} agonists, such as D-lysergic acid diethylamide (LSD) and 2,5-dimethoxy-4-iodoamphetamine (DOI), have been shown to induce mainly hyperlocomotion and impaired PPI in non-clinical animal models (Nordquist et al. 2008; Betti et al. 2017), whereas their effects on cognition have been negligible (Ko and Evenden 2009; Talpos et al. 2014). Instead, chronic administration has been shown to reduce social interaction, although no pharmacological treatments have been assessed against asociality (Marona-Lewicka et al. 2011).

GABAergic

Evidence of GABAergic dysfunction in schizophrenia has led to the concept of using GABA receptor antagonists in modeling schizophrenia (Steeds et al. 2015). However, the reduced activity of primarily cortical parvalbumin-containing GABAergic interneurons suggests that a general GABA_A receptor blockade may not represent a valid model (Pratt et al. 2012). Thus, an indirect way to induce GABAergic dysfunction with NMDA antagonists interfering with the inhibitory activity of the interneurons has been used in rodents.

Cholinergic

Antimuscarinic agents, such as scopolamine, are perhaps best known in the modeling of memory impairments encountered in Alzheimer's disease. Nonetheless, scopolamine has been used to model cognitive dysfunction in schizophrenia (Barak and Weiner 2011; Steeds et al. 2015). However, it has also been shown to disrupt PPI (Barak 2009). Cholinergic agents and also 5-HT₆ receptor antagonists have been shown to ameliorate the scopolamine-induced cognitive impairments (Barak and Weiner 2011).

Endocannabinoids

THC has been used to induce schizophrenia-like symptoms in rodents with some reports of impaired PPI, reduced social interaction and cognitive dysfunction (Boucher et al. 2007; Tournier and Ginovart 2014; Murphy et al. 2017; Renard et al. 2017; Lloyd et al. 2018). Interestingly, a low affinity antagonist of the CB₁ and CB₂ receptors, cannabidiol, has shown antipsychotic-like effects in non-clinical and

clinical studies (Elsaid et al. 2019; Batalla et al. 2019). However, cannabidiol has several other targets including 5-HT_{1A} receptors.

2.4 NOVEL DRUGS TO COMBAT THE COGNITIVE AND NEGATIVE SYMPTOMS OF SCHIZOPHRENIA

Recently, improving the treatment of cognitive dysfunction and negative symptoms has gained a central role in schizophrenia research (Green and Harvey 2014; Millan et al. 2014). This is due to the emerging awareness of the inability of the currently available antipsychotic drugs to alleviate cognitive deficits and negative symptoms, resulting in unsatisfactory social, cognitive and occupational functioning in the patients. In fact, only about 15% of patients achieve recovery, i.e. minimal symptomatology accompanied by self-care, social and educational functioning for at least two years (Carbon and Correll 2014). Additionally, roughly only every third patient responds to treatment with antipsychotic drugs achieving remission with only mild symptomatology for at least 6 months. Another third shows some response with evident residual symptoms, while the remainder fail to respond to the antipsychotic drug treatment. Hence, novel treatment strategies to alleviate cognitive deficits and negative symptoms are topics of extensive research (Green and Harvey 2014; Millan et al. 2014). Methods for achieving improvement in affected patients' cognitive abilities and negative symptoms, include the development of novel effective pharmacotherapies, development and better utilization of behavioral and psychotherapies, and refinement of the lately introduced methods of neurostimulation.

2.4.1 Antipsychotic drugs

The common feature for all antipsychotic drugs (Table 4) is binding to the dopaminergic D₂ receptors to varying degrees (Kapur and Mamo 2003; Pratt et al. 2012; McCutcheon et al. 2020). The difference between the groups of antipsychotic drugs is that the typical (first generation) and atypical (second generation) antipsychotic drugs block the D₂ receptors but the novel atypical (third generation) antipsychotic drugs such as aripiprazole, brexpiprazole and cariprazine act as partial agonists at D₂ receptors.

Typical antipsychotics

The blockade of D₂ receptors in subcortical regions is considered essential for alleviating the psychotic symptoms of schizophrenia (Kapur and Mamo 2003; McCutcheon et al. 2020), and thus, typical antipsychotic drugs are effective against these symptoms. However, blockade of the D₂ receptors in the nigrostriatal dopamine pathway gives rise to the typical extrapyramidal adverse effects of antipsychotic drugs, such as parkinsonian-like symptoms, akathisia and dystonias. More severe side effects of the blockade of D₂ receptors outside the nigrostriatal

pathway are tardive dyskinesia and even malignant neuroleptic syndrome. Hyperprolactinemia is a consequence of blockade of the D₂ receptors in tuberoinfundibular pathway, where dopaminergic tone regulates prolactin secretion from the pituitary gland. Typical antipsychotic drugs have high affinity for D₂ receptors, and thus, a liability to cause the above mentioned adverse effects. There may be a therapeutic window for these effects; the clinical response has been related to D₂ receptor occupancy of at least 65%, and the likelihood of hyperprolactinemia and extrapyramidal adverse effects have been related to D₂ receptor occupancy of higher than 72% and 78%, respectively (Kapur et al. 2000).

Atypical antipsychotics

All atypical antipsychotic drugs have their own characteristic receptor binding profiles. The atypicality of antipsychotics is a constant subject of debate. It has been suggested that the antagonistic 5-HT₂/D₂ ratio would form a basis for the atypicality. Nevertheless, not all atypical antipsychotic drugs have 5-HT₂/D₂ ratio >1, while also the typical antipsychotic drugs bind to 5-HT₂ receptors (Kapur and Mamo 2003). Most of the atypical antipsychotics have relatively high affinities for 5-HT₂ receptors (Meltzer et al. 2003). 5-HT_{2A} antagonism and agonism of the counteracting 5-HT_{1A} receptors are proposed to contribute to the antipsychotic action by increasing cortical dopamine release. Furthermore, 5-HT_{2A} antagonists typically bind also to the structurally related 5-HT_{2C} receptors, which have been shown to enhance dopamine release in nucleus accumbens and PFC. However, 5-HT_{2C} receptor antagonism is linked to the risk of weight gain and metabolic syndrome which are common adverse effects of both typical and atypical antipsychotic drugs (Meltzer et al. 2003; Pratt et al. 2012). 5-HT₆ receptor antagonism has been proposed to facilitate cortical and hippocampal glutamatergic function and possibly acetylcholine release, and thus, improve cognition (Meltzer et al. 2003). If one considers binding at receptors other than 5-HT receptors, the high antagonistic adrenergic α_{2C} /D₂ ratio of some atypical antipsychotic drugs has been postulated to result in favorable effects on cognitive and negative symptoms (Kalkman and Loetscher 2003; Brosda et al. 2014), while antagonism of mAChRs and histamine H₁ receptors has been related to anticholinergic and sedative adverse effects (Pratt et al. 2012).

A novel atypical antipsychotic, aripiprazole, combines partial agonism of D₂ and 5-HT_{1A} to antagonism of 5-HT_{2A} receptors (Meltzer et al. 2003). Unlike antipsychotic drugs with a D₂ receptor antagonistic profile, over 90% D₂ receptor occupancy is needed to yield a clinical effect by aripiprazole. Because of its partial agonism, however, some intrinsic dopamine activity is preserved while the hyperdopaminergic states are alleviated by aripiprazole treatment.

Two other aripiprazole-like antipsychotic drugs, brexpiprazole and cariprazine, were approved for the treatment of schizophrenia in 2015 in the USA, and a few years later (2018 and 2017, respectively) also in Europe. They act as partial agonists at D_{2/3} and 5-HT_{1A} receptors similarly as aripiprazole, but cariprazine binds preferentially

to D₃ receptors (Girgis et al. 2019). Additionally, cariprazine has antagonistic effect on 5-HT_{2B} receptors, whereas brexpiprazole has wider affinity for other receptors, such as 5-HT_{2A}, 5-HT_{2B}, α_1 , and α_2C , where it acts as an antagonist (Maeda et al. 2014).

Although the receptor profiles of atypical antipsychotic drugs are more favorable than the typical antipsychotic drugs regarding treatment of negative and cognitive symptoms, they have failed to provide robust clinical efficacy (Meltzer and McGurk 1999; Kirkpatrick et al. 2006; Davidson et al. 2009; Hanson et al. 2010; Citrome 2014; Sarkar et al. 2015; Veerman et al. 2017).

Table 4. Antipsychotic medicine available in Finland year 2020

Typical		Atypical	Novel atypical
Chlorprothixene	<i>Amisulpride</i>	Asenapine	Aripiprazole
Flupentixol	<i>Chlorpromazine</i>	Clozapine	Brexpiprazole
Haloperidol	<i>Melperone</i>	Lurasidone	Cariprazine
Levomepromazine	<i>Periciazine</i>	Olanzapine	
Perphenazine	<i>Pimozide</i>	Paliperidone	
Prochlorperazine	<i>Promazine</i>	Quetiapine	
Sulpiride	<i>Thioridazine</i>	Risperidone	
Zuclopendixol	<i>Tiapride</i>	Sertindole	
	<i>Trifluoperazine</i>	Ziprasidone	

Only available as medicinal products requiring a special permit (italics)

2.4.2 Novel drugs in clinical development phase and non-clinical testing in NMDA antagonist models

Several novel drugs are being studied for the treatment of cognitive and negative symptoms of schizophrenia in clinical trials. Novel agents are targeting neurotransmitter systems compromised in schizophrenia, i.e. dopaminergic, serotonergic, glutamatergic, GABAergic, cholinergic, noradrenergic and endocannabinoid systems. In addition, several compounds such as anti-inflammatory agents, antioxidants, antibiotics, hormones, neuropeptides and agents modulating intracellular processes have been investigated. Here, those drugs acting on neurotransmitter systems, addressing especially the cognitive and negative symptoms of schizophrenia recently approved for human use or currently in the pipeline, are briefly summarized. A comparison, where appropriate, is made with the results of non-clinical studies with NMDA antagonist rodent models of schizophrenia-like cognitive and negative symptoms. The information of ongoing clinical trials has been gathered from EU Clinical Trials Register (2020a) and a clinical trials database maintained by the U.S. National Library of Medicine (2020b).

Novel atypical antipsychotic drugs recently approved for treatment of schizophrenia

Cariprazine was shown to alleviate several subdomains of negative symptoms significantly better than risperidone in a 26-week trial in patients with predominant negative symptoms (Németh et al. 2017; Fleischhacker et al. 2019). It showed some improvement in negative but not positive symptoms also in a 6-week proof-of-concept trial (Durgam et al. 2016). Brexpiprazole did not demonstrate any significant effect on negative or cognitive symptoms in patients with schizophrenia (Kane et al. 2015; Correll et al. 2015; Citrome et al. 2016; Fleischhacker et al. 2017). Both cariprazine and brexpiprazole have been examined also in non-clinical NMDA antagonist rodent models, and they have shown mainly promising results (Table 5). The effects of brexpiprazole on cognition have been connected with its actions on the 5-HT_{1A} receptor (Maeda et al. 2014; Yoshimi et al. 2014, 2015).

Dopaminergic agents

There have been attempts to enhance cortical dopaminergic activity with agents stimulating DA receptors. However, trials with apomorphine, L-DOPA, tyrosine and a D₁ receptor agonist have not demonstrated efficacy in the treatment of cognitive and negative symptoms (Girgis et al. 2019).

Dopamine's effect in synapses is terminated via uptake by the dopamine transporter (DAT). However, there is low expression of dopamine transporter in the frontal cortex, and therefore metabolism via catechol-*O*-methyltransferase (COMT) is of greater importance. Thus, COMT inhibition could selectively elevate dopamine levels in the frontal cortex while leaving striatal dopamine levels unaffected (Talpos 2017). Two COMT inhibitors, tolcapone and entacapone, better known as antiparkinsonian drugs, are under investigation in the treatment of schizophrenia. At present, a preliminary result from a 12-week add-on treatment study with a COMT inhibitor, entacapone, suggested that there were no beneficial effects on any of the symptom domains (Kaphzan et al. 2014). However, entacapone does not readily cross the blood-brain barrier. No clinical trials with entacapone are ongoing. Tolcapone, but not entacapone, has been evaluated also in NMDA antagonist rodent models for cognition (Table 5).

Agents acting on dopamine and serotonin receptors

Lumateperone (ITI-007) is novel molecule combining monoamine modulation with phosphorylation of intracellular signaling proteins (Lieberman et al. 2016). It is a high-affinity 5-HT_{2A} receptor antagonist with significantly lower affinity for D₂ and other receptors typically seen with antipsychotic drugs. Thus, it can fully saturate 5-HT_{2A} receptors with only modest binding to D₂ receptors. In addition, lumateperone acts as a partial agonist for presynaptic and as an antagonist for postsynaptic D₂ receptors allowing functional inhibition of dopamine neurotransmission, which

should prevent adverse motor effects. Lumateperone does not have significant activity on H₁, muscarinic and 5-HT_{2C} receptors, which are receptors associated with many of the typical adverse effects of antipsychotic drugs, such as weight gain, sedation and cognitive impairments. A 4-week trial reported that lumateperone improved negative, especially social and depressive symptoms of patients with a well-tolerable adverse effect profile (Lieberman et al. 2016). A phase II trial for patients poorly responding or tolerating approved medications is ongoing. There are no published data on the effects of lumateperone in non-clinical NMDA antagonist models of schizophrenia.

RP5063 is a dopamine-serotonin system stabilizer acting as partial agonist at D₂, -₃, -₄, 5-HT_{1A} and -_{2A} receptors and antagonist at 5-HT_{2B}, -_{2C}, -₆ and -₇ receptors with a weaker affinity for 5-HT_{2C} and -₆ (Cantillon et al. 2017). It has shown efficacy against both the positive and negative symptoms, and also some prosocial effects with an adverse effect profile comparable to placebo. However, no clinical trials are ongoing. One study has evaluated the effects of RP5063 against PCP-induced deficits on cognition (Table 5).

Glutamatergic agents

The NMDA receptor hypofunction hypothesis of excessive pyramidal cell glutamatergic action has led to expectations of beneficial effects resulting from NMDA receptor stimulation. However, NMDA receptor agonists at glutamate binding site are neurotoxic, hence alternative mechanisms have been explored. NMDA receptor stimulation via the glycine binding site has been under extensive research in clinical trials since the late 1990s. Direct agonists, such as glycine, D-alanine, D-serine and partial agonist, D-cycloserine (NMDA receptor co-agonists), have demonstrated contradictory findings for negative and cognitive symptoms (Singh and Singh 2011; for a review, see Girgis et al. 2019). Only D-serine and a small sample of D-alanine were proven effective against negative symptoms in a meta-analysis (Singh and Singh 2011), while in a more recent review, glycine and sarcosine, but not D-serine, were associated with a modest improvement in the negative symptoms (Girgis et al. 2019). Several clinical trials with D-serine are ongoing or being evaluated. Glycine binding site ligands have been assessed also in NMDA antagonist models with encouraging effects on negative and cognitive symptoms (Table 5).

Despite acting as co-agonist at NMDA receptor, glycine is also an inhibitory neurotransmitter exerting its effects via binding at glycine receptors. Synaptic levels of glycine can be elevated by inhibiting glycine reuptake via glycine transporters (GlyT) or glycine metabolism with D-amino acid oxidase (DAAO) inhibitors. Sarcosine (*N*-methylglycine) is an endogenous antagonist of glycine transporter GlyT₁. It has shown efficacy against the cognitive and negative symptoms of schizophrenia as an adjunctive therapy with antipsychotic drugs (Tsai et al. 2004; Lane et al. 2005, 2010), as well as with the DAAO inhibitor sodium benzoate (Lin et

al. 2017) but not with clozapine (Lane et al. 2006) or as monotherapy (Lane et al. 2008; Lin et al. 2017). In a non-clinical MK-801 model, sarcosine displayed some beneficial effect in a fear conditioning task (Table 5). A GlyT₁ antagonist, bitopertin, (RG1678, RO4917838) has been shown to improve negative symptoms as an add-on (adjunctive) therapy with antipsychotics in patients with persistent predominant negative symptoms (Umbricht et al. 2014a; Hirayasu et al. 2016), but this effect was not replicated in larger trials (Bugarski-Kirola et al. 2016, 2017) and also monotherapy was found to be ineffective (Bugarski-Kirola et al. 2014). No actively recruiting clinical trials are ongoing. An add-on treatment with a competitive GlyT₁ inhibitor Org 25935 (SCH 900435, MK-8435) did not differ from placebo in patients with predominant persistent negative symptoms (Schoemaker et al. 2014). There are no published data available on the effects of bitopertin or Org 25935 in the NMDA antagonist rodent models.

Another way to attenuate excessive glutamate neurotransmission is to utilize presynaptic mGluR_{2/3} agonists or to modulate mGluR_{1/5} and AMPA receptors. Two metabotropic mGluR_{2/3} agonists, pomaglumetad methionil (LY404039) and its prodrug, LY2140023, have been assessed in clinical trials with contradictory results. Although an initial study showed improvements in positive and negative symptoms (Patil et al. 2007), later studies found no effect as a mono- (Kinon et al. 2011; Adams et al. 2013; Downing et al. 2014) or an add-on therapy with antipsychotics (Stauffer et al. 2013). Pomaglumetad methionil is currently being assessed in patients with a high psychosis risk in a phase I trial. LY404039 did not exhibit any procognitive effects in a ketamine rodent model (Table 5).

CX516 is an AMPA receptor positive allosteric modulator. While an initial trial showed some promising effects as an add-on therapy to improve attention (Goff et al. 2001), no effect was shown in subsequent phase II trials as add-on (Goff et al. 2008) or monotherapy (Marengo et al. 2002). Furthermore, CX516 displayed a more pronounced adverse effect profile. No clinical trials are ongoing. CX516 has been shown to improve PCP-induced cognitive deficits in rodents (Table 5).

Sodium benzoate, a DAAO inhibitor, acts by elevating synaptic levels of D-amino acids, such as glycine and D-serine. The effects of sodium benzoate (NaBen®) as an add-on therapy with antipsychotics have been evaluated in clinical trials showing improved symptomatology of patients with chronic schizophrenia (Lane et al. 2013) and clozapine-resistant schizophrenia (Lin et al. 2018). When sodium benzoate was combined with sarcosine, a GlyT₁ inhibitor, this adjunctive combination therapy improved cognitive function of chronic schizophrenia patients (Lin et al. 2017). Several phase II/III clinical trials are ongoing for assessing the treatment effects in social and cognitive functioning, and also overall efficacy in adult and adolescent patients with schizophrenia. In a non-clinical study, pretreatment with sodium benzoate prevented ketamine to induced cognitive deficits (Table 5).

One interesting treatment option is the competitive NMDA antagonist memantine which is more commonly known as a treatment for Alzheimer's disease. Memantine has shown beneficial effects as an add-on therapy with antipsychotics to

combat the negative and positive symptoms (Krivoy et al. 2008; de Lucena et al. 2009; Rezaei et al. 2013), but later also against the cognitive symptoms with minimal adverse effects in several small clinical trials (Fakhri et al. 2016; Mazinani et al. 2017; Omranifard et al. 2017). Memantine improved negative and cognitive symptoms also in patients with clozapine-treated refractory schizophrenia (Veerman et al. 2016, 2017). In a recent study, add-on memantine therapy with antipsychotics improved cognition of both patients experiencing an acute psychotic episode, and in those with chronic schizophrenia with no effect on negative symptoms (Schaefer et al. 2020). However, one study found no beneficial effect of memantine as add-on treatment to atypical antipsychotic drugs among patients with the persistent residual psychopathology of schizophrenia (Lieberman et al. 2009). Memantine is in a phase IV clinical trial assessing its effect on cognition. Memantine has shown promising effects in one tentative ketamine model of social interaction deficit, but the drug's effects on locomotor activity may have affected the results (Uribe et al. 2013).

Agents acting mainly on serotonin receptors

Add-on treatment with the 5-HT_{2A} inverse agonist, pimavanserin (ACP-103), has been shown to potentiate the effect of a sub-effective dose of risperidone to the level of a standard dose of risperidone alone but with less adverse effects (Meltzer et al. 2012). A similar effect of pimavanserin was not seen in combination with haloperidol. Several phase II and III clinical trials are ongoing or being analyzed. Pimavanserin has shown procognitive effects as adjunctive therapy to second, but not first, generation antipsychotics, but not as a monotherapy; results confirmed also in a non-clinical NMDA antagonist model (Table 5). An add-on treatment with the 5-HT_{2A/2C} antagonist ritanserin has shown beneficial effects on negative symptoms (Duinkerke et al. 1993; Akhondzadeh et al. 2008), while one study reported no beneficial effect (Den Boer et al. 2000). No clinical trials are ongoing. In one non-clinical study, ritanserin was shown to possess procognitive effects in the MK-801 model (Table 5).

5-HT₃ receptors have been shown to modulate dopaminergic mesocortical and mesolimbic pathways. 5-HT₃ receptor antagonists, ondansetron, tropisetron and granisetron have been shown to improve negative (Khodaie-Ardakani et al. 2013; Noroozian et al. 2013) and also cognitive symptoms (Zhang et al. 2006; Akhondzadeh et al. 2009). The results of several completed phase I–III clinical trials with ondansetron and one with tropisetron (phase III) have not yet been published. There are no published data on the effects of these drugs in NMDA antagonist rodent models.

5-HT₆ receptors are highly expressed in hippocampus and cortex, and 5-HT₆ antagonism has been associated with cognition-enhancing effects in animal models (Hatcher et al. 2005; Hirst et al. 2006). A novel 5-HT₆ antagonist, AVN-211 (avisetron, CD-008-0173), was shown to boost antipsychotic and procognitive effects related to attention in patients receiving antipsychotic medication (Morozova et al. 2014). However, these results could not be replicated subsequently, although slightly better

results were achieved among the trial's female participants (Morozova et al. 2017). Thus no clinical trials are ongoing (Fellner 2017). There are no published data on the effects of AVN-211 in NMDA antagonist rodent models.

Roluperidone (MIN-101) is a novel selective antagonist at 5-HT_{2A} and σ_2 opioid receptors, but it has binding affinity also for α_1 receptors. It has shown effects to alleviate the negative symptoms and also caused some improvement of cognitive dysfunction, especially speed of processing, in a 12-week trial in patients with stable schizophrenia (Davidson et al. 2017; Keefe et al. 2018; Harvey et al. 2019). Phase III clinical trials assessing the efficacy of roluperidone on negative symptoms are ongoing. There are no published data on the effects of roluperidone in NMDA antagonist models.

SEP-353856 (SEP-856) acts mainly as an agonist at TAAR1 (trace amine-associated receptor 1) and 5-HT_{1A} receptors (Dedic et al. 2019). TAAR1 agonism has been connected with inhibition of firing in the VTA dopaminergic neurons (Koblan et al. 2020), while activation of postsynaptic 5-HT_{1A} receptors in PFC has been shown to activate the mesocortical projections, enhancing dopamine release in PFC (Celada et al. 2013). In a 4-week trial in patients with acute exacerbation of schizophrenia, SEP-363856 ameliorated symptoms better than placebo with a comparable adverse effect profile (Koblan et al. 2020). Several phase II and III clinical trials assessing the long-term efficacy, safety and tolerability of SEP-363856 in patients with schizophrenia are ongoing. SEP-363856 reversed subchronic PCP-induced social interaction deficits in rats (Dedic et al. 2019).

GABAergic agents

The partial dysfunction of GABAergic neurotransmission in schizophrenia has encouraged the development of novel GABAergic compounds. GABA does not readily cross the blood-brain barrier when administered peripherally, but instead, BL-1020 (also known as CYP-1020) is an orally bioavailable ester of GABA and typical antipsychotic drug, perphenazine, that aids GABA in gaining access to the CNS (Geffen et al. 2009). BL-1020 blocks D₂, 5-HT_{2A} and H₁ receptors, but it also acts as a GABA_A receptor agonist. BL-1020 showed significant improvements on cognitive functioning of chronic schizophrenia patients compared to risperidone in a 6-week trial (Geffen et al. 2012). However, no further studies have been reported. There are no published data on the effects of BL-1020 in NMDA antagonist rodent models.

MK-0777 (TPA-023) is a partial agonist at GABA_A receptor $\alpha_{2/3}$ subunits. It showed procognitive effects in a pilot study with patients with schizophrenia (Lewis et al. 2008), but no effect was seen in a subsequent 4-week clinical trial (Buchanan et al. 2011). MK-0777 reversed subchronic PCP-induced cognitive deficits in one study (Table 5).

Cholinergic agents

Many ligands activating α_7 nAChR have been developed for the treatment of cognitive symptoms of schizophrenia. Clinical data has been reported on EVP-6124 (encenicline), GTS-21 (or DMXB-A; anabaseine), ABT-126, RG3487 (or MEM3454), AQW051, TC-5619 and AVL-3288. To date, the most extensive clinical data is available on EVP-6124 (encenicline). It has shown some efficacy against cognitive dysfunction, but also against the negative symptoms in phase II studies (Lieberman et al. 2013; Keefe et al. 2015), but failed to replicate these effects in phase III trials (Fidler 2016; Keshavan et al. 2017). In addition, an instant release formulation of DMXB-A (GTS-21) showed some positive effects on cognition, especially in attention (Olincy et al. 2006), and negative symptoms (anhedonia and alogia) in phase II trials (Freedman et al. 2008), but an extended release formulation showed no clinical efficacy (Kem et al. 2018). ABT-126 tended to alleviate negative symptoms, but failed to exert procognitive effects in patients with schizophrenia regardless of their smoking status (Haig et al. 2016, 2018). RG3487 had no effect on cognition, but alleviated negative symptoms of patients expressing moderate negative symptoms (Umbricht et al. 2014b). AQW051 exhibited no effect on performance of patients with schizophrenia in working and episodic memory tasks (Barch et al. 2016). TC-5619 has shown some mixed results with respect to cognitive and negative symptoms in patients with schizophrenia (Lieberman et al. 2013; Walling et al. 2016). However, none of these α_7 nAChR ligands are currently in phase III or IV clinical trials evaluating their effect on cognitive or negative symptoms. Some of these α_7 nAChR ligands have also been examined in non-clinical NMDA antagonist rodent models. The results have been rather promising and are summarized in Table 5.

Acetylcholinesterase inhibitors donepezil, rivastigmine and galantamine, that is also a positive allosteric modulator at homomeric α_7 and heteromeric $\alpha_4\beta_2$ receptors, have not demonstrated any robust efficacy against the negative or cognitive symptoms (Girgis et al. 2019).

Noradrenergic agents

Most of the atypical antipsychotic drugs have antagonistic properties at α_2 ARs, which has been suggested to contribute to their atypicality, a high α_{2c}/D_2 ratio being claimed to be particularly beneficial (Kalkman and Loetscher 2003; Brosda et al. 2014; Langer 2015). Mirtazapine and mianserin are noradrenergic and specific serotonergic antidepressants; these drugs act as an antagonist at α_2 ARs in addition to several 5-HT receptors. Mirtazapine has been able to alleviate negative (Berk et al. 2001; Zoccali et al. 2004; Joffe et al. 2009; Abbasi et al. 2010; Terevnikov et al. 2010; Caforio et al. 2013) and/or cognitive symptoms (Delle Chiaie et al. 2007; Stenberg et al. 2010, 2011; Cho et al. 2011) as an add-on therapy to different antipsychotic drugs. However, one trial resulted with no effects attributable to add-on mirtazapine (Berk et al. 2009). Mianserin has shown beneficial effects on cognition (Poyurovsky et al. 2003) and

augmented the effect of typical antipsychotic drugs in treatment-resistant schizophrenia (Shiloh et al. 2002). No clinical trials are ongoing with these agents. Only reports on the effects of mirtazapine in NMDA rodent models have been published; beneficial effects on negative and cognitive symptoms were observed when it was used as an adjunct to atypical antipsychotic drugs, but not as monotherapy (Table 5).

Cannabinoid receptor antagonists

Cannabinoid receptor (CB₁ and CB₂) antagonists have been studied in phase II clinical trials for the treatment of schizophrenia. Cannabidiol is a low affinity CB₁ and CB₂ antagonist, it has agonistic properties at 5-HT_{1A} receptors, and it may prevent degradation of an endocannabinoid anandamide (Elsaid et al. 2019). Cannabidiol, has been well-tolerated, but it has shown controversial results in patients with schizophrenia. Leweke et al. (2012) found it to be as effective as amisulpridi, but with fewer adverse effects. Boggs et al. (2018) found no effect, while McGuire et al. (2018) observed efficacy against positive symptoms as an add-on therapy with antipsychotics. Several phase II and one phase III clinical trials are ongoing. Cannabidiol has also been studied in NMDA antagonist rodent models, but the results have also been controversial (Table 5).

Rimonabant (SR141716) is a CB₁ antagonist, which has no effect on symptoms as an add-on treatment (Meltzer et al. 2004; Kelly et al. 2011; Boggs et al. 2012). There are no data on the effects of rimonabant in the NMDA rodent model.

Table 5. The effects of novel drugs on cognitive and negative-like symptoms in non-clinical NMDA antagonist models of schizophrenia.

Treatment (mg/kg)	NMDA antagonist (mg/kg)	Species, sex	Behavioral test(s)	Results (dose mg/kg)	Reference
<i>Novel atypical antipsychotic drugs recently approved for treatment of schizophrenia (≤5 years)</i>					
Brexpiprazole	PCP 10 s.c., 1 x d, sch	♂ Mouse	NOR (ITI 24 h)	↔ 0.3, ↑ 1, 3 p.o., 1 x d, 14 d	(Yoshimi et al. 2014)
	PCP 5 i.p., 2 x d, sch	♂ Rat	NOR (ITI 1 h) ASST (EDS) ASST (EDR)	↔ 0.3, ↑ 1, 3 p.o. ↑ 1, ↔ 3 s.c. ↓ 1, ↔ 3 s.c.	(Maeda et al. 2014)
	MK 0.1 i.p., 1 x 3, acute	♂ Mouse	SR	↑ 0.01, 0.03, 0.1 p.o.	(Yoshimi et al. 2015)
Cariprazine	PCP 2 i.p., 2 x d, sch	♀ Rat	NOR ORL	↑ 0.05, 0.1, ↔ 0.25 p.o. ↔ 0.05, ↑ 0.1, 0.25 p.o.	(Neill et al. 2016)
	PCP 1 i.p., acute	♂ Mouse	DAT ASST (EDS) SR	↑ 0.005, 0.01, 0.02 i.p. ↔ 0.005, ↑ 0.01, 0.02 i.p. ↔ 0.005, ↑ 0.01, 0.02 i.p.	(Zimmisky et al. 2013)
	PCP 2 i.p., 2 x d, sch	♀ Rat	SIT (avoiding) SIT (following)	↑ 0.05, 0.1, 0.25 p.o. ↑ 0.05, ↔ 0.1, 0.25 p.o.	(Neill et al. 2016)
<i>Dopaminergic agents</i>					
Tolcapone	PCP 5 i.p.?, 2 x d, sch	? Rat	NOR (ITI 30 min)	↑ 7.5, 15, 30 i.p.	(Detrait et al. 2016)
	PCP 2 i.p., 2 x d, sch	♂ Rat	TM SST (EDS)	↑ 30 i.p.	(Troudet et al. 2016)
<i>Agents acting on dopamine and serotonin receptors</i>					
RP5063	PCP 10 i.p., 2 x d, sch	♂ Mouse	NOR ORL	↔ 0.3, ↑ 1 i.p. ↔ 1, 3 i.p.	(Rajagopal et al. 2017)

Table 5. (Continued)

Treatment (mg/kg)	NMDA antagonist (mg/kg)	Species, sex	Behavioral test(s)	Results (dose mg/kg)	Reference
<i>Glutamatergic agents</i>					
Glycine	MK 0.05 i.p., acute	♂ Rat	LI	↑ 800 i.p.	(Gaisler-Salomon et al. 2008)
D-serine	MK 0.15 i.p., acute	♂ Mouse	LI	↑ 600 s.c.	(Lipina et al. 2005)
	MK 0.5, ?, 2 x d, sch	♂ Mouse	NOR (ITI 4 h)	↑ 50, ?, rep	(Bado et al. 2011)
	MK 0.3 i.p., acute	♂ Rat	NOR (ITI 24 h)	↔ 320, 640, ↑ 1280 s.c.	(Smith et al. 2009)
	MK 0.1 i.p., acute	♂ Rat	NOR (ITI 2 h)	↔ 400, ↑ 800 i.p.	(Karasawa et al. 2008)
	PCP 10 s.c., 1 x d, sch	♂ Mouse	NOR (ITI 24 h)	↑ 600 i.p. sch	(Hashimoto et al. 2008)
	MK 0.3 i.p., acute	♂ Rat	SPT	↑ 1280 s.c.	(Vardigan et al. 2010)
	MK 0.05 i.p., acute	♂ Rat	LI	↔ 3, ↑ 15, 30 i.p.	(Gaisler-Salomon et al. 2008)
D-cycloserine	MK 0.05 i.p., acute	♂ Rat	VSDT	↔ 3, ↑ 10 i.p.	(Howe and Burk 2007)
Sarcosine	MK 0.2 i.p., acute	♂ Mouse	Fear conditioning (cue test)	↔ 500, ↑ 1000 i.p.	(Pei et al. 2019)
Pomalgumetad methionil (LY404039, LY2140023)	KET 10 i.p., 1 x d, sch	♂ Rat	OST	↔ 0.3, 1, 3, 10 s.c.	(Rushforth et al. 2011)
CX516	PCP 2 i.p., 2 x d, sch	♀ Rat	NOR	↔ 0.5, 2.5, ↑ 10, 40, 80 s.c.	(Damgaard et al. 2010)
	PCP 5 i.p., 2 x d, sch	♂ Rat	ASST (EDS)	↔ 5, 40, ↑ 10, 20 s.c., 2 x d	(Broberg et al. 2009)
Sodium benzoate	KET 25 i.p., acute	♂ Rat	RAWM	↑ 0.01 p.o. rep	(Mahmoud et al. 2019)

Table 5. (Continued)

Treatment (mg/kg)	NMDA antagonist (mg/kg)	Species, sex	Behavioral test(s)	Results (dose mg/kg)	Reference
<i>Agents acting mainly on serotonin receptors</i>					
Pimavanserin (ACP-103)	PCP 2 i.p., 2 x d, sch	♀ Rat	NOR (ITI 1 min)	↔ 3.0 i.p. ↑ 3.0 i.p. with subeff. SGAs	(Snigdha et al. 2010)
Ritanserin	MK 0.1 i.p., 1 x 4, rep	♂ Rat	AAPA	↑ 2.5, 5 i.p., 1 x 4, rep	(Bubenikova-Valesova et al. 2008)
SEP-363856	PCP 2.5 s.c., 2 x d, sch	♂ Rat	SIT	↑ 1, 3, 10, p.o.	(Dedic et al. 2019)
<i>GABAergic agents</i>					
MK-0777 (TPA-023)	PCP 10 i.p., 2 x d, sch	♂ Mouse	NOR (ITI ?)	↔ 0.03 ↑ 0.1 i.p. ↑ 0.03 with subeffective SGA ↑ 2 i.p., sch, before/during PCP ↑ 1 ↔ 2 i.p., sch, after PCP ↔ 0.03, 1, ↑ 2 i.p.	(Rajagopal et al. 2018)
<i>α₇ nAChR ligands</i>					
EVP-6124	PCP 1.5 s.c., acute	♂ Rat	SIT	↔ 0.3 s.c.	(Savolainen et al. 2018)
	PCP 13.0 s.c., 1 x d, sch	♂ Mouse	SIT	↑ 1 p.o.	(Pedersen et al. 2014)
TC-5619	PCP 13.0 s.c., 1 x d, sch	♂ Mouse	SIT	↑ 1 p.o.	(Pedersen et al. 2014)
GTS-21 (DMXB-A)	KET 30 i.p., 2 x d, sch	♂ Rat	CMOR	↔ 0.01, 0.03, 0.1 i.p.	(Cloeke and Winters 2015)
	MK 0.08 i.p., acute	♂ Rat	NOR (ITI 3 h)	↔ 1 ↑ 3, 10 i.p.	(Callahan et al. 2014)
	KET 20 i.p., acute	♂ Rat	NOR (ITI 1 h)	↑ 0.3, 1 i.p.	(Potasiewicz et al. 2017)
	KET 10 s.c., acute	♂ Rat	ASST (EDS)	↑ 0.3, 1 i.p.	
	MK 0.03 i.p., acute	♂ Rat	TM SST (EDS)	↔ 3, 10 ↑ 30 i.p.	(Jones et al. 2014)
	KET 20 i.p., acute	♂ Rat	SIT	↑ 1 i.p.	(Potasiewicz et al. 2017)

Table 5. (Continued)

Treatment (mg/kg)	NMDA antagonist (mg/kg)	Species, sex	Behavioral test(s)	Results (dose mg/kg)	Reference
<i>Noradrenergic agents</i>					
Mirtazapine	MK 0.2 i.p., 1 x d, sch	♂ Rat	MWM	↔ 6 i.p. sch	(Tao et al. 2016)
	MK 0.2 i.p., acute	♂ Mouse	NOR (ITI 90 min)	↔ 2.5, 5 i.p. ↑ 2.5, 5 i.p. with subeff. TGA	(Rogóż et al. 2018)
	MK 0.2 i.p., acute	♂ Mouse	NOR (ITI 90 min)	↔ 2.5, 5 i.p. ↑ 2.5, 5 i.p. with subeff. SGA	(Rogóż 2013)
	MK 0.1 s.c., acute	♂ Rat	SIT	↔ 2.5 ↑ 5 i.p. with subeff. TGAs	(Hereta et al. 2019)
	MK 0.1 s.c., acute	♂ Rat	SIT	↔ 2.5, 5 i.p. ↑ 2.5, 5 i.p. with subeff. SGA	(Kamińska and Rogóż 2015)
<i>Cannabinoid receptor antagonists</i>					
Cannabidiol	MK 0.08 i.p., acute	♂ Rat	SR	↔ 5, 12, 30 i.p. ↔ 5, 12 i.p. with TGA	(Deiana et al. 2015)
	MK 0.3 i.p., acute	♂ Rat	SIT	↔ 1, ↑ 3 i.p.	(Gururajan et al. 2012)
	MK 0.6 i.p., acute	♂ Rat	SIT	↔ 3, 10, 30 i.p.	(Gururajan et al. 2011)

↑ = improved performance; ↓ = impaired performance; ↔ = no effect

Abbreviations: ? – not specified, AAPA – active allothetic place avoidance task, ASST – attentional set-shifting task, CMOR – cross-modal object recognition task, DAT – delayed alternation task, EDR – extra-dimensional reversal, EDS – extra-dimensional set-shifting, ITI – inter-trial interval, KET – ketamine, LI – latent inhibition, MK – MK-801 (dizocipine), MWM (RM) – Morris water maze (reference memory / acquisition), NOR – novel object recognition task, ORL – operant reversal learning, OST – odor span task, PCP – phencyclidine; PND – postnatal day, RAWM – radial arm water maze, rep – repeated (acute) administration, sch – subchronic administration with washout, SGA – second generation antipsychotic drug, SIT – social interaction task, SPT – sucrose preference test, SR – social recognition memory, SST – set-shifting task, subeff. – subeffective dose, TGA – third generation antipsychotic drug, TM – T-maze, TUNL – trial unique non-match to location, VSdT – visual signal detection task

Overall, many of the novel drugs seem to have some alleviating effect on negative, and to some extent also on cognitive symptoms. The poor predictive validity of non-clinical NMDA antagonist models results in some false positives, e.g. in the efficacy of brexpiprazole and CX516, when these drugs have failed in clinical trials. Nevertheless, the non-clinical results for example with pimavanserin and mirtazapine correlated with clinical observations, where an effect was found when the trial drug was used as an adjunctive therapy with antipsychotics. However, the challenges remain concerning the overall predictive validity of non-clinical schizophrenia models.

There are also some translational challenges. Experimental animals in their standardized laboratory conditions presumably are a more homogenic population than patients even in clinical trials, since regardless of inclusion criteria, every participant has his/her unique history and situation in life. These include phase of illness, symptomatology, underlying pathophysiology, medications, other stressors and personal expectations among others, and they may affect also the response to the experimental medications. Another confounding factor causing heterogeneity is the suggested sexual dimorphism in neurobiological pathophysiology and performance in cognitive domains encountered in patients with schizophrenia (Leger and Neill 2016; Tiihonen et al. 2019) while animal studies typically focus on one sex at a time. Therefore, both non-clinical experiments and clinical trials should be conducted, and results analyzed with both sexes to reliably assess the efficacy of novel treatments.

3 AIMS OF THE STUDY

The non-clinical development of novel pharmacotherapies for cognitive and negative symptoms of schizophrenia utilizes valid animal models. The general objective of this doctoral thesis was to develop and validate animal models mimicking cognitive and negative symptoms of schizophrenia for assessing novel drugs. The NMDA antagonist, PCP, was used to induce cognitive and negative symptoms in rats. The suitability of these animal models in the assessment of novel drugs was evaluated by testing both clinically available atypical antipsychotics and novel drugs.

The specific aims of this study were:

1. To adjust the PCP administration protocol such that it would cause deficits in cognition and social interaction without inducing non-specific behavioral effects (I–III).
2. To compare the effects of repeated PCP administration on visuo-spatial learning and memory between two rat strains, albino Wistar and pigmented Lister Hooded (LH), using the Morris swim navigation test (I).
3. To investigate the effects of repeated PCP administrations on cognitive flexibility using the touchscreen-based pairwise visual discrimination and reversal test (II).
4. To evaluate the effects of acute single-dose PCP on social interaction (III).
5. To assess the efficacy of established atypical antipsychotic drugs and novel drugs in alleviating PCP-induced schizophrenia-like cognitive and negative symptoms (I–III).

4 SUBJECTS AND METHODS

4.1 EXPERIMENTAL ANIMALS

Male Wistar rats were delivered from Lab Animal Centre, University of Eastern Finland (HsdHan:WIST (I) and RccHan:WIST (III); Kuopio, Finland) and Envigo (formerly Harlan Laboratories) (RccHan:WIST; the Netherlands and USA) (III). Male LH rats (HsdOla:LH) were delivered from Envigo (UK and the Netherlands (I) and Italy(III)). The rats were housed in groups of two in stainless steel cages (285 × 485 × 200 mm, Franke Finland Oy, Naarajärvi, Finland) with aspen woodchip bedding (Tapvei, Kaavi, Finland) and enrichments (wooden sticks to chew and plastic tubes) in controlled laboratory conditions with 12:12 h light/dark cycle (lights on at 7.00 a.m., temperature 21 ± 2 °C, humidity 55 ± 15%) except for an isolation period of 4–7 days before the social interaction test, when the animals were single-housed (III). Food (2016S, Teklad, Envigo, Indianapolis, USA) and water were available ad libitum except during training (II) and the test sessions (I–III). The animals were allowed to acclimatize to housing conditions for at least 7 days and were fully habituated to handling before starting the behavioral tests. All experiments were conducted in the light phase of the day.

All experiments were performed in accordance with European Union guidelines (directive 2010/63/EU and guidelines 2007/526/EC) and approved by the Animal Experiment Board in Finland.

4.2 DRUGS AND TREATMENTS

A noncompetitive NMDA antagonist, phencyclidine hydrochloride (PCP; 1-(1-phenylcyclohexyl)piperidine hydrochloride, Bio-Techne Ltd., Abingdon, UK, formerly Tocris Bioscience, Bristol, UK), was dissolved in physiological saline (doses refer to the hydrochloride form). PCP solution or corresponding vehicle were administered in a volume of 5 ml/kg (I, III) or 2 ml/kg (II). Acute and repeated PCP administration was used to induce schizophrenia-like cognitive and negative symptoms to experimental animals in the studies of this thesis.

Atypical antipsychotic drugs clozapine (Bio-Techne/Tocris Bioscience) and olanzapine (Merck, Darmstadt, Germany, formerly Sigma-Aldrich, St. Louis, USA) were dissolved in physiological saline with a minimum amount of 0.1 M HCl (III). Sertindole (Merck) was dissolved in phosphate buffer solution (United States Pharmacopeia, USP; pH 6.0) containing 2% Tween® 80 (I). Risperidone (Sigma-Aldrich) was dissolved either in USP phosphate buffer solution (pH 6.0) with 2% Tween® 80 (I) or physiological saline with a minimum amount of 0.1 M HCl (III). Sertindole, risperidone and olanzapine with their corresponding vehicle solutions were administered in a volume of 5 ml/kg; clozapine in a volume of 2 ml/kg (I, III).

A selective α_{2C} AR antagonist ORM-13070 (1-[(S)-1-(2,3-dihydrobenzo[1,4]dioxin-2-yl)methyl]-4-(3-methoxymethylpyridin-2-yl)-piperazine; $C_{20}H_{25}N_3O_3$; MW: 355.44; provided by Orion Pharma, Espoo, Finland; Figure 3A) was dissolved in a mixture of 15% polyethylene glycol 400 and 85% Glucosteril 50 mg/ml. The pH of the solution was adjusted to 4–5 with 1 M HCl (II, III). ORM-13070 is a brain penetrating and highly selective α_{2C} AR antagonist (binding affinity for α_{2C} over α_{2A} is over 28 fold), which has been screened for binding to more than 100 other potential receptors and targets; on them, ORM-13070 displayed either weak or no activity (Arponen et al. 2014). An α_7 nAChR partial agonist EVP-6124 hydrochloride, also known as encenicline hydrochloride ((*R*)-7-chloro-*N*-(quinuclidin-3-yl)benzo[*b*]thiophene-2-carboxamide hydrochloride; $C_{16}H_{17}ClN_2OS$; MW: 320.84; MedChem Express, Sollentuna, Sweden; Figure 3B), was dissolved in physiological saline (doses refer to the hydrochloride form) (II). EVP-6124 is also a brain penetrating compound which has been screened for binding to more than 60 other receptors and targets. EVP-6124 showed weak or no activity for all but one; a similar affinity was observed for 5-HT₃ receptors, which is typical also for other α_7 quinuclidine agonists (Prickaerts et al. 2012). ORM-13070 and EVP-6124 with their corresponding vehicle solutions were administered in volumes of 1 ml/kg and 2 ml/kg, respectively (II, III).

All treatments with their corresponding vehicles were administered via the subcutaneous route except clozapine, which was administered intraperitoneally. The PCP doses for the experiments were selected on the basis of previous rat studies on Morris water navigation (Podhorna and Didriksen 2005; Wass et al. 2006a, b; Didriksen et al. 2007), pairwise visual discrimination and reversal learning (Talpos et al. 2012; Fellini et al. 2014), social interaction behavior (Corbett et al. 1995; Sams-Dodd 1996; Boulay et al. 2004; Sallinen et al. 2013) and our task-specific pilot studies so that non-specific behavioral effects could be avoided (Castellani and Adams 1981). Furthermore, our functional magnetic resonance imaging data confirmed the suitability of the selected PCP doses (Paasonen et al. 2017).

The doses of sertindole and risperidone were selected on the basis of previous results (Didriksen et al. 2007) (I). The doses of sertindole (1.6 mg/kg/day, s.c., for 5 days) and risperidone (0.04 mg/kg/day, s.c., for 5 days) were within the window that should reverse PCP-induced deficits in water maze performance, but clearly below the doses causing deficits in MWM navigation in naïve rats (Skarsfeldt 1996). The dose of the α_{2C} AR antagonist ORM-13070 was selected on the basis of pilot experiments (II, III). The doses of clozapine, risperidone and olanzapine and the α_7 nAChR partial agonist EVP-6124 were selected on the basis of previous literature (Corbett et al. 1995; Sams-Dodd 1997; Maehara et al. 2011; Prickaerts et al. 2012; Sallinen et al. 2013; Pedersen et al. 2014; Huang et al. 2014) (II).

The treatment groups and the number of animals in Studies I–III are shown in Table 6 and the treatment regimens for all compounds in Table 7.

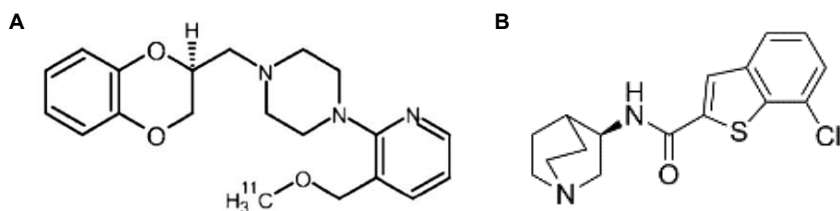


Figure 3. Chemical structures of ORM-13070 (^{11}C labeled) (A) and EVP-6124 (B) (Prickaerts et al. 2012; Arponen et al. 2014).

Table 6. Treatment groups and number of animals in Studies I–III.

Study I			Study II		Study III	
Treatments (mg/kg)	LH (n)	WI (n)	Treatments (mg/kg)	LH (n)	Treatments (mg/kg)	WI (n)
<i>PCP dose-response</i>			<i>PCP</i>		<i>PCP dose-response</i>	
SAL	12	16	SAL	9–13	SAL	14
PCP 1.3	18	16	PCP 1.5	10–12	PCP 1.15	14
PCP 1.6	18	8			PCP 1.5	14
PCP 2.0	8	16	<i>ORM-13070</i>		<i>ORM-13070</i>	
<i>Sertindole</i>			SAL + VEH	8	SAL + VEH	14+20+18
SAL + VEH	30	35	PCP 1.5 + VEH	8	PCP 1.5 + VEH	14+18+18
PCP 2.0 + VEH	45	35	PCP 1.5 + ORM 1.0	8	PCP 1.5 + ORM 0.3	14+0+0
PCP 2.0 + SERT 1.6	45	35			PCP 1.5 + ORM 1.0	14+18+18
<i>Risperidone</i>					<i>EVP-6124</i>	
SAL + VEH	6	19			SAL + VEH	18
PCP 2.0 + VEH	16	19			PCP 1.5 + VEH	18
PCP 2.0 + RISP 0.04	16	19			PCP 1.5 + EVP 0.3	18
					<i>Clozapine</i>	
					SAL + VEH	18
					PCP 1.5 + VEH	18
					PCP 1.5 + CLO 2.5	18
					<i>Risperidone</i>	
					SAL + VEH	28
					PCP 1.5 + VEH	28
					PCP 1.5 + RISP 0.04	14
					PCP 1.5 + RISP 0.08	14
					<i>Olanzapine</i>	
					SAL + VEH	26
					PCP 1.5 + VEH	28
					PCP 1.5 + OLA 0.125	14
					PCP 1.5 + OLA 0.5	14

Abbreviations: CLO – clozapine, EVP – EVP-6124 (encenicline), LH – Lister Hooded, OLA – olanzapine, ORM – ORM-13070, PCP – phencyclidine, RISP – risperidone, SAL – saline (control), SERT – sertindole, VEH – vehicle, WI – Wistar

Table 7. Treatment regimens in studies I–III.

Study	PCP (mg/kg)	Treatment regimen	Other treatments (mg/kg)	Treatment regimen
I	1.3 1.6 2.0	30 min before test - 1 × d for 8 d - Starting 3 d before test	Sertindole - 1.6	60 min before test - 1 × d for 5 d - On test days
			Risperidone - 0.04	As above
II	1.5	40 or 45 min before test - 1 × d for 4 d - Starting on 2 nd reversal d	ORM-13070 - 1.0	60 min before test - 1 × d for 4 d - Starting on 2 nd reversal d
III	1.15 1.5	45 min before test - Single dose	ORM-13070 - 0.3 - 1.0	45 min before test - Single dose
			EVP-6124 - 0.3	60 min before test - Single dose
			Clozapine - 2.5	45 min before test - Single dose
			Risperidone - 0.04 - 0.08	75 min before test - Single dose
			Olanzapine - 0.125 - 0.5	45 min before test - Single dose

Abbreviations: d – day, PCP – phencyclidine

4.3 BEHAVIORAL TESTS

4.3.1 Morris swim navigation (I)

Morris swim navigation (Morris ‘water maze’, MWM) task assesses visuo-spatial learning and memory of rodents (Morris 1984). In MWM, an experimental animal must use external visual cues to find a hidden platform in a pool filled with water. In this study, a black circular pool (ø 150 cm, height 76 cm) was used in experiments with albino Wistar rats while a white pool (ø 146 cm, height 70 cm) was used with black/white LH rats to ensure optimal contrast for video tracking. The water temperature was 19 ± 1 °C. A rectangular escape platform (10 × 10 cm) was located 2 cm below water surface in the north-east pool quadrant. There were no cues inside the pools, but instead three extra-maze visual cues (two black screens with white figures, a triangle and a striped square, and a black circle on a light wall) were placed around the pool to help in spatial navigation. The behavior of the animals was recorded by an overhead video camera and analyzed subsequently using EthoVision XT v. 7.1 software (Noldus Information Technology, Wageningen, the Netherlands).

Before the first and then after each successive trial, the rat was placed (or permitted to stay) on the hidden platform for 10–15 s to allow the animal to be aware of the presence and location of the platform. In each trial, the rat was placed in the pool at one of the five starting positions (south-east, south, south-west, west and north-west), which were used in the same order for all rats. The rats performed three trials (max 60 s) per day for four consecutive days. On day five, rats performed one 60-s probe trial without the platform. Each rat was tested only once in the MWM task.

The following variables were calculated during each trial: latency to reach platform (s), swimming speed (cm/s) and the time spent in the wall zone (the most peripheral area of the pool, radius 15 cm from the wall) (s). The following variables were calculated during probe trials: Time spent in platform area (30 cm circular area centered on the previous platform location) (s) and time spent in the start and target quadrants (s).

4.3.2 Visual reversal learning test (II)

The reversal of a two-choice visual discrimination test assesses cognitive flexibility in rodents (Chudasama and Robbins 2003; Young et al. 2009; Gilmour et al. 2013). The task was carried out using six automated Bussey-Saksida rat operant chambers equipped with a touchscreen system. (Campden Instruments Ltd, Loughborough, UK; h×w×l: 29 cm × 13 cm back/20 cm front × 34 cm; Figure 4C). The back wall of the chamber was equipped with a pellet dispenser. The front wall of the chamber consisted of a wall-size touch-sensitive computer monitor. The monitor was covered with a mask that restricted the touch responses to two open areas on the screen, in which the images were displayed. A shelf was placed in front of the screen to facilitate the rat's attention to the stimuli and to prevent impulsive choices on the touchscreen (Bussey et al. 2008). The floor consisted of an aluminum grid. All operant boxes were housed within sound-attenuated chambers fitted with a ventilation fan. The boxes were controlled by ABET II software (Animal Behavior Environment Test system version 2.15, Whisker 4.4, Lafayette Instrument Co, Lafayette, IN, USA).

Male LH rats were used due to the better vision of these pigmented rats compared to albino strains (Prusky et al. 2002; Kumar et al. 2015b). The animals were kept on a restricted diet (maintained at 85–90% of the free feeding body weight) to increase the motivation to perform the food-rewarded test. The rats were habituated to operate in test chambers following the manufacturer's manual 'Rat Touch PD ABET II Manual V2 / Pairwise (Visual) Discrimination (PD) Task for Rat Touch Screen Systems and ABET II' (Campden Instruments; based on Bussey et al. 2008). The pre-training was used to shape the rats to touchscreen behavior and consisted of five stages: habituation, initial touch, must touch stimuli, must initiate and punish incorrect training.

Once the rats had completed the pre-training phase, they were trained on a two-choice visual discrimination, where one stimulus was the correct S+ and the other the was incorrect S-. A nose-poke to a correct stimulus resulted in a tone, tray light, and a 45 mg reward pellet (5TUL, unflavored, TestDiet, St. Louis, MO, USA). An incorrect response resulted in a 5-sec time-out period (house light on) followed by the

correction procedure. The correction trials were not counted as completed trials in the data analysis. The inter-trial interval (ITI) time after correct or incorrect response was 20 s. The left-right arrangement of the stimuli was determined pseudorandomly, with a constraint that a given stimulus could not appear on the same side of the screen on more than three consecutive trials. The criteria were 100 trials completed in 60 min (PCP study) or 70 trials in 40 min (ORM-13070 study), and 80% or more choice accuracy in two consecutive test sessions.

Once the rats reached the criterion of at least 80% choice accuracy, a reversal learning phase of 5 sessions was introduced. The reversal learning test was done in a similar way as visual discrimination, but S+ and S- were reversed (the previously correct image was now incorrect, and vice versa) and this order of stimuli was kept throughout the reversal learning phase. Before the start of the reversal learning phase, the rats were ranked on the basis of training sessions needed to reach the criterion of 80% choice accuracy. Then the rats were evenly assigned to treatment groups based on their ranking order. If a rat did not reach the criterion, it was discarded from the reversal learning experiment. A scheme of the study design is presented in Figure 4A,B. Each animal was tested in several reversal learning experiments. The animals of ORM-13070 study had been pre-exposed to PCP in a prior reversal learning experiment similarly as in PCP study.

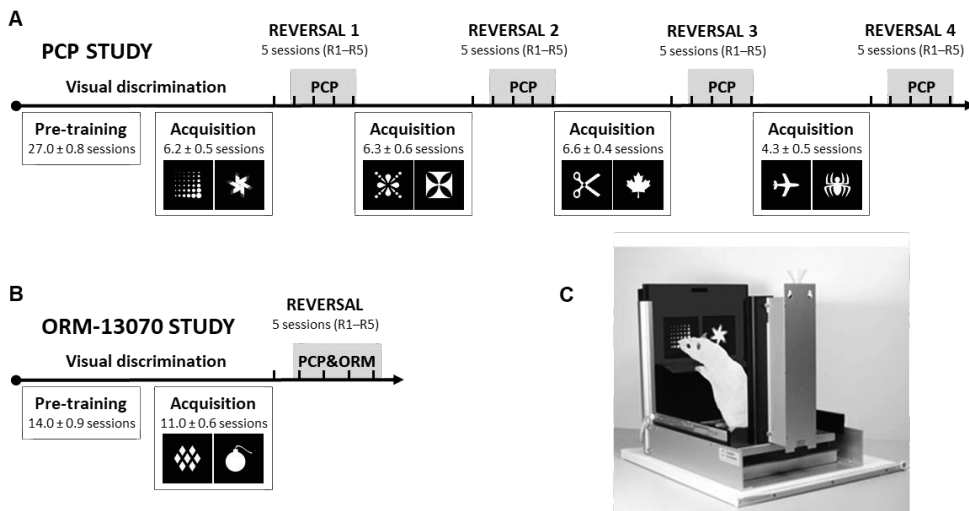


Figure 4. (A) PCP study: A scheme of the visual discrimination and reversal learning test. Four reversal learning experiments (reversals 1–4) were conducted using the same rats in every experiment. Each reversal learning experiment consisted of one daily test session and lasted 5 days. The rats were injected daily either with saline (2 ml/kg) or PCP 1.5 mg/kg (s.c.) starting on the 2nd reversal learning day (reversal learning sessions 2–5; R2–R5). The washout periods between the four PCP treatments were as follows: 31, 17 and 17 days, respectively. A new pair of images was used in every reversal learning experiment. (B) ORM-13070 study: A scheme of the experiment where the effect of a selective α_{2C} AR antagonist on PCP-induced reversal learning deficits was assessed. The PCP washout period before the experiment was 24 days. (C) The rat touchscreen apparatus with the trapezoidal-shaped side walls removed (Campden instruments Ltd; <https://www.campdeninstruments.com>).

The following variables were analyzed from reversal learning experiments: choice accuracy (% correct; correct trials / [correct + incorrect trials] *100), selection trials (correct + incorrect trials), correction trials, total trials completed (correct + incorrect + correction trials), response latency (correct touch latency), reward collection latency and ITI touches.

4.3.3 Social interaction test (III)

The social interaction test (File and Hyde 1978) is commonly utilized as a non-clinical model for the assessment of social functioning in rodents (Wilson and Koenig 2014). Male Wistar rats were transferred into single cages 4–7 days before testing to increase the social interaction behavior (Niesink and van Ree 1982). The social interaction test was performed in open field arenas (60 cm × 60 cm × 40 cm, illumination at the floor level 55–65 lx; Samplastic Oy, Kuopio, Finland) with video cameras mounted above each of the four arenas. On the test day, a pair of unfamiliar rats (matched body weight within 15 g) receiving the same pharmacological treatment, were placed in the opposite corners of an unfamiliar open field arena and their behavior was recorded for 10 min (Media Recorder, Noldus Information Technology). The animals were randomly assigned into treatment groups. With ORM-13070, three independent experiments were conducted. Each animal was tested only once in the social interaction test.

An experimenter blind to the treatments counted the number of preselected patterns of social interaction. These included sniffing the conspecific's snout or parts of the body (including anogenital region), following, walking around partner, climbing over or under, and mutual grooming were considered as social interaction whereas passive social contact or aggressive behavior were not counted as this kind of behavior. The locomotor activity of individual animals was analyzed automatically with EthoVision XT v. 8.5 software.

4.4 DATA ANALYSIS AND STATISTICS

IBM SPSS Statistics software v. 21 (I, III) and 25 (II) (IBM Finland, Helsinki, Finland) were used in statistical analyses. The results are expressed as mean ± SEM. The differences between treatment groups were considered statistically significant at the $p < 0.05$ level. The reported p values are from post hoc tests where appropriate, that is, when the main group comparison effect of ANOVAs are significant. More detailed statistical analyses can be found in the original articles (I–III).

4.4.1 Morris swim navigation (I)

The effect of treatments (PCP and test compounds) on task acquisition across four test days were assessed using analysis of variance for repeated measures (rmANOVA). Treatment effects on the search bias on the probe trial on day 5 were measured using one-way analysis of variance (ANOVA) or t -test. Tukey's test was

used in post-hoc comparisons. In the PCP dose-response experiment, the control groups of both rat strains were compared also separately to reveal strain-dependent differences in baseline performance.

4.4.2 Visual reversal learning test (II)

The main effect of drug treatment on test variables over reversal learning sessions (R2–R5; the sessions when the rats received pharmacological treatments) was analyzed with rmANOVA with a test session as a within-subjects factor and treatment as a between-subjects factor. The treatment effects on test variables in single reversal learning test sessions (R2–R5) were assessed using independent samples *t* test in the PCP study and ANOVA in ORM-13070 study. Tukey's test was used in post-hoc comparisons. The sessions needed to acquire new stimulus pairs were compared with independent samples *t*-test. The session by session analysis with *t*-test or ANOVA was performed only when the between-subjects effect of rmANOVA was significant.

4.4.3 Social interaction test (III)

A 7-min period between 3 and 10 min of each trial was analyzed since there were no differences in social interaction duration between treatment groups (SAL, PCP 1.15 and PCP 1.5 mg/kg) in the first 3 min of the 10-min test session in the initial analysis. The effect of treatments (PCP and test compounds) on social interaction duration and locomotor activity were assessed using ANOVA followed by Tukey's post-hoc test.

5 RESULTS

5.1 PCP-INDUCED COGNITIVE DEFICITS (I, II)

5.1.1 Visual learning and memory (I)

PCP induced spatial navigation deficits at lower doses in LH than in Wistar rats. Repeated PCP administration at 1.3, 1.6 or 2.0 mg/kg for 8 days dose-dependently impaired the task acquisition in LH rats (Figure 5). Already the lowest PCP dose (1.3 mg/kg/day) increased escape latency ($p<0.01$) during task acquisition (Figure 5A) and decreased the time spent in the platform area by 42% ($p<0.01$) during the probe test compared to controls (Figure 5E). The two higher doses (1.6 and 2.0 mg/kg/day) increased the time spent in the pool periphery compared to controls ($p<0.001$ and $p<0.01$, respectively) (Figure 5C) indicative of difficulties in adopting an efficient visuo-spatial learning strategy.

In contrast, only the highest PCP dose (2.0 mg/kg/day) impaired task acquisition in Wistar rats, increasing escape latency ($p<0.001$) (Figure 5B) and time spent in the pool periphery ($p<0.001$) (Figure 5D) but having no effect on search bias on the probe day (Figure 5F) compared to controls.

Since only the highest dose of PCP impaired MWM performance in both rat strains, a PCP dose 2.0 mg/kg/day was selected for studies where the effects of two atypical antipsychotic drugs, sertindole and risperidone, in reversing PCP-induced deficits were compared in LH and Wistar rats.

LH rats showed a better spatial memory compared to Wistar rats in MWM. There were no differences in the escape latency to the hidden platform ($p>0.05$) (Figure 5A,B) or in the time spent in the pool periphery ($p>0.05$) (Figure 5C,D) between saline-treated LH and Wistar rats during task acquisition. In contrast, there was a significant difference between LH and Wistar rats in the probe test where only LH rats displayed a clear search bias; LH rats spent 2.1-fold longer time in the platform area than Wistar rats ($p<0.001$) (Figure 5E,F).

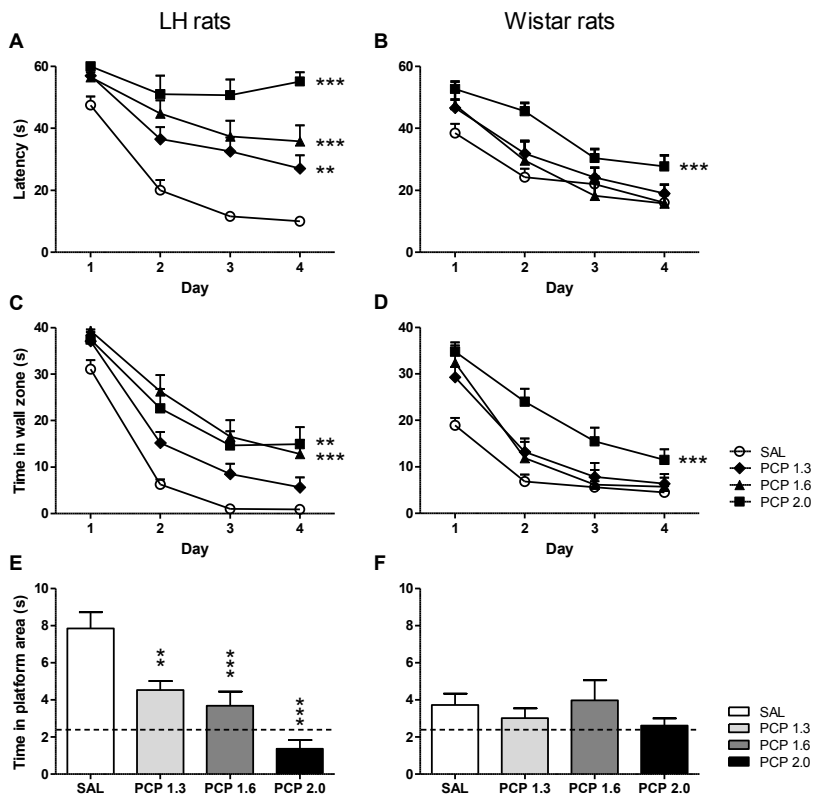


Figure 5. PCP-induced (s.c.) deficits in escape latency (A–B) and time spent in the pool periphery (C–D) during task acquisition, and time spent in the former platform area (diameter 30 cm) during probe trial (E–F) in Lister Hooded and Wistar rats. Task acquisition data (A–D) are presented as the mean of the three daily trials \pm SEM. Group differences are indicated with asterisks next to the last data point (rmANOVA). Probe trial data (E–F) are presented as mean \pm SEM. Group differences are indicated with asterisks above group data (ANOVA). *** $p < 0.001$, ** $p < 0.01$ vs. control group (Tukey post hoc). The horizontal dashed lines indicate the level of random performance. Group sizes are presented in Table 6. Abbreviations: LH – Lister Hooded, PCP – phencyclidine, SAL – saline (control).

5.1.2 Cognitive flexibility (II)

The acquisition and reversal of visual discrimination were repeated four times with the same animals using a new stimulus pair in each experiment (Figure 4A). This was done in order to evaluate whether PCP-induced deficits could be seen upon repetition which would increase the throughput and thus, feasibility of the test. Repeated PCP administration (1.5 mg/kg/day) for 4 days significantly decreased the choice accuracy of LH rats in all four reversal learning experiments ($p < 0.001$, $p < 0.001$, $p < 0.01$, $p < 0.01$, respectively) (Figure 6A–D). PCP significantly impaired choice accuracy in the fifth reversal test session by 24–50% (reversals 1–4: $p < 0.001$ –0.01) (Figure 6A–D) in every reversal learning experiment compared to the control group. The significant effect of PCP was consistently shown already in the fourth reversal session (reversals 1–4: $p < 0.001$ –0.01).

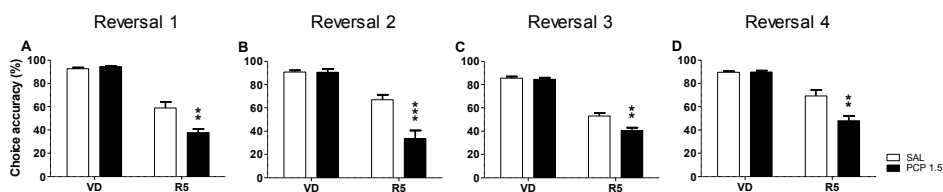


Figure 6. The effect of PCP (1.5 mg/kg/day, s.c.) on choice accuracy in reversal day 5 (A–D) during four reversal learning experiments in Lister Hooded rats. The 5-days' reversal of visual discrimination was repeated in four separate experiments using a new stimulus pair in each experiment. PCP and saline treatments were started on the second reversal session. Data are expressed as mean \pm SEM. Group differences are indicated with asterisks above group data. *** $p < 0.001$, ** $p < 0.01$ vs. control group (t -test). Group sizes are presented in Table 6. Abbreviations: PCP – phencyclidine, R5 – reversal session 5, SAL – saline (control), VD – mean value of the last two acquisition sessions before reversal (II).

Repeated PCP treatments had no effect on the acquisition of new stimulus pairs as there were no differences between the treatment groups in the numbers of sessions needed to learn new stimulus pairs (experiments 1–4: $p > 0.2$).

5.1.3 PCP-induced non-specific behavioral effects (I, II)

Morris swim navigation

During MWM task acquisition, the lowest dose of PCP (1.3 mg/kg/day for 8 days) slightly increased swimming speed compared to controls in LH and Wistar rats ($p < 0.05$).

Saline-treated LH rats swam faster than saline-treated Wistar rats ($p < 0.001$) during task acquisition while no difference was observed in the probe test ($p > 0.05$).

Visual reversal learning

Overall, PCP-induced non-specific behavioral effects were observed in the first 1–2 reversal learning sessions but not in the last two sessions in the visual reversal learning experiments. PCP decreased the number of total trials in the first three reversal learning experiments ($p < 0.01$ – 0.05) (Figure 7A–D). Furthermore, a significant increase in correct response latencies across test sessions was found in experiments 1 and 2 ($p < 0.01$ and $p < 0.05$, respectively). Instead, PCP had no effect on the reward collection latencies ($p > 0.06$) (Figure 7E–H) or on the number of screen touches during the ITI period ($p > 0.06$) indicating that there was no motivational impairment nor impulsive behavior in any of the visual reversal learning experiments.

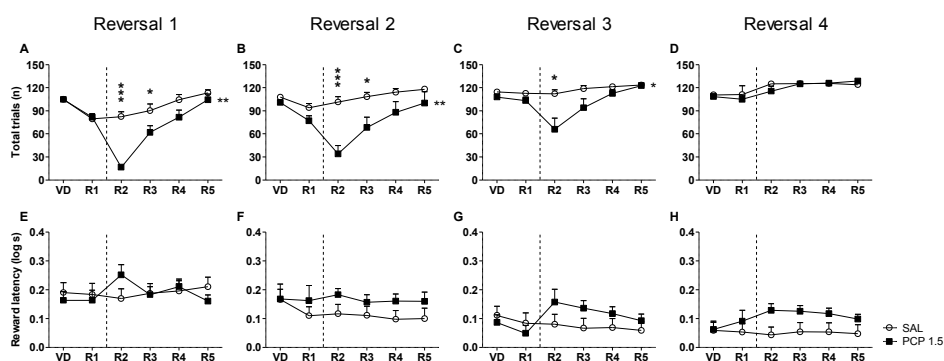


Figure 7. The effect of PCP (1.5 mg/kg/day, s.c.) on the number of total trials (A–D) and reward collection latencies (E–H) during four reversal learning experiments in Lister Hooded rats. PCP and saline treatments were started on the second reversal session (the vertical dashed lines). Data are expressed as mean \pm SEM. Group differences are indicated with asterisks next to the last reversal session data point (rmANOVA). Differences within single reversal learning test sessions are indicated with asterisks above data points (t -test). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. control group. Group sizes are presented in Table 6. Abbreviations: PCP – phencyclidine, R1–5 – the reversal sessions 1–5, SAL – saline (control), VD – mean value of the last two acquisition sessions before reversal.

5.2 PCP-INDUCED SOCIAL INTERACTION DEFICITS (III)

There were no group differences in the social interaction duration between PCP-treated and control rats in the first 3 min of the 10-min test session ($p>0.1$) (Figure 8A). During the 7-min period between 3 and 10 min, the lower PCP dose (1.15 mg/kg) decreased social interaction by 32% ($p<0.05$); at the dose of 1.5 mg/kg, the effect was more pronounced, i.e., a 47% reduction ($p<0.01$) was observed compared to the control group (Figure 8A). PCP had no effect on locomotor activity as indicated by the distance travelled during the social interaction task either during the first 3 min ($p>0.2$) or in the last 7 min period ($p>0.9$) (Figure 8B).

Since the PCP dose 1.5 mg/kg induced a more prominent defect on social interaction without significantly affecting locomotor activity, this dose was selected for further experiments with the novel drugs and the antipsychotics. These subsequent experiments also confirmed that the PCP-induced deficits at the dose level of 1.5 mg/kg were robust and repeatable throughout the experiments (reduction of social interaction time by 29–49% compared to controls) (Figure 11 and 12).

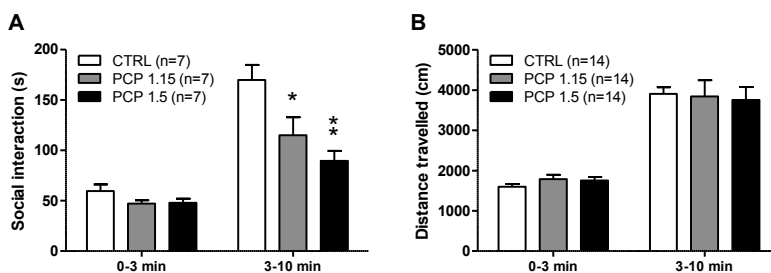


Figure 8. The effect of PCP (1.15 and 1.5 mg/kg, s.c.) on the social interaction behavior (A) and locomotor activity (B) between 0–3 and 3–10 min in Wistar rats. Data are expressed as mean \pm SEM. Group differences are indicated with asterisks above group data (ANOVA). ** $p < 0.01$, * $p < 0.05$ vs. control group (Tukey post hoc). Group sizes are presented in Table 6. Abbreviations: SAL – saline (control), PCP – phencyclidine.

5.3 REVERSAL OF THE PCP-INDUCED COGNITIVE DEFICITS (I, II)

5.3.1 Visual learning and memory (I)

An atypical antipsychotic, sertindole, partly reversed PCP-induced deficits in spatial navigation in LH rats. The sertindole-treated (1.6 mg/kg/day for 5 days) group had a shorter escape latency than the PCP-treated (2.0 mg/kg/day for 8 days) group ($p<0.01$) during the task acquisition (Figure 9A). In the probe test, the sertindole-treated group spent 2.2-fold more time in the platform area ($p<0.01$) (Figure 9C). Sertindole (1.6 mg/kg/day for 5 days) did not reverse PCP-induced deficits in Wistar rats (Figure 9B,D).

An atypical antipsychotic, risperidone (0.04 mg/kg/day for 5 days), did not reverse the PCP-induced (2.0 mg/kg/day for 8 days) deficits in any of the measured variables in LH (Figure 9E,G) or Wistar rats (Figure 9F,H).

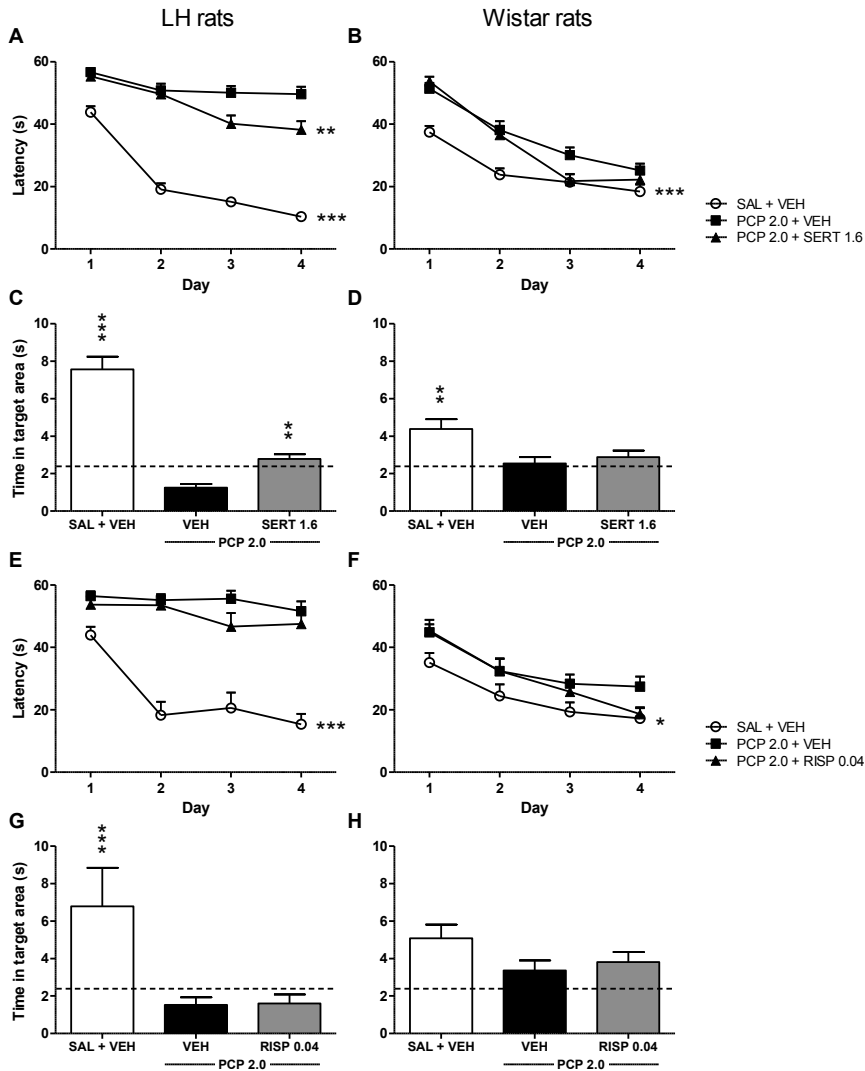


Figure 9. The effect of sertindole (1.6 mg/kg/day, s.c.) and risperidone (0.04 mg/kg/day, s.c.) on PCP-induced (2.0 mg/kg/day, s.c.) deficits in escape latency (A–B, E–F) and time spent in the former platform area (diameter 30 cm) during the probe trial (C–D, G–H) in Lister Hooded and Wistar rats. Task acquisition data (A–B, E–F) are expressed as mean of the three daily trials \pm SEM. Group differences are indicated with asterisks next to the last data point (rmANOVA). Probe trial data (C–D, G–H) are presented as mean \pm SEM. Group differences are indicated with asterisks above group data (ANOVA). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. PCP-treated group (Tukey post hoc). The horizontal dashed lines indicate the level of random performance. Group sizes are presented in Table 6. Abbreviations: LH – Lister Hooded, PCP – phencyclidine, RISP – risperidone, SAL – saline (control), VEH – vehicle.

5.3.2 Cognitive flexibility (II)

In the visual reversal learning test, an α_{2C} AR antagonist ORM-13070 (1.0 mg/kg/day for 4 days), ameliorated PCP-induced deficits in choice accuracy by 83% in comparison to the PCP-treated group ($p < 0.05$) on the fifth reversal day (Figure 10). Additionally, ORM-13070 increased ITI touches compared to saline-treated controls ($p < 0.05$), but not compared to the PCP-treated group on the fifth reversal day indicating increased impulsivity. ORM-13070 had no effect on any other measured variables.

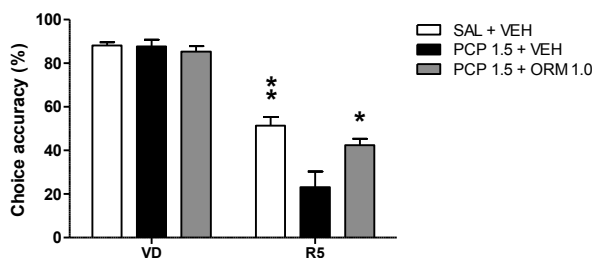


Figure 10. The effect of an adrenergic α_{2C} receptor antagonist, ORM-13070 (1.0 mg/kg/day, s.c.), on PCP-induced (1.5 mg/kg/day, s.c.) impairments in the last reversal learning session (R5) in Lister Hooded rats. PCP and saline treatments were started on the second reversal session. Data are expressed as mean \pm SEM. Group differences are indicated with asterisks above group data (ANOVA). ** $p < 0.01$, * $p < 0.05$ vs. PCP-treated group (Tukey post hoc). Group sizes are presented in Table 6. Abbreviations: ORM – ORM-13070, PCP – phencyclidine, R5 – the 5th reversal session, SAL – saline (control), VD – mean value of the last two acquisition sessions before reversal, VEH – vehicle.

5.4 REVERSAL OF THE PCP-INDUCED SOCIAL INTERACTION DEFICITS (III)

Atypical antipsychotics

None of the three selected atypical antipsychotic drugs, clozapine (2.5 mg/kg), risperidone (0.04 and 0.08 mg/kg) or olanzapine (0.125 and 0.5 mg/kg), were able to reverse the PCP-induced (1.5 mg/kg) deficits in the social interaction test ($p > 0.2$) (Figure 11). Furthermore, clozapine (2.5 mg/kg) and the higher doses of risperidone (0.08 mg/kg) and olanzapine (0.5 mg/kg) significantly decreased locomotor activity compared to the corresponding PCP groups by 63% ($p < 0.001$), 47% ($p < 0.001$) and 48% ($p < 0.01$), respectively.

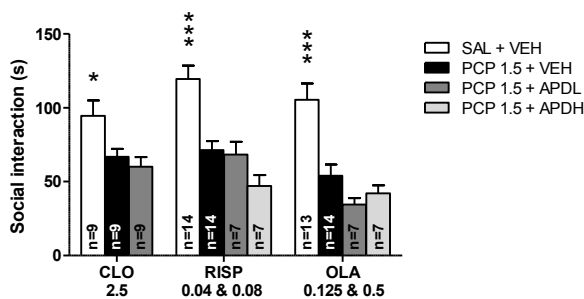


Figure 11. The effect of clozapine (2.5 mg/kg, i.p.), risperidone (0.04 and 0.08 mg/kg, s.c.) and olanzapine (0.125 and 0.5 mg/kg, s.c.) on PCP-induced (1.5 mg/kg, s.c.) social interaction deficits in Wistar rats. Data are expressed as mean \pm SEM. Group differences are indicated with asterisks above group data (ANOVA). *** $p < 0.001$, * $p < 0.05$ vs. PCP-treated group (Tukey post hoc). Group sizes are presented in Table 6. Abbreviations: APDH – higher dose of antipsychotic drug, APDL – lower dose of antipsychotic drug, CLO – clozapine, OLA – olanzapine, PCP – phencyclidine, RISP – risperidone, SAL – saline (control), VEH – vehicle.

Novel drugs

The higher dose of the α_2c AR antagonist ORM-13070 (1.0 mg/kg) significantly ameliorated PCP-induced (1.5 mg/kg) social interaction deficits by increasing the social interaction time by 49% ($p < 0.01$) but the lower dose (0.3 mg/kg) had no effect ($p > 0.7$) (Figure 12A). The α_7 nAChR partial agonist EVP-6124 (0.3 mg/kg) failed to reverse the PCP-induced deficits ($p > 0.8$) (Figure 12B). Neither ORM-13070 nor EVP-6124 had any effect on locomotor activity.

The effect of ORM-13070 1.0 mg/kg to reverse the PCP-induced social interaction deficits was assessed in three independent experiments to confirm the repeatability of the initial finding. These replicates confirmed that the effect of ORM-13070 was highly robust and repeatable, since it ameliorated the PCP-induced deficits by

increasing the social interaction time by 53% ($p < 0.05$), 53% ($p < 0.05$) and 40% ($p < 0.05$), respectively, compared to the corresponding PCP groups.

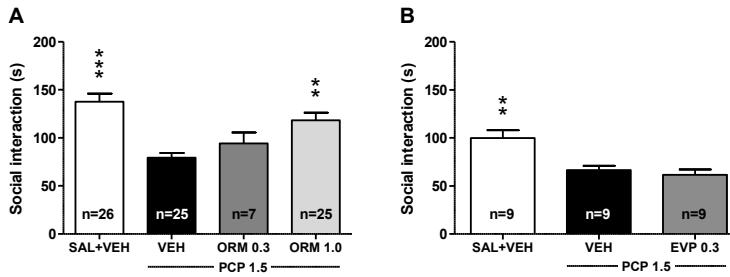


Figure 12. The effects of an adrenergic α_{2C} receptor antagonist ORM-13070 (0.3 and 1.0 mg/kg, s.c.) (A) and an α_7 nicotinic acetylcholine receptor partial agonist EVP-6124 (0.3 mg/kg, s.c.) (B) on PCP-induced (1.5 mg/kg, s.c.) social interaction deficits in Wistar rats. Data are expressed as mean \pm SEM. Group differences are indicated with asterisks above group data (ANOVA). *** $p < 0.001$, ** $p < 0.01$ vs. PCP-treated group (Tukey post hoc). Group sizes are presented in Table 6. Abbreviations: EVP – EVP-6124, ORM – ORM-13070, PCP – phencyclidine, VEH – vehicle.

6 DISCUSSION

6.1 GENERAL ASPECTS OF THE PCP ANIMAL MODEL (I–III)

6.1.1 PCP dosing regimen (I–III)

Administration protocol

It has been suggested that chronic PCP administration to rodents induces persistent alterations mimicking the long-term impairments of schizophrenia such as cognitive defects and negative symptoms (Jentsch and Roth 1999). However, the subchronic PCP model has proven challenging as it has not induced impairments in all behavioral tests, but instead, an acute effect has been needed. For example, repeated PCP induces visual learning and memory deficits in Morris swim navigation task in rodents when administered before the daily trials (Podhorna and Didriksen 2005; Wass et al. 2006a, b; Didriksen et al. 2007), but not when administered after the last daily trial (Podhorna and Didriksen 2005) or when given subchronically (Janhunen et al. 2015). Furthermore, repeated PCP administrations impaired cognitive flexibility in a visual discrimination and reversal learning task in rodents (Fellini et al. 2014), whereas subchronic PCP treatment has systematically failed to induce impairments in this task (Brigman et al. 2009; Fellini et al. 2014; Janhunen et al. 2015; McAllister et al. 2015). However, both subchronic (Sams-Dodd 1996; Pedersen et al. 2014; Neill et al. 2016; Tarland et al. 2018) and acute PCP administration as a single dose (Corbett et al. 1995; Sallinen et al. 2013) or as a single dose combined with a 2-day pretreatment (Sams-Dodd 1997; Bruins Slot et al. 2005) were reported to impair social interaction in rodents, whereas mainly acute administration of ketamine and MK-801 have been used (Maehara et al. 2011; Nikiforuk et al. 2016; Potasiewicz et al. 2017). In summary, repeated PCP administration seems to represent a feasible method for impairing especially the cognitive performance of rodents. It is suitable for many behavioral test protocols, and it has only minor non-specific behavioral effects which could hinder its use in non-clinical modeling.

Dosing

PCP is most often administered via the i.p. or s.c. route in rodents. However, the administration route has a considerable effect on the PCP exposure. It has been shown that subcutaneous PCP administration results in 3- to 5-fold higher brain concentrations, 3-fold higher areas under the time-concentration curve (AUC) in brain and plasma, and a 2-fold longer half-life ($T_{1/2}$) in the brain compared to intraperitoneal administration (Kalinichev et al. 2008). This is most likely due to extensive cytochrome P450-dependent metabolism in the liver. In our experiments,

the subcutaneous route was selected in order to achieve better bioavailability and brain exposure of PCP.

The PCP dose was selected based on previous studies with PCP (Corbett et al. 1995; Sams-Dodd 1996; Boulay et al. 2004; Podhorna and Didriksen 2005; Wass et al. 2006a, b; Didriksen et al. 2007; Talpos et al. 2012; Sallinen et al. 2013; Fellini et al. 2014) and our pilot experiments. Our aim was to achieve a robust impairment in selected behavioral tests assessing cognitive and negative symptoms without inducing confounding non-specific behavioral effects such as changes in sensorimotor functions or motivation. These non-specific behavioral effects have been most readily detected after acute single dose PCP administration (Sams-Dodd 1996; Gilmour et al. 2009; Dix et al. 2010b; Smith et al. 2011a). Nevertheless, many fully-automated, such as touchscreen-based tests, provide a wide range of measures to help also in the assessment of more subtle behavioral changes (Horner et al. 2013; Mar et al. 2013).

6.1.2 PCP-induced non-specific behavioral changes (I–III)

Morris swim navigation

In MWM, sensorimotor functions can be assessed by measuring swimming speed, excessive thigmotaxis (swimming around the pool in close contact to the wall), jump-offs from platform, and not climbing to or staying on platform when reached (Vorhees and Williams 2006). In our MWM study, swimming speed was used to assess differences in sensorimotor functions between PCP and control animals.

Higher PCP doses (1.6 and 2.0 mg/kg/day) had no effect on swimming speed of LH and Wistar rats. Regardless of the slight increase in swimming speed in the lowest (1.3 mg/kg/day) dose indicating a subtle stimulatory effect, PCP did not hamper the performance in MWM task. However, the results of previous studies differ, as PCP doses ≤ 2.0 mg/kg/day have had no effect on swimming speed in Wistar or Sprague Dawley rats (Podhorna and Didriksen 2005; Wass et al. 2006a; Didriksen et al. 2007). Only PCP 2.5 mg/kg/day increased the swimming speed of Wistar rats in the study conducted by Podhorna and Didriksen (2005), but the effect was lost when a 3-day pretreatment prior to the first acquisition day was used.

Visual reversal learning

As well as being useful in the assessment of cognitive functioning, automated touchscreen-based operant systems enable the monitoring of several different behavioral measures. Choice accuracy is a measure of cognitive flexibility in the visual discrimination and reversal test (Young et al. 2009; Mar et al. 2013; Gilmour et al. 2013). In addition, impulse control, motivation, and other cognitive domains, such as processing speed can be measured (Bussey et al. 1994; Robbins 2002; Horner et al. 2013; Mar et al. 2013). Furthermore, a close observation of the effects of food restriction helps to reduce variation in performance (Mar et al. 2013). In the present study, measures of all of the above-mentioned factors were assessed.

In reversal of visual discrimination, some suppression of behavioral activity was observed in the first couple of days of the PCP treatments, as indicated by the decreased total trials and the increased correct response latency compared to controls. PCP had no effect on reward collection latency indicating that there was no motivational impairment nor signals of impulsive behavior as there were no changes in ITI touches on the screen. This result is in line with the findings in the study of Fellini et al. (2014), where PCP (1.0 mg/kg/day) decreased the amount of total responses and increased response latency on the first day of administration but had no effect on reward collection latency.

These effects of PCP were diminished within one or two days leading to comparable results between treatment groups on the last two reversal days and were totally absent in the fourth reversal learning experiment. The effects could be explained by the transient non-specific behavioral suppression induced by acute PCP (Amitai et al. 2007; Amitai and Markou 2009a, b; Gilmour et al. 2009; Dix et al. 2010a; Smith et al. 2011b), which have been shown to attenuate with a 2- to 3-day pretreatment with PCP prior to behavioral testing (Podhorna and Didriksen 2005; Amitai et al. 2007). In conclusion, although the repeated PCP model induces transient non-specific behavioral suppression, a robust impairment in choice accuracy is evident also upon repetition.

Social interaction

In the social interaction test, non-specific behavioral effects of PCP can be determined by measuring locomotor activity of the rats, and by assessing the incidence of stereotyped behavior and ataxia (Sams-Dodd 1996). Our pilot experiments revealed no stereotyped behavior or ataxia in the open field test in male Wistar rats with the PCP doses 1–1.5 mg/kg (s.c.) used in our social interaction studies, and thus, only the locomotor activity was assessed.

Neither of the selected PCP doses induced changes in locomotor activity of the animals, and thus, did not confound the analysis of social interaction. Similar observations have been made in study of Wass et al. (2009), where acute subcutaneous injections of PCP 2.0 mg/kg did not affect locomotor activity in the social interaction test. However, also divergent results exist, as Sams-Dodd (1996) found a decrease in locomotor activity with two PCP doses i.e. 1.0 and 4.0 mg/kg. However, the higher PCP dose 4.0 mg/kg substantially increased both ataxia and stereotyped behavior of the rats, explaining that result.

6.1.3 Strain selection (I–III)

Morris swim navigation

Pigmented rat strains are commonly used to model schizophrenia-like cognitive deficits in tasks requiring good vision such as novel object recognition (Vigano et al. 2009; Redrobe et al. 2010; Snigdha et al. 2011; Savage et al. 2011) and touchscreen-

based cognitive tasks (Talpos et al. 2012, 2015; Fellini et al. 2014; Alsiö et al. 2015; Janhunen et al. 2015). Notwithstanding, all previous MWM studies to assess the effects of PCP on visual learning and memory have been conducted with albino Wistar or Sprague Dawley rat strains (Podhorna and Didriksen 2005; Wass et al. 2006a, b; Didriksen et al. 2007), which have much poorer vision compared to their pigmented counterparts (Prusky et al. 2000, 2002; Redfern et al. 2011; Entlerova et al. 2013; Kumar et al. 2015). Therefore, two outbred rat strains, the pigmented LH and commonly used albino Wistar, were compared to assess the suitability of the rat strains in PCP-induced model of schizophrenia-like visuo-spatial learning and memory deficits in Study I.

The results indicate that the performance of LH rats was superior to Wistar rats in MWM. During the probe test, LH rats focused their search on the platform quadrant and the former platform area, whereas Wistar rats showed no such preference indicating a lack of memory trace formation. Furthermore, Wistar rats could not improve their performance in the manner of the LH rats in the last two days of acquisition phase. A similar finding has been made also between pigmented Long Evans and albino Sprague Dawley rats (Tonkiss et al. 1992).

The acquisition phase of MWM not only assesses spatial learning and memory but rats also develop non-spatial learning strategies for platform finding (Whishaw 1985; Day and Schallert 1996). In contrast, in the probe test, rats relying on a spatial strategy (utilize the extra-maze landmarks) search for the platform from its original location but rats using non-spatial search strategies, like swimming around the pool at a certain distance from the wall to find the hidden platform, show no such preference (Whishaw 1985; Day and Schallert 1996). Even though Wistar rats learned to find the platform location during task acquisition, they were not able to utilize the extra-maze cues to devise a spatial search strategy.

Albino strains have also shown a preference for the former platform location during probe trials (Wass et al. 2006a; Quan et al. 2010; Ghotbi Ravandi et al. 2019; Sepehri et al. 2019). However, in general, a more intensive training during acquisition phase or a shorter interval between the last acquisition trial and the probe trial has been used. In addition, it is impossible to compare the visual cues used in different studies. Nonetheless, there are results where the facilitations of the test protocol have not resulted in any clear preference for the former target quadrant (Mohammadi et al. 2014; Zhang et al. 2018).

One aspect affecting the differences in baseline performance of the intact rats may be the poorer visual acuity of the albino Wistar rats in comparison with their pigmented LH counterparts (Prusky et al. 2000, 2002; Redfern et al. 2011; Entlerova et al. 2013; Kumar et al. 2015b; Leinonen and Tanila 2018). For example, in the study of Kumar et al. (2015b), albino strains had a poorer performance in a visually demanding touchscreen-based cognitive test when compared to pigmented strains but no impairment was detected between the strains in a visually less demanding spatial task. However, Harker and Whishaw (2002) found that poor water maze performance was not simply due to visual acuity of the animals, but was related also

to their inbreeding status, and thus, baseline cognitive abilities should not confound the comparison between outbred strains like LH and Wistar.

In MWM, saline-treated LH rats swam significantly faster than the saline-treated Wistar rats during the acquisition phase, that was a case also in another MWM study (Zhang et al. 2016). One explanation could be a naturally higher state of activity, as LH rats have shown a tendency for higher locomotor activity than their Wistar counterparts in open field tests (Broersen and Uylings 1999; Weiss et al. 2000).

Altogether, these findings challenge the widespread use of albino Wistar rats in MWM tasks due to their poorer baseline performance, which complicates the assessment of changes in spatial learning and memory after divergent manipulations. However, our assessment is restricted to only the Wistar strain in one test protocol, and thus whether this qualification extends to the use of other albino strains or protocols including more intensive training during the acquisition phase cannot be directly stated, since baseline cognitive abilities may vary between strains and also compensate for the weakness in their visual abilities.

Visual reversal learning

Pigmented rat strains have been shown to outperform albino strains in visually demanding touchscreen-based cognitive tasks like visual discrimination and reversal learning. It took more sessions for Wistar than LH and Long Evans rats to acquire the visual discrimination and also the reversal task, while Sprague Dawley rats failed to learn to discriminate between stimuli (Kumar et al. 2015b; Martis et al. 2018). Touchscreen-based tests have been typically conducted with pigmented LH rats (Talpos et al. 2012, 2014, 2015a, b; Fellini et al. 2014; Alsiö et al. 2015; Janhunen et al. 2015; Kumar et al. 2015a), and thus, pigmented LH rats were selected for the visual reversal learning experiments.

Social interaction

Albino rat strains have been commonly used to model schizophrenia-like negative symptoms in social interaction tests (Sams-Dodd 1997; Maehara et al. 2011; Sallinen et al. 2013; Kamińska and Rogóż 2015; Potasiewicz et al. 2017). Wistar and Sprague Dawley strains have been utilized in ketamine and MK-801 models (Maehara et al. 2011; Kamińska and Rogóż 2015; Potasiewicz et al. 2017), while in PCP models, also pigmented strains such as LH have been used (Sams-Dodd 1997; Snigdha and Neill 2008; Sallinen et al. 2013; Neill et al. 2016). The social interaction test is not visually demanding, and due to the fact that Wistar rats have been shown to be sufficiently sociable for the demands of the test, that strain was selected for the experiments in Study III.

6.2 PCP-INDUCED COGNITIVE DEFICITS (I–II)

Visual learning and memory are one of the key cognitive domains disturbed in schizophrenia (Nuechterlein et al. 2004). MWM (Morris 1984) is a commonly used behavioral test to assess visuo-spatial learning and memory in rodents. In Study I, PCP-induced deficits in spatial learning and memory in MWM were assessed and compared between two commonly used rat strains, pigmented LH, and albino Wistar rats.

Cognitive flexibility is another key cognitive domain disturbed in schizophrenia (Nuechterlein et al. 2004). Cognitive flexibility can be assessed in reversal learning paradigms in rodents (Young et al. 2009; Gilmour et al. 2013). Automated touchscreen-based pairwise visual discrimination and reversal task (Bussey et al. 2008; Mar et al. 2013; Gilmour et al. 2013) was used in the present studies. In this task, the effects of repeated acute PCP on visual reversal learning were delineated in LH rats (II). Despite the translationality of the touchscreen paradigm, the task has been considered time- and resource-consuming, partly due to the long pre-training phase. Repeated reversals of visual discrimination could make the test more feasible in testing novel drugs as the extensive pre-training phase could be utilized more thoroughly with an opportunity to compare within-subjects drug effects. Therefore, it was assessed whether repeated acute PCP impaired visual reversal learning in LH rats already after four days of administration, whether some confounding non-specific behavioral effects occurred and whether PCP-induced cognitive deficits could be seen upon repetition in the same animals.

6.2.1 PCP impairs visual learning and memory (I)

In the MWM test, when administered before the first daily trial, repeated PCP impaired task acquisition and probe trial performance in LH rats already with the lowest dose used (1.3 mg/kg/day, s.c.). Only the highest dose (2.0 mg/kg) affected the Wistar rats' performance in task acquisition, while there was no effect on the performance in probe trial in the PCP dose-response study. The increase in the time spent in the pool periphery with higher doses correlated with PCP-induced impairment in visual learning with both strains. Our results indicating that a PCP dose of at least 2.0 mg/kg in Wistar rats is required to induce impairments in navigation during the acquisition phase, but not in the probe test in MWM are consistent with previous findings (Podhorna and Didriksen 2005; Wass et al. 2006a, b). A PCP dose of 1.3 mg/kg (s.c.) was enough to increase the swimming distance to the hidden platform in one previous study (Didriksen et al. 2007). Wistar rats showed no memory trace formation after four days of acquisition, and thus, PCP's inability to affect probe performance is likely attributable to difficulties in acquiring the initial task. One plausible explanation for the divergent effects of PCP between strains in the dose-response study could be the differences in NMDA receptor occupancy and pharmacokinetics. There is some evidence that maximum plasma and brain tissue concentrations 15–30 min after administration of PCP 2.5 mg/kg (s.c.) are higher in

LH than Wistar rats (Gastambide et al. 2013). The results of the present study indicate that repeated PCP induces a robust deficit in spatial learning and memory of the LH rats, while it does not recommend the use of Wistar rats due to the poor baseline performance of these animals.

6.2.2 PCP impairs cognitive flexibility (II)

When administered before the daily reversal session, repeated PCP (1.5 mg/kg/day, administered from the second reversal session onward) impaired cognitive flexibility of LH rats in our visual reversal learning experiments already within four days of administration. This is in accordance with previous studies, where repeated or acute PCP induced deficits in choice accuracy in touchscreen visual reversal learning (Fellini et al. 2014) and classical operant reversal learning paradigms (Abdul-Monim et al. 2003; Idris et al. 2005, 2009). The control animals reached a 60–70% level of choice accuracy during five reversal sessions whereas 18 sessions were needed to reach the 85% choice accuracy level in the study of Fellini et al. (2014). Nevertheless, the magnitude of PCP-induced impairment in the choice accuracy remained constant between a 60% and 85% level of control performance, suggesting that PCP-induced deficits in cognitive flexibility could be investigated already when control animals had reached the 60% level of choice accuracy. Shorter reversals together with shorter PCP treatment periods partly diminish the risk of carry-over effects of PCP between repeated reversals.

In this study, we assessed the effects of PCP on repeated reversals of visual discrimination in rat touchscreen operant system using different stimulus pairs. PCP (1.5 mg/kg/day) induced a robust impairment in every four reversal learning experiments. A similar finding was made also by van der Meulen et al. (2003), where acute MK-801 (0.1 mg/kg) impaired serial operant reversal learning. However, repeated and serial reversal learning tasks are not completely parallel tests, nor are the effects of different NMDA antagonists directly comparable (Gilmour et al. 2009; Dix et al. 2010a; de Bruin et al. 2013). The question remains whether the transient behavioral suppression encountered in the first trials of the first reversal learning experiments could have been further diminished by a 2- to 3-day pretreatment period with PCP (Podhorna and Didriksen 2005; Amitai et al. 2007), and would this have affected reversal learning or acquiring with new stimulus pairs. Most importantly, the PCP-induced deficits remained stable indicating that a similar protocol could be utilized in a cross-over study design with novel compounds, which would improve the throughput of the test, and thus, the feasibility of using the visual discrimination and reversal test in these kinds of trials.

6.3 PCP-INDUCED SOCIAL INTERACTION DEFICITS (III)

Asociality is a common feature of patients with schizophrenia (Millan et al. 2014). A social interaction test (File and Hyde 1978) is commonly utilized as a non-clinical model of the asociality encountered in schizophrenia (Wilson and Koenig 2014). In the present study, the effects of acute PCP on social interaction behavior and locomotor activity were assessed in Wistar rats.

The acute PCP dose was optimized to induce robust social interaction deficits without affecting locomotor activity of the rats. Single PCP doses between 1.0–2.0 mg/kg have been used to induce social interaction deficits in rats (Corbett et al. 1995; Boulay et al. 2004; Savage et al. 2011; Sallinen et al. 2013). Thus far, acute single PCP doses lower than 1.5 mg/kg have been used only in experimental set-ups with a reversed light-dark cycle (Sams-Dodd 1996; Boulay et al. 2004; Sallinen et al. 2013). However, PCP has been shown to induce non-specific effects, such as stereotypy, ataxia and locomotor activity interfering with the social interaction of the animals already at a dose of 2.0 mg/kg (Castellani and Adams 1981; Sams-Dodd 1996; Boulay et al. 2004).

Both selected PCP doses (1.15 and 1.5 mg/kg) significantly reduced the time spent in social interaction but had no effect on locomotor activity. The higher dose was selected to maintain a robust effect due to some variation in the performance of the control group in subsequent experiments. In this study, neither reversal of light-dark cycle nor habituation to the test environment were used in order to increase the throughput of the test. The min-to-min analysis revealed that there were no differences between treatment groups in the social interaction nor in locomotor activity during the first minutes of the test. Thus, only the period between 3–10 min was used in the data analysis to improve the test's sensitivity.

We also repeated the social interaction test with the same animals after a few weeks but it seems, however, that control animals lose their interest towards the conspecifics which means that the PCP-induced defect is lost (data not shown). Accordingly, one preliminary finding was that with this protocol, social interaction tests cannot be repeated with the same animals. Wass et al. (2009) made a similar finding with control and PCP-treated rats. However, they conducted the second social interaction test already 24 h after the first one, and PCP treatment was given only during the first test. Nevertheless, this is an unfortunate limitation, because it prevents the possibility to make within-subject comparisons hindering the feasibility of the test protocol in non-clinical drug development.

Taken together, acute PCP administration can be used as a straightforward model to achieve robust impairments in social behavior without encountering confounding non-specific effects in locomotion.

6.4 REVERSAL OF THE PCP-INDUCED COGNITIVE AND SOCIAL INTERACTION DEFICITS (I–III)

The abilities of atypical antipsychotics (I, III) and novel drugs (II, III) to reverse the PCP-induced cognitive and social interaction deficits were assessed in the pharmacological schizophrenia animal models used in this thesis.

6.4.1 Atypical antipsychotics (I, III)

Visual learning and memory

Sertindole (1.6 mg/kg/day) partly reversed PCP-induced spatial navigation deficits during task acquisition in LH rats but not in Wistar rats. Similar findings have been made in previous studies, where sertindole reversed NMDA antagonist-induced spatial learning impairment in Wistar rats (Didriksen et al. 2007) and in mice (Mutlu et al. 2011) during task acquisition. It should be noted however, that even though sertindole significantly improved search bias during the probe trial as compared to LH rats receiving PCP alone, the performance only reached the random level and was well below that of the controls. Thus, in our study, sertindole had only a limited efficacy in reversing PCP-induced deficits in spatial learning and memory.

In contrast, risperidone (0.04 mg/kg/day) failed to reverse PCP-induced deficits in either strain during MWM task acquisition and probe trial. The risperidone doses of 0.02 and 0.08 mg/kg/day were also tested with no effect on PCP-induced deficits. Previous studies with risperidone have shown both efficacy (Didriksen et al. 2007; Celikyurt et al. 2011) and inefficacy (Enomoto et al. 2008) against NMDA antagonist-induced spatial learning and memory deficits. However, one can speculate whether a lower PCP dose would have resulted in a more favorable effect of risperidone in LH rats.

Sertindole and risperidone have multiple receptor targets, and their significant antagonistic effect on serotonin 5-HT_{2A} and 5-HT_{2C} receptors has been proposed to induce procognitive effects in patients with schizophrenia (Meltzer et al. 2003) and in animal models of schizophrenia (Bubenikova-Valesova et al. 2008). Differences in their receptor binding profiles may explain the better efficacy of sertindole in reversing PCP-induced visual learning and memory deficits. For example, sertindole has higher affinity for 5-HT₆ receptors (Arnt and Skarsfeldt 1998) which are highly expressed in hippocampus and cortex, and 5-HT₆ antagonism has been associated with cognition-enhancing effects (Meltzer et al. 2003; Hatcher et al. 2005; Hirst et al. 2006).

The present results further support the superiority of pigmented LH rats over albino Wistar rats in modeling visuo-spatial learning and memory deficits in MWM related to impairments encountered in patients with schizophrenia.

Social interaction

None of the three atypical antipsychotic drugs tested, clozapine (2.5 mg/kg, i.p.), risperidone (0.04 and 0.08 mg/kg, s.c.) or olanzapine (0.125 and 0.5 mg/kg, s.c.), ameliorated PCP-induced social interaction deficits in Wistar rats. Instead, higher doses of olanzapine and risperidone tended to further reduce the durations of social interaction while also exerting a detrimental effect on locomotor activity. As clozapine significantly decreased locomotor activity already at the first tested dose without affecting social interaction, higher doses were not tested. The effects of clozapine, risperidone and olanzapine on PCP-induced social interaction deficits have been shown to be variable (Corbett et al. 1995; Sams-Dodd 1997, 1998; Boulay et al. 2004; Sallinen et al. 2013). In addition, clinical findings have revealed the inadequate efficacy of atypical antipsychotic drugs against the negative symptoms of schizophrenia (Kirkpatrick et al. 2006; Sarkar et al. 2015). Nevertheless, it is not known which mechanisms of the multimodal atypical antipsychotics contribute to their efficacy against the negative symptoms.

6.4.2 Novel drugs (II, III)

An α_{2C} AR antagonist, ORM-10921, has shown some promising effects in ameliorating schizophrenia-like social interaction deficits, and visual learning and memory deficits in MWM test in NMDA antagonist rat models of schizophrenia (Sallinen et al. 2013). It also ameliorated visual learning and memory deficits in a novel object recognition task in a neurodevelopmental rat model of schizophrenia (Uys et al. 2016). Another α_{2C} AR antagonist, ORM-12741, has demonstrated some beneficial effects on episodic memory as an add-on treatment with acetylcholinesterase inhibitors in a proof-of-concept trial in Alzheimer's disease patients (Rinne et al. 2017). However, the effect of selective α_{2C} AR antagonist in reversing PCP-induced cognitive deficits in reversal of visual discrimination had not been investigated previously.

Full and partial agonists together with type I and II positive allosteric modulators at α_7 nAChRs have either reversed or alleviated the NMDA-antagonist-induced schizophrenia-like social interaction deficits in rodent models (Pedersen et al. 2014; Nikiforuk et al. 2016; Potasiewicz et al. 2017). The effect of a partial α_7 nAChR agonist on acute PCP-induced social interaction deficits had not been investigated previously.

Cognitive flexibility

The α_{2C} AR antagonist, ORM-13070, significantly ameliorated PCP-induced visual reversal learning deficits on the last reversal day. A trend towards an ameliorating effect of ORM-13070 was observed already on the first administration day and the effect was sustained throughout the first three days of the treatment period. ORM-13070 also increased the ITI touches compared to saline-treated control group which, however, did not prevent the improvement in choice accuracy. ORM-13070 had no effect on the other measures reflecting non-specific behavioral effects.

In humans, a dysfunctional PFC has been associated with impairments in cognitive flexibility in patients with schizophrenia (for a review, see Waltz 2017), while in rodents, especially orbitofrontal cortex (Bohn et al. 2003; Chudasama and Robbins 2003; McAlonan and Brown 2003; for a review, see Dalley et al. 2004) and corticostriatal circuitry (Brigman et al. 2013) are involved. Possible mechanisms underlying the procognitive effects of α_{2c} AR antagonists might be the facilitation of firing of the dopaminergic neurons in the VTA, where the mesocortical projections originate (Rosin et al. 1996; Inyushin et al. 2010; Sallinen et al. 2013). On the other hand, α_{2c} ARs can directly regulate DA release at the terminal level in the PFC (Ihalainen and Tanila 2002). It has also been suggested that in cortical and hippocampal regions, α_{2c} autoreceptors modulate dopamine and noradrenaline synthesis by inhibiting tyrosine hydroxylase (Esteban et al. 1996; Uys et al. 2017). Tyrosine hydroxylase is the enzyme converting tyrosine into 3,4-dihydroxyphenylalanine (DOPA), a precursor of dopamine, which can be converted into noradrenaline subsequently. Thus, α_{2c} AR antagonists could facilitate dopamine and noradrenaline synthesis. The enhanced DA turnover in these regions could ameliorate cognitive deficits resulting from dopaminergic hypofrontality. Furthermore, α_{2c} ARs have been postulated to modulate cholinergic and GABAergic neurotransmission in striatal regions (Holmberg et al. 1999; Zhang and Ordway 2003). However, the precise mechanism by which α_{2c} AR antagonists exert their procognitive effects remains to be resolved.

These results support the previous non-clinical and clinical findings suggesting that α_{2c} AR antagonism may possess procognitive potential in the treatment of schizophrenia.

Social interaction

The α_{2c} AR antagonist ORM-13070 significantly ameliorated PCP-induced social interaction deficits similarly as in a previous study conducted by Sallinen et al. (2013), whereas the α_7 nAChR partial agonist EVP-6124 had no such effect. The dopaminergic hypofunction in cortical regions has been closely associated with pronounced negative symptoms in patients with schizophrenia (Winograd-Gurvich et al. 2006; Schwartz et al. 2012). Thus, similar mechanisms could underlie the efficacy of α_{2c} AR antagonist against cognitive as well as negative symptoms, although the precise mechanism remains unknown. There is a discrepancy between the present results with the α_7 nAChR partial agonist and the previous literature of the α_7 nAChR modulating agents (Pedersen et al. 2014; Nikiforuk et al. 2016; Potasiewicz et al. 2017). One possible explanation is that the dose selection in the present study was not optimal, as evidence for an inverted U-shaped dose-response profile for EVP-6124 was found in a rat microdialysis study (Huang et al. 2014).

Taken together, these results further support the hypothesis that α_{2c} AR antagonism could have a role in ameliorating the asociality evident in schizophrenia.

7 CONCLUSIONS

Based on the results of the experimental section (Studies I–III), the following specific conclusions can be drawn:

1. The lowest possible PCP dose inducing cognitive and negative symptoms with minimal confounding non-specific behavioral effects in different models needs to be adjusted in laboratories before conducting any experiments. However, the method must also be robust enough to reach repeatability, thus making the model feasible for testing novel drugs.
2. Pigmented LH rats show better visuo-spatial learning and memory in MWM and lower PCP doses are needed to impair their performance compared to albino Wistar rats with known visual defects. In addition, sertindole was able to ameliorate the PCP-induced deficits only in LH rats. Thus, the visual learning and memory deficits encountered in patients with schizophrenia are better modeled by repeated PCP in LH rats than in Wistar rats.
3. Short, 4-day repeated administrations of PCP robustly induce impaired cognitive flexibility in LH rats in at least four consecutive repeated reversal learning experiments with novel stimulus pairs. This protocol improves the throughput of the test and provides an opportunity for a cross-over study design making it more feasible for testing novel drugs.
4. Acute low-dose PCP induces a robust impairment in social interaction without the need for prior habituation or a reversed light-dark cycle. This seems to provide a straightforward method for testing novel drugs.
5. All the models revealed that the PCP-induced cognitive and social defects can be reversed, at least partially. As there is no “gold standard” or positive control in the treatment of cognitive or negative symptoms of schizophrenia, it is impossible to thoroughly validate the present PCP schizophrenia models. As the effects of available antipsychotic drugs on cognitive and negative symptoms in patients with schizophrenia are limited, one should not expect significant efficacy in non-clinical models. However, the feasibility of the models, especially in spatial learning and memory, and visual reversal learning, have taken a step further to help in the assessment of novel drugs. Furthermore, the results of this thesis have strengthened the previous findings of the potential of an α_2c AR antagonism to ameliorate schizophrenia-like cognitive and negative symptoms.

The further validation of the models used in this thesis would require an assessment of their reproducibility in different laboratories; a feature listed as a strength of fully-automated touchscreen-based tests. In repeated visual reversal learning tasks, PCP administration with a 2- to 3-day pretreatment should be tested to further diminish the non-specific suppressive effect of PCP on rodent behavior. No carry-over effects

were observed in acquisition or reversal phases with our 4-day treatment, and this should be evaluated also with the longer treatment period. The MWM task could be further refined by using a slightly lower PCP dose, which was sufficient to induce spatial learning and memory deficits in LH rats. Furthermore, the effect of α_{2C} AR antagonism on other cognitive domains beyond cognitive flexibility should also be assessed. Finally, as it has been suggested that there is sexual dimorphism in the cognitive abilities, the experiments should be conducted with male and female rats.

Overall, novel drugs should be tested against deficits in different neurocognitive or negative symptom domains to assess their specific activity profile, and the results should be accompanied with relevant neuropathological data. Furthermore, clinical trials should assess the effects of the drugs in separate cognitive and negative symptom domains. Thus, also the predictive validity of different animal models could be improved.

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Currently available pharmacotherapies for schizophrenia mainly affect the positive symptoms, while there is no effective treatment for the cognitive and negative symptoms. Valid animal models are needed in the non-clinical development of novel drugs. Schizophrenia-like symptoms can be induced by NMDA antagonists such as phencyclidine (PCP). In this thesis, PCP animal models were developed and validated for the assessment of novel drugs for cognitive and negative symptoms of schizophrenia.



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